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Development of non-contacting high throughput sensing to determine drought stress in wheat and maize

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AB:	Aerial biomass
CT:	Canopy temperature
CWC:	Canopy water content
CWM:	Canopy water mass
DW:	Dry weight
EWT:	Equivalent water thickness
F690/F730:	Fluorescence ratio
F690:	Fluorescence intensity at 690 nm
F730:	Fluorescence intensity at 730 nm
F_m :	Maximum chlorophyll fluorescence
F_S:	Steady state chlorophyll fluorescence
FW:	Fresh weight
GIS:	Geographical Information System
GPS:	Geographical positioning system
LAI:	Leaf area index
LWC:	Leaf water content
LWP:	Leaf water potential
MIR:	Middle infrared
NDVI:	Normalized difference vegetation index
NDWI:	Normalized difference water index
NIR:	Near infrared
PRI:	Photochemical reflectance index
PSI:	Photosystem I
PSII:	Photosystem II
R:	Reflectance
R^2 :	Coefficient of determination
REIP:	Red edge inflection point
R_{fd}:	Variable chlorophyll fluorescence
RWC:	Relative water content
SD	Standard deviation
SWC	Soil water content
SWIR:	Shortwave Infrared
VIS:	Visible spectra
WI:	Water index

1 INTRODUCTION

Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world (Passioura, 2007; Richards et al., 2010). Plant drought stress refers to the condition in which plant cells and tissues are at less than full turgor. This occurs whenever the loss of water by transpiration exceeds the rate of water absorption (Kramer and Boyer, 1995). It can happen when soil moisture availability is low, limiting the supply of water to the roots; it can also happen when environmental temperature or irradiance level is high, causing high evaporative load (Schmidhalter et al., 1998a; Timergalina et al., 2007). With the occurrence of drought stress, almost all of the processes associated with plant growth are affected. The effects may vary with the degree and the duration of drought as well as with the growth stage of the plant (Bradford and Hsiao, 1982; Siddique et al., 2000).

Plant water status provides information that can be used to prevent crop water deficit through irrigation (Koksal, 2008), to select genotypes in breeding (Munjal and Dhanda, 2005), and to assess crop growth under drought conditions (Tucker, 1980; Penuelas et al., 1993). Different methods can be used to determine the water status in plants such as leaf water potential, relative water content, leaf water content, canopy water content, canopy water mass, aerial biomass and canopy temperature (Boyer et al., 2008; Linke et al., 2008; Feng et al., 2010; Gutierrez et al., 2010; Wang et al., 2010). All these plant physiological parameters were used to estimate the water status in crops in the present study.

As plants are exposed to drought, this leads to noticeable decreases in leaf water potential and water content followed by a concurrent increase in leaf and canopy temperatures.

This is the associated with decreased photosynthetic rate resulting from stomatal closure (Bradford and Hsiao, 1982; Schmidhalter et al., 1998b; Siddique et al., 2000).

The early detection of these stress factors with non-destructive methods is crucial because it could help to identify stress status at larger temporal and spatial scales before any damage is clearly visible (Zarco-Tejada, 2002). In this regard, precision agriculture and precision phenotyping technologies for crop management have the potential to provide more information for making more informed management decisions on a canopy scale in real time (Bredemeier and Schmidhalter, 2005; Mistele and Schmidhalter, 2008a & 2010; Thoren and Schmidhalter, 2009; Gutierrez et al., 2010; Winterhalter et al., 2011). This is in stark contrast to classical methods such as pressure chambers and oven drying, which are time-consuming and require numerous observations to characterise a field. Similar to that, for detecting water relation in the soil, numerous observations are required to characterize a field. For the same reasons, classical methods are unsuited to tracking frequent changes in environmental conditions, which requires rapid measurements.

The simplified, rapid assessment of the plant water status or related properties such methods enable are not only useful for irrigation management purposes, but would also allow for the efficient screening of large populations of plants as part of a high-throughput system to precisely evaluate the phenotype for breeding purposes (Schmidhalter, 2005a; Sirault et al., 2009; Winterhalter et al., 2011).

Over the past few years, proximal/remote sensing techniques have been used as very useful tools to precisely monitor crops throughout their growing period to support decisions for good agricultural practices by taking advantages of numerous available technologies, such as geographic positioning system, electromagnetic induction, aerial imagery, thermography, reflectance sensing and laser-induced chlorophyll fluorescence sensing (Schmidhalter et al., 2006; Mistele and Schmidhalter, 2008 a & b, 2010; Thoren and Schmidhalter, 2009; Thoren et al., 2010). These techniques could potentially contribute to enhance selection procedures of water status in plants because they are very cost-effective, allow for rapid vegetation measurements with non-invasive sampling, and provide detailed spatial data on the variability of plant development (Bredemeier and Schmidhalter, 2001, 2003; Schmidhalter, 2005a).

In the present study, we have used non-contacting techniques such as a passive reflectance sensing, laser induced chlorophyll fluorescence sensing, thermal near infrared sensing and geographic positioning system (GPS) for detecting plant drought stress of wheat and maize under controlled growth chamber conditions as well as wheat under field conditions.

1.1 Spectral reflectance measurements

Plant water status can be assessed remotely by measuring canopy reflectance indices, since they change in response to crop water content (Peñuelas et al., 1997; Ustin et al., 1998; Stimson et al., 2005). Several spectral regions are useful for the detection of water stress. In one of the earliest reports, Wolley (1971) identified the visible spectra (VIS; 400-700 nm) as being suitable for this purpose. Reflectance changes in the near infrared region (NIR; 700-1300 nm) can also be used for the detection of water in biological samples because the NIR penetrates more deeply into the measured structures than middle infrared (MIR; 1300-2500 nm). As such, the reflectance indicates the water content more of the entire sample rather than of water located in the uppermost layers

(Peñuelas et al., 1993). In the MIR, the strongest absorption properties of water molecules are found at 1450, 1940 and 2500 nm (Carter, 1991).

Canopy reflectance data have been proven to be a potential source to estimate several canopy variables related to physiological parameters. Previous research has shown that spectral measurements can estimate water status in plants (Peñuelas and Inoue, 1999; Ruthenkolk et al., 2001; Graeff and Claupein, 2007; Kakani et al., 2007; Yonghong et al., 2007; Seelig et al., 2008; Wu et al., 2009). But results from the literature for the remaining spectral indices are mixed and the assessment of their potential for measuring plant water status is complicated by the use of different plant species and experimental conditions. Spectral reflectance is affected by many factors under field conditions because crop reflectance 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; Mistele and Schmidhalter, 2008 a & b). Additionally, spectral characteristics of plants vary across plant development. Thus, it remains unclear whether changes in leaf water potential per se can reliably be detected spectrometrically or whether such measurements also reflect autocorrelated changes in the leaf water content (LWC) or the aerial plant biomass. Therefore, we tested the ability of spectrometric reflectance measurements in this context under controlled conditions that minimized perturbing influences but allow for significant changes in the leaf water potential of wheat and maize. Although a lot of studies were established to detect water status in plants by spectral measurements, it was found that water stress has to be well developed in order to be detectable by spectral reflectance (Carter, 1991; Cohen, 1991; Penuelas et al., 1993, 1997; Pu et al., 2003; Graeff and Claupein, 2007). Therefore, we tested the ability of spectrometric reflectance measurements at darkroom to detect water status in wheat and maize under different water regimes at leaf and canopy level either under high stress in wheat or low stress in maize at leaf and canopy level.

Several studies evaluated relationships between spectral indices and leaf or canopy water content. Some indices showed great potential to detect leaf or canopy water content such as the normalized difference water index $NDWI_{1640}$ and the normalized difference water index $NDWI_{2130}$ (Yonghong et al., 2007), water index (R_{900}/R_{970}) (Peñuelas et al., 1993), normalized difference vegetation index (NDVI) and normalized difference water index $NDWI_{1200,}$ $NDWI_{1450,}$ $NDWI_{1940}$ (Wu et al., 2009) the wavelength range 510 - 780, 540 - 780, 490 - 1300, 540 - 1300 nm (Graeff and Claupein, 2007), R_{850}, simple ratio (R_{810}/R_{560}) and red edge inflection point (REIP) (Behrens et al., 2006).

Few studies have evaluated relationships between spectral indices and leaf water potential. The spectral reflectance of wheat leaves has been reported to be closely related to changes in leaf water potential (LWP) under growth chamber conditions, with the best correlation of LWP to reflectance being found for the normalized difference vegetation index (NDVI; global $R^2 = 0.81$), and at wavelengths of 1450 nm ($R^2 = 0.92$) (Ruthenkolk et al., 2001). Kakani et al. (2007) found that the simple reflectance ratio, R_{1689}/R_{1657} was significantly related to LWP in cotton ($R^2 = 0.68$). 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). By contrast, the photochemical reflectance index (PRI) did not exhibit consistent relationships with the LWP of olive (Suárez et al., 2008). Weak relationships were also observed between LWP in *Populus* ssp. Either the water index (WI) or red edge inflection point (REIP) at the leaf and canopy level under controlled conditions (Eitel et al., 2006).

Overall spectral reflectance and spectral indices, have been conducted by using handheld sensors on the leaf level or on canopies grown under controlled conditions in growth chamber, greenhouse and under field conditions with different cultivars and environmental conditions. As well as, measurements in the nadir are commonly used for all canopy reflectance measurements with different sensor configurations. In addition, for measuring in the nadir, scientists often use a spectralon reflectance standard to the sun radiation instead of simultaneous measurements. There is always a time shift between sun radiation measurements and canopy reflectance measurements. If the radiation conditions are not totally stable, it may result in an error within the measurements. Thus, it is necessary to develop a potentially universal method and to identify physiological parameter that can be used for the evaluation of drought stress of wheat under field conditions. The effects of external factors must be decreased because the climate under German field conditions is unstable and rapidly changing. In this study, a passive reflectance sensor linked to four optics in one light fiber to create an optical mixed signal from four fields of view at different directions was used in 2006 and 2007. Reflectance signals of the four optics were averaged, so it may be nearly constant at any solar zenith angle. In addition, in 2008, a passive sensor was used to measure canopy reflectance and sun reflectance under the same conditions, either sunny or cloudy, to prevent the error within the measurements. The data were combined from two passive sensors to show the best indices which can be used under changing field conditions.

1.2 Laser-induced chlorophyll fluorescence sensing

Proximal remote sensing systems depending on laser-induced chlorophyll fluorescence 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 and Lichtenthaler; Buschmann and Lichtenthaler, 1998). Fluorescence has been used successfully to detect both water stress (Lichtenthaler, 1990; Dahn et al., 1992; Günther et al., 1994; Schweiger et al., 1996; Lichtenthaler et al., 2000; Apostol et al., 2003; Bredemeier and Schmidhalter, 2005; Zhang et al., 2005) and ozone stress (Rosema et al., 1992, 1998) in plants. However, while informative, the results of the majority of these studies are often not directly transferable to the goal of detecting water stress at the canopy level in the field because they focused on CO_2 fixation, photosynthetic activity, or the daily cycle of fluorescence and/or because the results were dependent on the change in nitrogen levels under water stress. More importantly, 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 as well as the mean lifetime of the laser pulse (which indicates faster energy transfer in water-stressed plants at the leaf level) are good indicators of the water content and water potential of maize.

Similarly, few studies have investigated the ability of chlorophyll fluorescence to detect dry biomass at different levels of nitrogen fertilisation under field conditions (Bredemeier and Schmidhalter, 2003; Thoren and Schmidhalter, 2009). There have been a number of studies done to determine the relationships between the fluorescence red/far red ratio and temperature (between 5°C to 25°C) under controlled conditions (Agati et al., 1996; Cerovic and Moya, 1999; Agati et al., 2000; Bredemeier and Schmidhalter, 2003).

In this study, we attempted to link changes in both chlorophyll fluorescence (based on two peaks at 690 nm and 730 nm) and the biomass index (Thoren et al., 2009) with physiological parameters indicating stress in plants in either the short (leaf water potential, LWP; canopy temperature, CT) or the long term (canopy water content, CWC; canopy water mass, CWM; aerial biomass, AB) under field conditions. A particular concern was the study of the stability of proximal sensing measurements of laser induced chlorophyll fluorescence to determine drought stress in wheat under field conditions throughout different development stages of cultivars and to determine which chlorophyll fluorescence and physiological parameters provided the most accurate estimates of plant drought stress.

1.3 Thermal near infrared sensing based on canopy temperature

Canopy temperature is a useful indicator of crop water stress and can also be used for making timely irrigation scheduling decisions. The use of canopy temperature to detect drought stress in plants is based upon the assumption that transpired water evaporates and cools the leaves to a level below the temperature of the surrounding air. As water becomes limited, transpiration is reduced, and leaf surface temperature will gradually become warmer than the air temperature because of absorbed radiation (Jackson et al., 1981; O'Toole et al., 1984).

Extensive work has been done on the relationships between plant temperatures and plant water stress. Leaf-canopy temperatures were found to be a reliable indicator of plant water stress (Ehrler, 1973; Blad and Rosenberg, 1976; Sandhu and Horton, 1978; Hatfield, 1979; Blum et al., 1982; Kumar and Tripathi, 1990; Cohen et al., 2005; Gutierrez et al., 2010). Cohen et al., (2004) found good relationships between LWP and leaf temperature during two months (July, R^2 = 0.73 and August, R^2 = 0.87) in cotton. In addition, Gutierrez et al., (2010) found, that there was a strong relationship between canopy temperature and leaf water potential of the investigated cultivars across a broad range of values (-2.0 to - 4.0 MPa).

Leaf water potential and plant water content can reflect the water stress level of plants by classical measurements, but they require numerous observations and are time-consuming. Therefore, if the changes in leaf water potential and plant water content can be assessed by measuring the changes in canopy temperature to distinguish between water stress treatments and cultivars under temperate field condition, we may substitute these methods by using easy and quick canopy temperature measurements.

In this study, thermal near infrared temperature sensing was used to estimate leaf water potential, canopy water content and canopy water mass of four winter wheat cultivars under field conditions to see, if these parameters can be reflected by the change in canopy temperature.

1.4 The objectives of this study were;

- Testing spectral reflectance measurements to obtain changes in leaf water potential without changes in aerial biomass;
- Development of high throughput spectral reflectance methods that can be used for the determination of drought stress of winter wheat under field conditions and to identify, which spectral indices and physiological parameter can provide an estimate of plant drought stress;
- Assess the accuracy of spectral reflectance indices for estimating small changes in the water status of plants;
- Studying the stability of proximal sensing measurements of laser induced chlorophyll fluorescence to determine drought stress in wheat under field conditions throughout different development stages of winter wheat and to identify, which chlorophyll fluorescence parameters and physiological parameter can provide an estimate of plant drought stress;
- Testing measurements of canopy temperature to detect changes in leaf water potential and plant water content under field conditions.

2 MATERIAL AND METHODS

The experiments were conducted between 2005 - 2009 under controlled conditions (growth chamber, greenhouse and dark room) and field conditions (mobile rain-out shelter) at the research station of the Chair of Plant Nutrition at the Technische Universität München in Dürnast. Non-contacting high throughput sensing methods such as passive reflectance sensors, laser induced chlorophyll fluorescence sensor, near infrared temperature sensor linked with GPS unit were used in this study to determine drought stress in wheat and maize by measuring the leaf water potential, leaf water content, relative water content, canopy water content, canopy water mass, aerial biomass and canopy temperature.

2.1 Growth chamber experiments to measure the change in leaf water potential and leaf water content of wheat and maize by using spectral reflectance measurements

Spectral reflectance measurements of wheat and maize were taken under control conditions to measure the change in leaf water potential without change in biomass, and small changes in leaf water content as well as to minimize the effects of environmental factors.

2.1.1 Experimental setup

Wheat (*Triticum aestivum* cv. Triso) and maize (*Zea mays* cv. Agromax) were previously cultivated in large containers (H x L x W = 70 x 100 x 55 cm) under greenhouse conditions until both had reached growth stage BBCH 33 (as characterized by 3rd nodes being detectable above crown node stem in both plant species and also by the stem of wheat (rosette) reaching 30% of its final length (diameter)) corresponding to the phase of stem elongation (Zadoks *et al.* 1974). A greenhouse equipped with removable roofs that were opened whenever no rainfall occurred. Therefore, except on rainy days, the spectral characteristic of the incident radiation was comparable to outside conditions. This allowed obtaining plants that were resembling field-grown plants. For the second and third measurement cycle plants were used that were 6 and 19 days, respectively, advanced, reaching growth stage BBCH 37 at the third measurement cycle. At the respective developmental stages, the containerized plants were moved three hours prior to the measurements into the growth chamber where controlled conditions were established and kept there for two to three hours depending on the experimental conditions (Table 1). Therefore, we expect that no morphological changes would occur.

The seeding rates for wheat and maize were increased to 636 and 218 per m^2, respectively, to cover the sensed area and decrease the reflectance caused by soil background. Conventional seeding rates under field conditions frequently vary in Western Europe between 200 - 450 and 8 - 12 per m^2 for wheat and maize, respectively. The soil used for the experiment was characterized as a silty loam with pH (CaCl2) 6.6. Based on previous determinations of the soil residual nitrogen content (Schmidhalter, 2005b), 120 kg ha^{-1} N was applied as calcium ammonium nitrate before sowing. All other nutrients were supplied in amounts to ensure adequate growth.

Spectral measurements as well as the determination of both leaf water content (LWC) and leaf water potential (LWP) were then carried out under growth chamber conditions. Before the onset of the measurements, plants were dark-adapted for three hours. For each measurement cycle, two containers with either wheat or maize were used, one for the non-destructive spectral reflectance measurements and the other, identically planted one for the determination of LWP and LWC. Previous testing confirmed that identical information with regard to the water status was provided by the two containers. For the LWP and LWC measurements, fully developed upper leaves that had been fully exposed to the light were used. It was tacitly assumed that possible changes in the aerial plant biomass of either well-watered or dehydrated wheat or maize plants occurring during the two/three-hour measurement cycle were negligible for the concurrent spectral assessments.

Measurements were conducted with either well watered or drought stressed plants at six dates of different conditions of air temperature and light intensity. For all measurements, the plants were exposed to temperatures of 18 or 25°C (wheat) or 18 or 28°C (maize). For a given measurement cycle, however, the temperature and relative humidity within the growth chamber were kept constant and only the light levels were incrementally increased or decreased. All measurements were conducted under a constant relative humidity of 60%. Full details of the experimental conditions, including the resultant changes in LWC and LWP, are presented in Table 1. Short-term changes in the light intensities are indicated in Figure 1.

Drought stress was induced by withholding watering from both wheat and maize before measurement for six days. Light was provided by using metal halide lamps (MT 400 DL, Osram, Germany). Lamp heat was removed by a refrigeration system separated from the growth area by a barrier with openings. Airflow passes uniformly upward through the entire walk-in area. Light intensities were incrementally increased or decreased throughout the measurement cycle of about three hours, thereby allowing the plants to adjust to the new conditions. In the first measurement cycle, the light intensity was increased through six levels only. In the second and third cycles, the light intensity was increased through five levels and then decreased through four so as to reach the first level again (Fig. 1). Each level was maintained for 20 minutes. The constant direct radiation that was incrementally increased was not perturbed by other confounding effects. Viewing always the same plant canopy in the same fixed position did not change the fraction of fully irradiated leaves as compared to less irradiated leaves. Incident light was provided within the growth chamber vertically down to the plant canopy.

Table 1. Description of the experimental conditions and changes induced in plant water status.

Crop and treatment	Date	Temperature (°C)	Light intensity (μmol m^{-2} s^{-1})	Light level duration (hours)	Leaf water content (%)	Leaf water potential (bar)
Wheat, well-watered	Sept 26, 2007	18	6 incremental light levels increasing from 101 to 574	2	81.3 - 83.4	(-4.7) - (-8.6)
Wheat, well-watered	Oct 2, 2007	25	5 incremental light levels increasing from 99 to 574; 4 incremental light levels decreasing from 574 to 97	3	79.9 - 84.6	(-4.7) - (-9.3)
Wheat, drought-stressed	Oct 15, 2007	25	5 incremental light levels increasing from 96 to 505; 4 incremental light levels decreasing from 505 to 91	3	76.2 - 79	(-10.8) - (-15.6)
Maize, well-watered	Sept 27, 2007	18	6 incremental light levels increasing from 100 to 582	2	88.2 - 89.2	(-1.1) - (-4.7)
Maize, well-watered	Oct 1, 2007	28	5 incremental light levels increasing from 107 to 587; 4 incremental light levels decreasing from 587 to 107	3	87.6 - 88.2	(-0.6) - (- 4.8)
Maize, drought-stressed	Oct 17, 2007	28	5 incremental light levels increasing from 86 to 489; 4 incremental light levels decreasing from 489 to 88	3	86.4 - 88.1	(-1.0) - (-6.4)

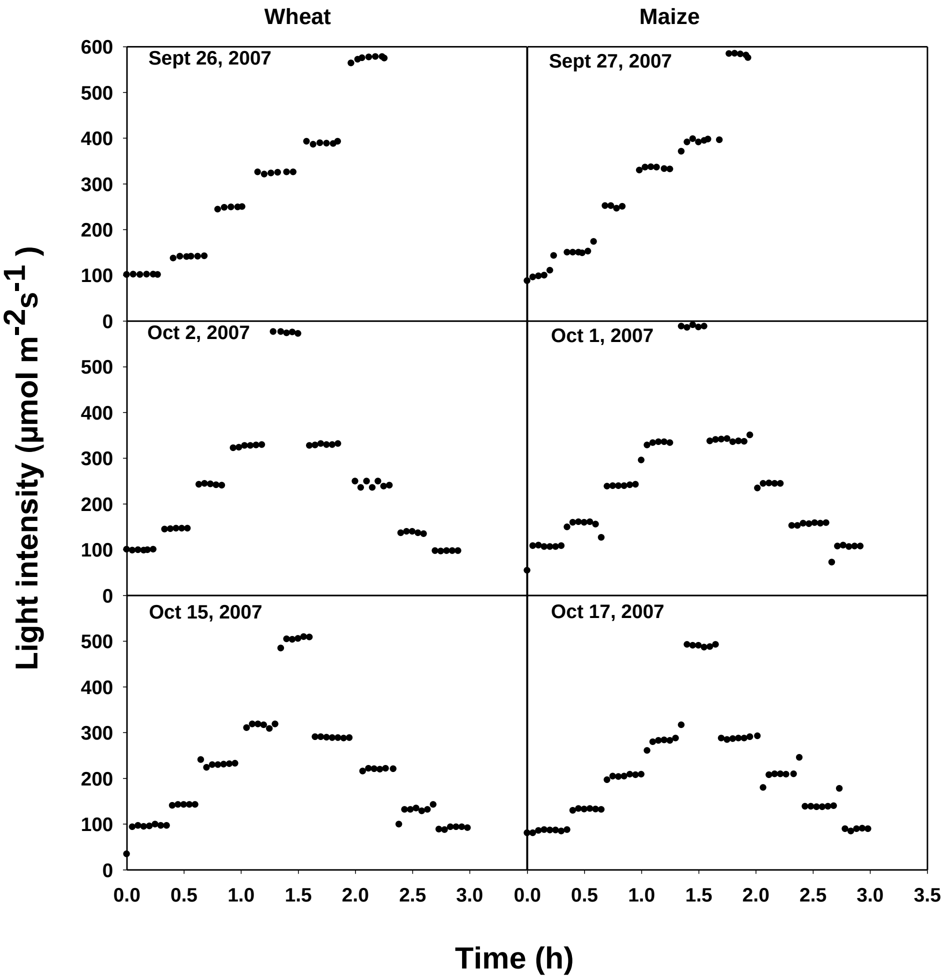

Figure 1. Course of incremental increases or decreases in light intensity experienced by wheat and maize plants grown in a climate chamber.

2.1.2 Spectral reflectance measurements

Passive reflectance was measured at wavelengths between 300 - 1100 nm with a peak to peak bandwidth of 3.3 nm. The sensors contained two units. One unit was linked with a diffuser and measured the light radiation as a reference signal. The second unit simultaneously measured the canopy reflectance with a fiber optic (Mistele and Schmidhalter, 2008a) positioned at the nadir direction about 1 m above the plants in the center of the container. The aperture of the optics was 12° and the field of view was 0.2 m^2. With the readings from both spectrometer units, the canopy reflectance was calculated and corrected with a calibration factor estimated using a $BaSO_4$ reflectance standard at the beginning and after its measurement cycle. Using the bidirectional sensor no further calibration is required and this allows tracking rapidly occurring changes in LWP.

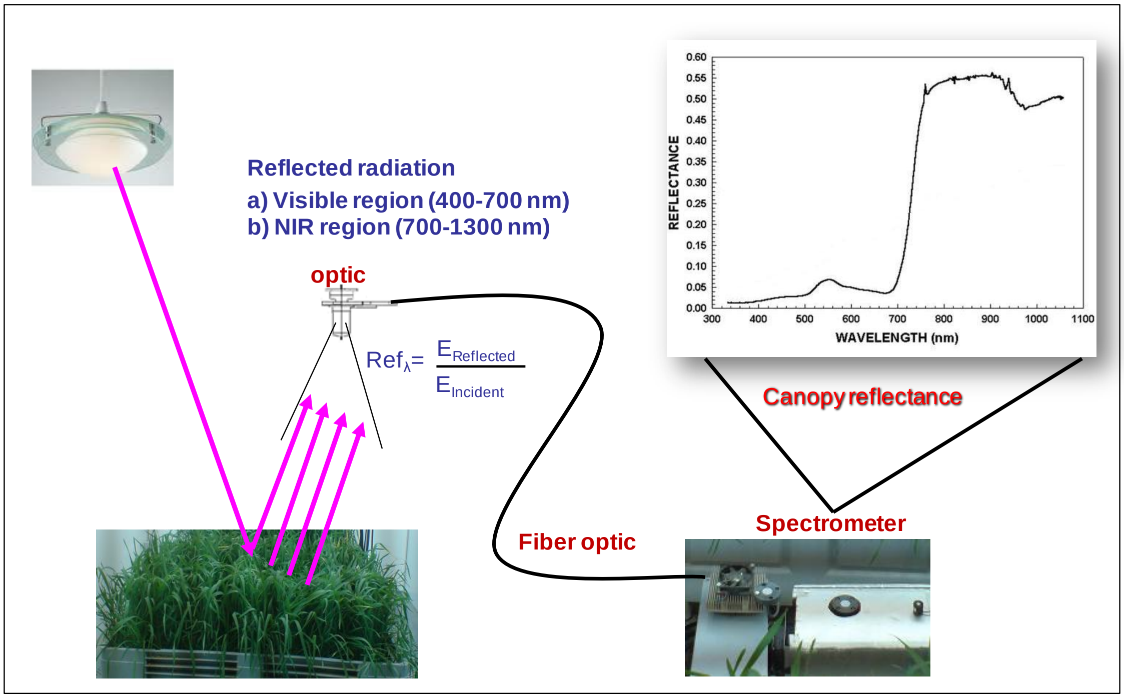

Figure 2. Passive reflectance sensor measuring at wavelengths 300 - 1100 as used to estimate leaf water potential of wheat under growth chamber conditions.

2.1.3 Spectral reflectance indices

In this study, we calculated and tested both known and novel indices. All possible dual wavelengths combinations were evaluated within this study, but only the best performing ones are described (Table 2). From the hyperspectral reflectance readings, twelve wavelengths (410, 490, 510, 531, 570, 600, 670, 780, 940, 960, 1000 and 1100 nm) were therefore used to calculate reflectance indices. Sensing information in the VIS/near infrared region was used since previous testing indicated such information reflected most sensitively the small changes in LWP and LWC expected within this experiment. At small changes of LWC increased signal noise ratios in the SWIR range may perturb measurements. In previous investigations it was found that the VIS/NIR range best reflected subtle changes in the plant water content. Current applications in Precision Farming or Precision Phenotyping using high-throughput active or passive sensors do mostly involve the VIS/NIR range. Therefore, this range offers currently more opportunities for a direct transfer to practice.

Table 2. Spectral reflectance indices examined in this study.

Spectral reflectance indices	Formula	Function	Reference
Normalized difference vegetation index (NDVI)	$(R_{780} - R_{670})/(R_{780} + R_{670})$	* Estimation of leaf area index * * Estimation of water potential	* Aparicio et al.,(2002) ** Gloser and Gloser, (2007)
Photochemical reflectance index (PRI)	$(R_{531} - R_{570})/(R_{531} + R_{570})$	Estimation of water status and photosynthetic	Suaréz et al., (2007)
Ratio of reflectance between 940 and 960 nm	R_{940}/R_{960}	Estimation of water status	this work
Ratio of reflectance between 1000 and 1100 nm	R_{1000}/R_{1100}	Estimation of water status	this work
Ratio of reflectance between 940/960 nm and NDVI	(R_{940}/R_{960})/NDVI	Estimation of water status	this work
Ratio of reflectance between 410-780 and 410+780 nm	$(R_{410} - R_{780})/(R_{410} + R_{780})$	Estimation of water status	this work
Ratio of reflectance between 490-780 and 490+780 nm	$(R_{490} - R_{780})/(R_{490} + R_{780})$	Estimation of water status	this work
Ratio of reflectance between 510-780 and 510+780 nm	$(R_{510} - R_{780})/(R_{510} + R_{780})$	Estimation of water status	this work
Ratio of reflectance between 600 and 780 nm	R_{600}/R_{780}	Estimation of water status	this work

2.1.4 Leaf water potential measurements

To measure LWP in both wheat and maize, a pressure chamber (PMS Instrument., Corvallis, Oregon, USA) was used (Schmidhalter et al., 1998a). Pressure was read within 1 min of leaf removal from the plant and LWP was determined from the average value obtained from five fully expanded leaves of similar age per level of artificial light intensity.

2.1.5 Leaf water content

The same leaf used for the pressure chamber readings was used for the determination of LWC. Fresh weight of the sample was recorded before the leaves were dried at 105 ^{0}C until no further change in dry weight was observed. The LWC was calculated by the following equation: LWC = (FW - DW/FW) * 100 (%), where FW and DW are the fresh and dry weights of the leaf, respectively.

2.2 Field experiments to measure the change in leaf water potential, canopy water content, canopy water mass and aerial biomass of wheat under four water treatments by using passive reflectance sensor, active laser sensor and near infrared temperature sensor

The experiments were conducted under a mobile rain-out shelter in 2005, 2006, 2007 and 2008 at the research station Dürnast, Chair of Plant Nutrition from the Technische Universität München. In this field experiment, five cultivars of winter wheat (Ludwig, Ellvis, Empire, Cubus and Mulan) were used. Four cultivars (Ludwig, Ellvis, Empire and Cubus) were used in 2005, 2006 and 2007 and two cultivars (Cubus and Mulan) were used in 2008. Winter wheat was sown in the middle of October, 2004, 2005, 2006 and 2007 at a seeding rate of 300, 250, 320 and 320, respectively, seeds per m^2. Liquid fertilizer as urea ammonium nitrate was split into two portions of 80 kg N ha^{-1} in BBCH

20 and 40 kg N ha^{-1} in BBCH 30 in 2004. It was split into three portions of 75 kg N ha^{-1} in BBCH 25, 40 kg N ha^{-1} in BBCH 29 and 40 kg N ha^{-1} in BBCH 49 in 2005 and in 2006. It was split into three portions of 100 kg N ha^{-1} in BBCH 25, 60 kg N ha^{-1} in BBCH 29 and 60 kg N ha^{-1} in BBCH 37 in 2007. The soil at the research station is characterized as silt 60%, clay 25% and sand 15% with pH ($CaCl_2$) 6.2. The water holding capacity of the soil is high (330 mm down to 1.2 m depth).

To induce drought stress in wheat the establishment of conventional field trials can be difficult in humid and sub-humid environments, because untimely rain can negate the effects of the imposed irrigation treatments. To avoid this problem, we have used a removable rainout shelter (Fig. 3). To control for the amount of water received by plots under the non-rainfed regimes, a removable shelter that covered the experimental area automatically was used to exclude rainfall. For these plots, spray irrigation was used instead.

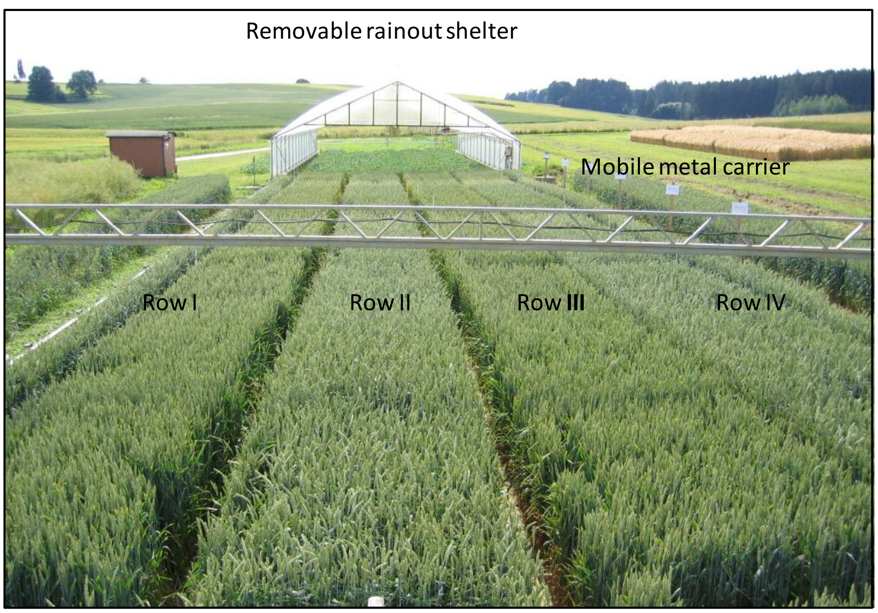

Figure 3. Removable rain-out shelter at the Dürnast research stations.

The experiment was a two factorial set up with four or two winter wheat cultivars and four water treatments (rainfed, irrigatied, early water stress and late water stress). Each regime was applied to two plots for each cultivar in 2005, 2006 and 2007 and was applied to four plots in 2008 for each cultivar. Except, the rainfed treatment that had only a single replicate in 2005, 2006 and 2007 and two replicates in 2008. The experiment included 28 plots, and each plot had the dimension of 4 m in length and 1.8 m in width (Fig. 4). The plants were grown under rainfed conditions and were then exposed to two cycles of water stress (early and late water stress) by withholding water at the gives period (see Table 3).

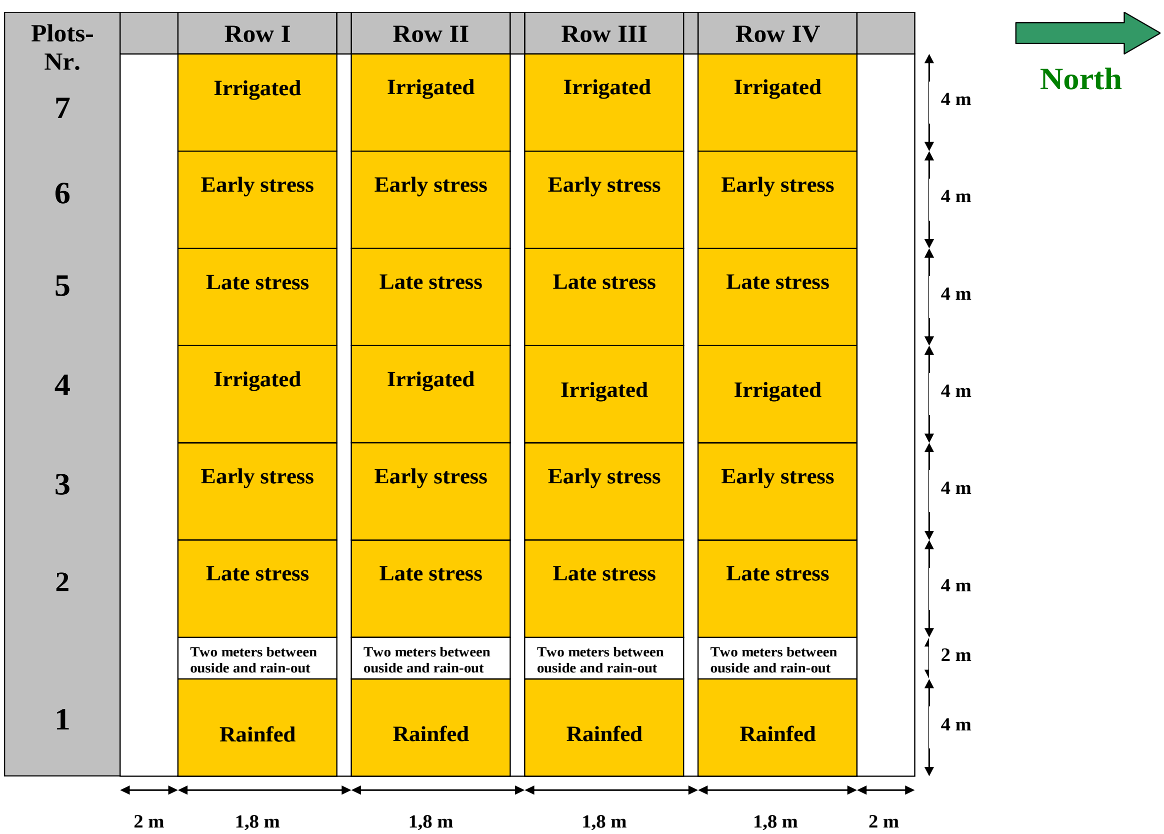

Figure 4. Experimental design with four or two cultivars, four treatments and two replicates, with exception of the rainfed treatmen.

Table 3. Water treatments, irrigation rate (mm m^{-2}) and stress period in years 2005, 2006, 2007 and 2008.

Treatments	Water added (mm) in 2005	Water added (mm) in 2006	Water added (mm) in 2007	Water added (mm) in 2008	Stress period 2005	Stress period 2006	Stress period 2007	Stress period 2008
Rainfed	203	197	289	314				
Irrigated	220	428	516	348				
Early stress	70	167	146	5	May 3 to June 14	May 24 to June 26	April 13 to June 19	April 29 to June 19
Later stress	100	223	256	105	May 28 to July 7	June 19 to July 20	May 22 to July 11	May 16 to July 14

Non-contacting high throughput sensing methods and destructive measurements were used for these investigations (Table 4).

Table 4. Cultivars, instruments and physiological parameters used and measured in the years 2005, 2006, 2007 and 2008.

Years	Cultivars	Instruments	Physiological parameters measured
2005	Ludwig, Empire, Ellvis and Cubus	Laser-induced chlorophyll fluorescence sensor measuring at 690 and 730 nm and near infrared temperature sensor	Leaf water potential, canopy water content, canopy water mass, aerial biomass and canopy temperature
2006 and 2007	Ludwig, Empire, Ellvis and Cubus	Passive reflectance sensor measuring at wavelength 300 - 1700 nm, GPS unit and near infrared temperature sensor	Leaf water potential, canopy water content, canopy water mass, aerial biomass and canopy temperature
2008	Cubus and Mulan	Passive reflectance sensor measuring at wavelength 300 - 1100 nm, GPS unit and near infrared temperature sensor	Leaf water potential, canopy water content, canopy water mass, aerial biomass and canopy temperature

2.2.1 Laser-induced chlorophyll florescence 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 (Fig. 5). The sensor was mounted at a height of 3 m with a zenith angle of 45° above the plant canopy. The sensor used a emission, which was measured at 690 nm (F690) and 730 nm (F730) 2000 times per second and averaged to one single value per second. A biomass index was calculated out of the frequency at which a green plant was hit by the laser beam and varied between 0 and 1 (Thoren and Schmidhalter, 2009). The index is based on the principle that chlorophyll fluorescence can only be induced from green leaves or another green plant part and that the frequency with which the laser contacts these structures (as opposed to dead leaves or soil) is related to biomass. Thus, by eliminating fluorescence values that are lower or higher than pre-defined threshold values for a green leaf from the data set, fluorescence signals only originality from green leaves can be selectively distinguished from dead leaves or soil measurements (Bredemeier and Schmidhalter, 2003). The fluorescence light was collected with a spherical mirror and detected by a photodetector. The data were transferred from the sensors to a portable computer and analysed with manufactured software to eliminate non-valid fluorescence values.

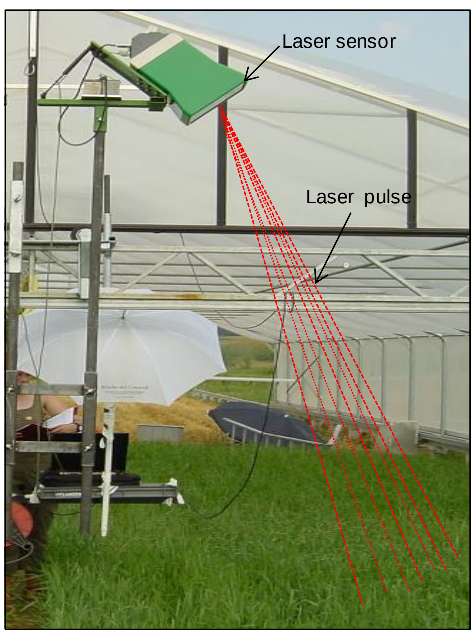

Figure 5. Laser-induced chlorophyll fluorescence sensor mounted on a mobile carrier frame, used to detect drought stress of wheat grown under rain-out shelter conditions.

Fluorescence measurements were taken several times during the growing period encompassing stem elongation until ripening (see Table 5). The different fluorescence parameters and the biomass index were compared with classically determined values of LWP, CWC, CWM, AB and CT.

Table 5. Laser-induced chlorophyll fluorescence and physiological parameters recorded at different growth stages, dates, and times.

Growth stage	Date	LICF Time of day	Physiological parameters measured
Stem elongation	May 25, 2005	12:45 - 13:19	Leaf water potential and canopy temperature
Stem elongation and inflorescence emergency	June 1, 2005	12:46 -13:40	Leaf water potential and canopy temperature
Inflorescence emergence and anthesis	June 8, 2005	14:11 -15:04	Canopy water content, canopy water mass and aerial biomass
Anthesis and milk development	June 21, 2005	13:41 - 14:14	Leaf water potential, canopy temperature, canopy water content, canopy water mass and aerial biomass
Anthesis and milk development	June 23, 2005	13:40 - 14:15	Leaf water potential and canopy temperature
Milk development and ripening	July 4, 2005	10:42 - 11:10	Leaf water potential, canopy water content, canopy water mass and aerial biomass

2.2.2 Spectral reflectance measurements

For spectral reflectance measurements, removable metal carrier-mounted passive reflectance sensors were connected with a portable computer and GPS antenna. Two different passive reflectance sensors were used in this study.

Firstly, a passive reflectance sensor for measuring at wavelengths between 300 - 1700 nm was used in 2006 and 2007 (Fig. 6). The sensor consists of four optics which were positioned on the edges of a metal frame (L x W = 1.9 x 1.9 m) with a zenith angle of 50^{o} at a height of 1.3 m above the wheat canopy to provide measurements independent of driving, viewing and solar zenith and azimuth and also to measure a large area. The aperture of the optics was 12° and the field of view was 0.28 m^2 for each optics. The sensor measured subsequently canopy reflectance and radiation as a reference signal with a shutter technique. Two detectors were used; one for the visible area with a bandwidth of 3.3 nm and another for the near infrared area with a bandwidth of 6 nm. They were linked to a four in one light fiber to create an optical mixed signal from four fields of view. A signal was measured as an average of four optics. So it was nearly constant at any solar zenith angle (Mistele and Schmidhalter, 2008). With the readings from the spectrometer units the canopy reflectance was calculated and corrected with a calibration factor, estimated with a $BaSO_4$ reflectance standard.

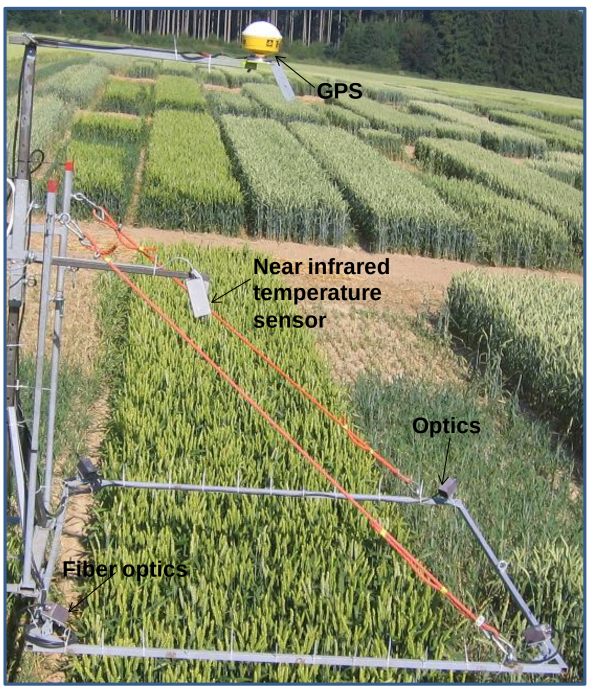

Figure 6. Passive reflectance sensor measuring at wavelengths between 300 -1700 nm connected with GPS were used to measure water status in wheat under rain-out shelter conditions.

Secondly, the same passive sensor that was used in the growth chamber as well was also used in 2008. This self constructed sensor was developed to measure canopy reflectance and sun reflectance under the same conditions. The sensor consists of one optics and an automatic reference 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 (Fig. 7). The angle of the fiber optic 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.

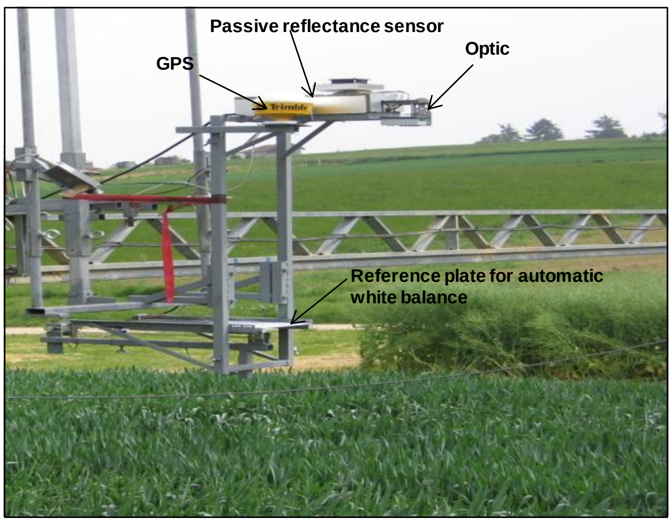

Figure 7. Passive reflectance sensor measuring at wavelengths between 300 -1100 nm with GPS used to measure water status in wheat under rain-out shelter conditions.

After the data were transferred from the sensors to a portable computer, the data were analysed by using a specific software coded in Lab View (National Instruments, Austin, Texas, USA) to extract spectral reflectance. The data were further analysed by using Arc View GIS version 3.3 (ESRI, Redlands, California, US) in order to compare spectral reflectance measurements with ground-truth evaluations exactly in the same area in the field (Fig. 8).

Spectral reflectance measurements were taken in different growth stages and related to physiological parameters as described in Table 6.

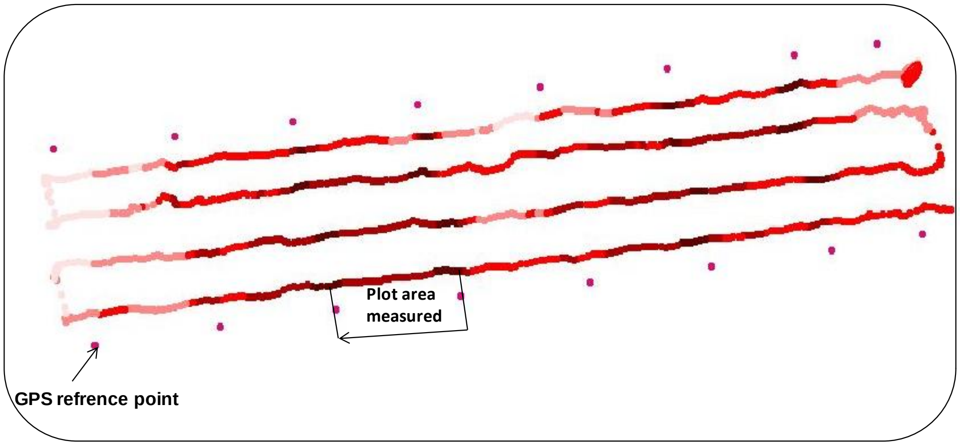

Figure 8. Spatial information of spectral reflectance measurements collected with GPS and analysed with GIS.

Table 6. Spectral reflectance measurements and physiological parameters at different growth stages, dates and times in 2006, 2007 and 2008.

Growth stages	Dates	Time (hour, minutes)	Physiological parameters
Heading and flowering	June 12, 2006	15:48 – 16:09	Leaf water potential and canopy temperature
Flowering	June 21, 2006	13:48 – 14:10	Leaf water potential, canopy temperature, canopy water content, canopy water mass and aerial biomass
Ripening	July 11, 2006	14:07 – 14:44	Leaf water potential, canopy temperature, canopy water content, canopy water mass and aerial biomass
Heading and flowering	May 30, 2007	14:35 – 14:55	Leaf water potential, canopy temperature, canopy water content, canopy water mass and aerial biomass
Development of fruit and ripening	June 14, 2007	15:40 – 16:00	Leaf water potential and canopy temperature
Development of fruit and ripening	June 19, 2007	14:50 – 15:15	Leaf water potential and canopy temperature
Heading	June 2, 2008	13:38 – 14:30	Leaf water potential, canopy water content, canopy water mass and aerial biomass
Heading and flowering	June 10, 2008	13:25 – 14:20	Leaf water potential
Milk and dough	July 2, 2008	13:56 – 15:50	Leaf water potential, canopy water content, canopy water mass and aerial biomass

2.2.3 Spectral reflectance indices

From the hyperspectral reflectance readings ten wavelengths at 410, 490, 510, 600, 670, 780, 1240, 840, 860 and 1650 nm were used to calculate reflectance indices. We calculated and tested known and new indices as described in Table 7.

Table 7. Formula, functions, and references of different previously developed and new spectral indices developed in this work being used in this study.

Spectral reflectance indices	Formula	Function	Reference
Normalized difference vegetation index (NDVI)	$(R_{780} - R_{670})/(R_{780} + R_{670})$	* Estimation of leaf area index ** Estimation of leaf water potential	*Aparicio et al., 2002 **Ruthenkolk et al., 2001
Normalized difference water index (NDWI2)	$(R_{840} - R_{1650})/(R_{840} + R_{1650})$	Estimation of water content	Clay et al., 2006
Ratio of reflectance between 1240 and 860 nm	R_{1240}/R_{860}	Estimation of water content	Zarco Tejada et al., 2003
Ratio of reflectance between 410-780 and 410+780 nm	$(R_{410} - R_{780})/(R_{410} + R_{780})$	Estimation of water status	this work
Ratio of reflectance between 490-780 and 490+780 nm	$(R_{490} - R_{780})/(R_{490} + R_{780})$	Estimation of water status	this work
Ratio of reflectance between 510-780 and 5100+780 nm	$(R_{510} - R_{780})/(R_{510} + R_{780})$	Estimation of water status	this work
Ratio of reflectance between 600 and 780 nm	R_{600}/R_{780}	Estimation of water status	this work

2.2.4 Canopy temperature measurement

Canopy temperature measurements were obtained using a near infrared temperature sensor (KT15.83, Heitronics, Germany) with a zenith angle of 45^{o} in the range of 8000 -12000 nm (Fig

6). Canopy temperature was measured concomitantly with spectral and fluorescence measurements. Measurements dates are presented in Tables 5 & 6.

2.2.5 Leaf water potential

LWP was measured with the method described in section 2.1.3. LWP was determined as averages of six fully expanded flag leaves. Four out of seven plots for each cultivar were used to measure LWP which represented all water treatments. Measurement dates are presented in Tables 5 & 6.

2.2.6 Biomass sampling

To determine aerial biomass, plants were cut above the ground within a 0.22 m^2 area (A) for all plots and aerial biomass weight was measured. Thereafter, a representative subsample was placed in an oven (105 ^oC) until there was no change in dry weight. Canopy water content (in %) was calculated as CWC = (FW – DW)/FW * 100. In addition, canopy water mass (in g/m^2) was calculated as CWM = (FW – DW) /A, where FW is the fresh weight, DW is dry weight, CWC is canopy water content, CWM is canopy water mass and A is the area of the biomass harvest. Measurements dates are presented in Tables 5 & 6.

2.2.7 Chlorophyll meter reading (SPAD values)

The relative chlorophyll content was taken to be the average value from ten fully expanded leaves of flag leaves as obtained from a portable chlorophyll meter (SPAD-502) in Figure 9. Measurements were taken throughout the growing period for all plots in 2005.

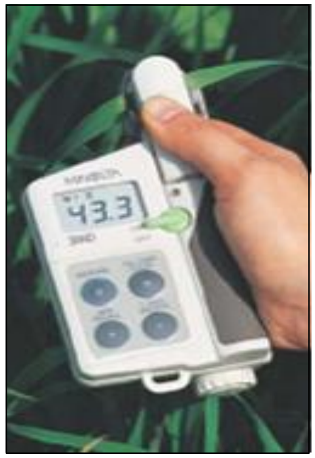

Figure 9. portable chlorophyll meter SPAD-502.

2.3 Darkroom experiments to measure leaf water potential, leaf water content, relative leaf water content and canopy water content of wheat and maize under six water treatments by spectral reflectance measurements at the leaf and canopy level

The experiments were conducted in a darkroom at the research station of the Chair of Plant Nutrition from the Technische Universität München at Dürnast. Spring wheat (*Triticum aestivum* cv. Star) and maize (*Zea mays* cv. Suzy) were previously cultivated at a density of 8 and 4 plants, respectively in small pots (D x H = 20 x 18 cm) containing 6 kg of dry loamy soil under greenhouse conditions. Nitrogen at the amount of 0.6 g N was applied as calcium ammonium nitrate per pot. All other nutrients were supplied in amounts to ensure adequate growth. Both wheat and maize were subjected to six water regimes (control, stress 1, stress 2, stress 3, stress 4 and stress 5) and each treatment had four replicates. The soil water content in well-watered treatments or control treatments was adjusted to 20% as gravimetric water content (θ_s) and corresponding to soil matric potential of about -0.2 bar. At BBCH 33 measurements were conducted.

2.3.1 Spectral reflectance measurements

A spectrometer (GER 3700) Geophysical Environmental Research Crop, New York, USA was used to measure at wavelengths between 350 nm - 2500 nm. Three detectors were used; one for the visible region 300 nm - 700 nm, the second for the near infrared region 700 nm – 1300 nm and the third for middle infrared region 1300 nm - 2500 nm.

At the canopy level, eight spectral measurements were made per pot for wheat and maize from different sides to cover all plant parts. For this purpose, the plants pots were put on a turntable and were rotated for each spectral measurement by 45^{o}. A fiber optics was positioned at a height of 0.4 m with a zenith angle of 60^{o} above the plant canopy.

At the leaf level, spectral measurements were made on both the adaxial and abaxial surfaces of wheat or maize leaves. The spectral measurements were made in two positions at the center and the lower third of the wheat leaf. For maize, they were made in three positions at the center, lower third and uper third of the leaf. To guarantee a complete coverage by leaves, a black sheet slit diaphragm was used to prevent spectral reflectance caused by background and the measurements were 4 mm for wheat leaves and 8 mm for maize leaves. The distance between the head of the fiber optic and the plant stand, the field of view and the optical angle at the canopy and leaf level of wheat and maize plants are shown in Table 8. With the readings from the both spectrometer units, the canopy reflectance was calculated.

Table 8. Descriptions of the optics height, field of view and optical angle at the canopy and leaf level of wheat and maize plants.

Measurements	Optics height (cm)	Field of view in (cm)	Optic angle(degree)
Canopy level	40	16.5	22°
Leaf level of maize	2.7	1.3	27°
Leaf level of wheat	1.65	1.1	37°

The spectral measurements were made under constant artificial light. As light source a 1000 W halogen lamp was used. The halogen lamp was positioned at a height of 1 m with a zenith angle of 40^{o} above the plant canopy opposite to the sensor.

2.3.2 Leaf water potential measurements

Leaf water potential of wheat and maize was measured as described in section 2.1.3 and was determined as the average value obtained from eight fully expanded leaves of similar age per treatment.

2.3.3 Relative water content, leaf water content, and canopy water content measurements

Relative water content (RWC) and leaf water content (LWC) was determined to describe the water status in wheat and maize under six water regimes. Cut leaves were weighed (fresh weight FW), then stored with the leaf base in water for four hours for saturation and their turgid weights (TW) were calculated. Then the samples were dried in an oven at 105 °C for 24 hours and weighed again (dry weight DW). The RWC (in %) was calculated as RWC = (FW-DW)/(TW-DW). In addition, LWC (in %) was calculated as LWC (%) = (FW-DW)/FW. Relative water content and leaf water content were determined as the average obtained from eight fully expanded leaves of similar age per treatment.

Canopy water content (CWC) was measured as described in section 2.2.6. It was determined from the average values obtained from sixteen plants for wheat and four plants for maize per treatment.

2.3.4 Soil water content

Soil water content (SWC) is expressed on a gravimetric basis. The gravimetric water content (θs) is the mass of water per mass of dry soil. It was measured by weighing a soil (m_{wet}) sample, drying the sample to remove the water, and then weighing the dried soil (m_{dry}). The samples were dried in an oven at 105 °C for 24 hours. Soil water content was determined as follows: SWC (%) = $m_{\mathbf{water}}/m_{\mathbf{soil}}$ = ($m_{\mathbf{wet}}$ – $m_{\mathbf{dry}}$)/$m_{\mathbf{dry}}$. Soil water content was determined as average of three replicates for wheat and maize per treatment. At the spectral reflectance measurement dates, the soil water content of wheat was for the control, stress 1, stress 2, stress 3, stress 4 and stress 5 treatments 10.7%, 8.6%, 8.7%, 8.2 %, 7.9% and 7.3%, respectively, and of maize 18.2%, 11.4%, 8.7%, 10.4 %, 10.6% and 9.4%, respectively.

2.3.5 Leaf growth

Leaf growth of wheat and maize was measured throughout five days for the six water treatments. Leaf growth was determined from the average values which were obtained from eight leaves of wheat and maize per treatment.

2.4 Statistical analysis

Sigmaplot for Windows v.10 (Systat software Inc., Chicago), SPSS 16 (SPSS Inc., Chicago, IL) and Microsoft Excel 2003 were used for the statistical analysis in this study. We calculated simple regressions to analyze the relationship between spectral indices, fluorescence parameters, the biomass index and the canopy temperature with each of LWP, RWC, LWC, CWC, CWM, AB. Coefficients of determination and significance were determined; a nominal alpha value of 0.05 was used.

3 RESULTS

3.1 Experiments under controlled conditions (growth chamber)

3.1.1 Changes in leaf water potential and content under increasing/decreasing light intensities

Short-term changes in LWP and LWC of wheat and maize determined at a constant temperature are shown in Tables 9 and 10. Leaf water potentials of both crops varied negatively with light intensity, with the lowest values generally being observed at the highest light-intensity level. Leaf water potentials declined from the lower to the higher level of light intensity, and increased due to decreasing light intensity. Whereas the difference in LWP between well-watered and drought stressed plants for wheat was marked, it was small in maize. There was little change in LWC across the different light intensities, with significant differences being limited to the second measurement cycle for wheat (Table. 9) and for the first and third measurement cycles for maize.

When relative humidity as well as temperature was held constant, a closer relation between light intensity level and either of LWP than LWC was found (Table 11). The induced changes however were larger for LWP than for LWC in both wheat and maize.

Table 9. Influence of increasing/decreasing light intensity at constant temperature in a two/three-hours measurement cycle (see Table 1) on the leaf water potential (LWP) and leaf water content (LWC) of wheat. Results from three measurement cycles are presented. Measured values at each light intensity level are derived from five measurements. Values with the same letter are not statistically different ($P \geq 0.05$) between light intensities. SD indicates standard deviation.

Wheat, Sept 26, 2007 at 18°C First measurement cycle					Wheat, Oct 2, 2007 at 25°C Second measurement cycle					Wheat, Oct 15, 2007 at 25°C Third measurement cycle				
Light intensity ($\mu mol\ m^{-2}s^{1}$)	LWP (bar)	SD (bar)	LWC (%)	SD (%)	Light intensity ($\mu mol\ m^{-2}s^{1}$)	LWP (bar)	SD (bar)	LWC (%)	SD (%)	Light intensity ($\mu mol\ m^{-2}\ s^{1}$)	LWP (bar)	SD (bar)	LWC (%)	SD (%)
101	-4.7 a	1.0	81.3 a	2.0	99	-4.7 a	1.6	82.7 abc	2.5	96	-10.8 a	1.4	78.2 a	1.5
140	-7.1 b	1.2	82.7 a	2.3	145	-7.8 c	0.8	81.6 bc	0.9	142	-12.9 b	0.8	77.6 a	2.4
248	-8.2 bc	0.8	82.9 a	1.1	242	-8.3 cd	0.8	80.8 bc	2.7	229	-12.5 ab	1.6	78.4 a	2.2
324	-8 bc	0.8	83.4 a	1.8	326	-9.0 cd	0.6	79.9 bc	1.3	316	-13.2 b	1.1	79.0 a	2.4
389	-8.02 bc	0.8	82.4 a	1.0	574	-9.3 d	0.8	81.0 bc	0.7	506	-15.6 c	1.6	78.5 a	3.5
574	-8.6 c	0.6	82.6 a	1.4	329	-8.1 cd	0.8	81.7 abc	1.8	288	-14.3 bc	1.0	78.1 a	1.0
					241	-8.2 cd	1.2	81.4 bc	2.4	220	-13.8 bc	1.7	77.4 a	2.5
					137	-6.3 b	0.9	83.7 ab	2.5	131	-13.0b	1.7	76.2 a	2.3
					97	-5.2 ab	1.2	84.6 a	1.5	91	-12.4 ab	1.3	77.7 a	1.6

Table 10. Influence of increasing/decreasing light intensity at constant temperature in a two/three-hours measurement cycle (see Table 1) on the leaf water potential (LWP) and leaf water content (LWC) of maize. Results from three measurement cycles are presented. Measured values at each light intensity level are derived from five measurements. Values with the same letter are not statistically different ($P \geq 0.05$) between light intensities. SD indicates standard deviation.

Maize, Sept 27, 2007 at 18°C First measurement cycle					Maize, Oct 1, 2007 at 28°C Second measurement cycle					Maize, Oct 17, 2007 at 28°C Third measurement cycle				
Light intensity ($\mu mol\ m^{-2}s^{1}$)	LWP (bar)	SD (bar)	LWC (%)	SD (%)	Light intensity ($\mu mol\ m^{-2}s^{1}$)	LWP (bar)	SD (bar)	LWC (%)	SD (%)	Light intensity ($\mu mol\ m^{-2}\ s^{1}$)	LWP (bar)	SD (bar)	LWC (%)	SD (%)
100	-1.1 a	0.2	88.8 abc	0.3	107	-0.6 a	0.2	88.1 a	0.3	86	-1.0 a	0.2	87.8 ab	0.4
153	-3.0b	0.7	89.2 a	0.2	157	-1.1 b	0.1	87.9 a	0.4	132	-1.8 a	0.5	88.1 a	0.6
253	-3.7 c	0.4	88.9 ab	0.3	240	-1.8 c	0.2	88.2 a	0.3	206	-3.7 b	0.8	87.9 ab	0.2
336	-3.9 c	0.3	88.7 abc	0.3	333	-2.8 d	0.2	88 a	0.2	283	-3.9 b	0.5	87.9 ab	0.5
396	-4.1 c	0.3	88.5 bc	0.4	588	-4.8 e	0.3	88 a	0.2	489	-5.9 c	0.7	87.2 bc	0.8
582	-4.7 d	0.3	88.2 c	0.5	340	-2.6 d	0.4	87.6 a	0.5	288	-6.4 c	1.1	86.8 c	0.2
					242	-2.6 d	0.4	87.8 a	0.8	208	-5.4 c	1.2	86.4 c	1.2
					156	-2.0 c	0.4	88 a	0.5	138	-4.0 b	1.3	87.2 bc	0.7
					107	-1.7 c	0.2	87.8 a	0.6	88	-3.9 b	1.7	87.2 abc	0.3

Table 11. Maximum differences in leaf water potential (LWP) and leaf water content (LWC) at constant temperature together with relationships of each with light intensity levels at different measurement dates for wheat and maize. Values for coefficients of determination (R^2-values) are indicated.

Measurement cycle	Crop/Measurement date	Temperature (°C)	LWP		LWC	
			Difference between min and max values (bar)	R^2	Difference between max and min values (%)	R^2
First	Wheat at Sept 26, 2007	18	-3.9	0.58	2.1	0.12
Second	Wheat at Oct 2, 2007	25	-4.6	0.64*	4.7	0.40
Third	Wheat at Oct 15, 2007	25	-4.8	0.70**	2.8	0.30
First	Maize at Sept 27, 2007	18	-3.6	0.75*	1.0	0.73*
Second	Maize at Oct 1, 2007	28	-4.2	0.88**	0.6	0.00
Third	Maize at Oct 17, 2007	28	-5.4	0.50*	1.7	0.07

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

3.1.2 The relationship between leaf water content and leaf water potential at different light intensities, temperatures and watering regimes

The relationship between LWC and LWP was determined at all measurement cycles for wheat and maize (Fig. 10). In two of the three measurement cycles for wheat, the two variables were significantly related with one another (R^2 = 0.66, P≤ 0.05 & R^2 = 0.70, P≤ 0.01; Fig. 10a). For maize, this was only true for one cycle (R^2 = 0.58, P≤ 0.05; Fig. 10b), possibly because the changes in water content were minimal in this crop.

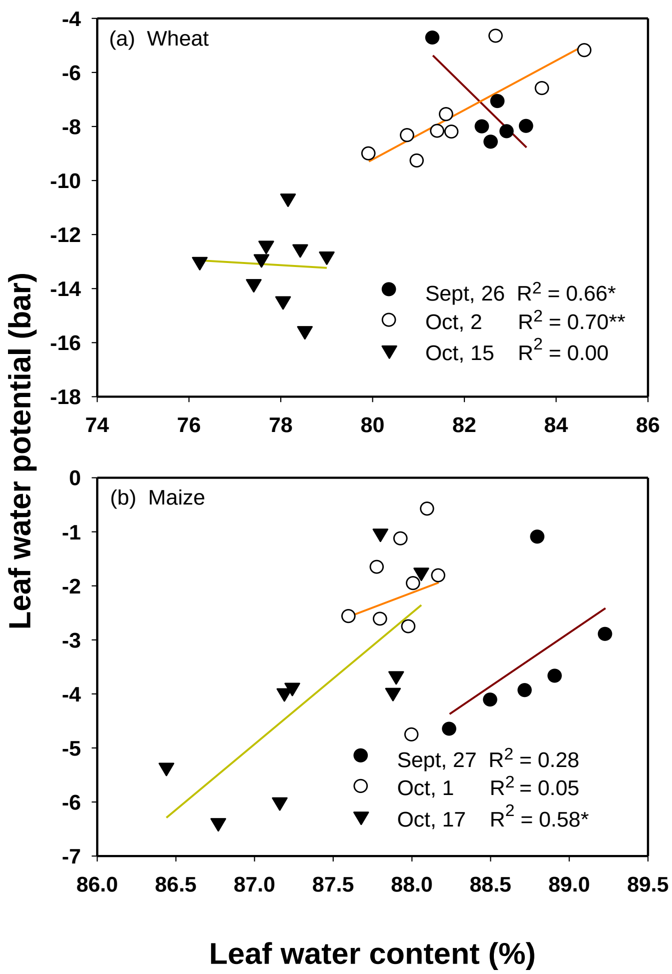

Figure 10. Light intensity induced changes in leaf water potential as a function of leaf water content in (a) wheat and (b) maize plants at constant temperatures and under two watering regimes.

3.1.3 The relationship between spectral reflectance indices and plant water status

Statistically significant relationships between all spectral reflectance indices derived from the VIS or NIR regions and LWP of wheat were obtained for the first two measurement cycles (Table 12), although the strength of the relationship varied greatly (R^2 = 0.55 - 0.91). Only the index (R_{940}/R_{960})/NDVI (Fig. 11) was significantly related to the LWP over all measurement cycles, although the precise relationship differed between the cycles. Statistically significant linear relationships between the spectral reflectance indices and the LWC were restricted to a few indices at the second measurement cycle only ($R^2 > 0.69$, $P \leq 0.01$; Table 12), with the index R_{1000}/R_{1100} displaying the strongest relationship ($R^2 = 0.80$).

Table 12. Coefficients of determination (R^2) between seven spectral indices and light induced changes in leaf water potential (LWP) and leaf water content (LWC) of wheat at three measurement dates.

Spectral indices	Wheat at Sept 26, 2007		Wheat at Oct 2, 2007		Wheat at Oct 15, 2007	
	LWP (bar)	LWC (%)	LWP (bar)	LWC (%)	LWP (bar)	LWC (%)
(R_{940} /R_{960})/NDVI	0.88**	0.43	0.84***	0.69**	0.75**	0.14
NDVI	0.77*	0.53	0.90***	0.74**	0.20	0.26
R_{600}/R_{780}	0.71*	0.66	0.63	0.46	0.13	0.19
R_{940}/R_{960}	0.85**	0.50	0.91***	0.75**	0.48	0.28
R_{1000}/R_{1100}	0.90**	0.61	0.91***	0.80**	0.37	0.30
PRI	0.74*	0.23	0.55*	0.20	0.41	0.15
(R_{410}-R_{780})/(R_{410}+R_{780})	0.11	0.55	0.02	0.00	0.25	0.00
(R_{490}-R_{780})/(R_{490}+R_{780})	0.70*	0.44	0.64**	0.41	0.20	0.16
(R_{510}-R_{780})/(R_{510}+R_{780})	0.76*	0.46	0.68**	0.45	0.21	0.18

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

For maize, statistically significant relationships between all spectral indices and LWP ($R^2 > 0.68$, $P < 0.05$) were sparse (Table 13). The strongest relationship was observed for the index R_{940}/R_{960} (R^2 = 0.92**) at the first measurement (Fig. 12). This index also was the only one to show a significant relationship for more than a single cycle. No significant relationship was observed between any spectral index and LWC at any measurement cycle (Table 13).

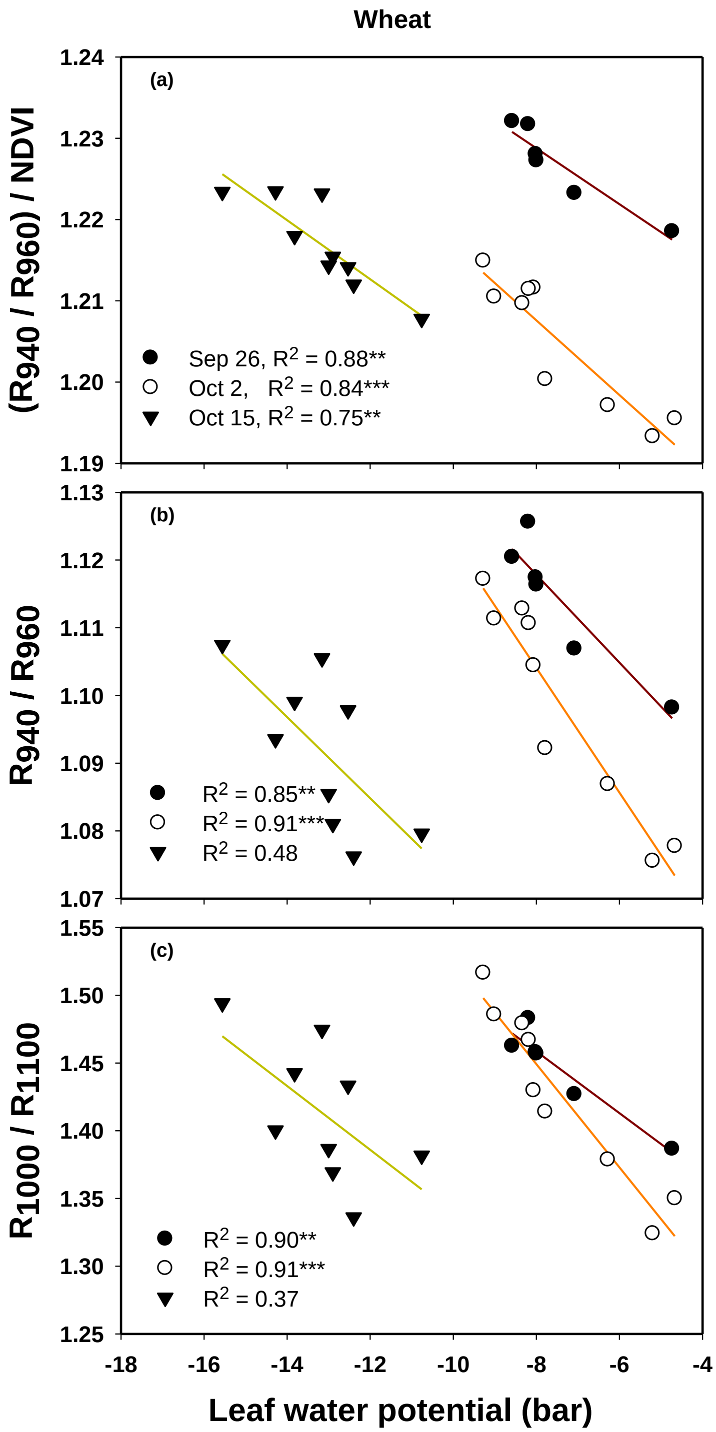

Figure 11. Relationship between three selected spectral indices (a) (R_{940}/R_{960})/NDVI, (b) R_{940}/R_{960} and (c) R_{1000}/R_{1100} and the leaf water potential of wheat subjected to two watering regimes at three measurement dates.

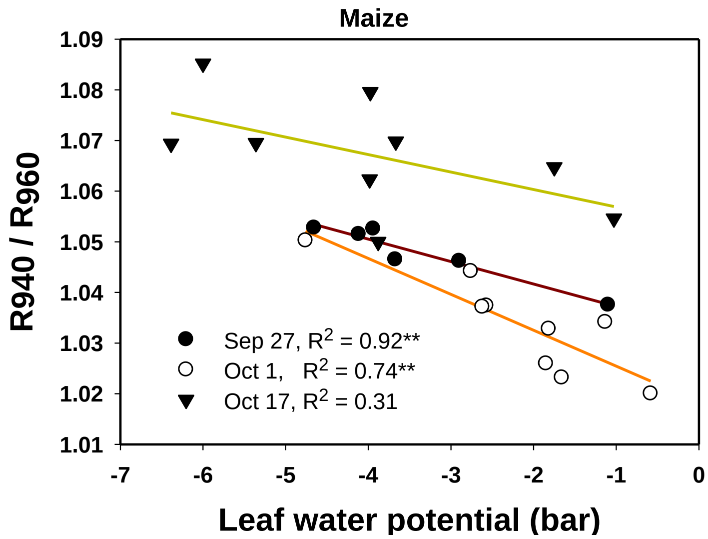

Figure 12. Relationship between the spectral index R_{940}/R_{960} and leaf water potential in maize subjected to two watering regimes at three measurement dates.

Table 13. Coefficients of determination between seven spectral indices and light induced changes in leaf water potential (LWP) and leaf water content (LWC) of maize at three measurement dates.

Spectral indices	Maize at Sept 27, 2007		Maize at Oct 1, 2007		Maize at Oct 17, 2007	
	LWP (bar)	LWC (%)	LWP (bar)	LWC (%)	LWP (bar)	LWC (%)
(R_{940}/R_{960})/NDVI	0.92**	0.47	0.17	0.15	0.14	0.06
NDVI	0.20	0.52	0.33	0.02	0.04	0.01
R_{600}/R_{780}	0.01	0.01	0.15	0.15	0.13	0.04
R_{940}/R_{960}	0.92**	0.25	0.74**	0.01	0.31	0.00
R_{1000}/R_{1100}	0.68*	0.00	0.50	0.05	0.16	0.00
PRI	0.17	0.06	0.17	0.12	0.02	0.02
$(R_{410} - R_{780})/(R_{410} + R_{780})$	0.15	0.66	0.50	0.00	0.29	0.00
$(R_{490} - R_{780})/(R_{490} + R_{780})$	0.04	0.00	0.11	0.09	0.06	0.00
$(R_{510} - R_{780})/(R_{510} + R_{780})$	0.06	0.00	0.08	0.01	0.03	0.00

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

3.2 Field experiments

3.2.1 Laser-induced chlorophyll fluorescence measurements and physiological parameters of winter wheat in 2005

3.2.1.1 Measurements of several fluorescence parameters and the biomass index as well as several physiological parameters of four wheat cultivars subjected to four watering regimes

Laser-induced chlorophyll fluorescence, the biomass index, canopy water content (CWC), canopy water mass (CWM), aerial biomass (AB), leaf water potential (LWP), and canopy temperature (CT) measurements were performed during the growing period of winter wheat, as indicated in Table 5. Table 14 shows the minimum, maximum and mean values for all parameters evaluated across all measurements dates. The cultivar Ludwig showed contrasting results compared to the other cultivars.

Table 14. Minimum, maximum, and mean values for fluorescence intensities at 690 nm and 730 nm, fluorescence ratio F690/F730, canopy water content (%), canopy water mass (g m^{-2}), aerial biomass (g m^{-2}), leaf water potential (bar), and canopy temperature (^{o}C) in wheat plants subjected to four water treatments, evaluated across all measurements.

Cultivars		CWC	CWM	AB	LWP	CT	F690	F730	F690/F730	Biomass index
		%	g m^{-2}	g m^{-2}	bar	^{o}C	counts	counts		
	Minimum	44.4	944.3	2127.3	-31.4	21.9	400.2	600.6	0.7	0.6
Ludwig	Maximum	79.9	4431.8	5555.5	-14.3	29.8	1238.8	1616.7	1.0	1.0
	Mean	68.5	2862.2	4066.5	-21.2	25.5	778.6	1069.0	0.8	0.9
	Minimum	53.7	1482.2	2580.0	-25.1	21.4	531.2	754.1	0.8	0.8
Empire	Maximum	82.8	4894.5	6253.6	-14.3	31.0	1639.0	1987.7	1.0	1.0
	Mean	70.5	3106.6	4280.0	-18.8	25.5	923.4	1197.8	0.8	0.9
	Minimum	46.4	1322.5	2847.8	-27.2	20.3	528.2	767.3	0.8	0.7
Ellvis	Maximum	82.7	5930.9	7171.8	-15.1	31.2	1490.0	1723.9	1.0	1.0
	Mean	70.4	3424.0	4769.2	-20.9	25.1	1008.5	1232.7	0.9	0.9
	Minimum	48.1	1353.0	2792.9	-27.4	20.0	456.1	622.7	0.7	0.6
Cubus	Maximum	82.0	5391.8	7056.4	-14.9	31.2	1632.0	1921.1	1.0	1.0
	Mean	69.3	3080.5	4350.4	-20.1	25.0	1041.5	1359.5	0.8	0.9

3.2.1.2 Relationship between canopy water content, canopy water mass, aerial biomass, leaf water potential, and canopy temperature

Across the entire sampling period and for the investigated parameters LWP, CWC, CWM, AB, and CT, the closest relationship was found between CWM and AB for each cultivar (Table 15). There was a moderate decrease in the coefficients of determination between CT and LWP among the cultivars. All other pairs of variables displayed lower coefficients of determination among each of the four cultivars.

Table 15. Interrelationships between selected pairs from canopy water content (%), canopy water mass (g m^{-2}), aerial biomass (g m^{-2}), leaf water potential (bar), and canopy temperature (°C) values for four cultivars under four water regimes and at all measurement dates. Values of coefficients of determination (R^2-values) are indicated.

Cultivar	Canopy water content and aerial biomass	Canopy water content and canopy water mass	Canopy water mass and aerial biomass	Canopy water content and leaf water potential	Leaf water potential and canopy temperature
Ludwig	0.70***	0.82***	0.97***	0.91***	0.76***
Empire	0.45**	0.70***	0.93***	0.79**	0.78***
Ellvis	0.57***	0.76***	0.95***	0.29	0.74***
Cubus	0.57***	0.74***	0.96***	0.20	0.79***

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

3.2.1.3 Relationship between canopy water content and the fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index

The relationships between CWC and several fluorescence parameters, as well as between CWC and the biomass index, were determined across three biomass harvests from BBCH 57 to 90 (Fig. 13). The fluorescence intensities at 690 nm and 730 nm and the biomass index all increased with increasing CWC, whereas the fluorescence ratio F690/F730 tended to decrease. The fluorescence intensities at 690 and 730 nm and the biomass index behaved similarly among the Empire, Ellvis, and Cubus cultivars. The fluorescence ratio F690/F730, however, showed large differences among all cultivars, being either strongly negatively related to CWC (Ludwig, R^2 = 0.78*** and Cubus, R^2 = 0.78***) or showing no relationship (Empire, R^2 = 0.21 and Ellvis, R^2 = 0.02). The relationships between CWC and the values of chlorophyll fluorescence at 690 nm and 730 nm and the biomass index were uniformly strong, both for each individual cultivar ($R^2 \geq$ 0.83***, 0.84***, and 0.82***, respectively) as well as when values were pooled across all cultivars (R^2 = 0.71***, 0.74***, and 0.74***, respectively).

3.2.1.4 Relationship between canopy water mass and the fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index

The CWM presented a linear relationship with all fluorescence parameters (when significant) and a polynomial one with the biomass index (Fig. 14). Otherwise, the results largely mirrored those for CWC. Significant positive relationships were found between CWM and chlorophyll fluorescence at 690 nm and 730 nm and between CWM and the biomass index, both for each individual cultivar and across all cultivars pooled together. By contrast, the relationship between CWM and the fluorescence ratio F690/F730 tended to be negative when significant (Ludwig and Cubus, as well as all cultivars pooled together).

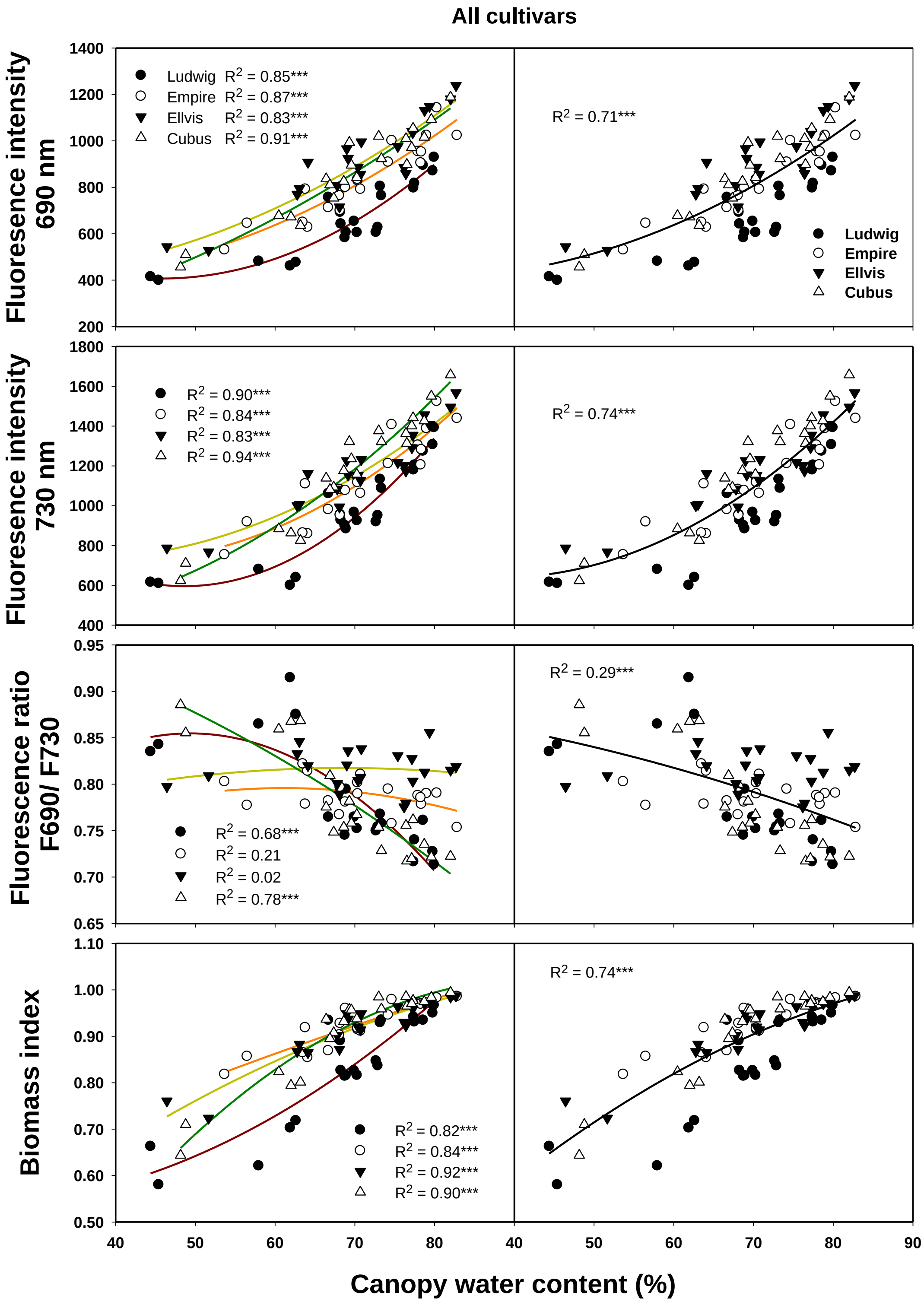

Figure 13. Relationship between canopy water content and fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index. Data were pooled across all watering regimes and all measurements and are presented for each individual cultivar (left) and for all cultivars together (right).

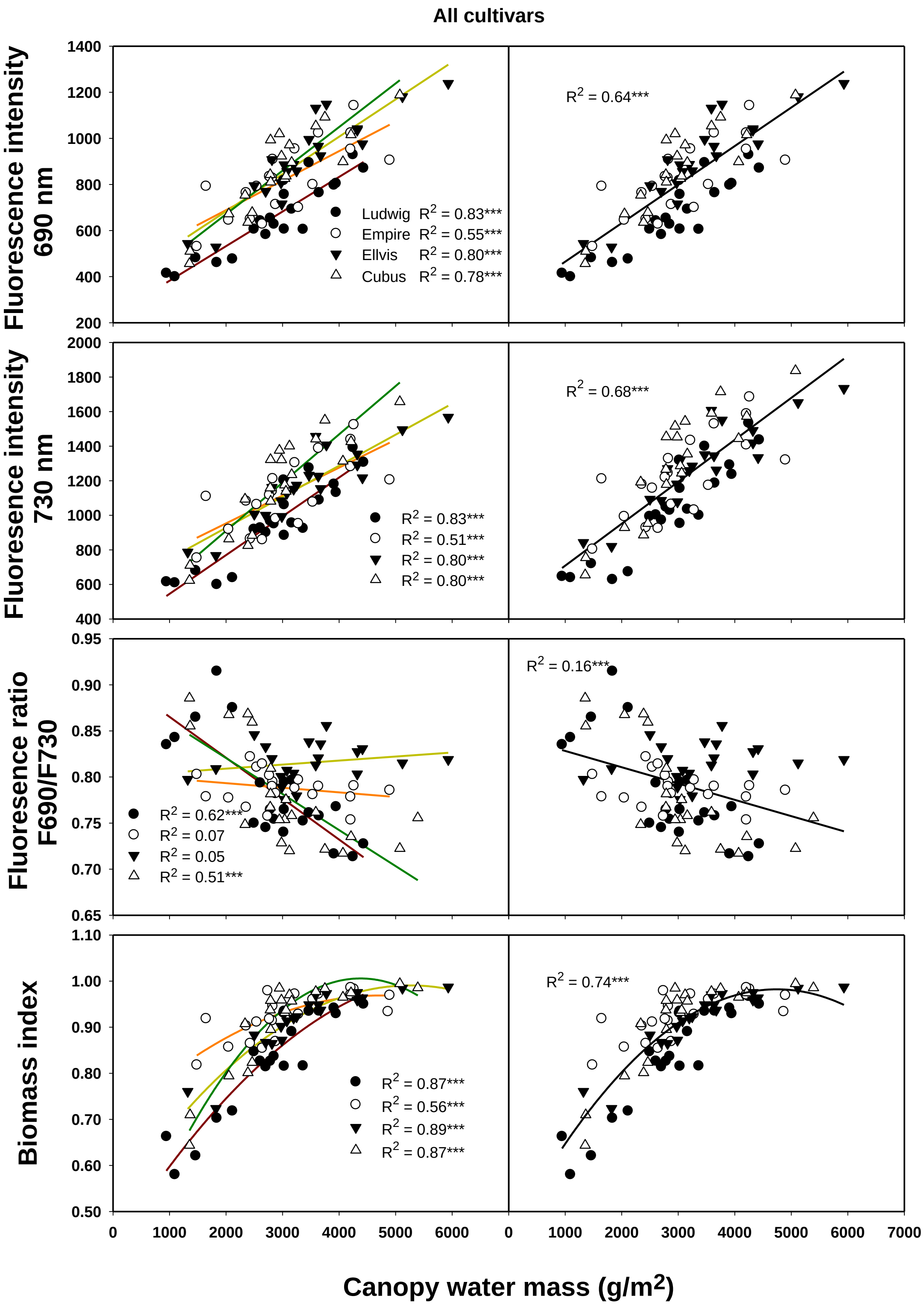

Figure 14. Relationship between canopy water mass and fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index. Data were pooled across all watering regimes and all measurements and are presented for each individual cultivar (left) and for all cultivars together (right).

3.2.1.5 Relationships between leaf water potential (bar) and the fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index

Linear relationships between leaf water potential and fluorescence parameters and the biomass index are shown in Fig. 15. There were stronger relationships between leaf water potential and fluorescence intensity at 690 nm than between leaf water potential and fluorescence intensity at 730 nm, the fluorescence ratio F690/F730, or the biomass index for each cultivar. The coefficients of determination for the relationships between leaf water potential and chlorophyll fluorescence at 690 nm, 730 nm, fluorescence ratio F690/F730, and the biomass index for each cultivar were $R^2 \geq 0.64$***, ≥ 0.13, ≥ 0.34*, and ≥ 0.44 ***, respectively. Average coefficients of determinations for all cultivars values of 0.46***, 0.29***, 0.39***, and 0.48***, respectively, were determined.

3.2.1.6 Relationship between aerial biomass and the fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730 and the biomass index

Coefficients of determination for the linear relationships between aerial biomass and fluorescence parameters and the biomass index are shown in Table 16. A significant relationship with aerial biomass could be shown for fluorescence parameters and the biomass index for each cultivar. However, their significant relationships varied considerably amongst the investigated indices (R^2 = 0.12 to 0.80). The aerial biomass and fluorescence parameters and biomass indices were significantly related to each other for each cultivar and for all cultivars pooled together, with the exception of the relationship between aerial biomass and fluorescence ratio F690/F730 for two cultivars (Empire and Ellvis). The fluorescence intensities at 690 nm and 730 nm and the biomass index proved to be reliable indicators to detect aerial biomass.

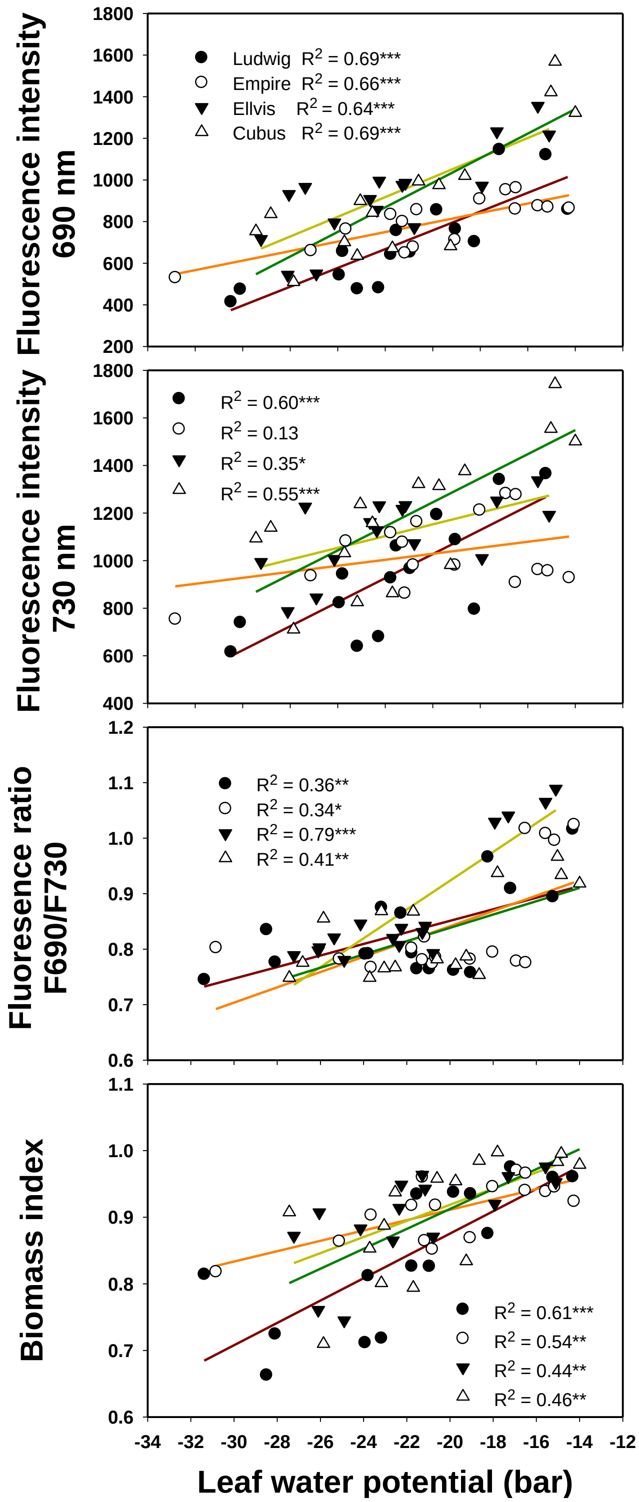

Figure 15. Relationships between leaf water potential and fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index. Data were pooled across all watering regimes and all measurements and presented for each individual cultivar.

Table 16. Coefficients of determination for the relationships between aerial biomass and the fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index. Data were pooled across four watering regimes and all measurements and are presented for each individual cultivar and for all cultivars together.

Cultivars	Fluorescence intensity at 690 nm	Fluorescence intensity at 730 nm	Fluorescence ratio 690/730	Biomass index
Ludwig	0.74***	0.72**	0.55***	0.80***
Empire	0.26*	0.12*	0.03	0.37**
Ellvis	0.68***	0.65***	0.01	0.60***
Cubus	0.64***	0.65***	0.37**	0.58***
All cultivars	0.55***	0.54***	0.11***	0.50***

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

3.2.1.7 The relationships between canopy temperature (°C) and the fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index

Canopy temperature showed stronger relationships with fluorescence intensities at 690 nm and 730 nm and the biomass index than with the fluorescence ratio F690/F730 (Table 17). It was not possible to fit one single regression curve across all measurements. Relationships between canopy temperature (°C) and chlorophyll fluorescence at 690 nm and 730 nm and the biomass index for all cultivars varied from R^2 = 0.62* to 97***. The two fluorescence parameters and the biomass index were inversely related to the fluorescence ratio F690/F730

3.2.1.8 Relative chlorophyll content as affected by four water treatments

Within a given cultivar, no significant changes in relative chlorophyll content were observed among the experimental treatments (Table 18)

3.2.1.9 The relationships between relative chlorophyll content and each of fluorescence intensity at 690 and 730 nm, fluorescence ratio F690/F730 and the biomass index

No significant relationship was observed between any fluorescence index and relative chlorophyll content at any measurement time (Table 19).

Table 17. Coefficients of determination for the relationship between canopy temperature (°C) and fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index for each cultivar. Data were pooled across four watering regimes.

Cultivars	Indices	Date			
		May 25	June 1	June 21	June 23
Ludwig	F690	0.78*	0.98***	0.86*	0.93**
	F730	0.73*	0.98***	0.83*	0.94**
	F690/F730	0.24	0.01	0.08	0.29
	Biomass index	0.95**	0.93**	0.97***	0.95**
Empire	F690	0.72*	0.81*	0.81**	0.81**
	F730	0.77*	0.86*	0.82*	0.80**
	F690/F730	0.47	0.44	0.25	0.47
	Biomass index	0.69*	0.79***	0.96**	0.79*
Ellvis	F690	0.80*	0.93**	0.78*	0.96**
	F730	0.79*	0.87*	0.79*	0.94**
	F690/F730	0.56	0.78*	0.54	0.53
	Biomass index	0.88*	0.92**	0.85*	0.95**
Cubus	F690	0.85*	0.79**	0.66*	0.91**
	F730	0.80*	0.88*	0.62*	0.88*
	F690/F730	0.04	0.11	0.05	0.01
	Biomass index	0.63*	0.84*	0.79*	0.80*

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

Table 18. Chlorophyll contents (SPAD values) for four cultivars under four watering treatments. Values with the same letter are not significantly different ($P \leq 0.05$).

Treatments	Cultivars			
	Ludwig	Empire	Ellvis	Cubus
Irrigated	45.0 a	42.8 a	43.2 a	47.4 a
Rainfed	44.8 a	44.4 a	43.9 a	45.7 a
Late stress	47.2 a	46.0 a	47.0 a	48.9 a
Early stress	47.7 a	46.3 a	47.2 a	50.5 a

Table 19. Coefficients of determination for the relationship between chlorophyll content (SPAD values) and fluorescence intensities at 690 and 730 nm, fluorescence ratio F690/F730, and the biomass index for each cultivar at individual measurements. Data were pooled across four watering regimes.

Cultivars	Indices	Dates					
		May 25	June 1	June 8	June 14	June 21	June 27
Ludwig	F690	0.04	0.26	0.32	0.20	0.10	0.02
	F730	0.02	0.18	0.29	0.20	0.08	0.03
	F690/F730	0.20	0.25	0.01	0.00	0.02	0.42
	Biomass index	0.34	0.07	0.14	0.06	0.11	0.05
Empire	F690	0.40	0.09	0.00	0.00	0.13	0.01
	F730	0.28	0.07	0.02	0.00	0.22	0.00
	F690/F730	0.00	0.04	0.37	0.26	0.44	0.58
	Biomass index	0.40	0.11	0.07	0.12	0.17	0.23
Ellvis	F690	0.00	0.11	0.09	0.23	0.00	0.00
	F730	0.00	0.10	0.07	0.10	0.00	0.00
	F690/F730	0.21	0.50	0.00	0.07	0.05	0.18
	Biomass index	0.00	0.05	0.10	0.40	0.01	0.00
Cubus	F690	0.02	0.10	0.72	0.05	0.50	0.02
	F730	0.01	0.02	0.65	0.00	0.44	0.03
	F690/F730	0.07	0.14	0.08	0.03	0.39	0.20
	Biomass index	0.03	0.01	0.52	0.38	0.25	0.10

3.2.2 Spectral reflectance measurements and physiological parameters of winter wheat in years 2006 and 2007

3.2.2.1 Destructively measured parameters of winter wheat

Minimum, maximum and mean values of destructively measured canopy water content, canopy water mass and leaf water potential of four winter wheat cultivars subjected to four water treatments are shown in Tables 20 & 21. The mean value of CWC within the individual measurements showed a large difference between the cultivars Empire, Ellvis and Cubus compared to Ludwig and the highest mean value was recorded on May 30, 2007 for Empire. There was a large variation in CWM between Empire, Ellvis and Cubus compared to Ludwig on

June 21, 2006. The mean values of LWP at the individual measurements among the cultivars at the same day showed only a small variation. The highest mean value of LWP was recorded on June 19, 2007 for Ellvis.

Table 20. Minimum, maximum and mean values for canopy water content and canopy water mass measured at three dates subjected to four watering treatments.

Cultivars	Dates	CWC (%)			CWM (g/m^2)		
		Min	Max	Mean	Min	Max	Mean
	June 21, 06	67.4	73.2	71.3	1178.0	2307.1	1790.2
Ludwig	July 12, 06	62.1	67.9	64.3	2112.2	3112.7	2482.8
	May 30, 07	70.1	76.9	74.0	2664.7	4558.3	3498.1
	June 21, 06	69.5	75.6	73.9	1751.9	2639.7	2247.1
Empire	July 12, 06	62.3	69.7	65.7	1684.9	2739.1	2399.7
	May 30, 07	73.0	79.8	77.5	2856.5	4997.4	4105.8
	June 21, 06	67.7	76.7	73.5	1496.5	2784.8	2142.0
Ellvis	July 12, 06	61.0	70.2	64.7	1831.6	3038.5	2292.5
	May 30, 07	71.3	79.1	76.6	3024.2	5509.2	4027.1
	June 21, 06	66.9	77.8	72.3	1702.6	2787.1	2301.4
Cubus	July 12, 06	62.7	69.2	65.4	1888.1	3086.2	2549.8
	May 30, 07	69.5	77.3	74.8	2761.5	4730.4	3694.8

Table 21. Minimum, maximum and mean values for canopy water content and canopy water mass subjected to four watering treatments, evaluated at individual measurement.

Cultivars	Dates	LWP (bar)		
		Min	Max	Mean
Ludwig	June 12, 06	-12.0	-15.2	-14.3
	June 21, 06	-14.0	-18.0	-15.2
	July 12, 06	16.8	-20.1	-18.0
	May 30, 07	-17.4	-20.1	-18.8
	June 14, 07	-18.6	-23.1	-20.9
	June 19, 07	-19.3	-20.4	-19.7
Empire	June 12, 06	-12.4	-16.0	-14.8
	June 21, 06	-14.2	-16.7	-15.1
	July 12, 06	-15.1	-19.7	-16.9
	May 30, 07	-18.0	-20.7	-19.0
	June 14, 07	-19.9	-22.1	-20.8
	June 19, 07	-19.3	-22.1	-20.7
Ellvis	June 12, 06	-12.7	-17.4	-15.9
	June 21, 06	-14.6	-18.5	-15.8
	July 12, 06	-14.4	-20.3	-17.4
	May 30, 07	-17.0	-21.6	-19.0
	June 14, 07	-19.7	-22.1	-21.2
	June 19, 07	-20.7	-25.8	-23.1
Cubus	June 12, 06	-12.8	-16.9	-15.7
	June 21, 06	-14.7	-19.9	-16.4
	July 12, 06	-12.7	-20.1	-16.6
	May 30, 07	-18.1	-21.1	-19.5
	June 14, 07	-19.4	-24.6	-21.7
	June 19, 07	-20.0	-24.0	-21.5

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

A significant relationship between canopy water content and spectral indices ($R^2 > 0.57$, $P \leq 0.05$) could be shown for all indices at all measurements. However, the strength of the relationship varied considerably amongst the investigated indices ($R^2 = 0.57 - 0.99$) as indicated in Table 22. NDVI, $R_{600}/R_{780,}$ $(R_{510} + R_{780})/(R_{510} - R_{780})$ were significantly related to CWC within individual measurements and across all measurement dates (Fig. 16), except some measurements with Empire in May 30 2007. On the other hand, the two indices $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$ were significantly related to CWC within individual measurements and across all measurements for each cultivar (Table 22 and Fig. 16). Coefficients of determination were calculated as linear relationships between canopy water content and spectral indices for each cultivar and date and the regression over all, expect NDVI, they were calculated by quadratic functions for the overall regressions.

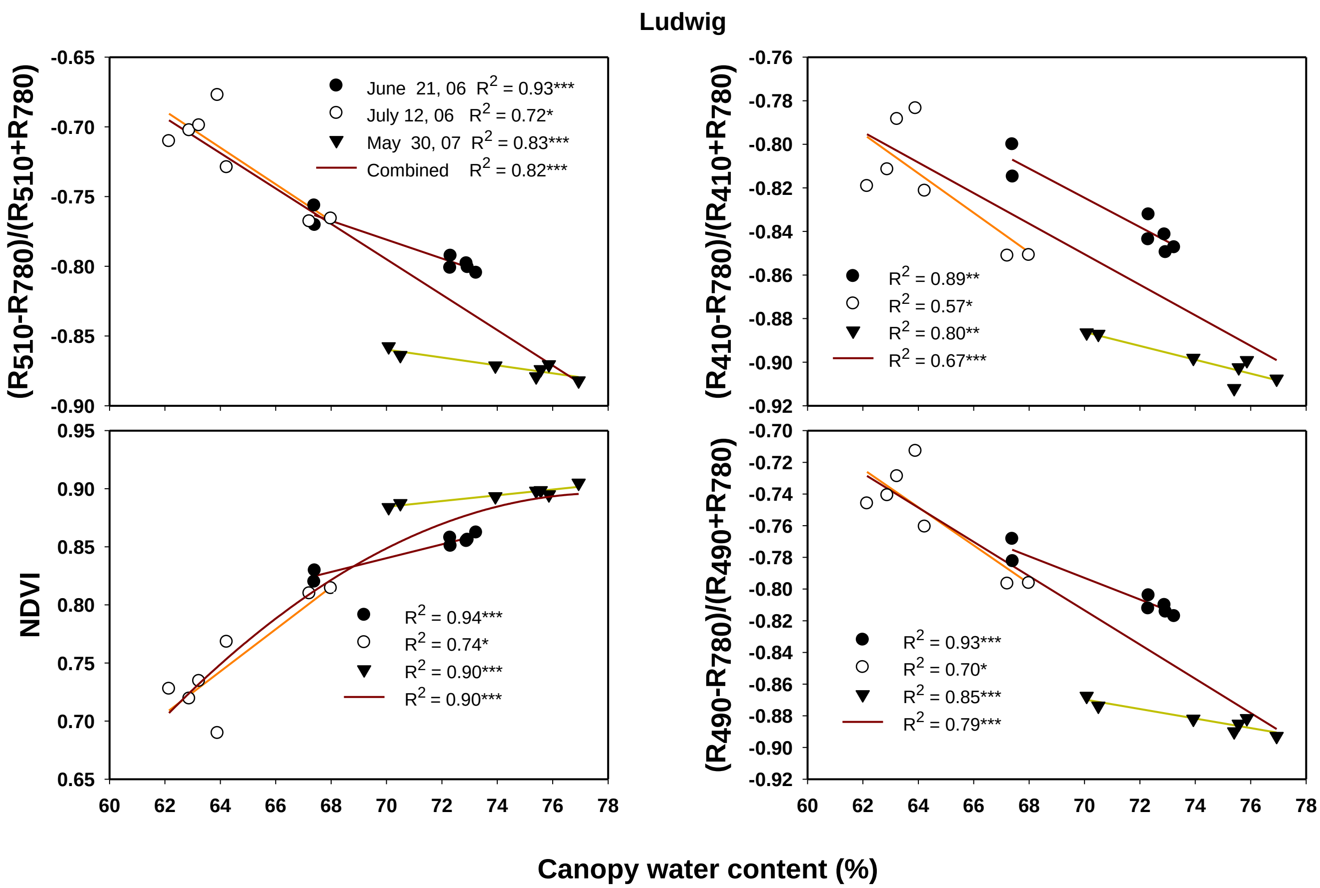

Figure 16. The relationship between canopy water content and four spectral indices for Ludwig subjected to four watering regimes. Data were pooled across four watering regimes. Measurements were taken at three dates and the regressions over all were fitted.

Table 22. Coefficients of determination of the relationship between canopy water content and spectral indices for four winter wheat cultivars subjected to four watering regimes at three 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}
Ludwig	June 21, 06	0.94***	0.93***	0.93***	0.89**	0.94***
	July 12, 06	0.74*	0.72*	0.70*	0.57*	0.76*
	May 30, 07	0.90***	0.83**	0.85**	0.80**	0.72*
	Combined dates	0.90***	0.82***	0.79***	0.67***	0.92***
Empire	June 21, 06	0.99***	0.99***	0.99***	0.98***	0.99***
	July 12, 06	0.87**	0.75*	0.72*	0.58*	0.74*
	May 30, 07	0.00	0.42	0.57*	0.80**	0.05
	Combined dates	0.94***	0.88***	0.87***	0.79***	0.91***
Ellvis	June 21, 06	0.92***	0.96***	0.97***	0.98***	0.93***
	July 12, 06	0.73*	0.77*	0.80**	0.81**	0.69*
	May 30, 07	0.84**	0.90***	0.91***	0.89**	0.72*
	Combined dates	0.88***	0.84***	0.82***	0.75***	0.86***
Cubus	June 21, 06	0.69*	0.68*	0.68*	0.64*	0.71*
	July 12, 06	0.58*	0.59*	0.60*	0.65*	0.58*
	May 30, 07	0.82**	0.73*	0.72*	0.58*	0.83**
	Combined dates	0.78***	0.78***	0.74***	0.66***	0.79***

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

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

The relationship between canopy water mass and four spectral indices were significant or non-significant at individual measurements within four winter wheat cultivars, but were significant when combining data for three dates (Table 23). Significant but weak relationships between spectral indices and CWM ($R^2 > 0.22$, $P \leq 0.05$) could be shown for all indices at all measurements. However, the strength of the relationships varied considerably amongst the investigated indices (R^2 = 0.22 - 0.84) with individual measurements and across all measurements for each cultivar. Coefficients of determination were calculated as linear relationships between canopy water content and spectral indices for each cultivar and date. The index $(R_{410} - R_{780})/(R_{410} + R_{780})$ generally showed stronger relationships with canopy water mass across all measurements than the other spectral indices (Table 23) and was found to be a reliable indicator to detect canopy water mass of winter wheat cultivars (Fig. 17)

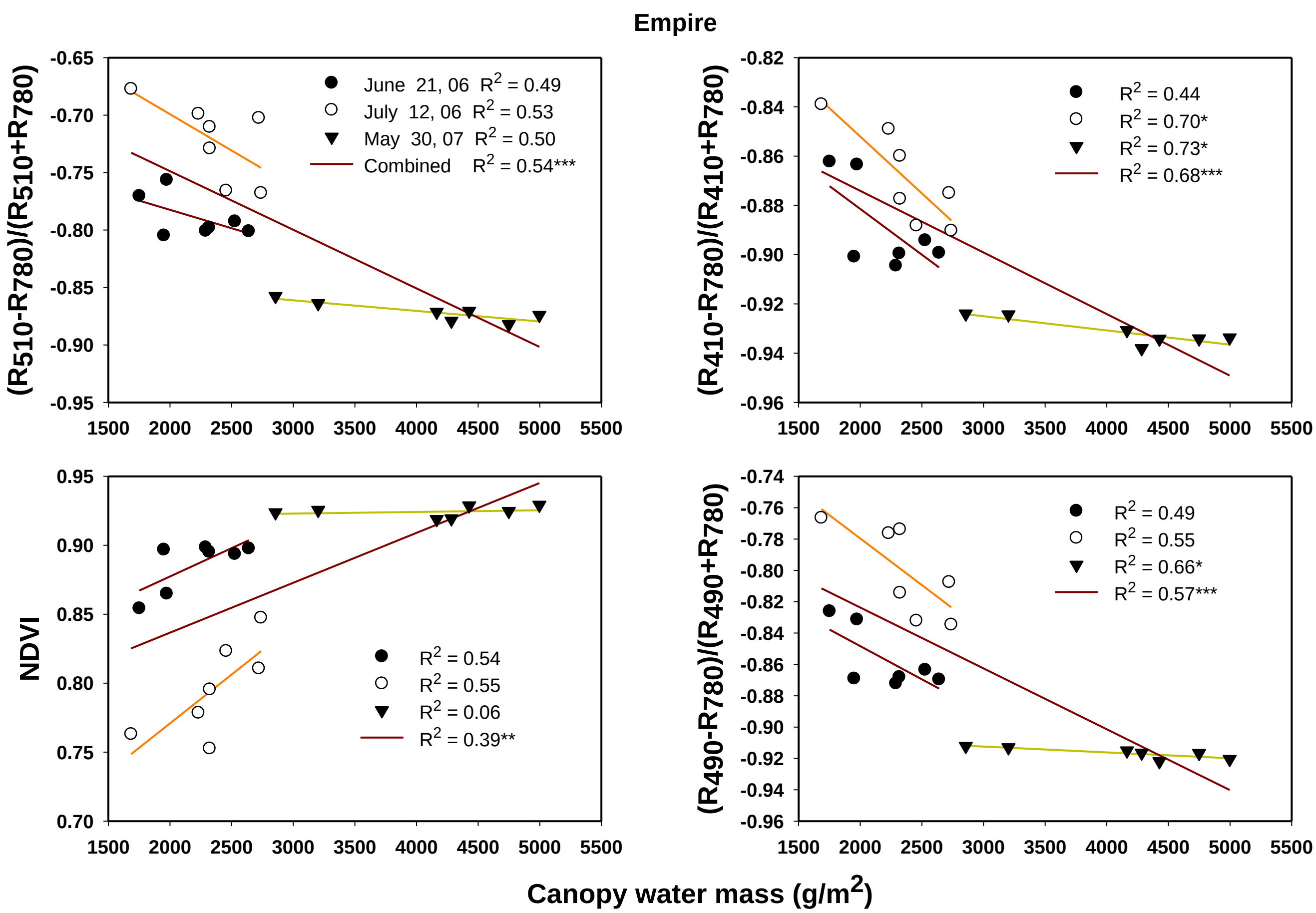

Figure 17. The relationship between canopy water mass and four spectral indices for Empire subjected to four watering regimes. Data were pooled across four watering regimes Measurements were taken at three dates and the regressions over all were fitted.

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Table 23. Coefficients of determination of the relationship between canopy water content and spectral indices for four winter wheat cultivars subjected to four watering regimes at three dates.

Cultivars	Dates	NDVI	$(R_{510}-R_{780})/(R_{510}+R_{780})$	$(R_{490}-R_{780})/(R_{490}+R_{780})$	$(R_{410}-R_{780})/(R_{410}+R_{780})$
Ludwig	June 21, 06	0.37	0.35	0.35	0.33
	July 12, 06	0.83**	0.84**	0.83*	0.78*
	May 30, 07	0.69*	0.54	0.55	0.50
	Combined dates	0.22*	0.39**	0.46***	0.59***
Empire	June 21, 06	0.54	0.49	0.49	0.44
	July 12, 06	0.55	0.53	0.55	0.70*
	May 30, 07	0.06	0.50	0.66*	0.73*
	Combined dates	0.39**	0.54***	0.57***	0.68***
Ellvis	June 21, 06	0.74*	0.68*	0.65*	0.54
	July 12, 06	0.03	0.00	0.01	0.02
	May 30, 07	0.74*	0.63*	0.59*	0.55
	Combined dates	0.37**	0.49***	0.54***	0.69***
Cubus	June 21, 06	0.28	0.26	0.25	0.21
	July 12, 06	0.06	0.04	0.05	0.06
	May 30, 07	0.67*	0.49	0.46	0.39
	Combined dates	0.19*	0.34**	0.38**	0.55***

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

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

Linear relationships between spectral reflectance indices derived from the visible (VIS) or near infrared (NIR) regions to the LWP are shown in Table 24. Significant relationships with $R^2 > 0.90$, $P \leq 0.05$ and with $R^2 > 0.56$, $P \leq 0.001$ to the LWP existed for all indices at the individual measurements or for two spectral indices R_{1240}/R_{840} and $(R_{840} - R_{1650})/(R_{840} + R_{1650})$ across all measurements, respectively. The highest coefficients of determination were obtained between R_{1240}/R_{840} and the leaf water potential of Ludwig cultivar (R^2 = 0.99; P $\leq$ 0.01) at individual measurements. About half of the values of the coefficients of determination for the relationship between three spectral indices and LWP at individual measurement showed $R^2 > 0.55$ without always being statistically significant. The two spectral indices R_{1240}/R_{840} and $(R_{840} - R_{1650})/(R_{840} + R_{1650})$ generally showed stronger relationships with LWP across all measurements and the coefficients of determination varied between (0.56; P $\leq$ 0.001 to 0.74; P $\leq$ 001). As well as there were strong relationships between theses indices and LWP after the data were collected through two years for all cultivars (Fig. 18) The coefficients of determination for both indices with LWP were (0.57; P $\leq$ 0.001).

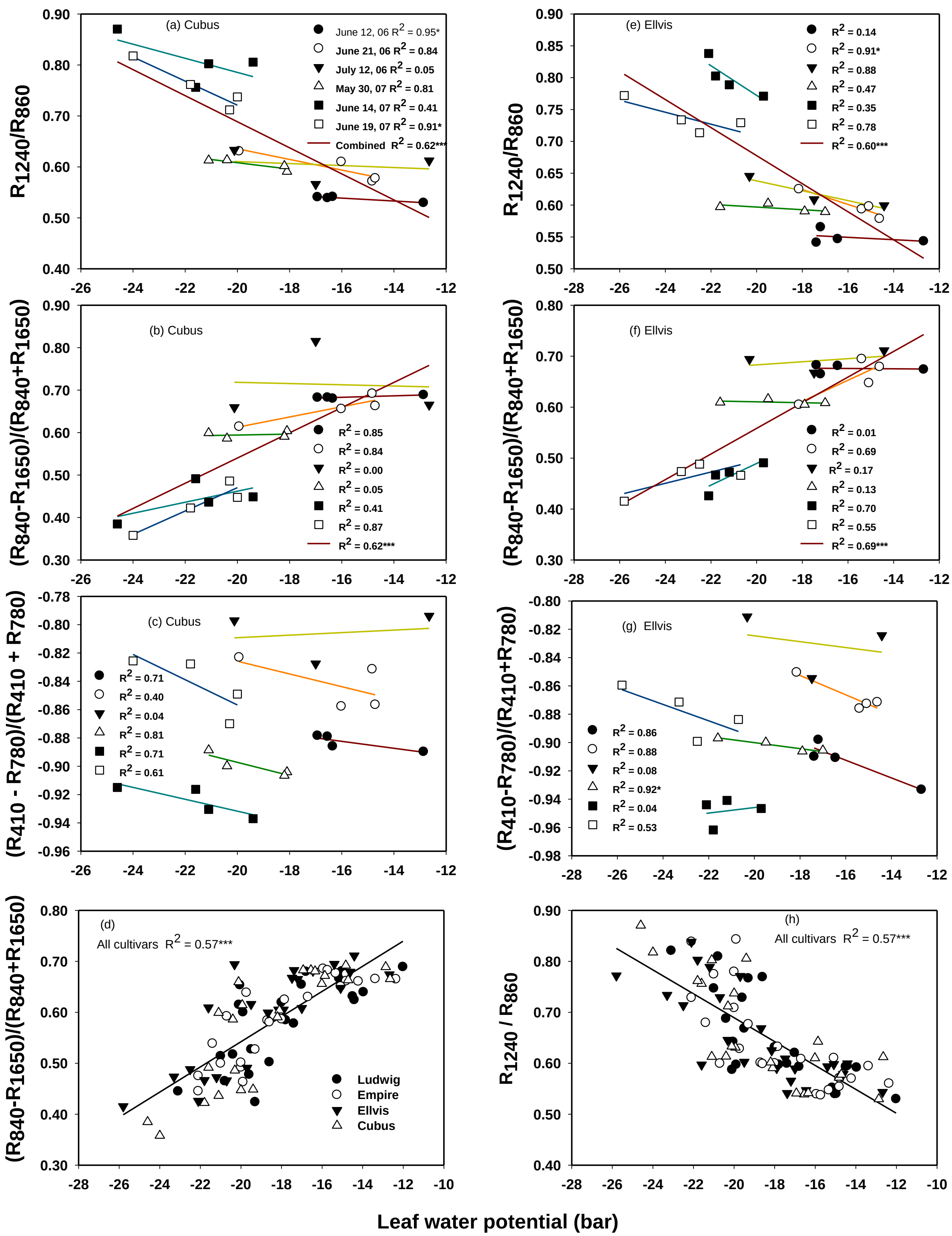

Figure 18. The relationship between LWP and three spectral indices (a,b,c,e,f & g) of Cubus and Ellvis and (d & h) between two spectral indices and LWP of all cultivars subjected to four watering regimes at two years**.**

Table 24 Coefficients of determination of the relationship between leaf water potential and three spectral indices for four winter wheat cultivars subjected to four watering regimes at six dates in two years.

Cultivars	Dates	R_{1240}/R_{860}	$(R_{840} - R_{1650})/(R_{840} + R_{1650})$	$(R_{410} - R_{780})/(R_{410} + R_{780})$
Ludwig	June 12, 06	0.66	0.60	0.39
	June 21, 06	0.99**	0.59	0.69
	July 12, 06	0.75	0.33	0.48
	May 30, 07	0.50	0.90*	0.74
	June 14, 07	0.33	0.48	0.97**
	June 19, 07	0.25	0.33	0.81
	Combined dates	0.60***	0.60***	-
Empire	June 12, 06	0.91*.	0.93*	0.26
	June 21, 06	0.75	0.74	0.97**
	July 12, 06	0.95*	0.90*	0.33
	May 30, 07	0.04	0.55	0.34
	June 14, 07	0.05	0.22	0.16
	June 19, 07	0.30	0.18	0.07
	Combined dates	0.56***	0.74***	-
Ellvis	June 12, 06	0.14	0.01	0.86
	June 21, 06	0.91*	0.69	0.88
	July 12, 06	0.88	0.17	0.08
	May 30, 07	0.47	0.13	0.92*
	June 14, 07	0.35	0.70	0.04
	June 19, 07	0.78	0.55	0.53
	Combined dates	0.60***	0.69***	-
Cubus	June 12, 06	0.95*	0.85	0.71
	June 21, 06	0.84	0.84	0.40
	July 12, 06	0.05	0.01	0.04
	May 30, 07	0.81	0.05	0.81
	June 14, 07	0.41	0.41	0.71
	June 19, 07	0.91*	0.87	0.61
	Combined dates	0.62***	0.62***	-

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

3.2.3 Spectral reflectance measurements and physiological parameters of winter wheat in the year 2008

3.2.3.1 Destructively measured parameter of winter wheat

Minimum, maximum and mean values of destructively measured canopy water content, canopy water mass and leaf water potential of four winter wheat cultivars subjected to four water treatments are shown in Table 25. The mean value of CWC at individual measurement showed very small difference between cultivars and the highest mean value was recorded at June 2, 2008 for Cubus. There was a clear difference in CWM between Cubus and Mulan at the second harvest in July 2, 2008. The mean value of LWP of Cubus was higher than LWP of Mulan at all individual measurements.

Table 25. Minimum, maximum and mean values for canopy water content and canopy water mass and leaf water potential subjected to four watering treatments, evaluated at individual measurements.

Cultivars	Dates	CWC (%)			CWM (g/m^2)			LWP (bar)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
	June 2,08	75.7	82.9	80.3	2913.3	4991.8	4169.5	-15.4	-20.9	-17.3
Cubus	June 15,08	-	-	-	-	-	-	-15.3	-22.1	-18.5
	July 2,08	54.6	68.8	63.2	1264.6	3769.8	2771.0	-15.9	-21.5	-19.0
	June 2,08	74.3	82.4	79.5	2707.6	4819.1	4141.5	-13.5	-19.1	-15.6
Mulan	June 15,08	-	-	-	-	-	-	-13.6	-18.8	-16.0
	July 2,08	55.7	71.0	64.2	1703.6	4269.5	3067.0	-13.3	-19.5	-16.8

(-) no measurements

3.2.3.2 Influence of four water regimes on destructively measured parameters of wheat

Figures 19 a & b show the change in canopy water content, canopy water mass and leaf water potential subjected to four watering treatments. At the first harvest, the CWC of Mulan and Cubus were greatly affected by the early stress treatments, but at the second harvest, the CWC and CWM of Mulan and Cubus were greatly affected by the early stress and late stress treatments.

Generally the early stress treatment existed significant effects on CWC and CWM compared to the other treatments.

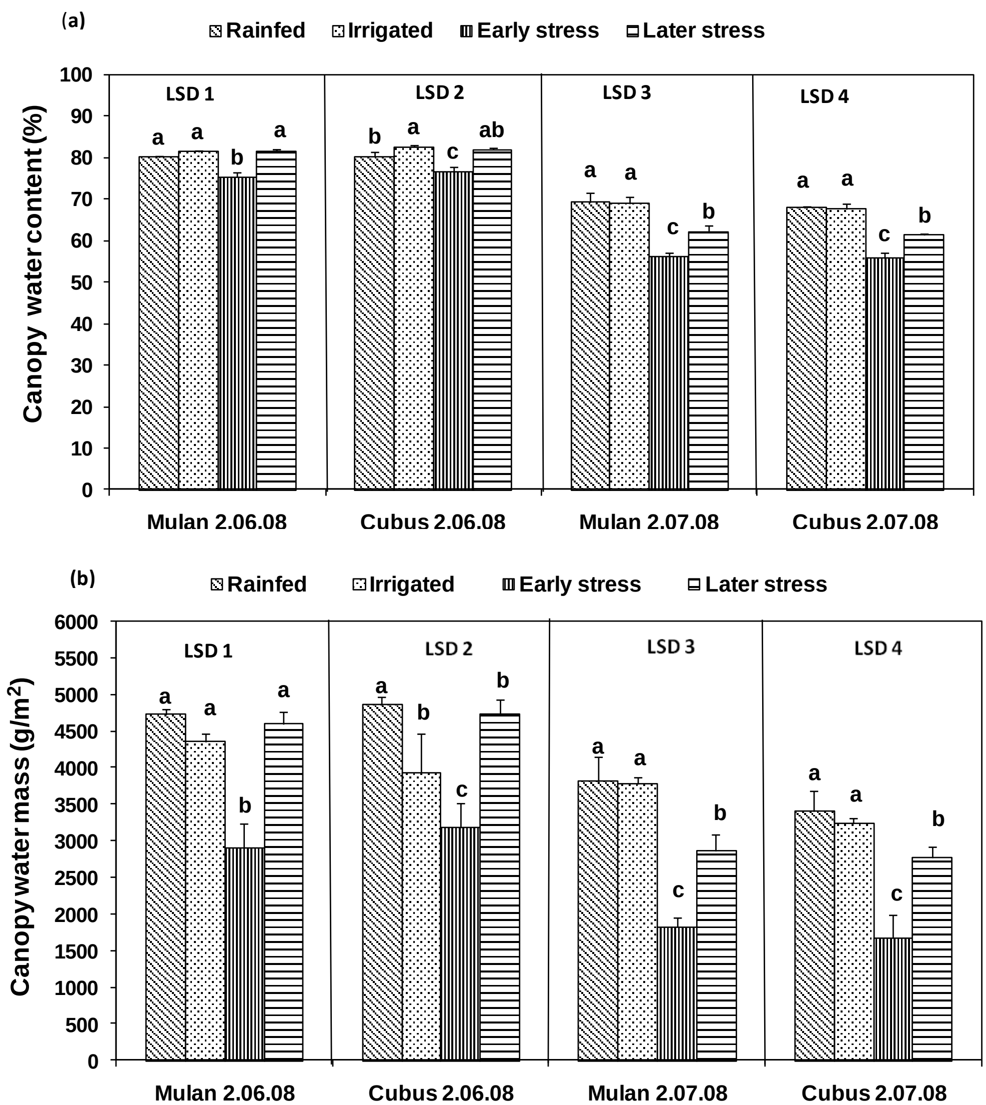

Figure 19. (a) Canopy water content and (b) canopy water mass in Mulan and Cubus as affected by four watering regimes at two harvests. Values with the same letter are not statistically different ($P \leq 0.05$) between the treatments.

The leaf water potential of Mulan was affected by the early stress treatment at all measurements days (Fig. 20). On the other hand, the LWP of Mulan and Cubus was affected by the early and later stress compared to other treatments. Cubus showed the highest LWP with in all water regimes at all individual measurements compared to the LWP of Mulan

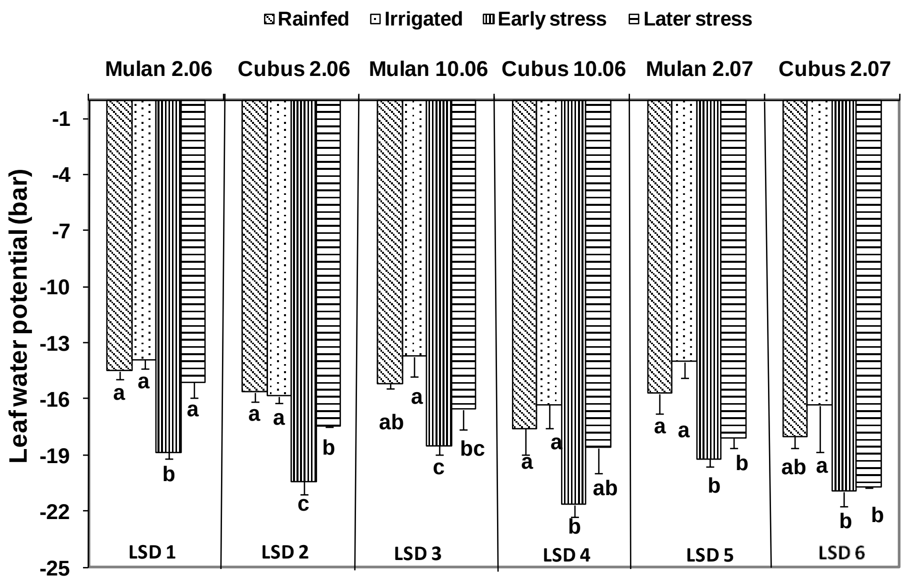

Figure 20. Leaf water potential in Mulan and Cubus as affected by four water regimes at three measurement days. Values with the same letter are not statistically different (P ≤ 0.05) between the treatments.

3.2.3.3 Influence of four water regimes on five spectral indices of wheat

The five spectral reflectance indices of Mulan were not affected by water treatments at the first measurement (Table 26). The four spectral reflectance indices of Cubus were generally affected by the early stress and later stress treatments compared to irrigated treatment at the first measurement. At the second and third measurement, the spectral reflectance indices of Cubus and Mulan were generally affected by the early stress, later stress and rainfed treatments. Four spectral reflectance indices were generally affected by the early stress treatments at all measurement times.

Table 26. Values of spectral reflectance indices as affected by four water treatments. Values with the same letter are not statistically different ($P \leq 0.05$) between the treatments.

Treatments	$(R_{410}-R_{780})/(R_{490}+R_{780})$	NDVI	$(R_{490}-R_{780})/(R_{490}+R_{780})$	$(R_{510}-R_{780})/(R_{510}+R_{780})$	R_{600}/R_{780}
		Cubus			
		First measurement at 2.06			
Irrigated	-0.92 d	0.91 a	-0.90 c	-0.89 c	0.07 b
Rainfed	-0.91 b	0.91 a	-0.89 b	-0.88 b	0.07 b
Early stress	-0.90 a	0.87 b	-0.87 a	-0.86 a	0.09 a
Later stress	-0.92 c	0.91 a	-0.90 c	-0.88 ab	0.07 b
		Second measurement at 10.06			
Irrigated	-0.93 c	0.91 a	-0.90 d	-0.89 d	0.07 b
Rainfed	-0.91 b	0.90 b	-0.89 c	-0.88 c	0.07 b
Early stress	-0.88 a	0.84 d	-0.85 a	-0.84 a	0.12 a
Later stress	-0.91 b	0.89 c	-0.88 b	-0.88 b	0.08 b
		Third measurement at 2.07			
Irrigated	-0.89 c	0.78 a	-0.83 b	-0.80 b	0.16 b
Rainfed	-0.80 c	0.80 a	-0.83 b	-0.79 b	0.15 b
Early stress	-0.84 a	0.77 a	-0.71 a	-0.67 a	0.30 a
Later stress	-0.87 b	0.57 b	-0.81 b	-0.77 b	0.17 b
		Mulan			
		First measurement at 2.06			
Irrigated	-0.91 a	0.90 a	-0.89 a	-0.87 a	0.08 a
Rainfed	-0.91 a	0.89 a	-0.88 a	-0.87 a	0.08 a
Early stress	-0.90 a	0.89 a	-0.88 a	-0.87 a	0.08 a
Later stress	-0.90 a	0.89 a	-0.88 a	-0.87 a	0.09 a
		Second measurement at 10.06			
Irrigated	-0.93 c	0.91 a	-0.90 c	-0.89 c	0.07 c
Rainfed	-0.91 b	0.90 b	-0.88 b	-0.87 b	0.08 b
Early stress	-0.89 a	0.85 c	-0.85 a	-0.84 a	0.10 a
Later stress	-0.91 b	0.89 b	-0.88 b	-0.88 b	0.08 b
		Third measurement at 2.07			
Irrigated	-0.91 c	0.83 a	-0.87 c	-0.83 c	0.14 c
Rainfed	-0.91 c	0.81 ab	-0.86 c	-0.86 d	0.11 d
Early stress	-0.89 b	0.77 b	-0.76 a	-0.72 a	0.26 a
Later stress	-0.85 a	0.61 c	-0.83 b	-0.80 b	0.16 b

3.2.3.4 The relationship between canopy water content and spectral indices of two wheat cultivars subjected to four watering regimes in 2008

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 27. 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 21. 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 Table 27.

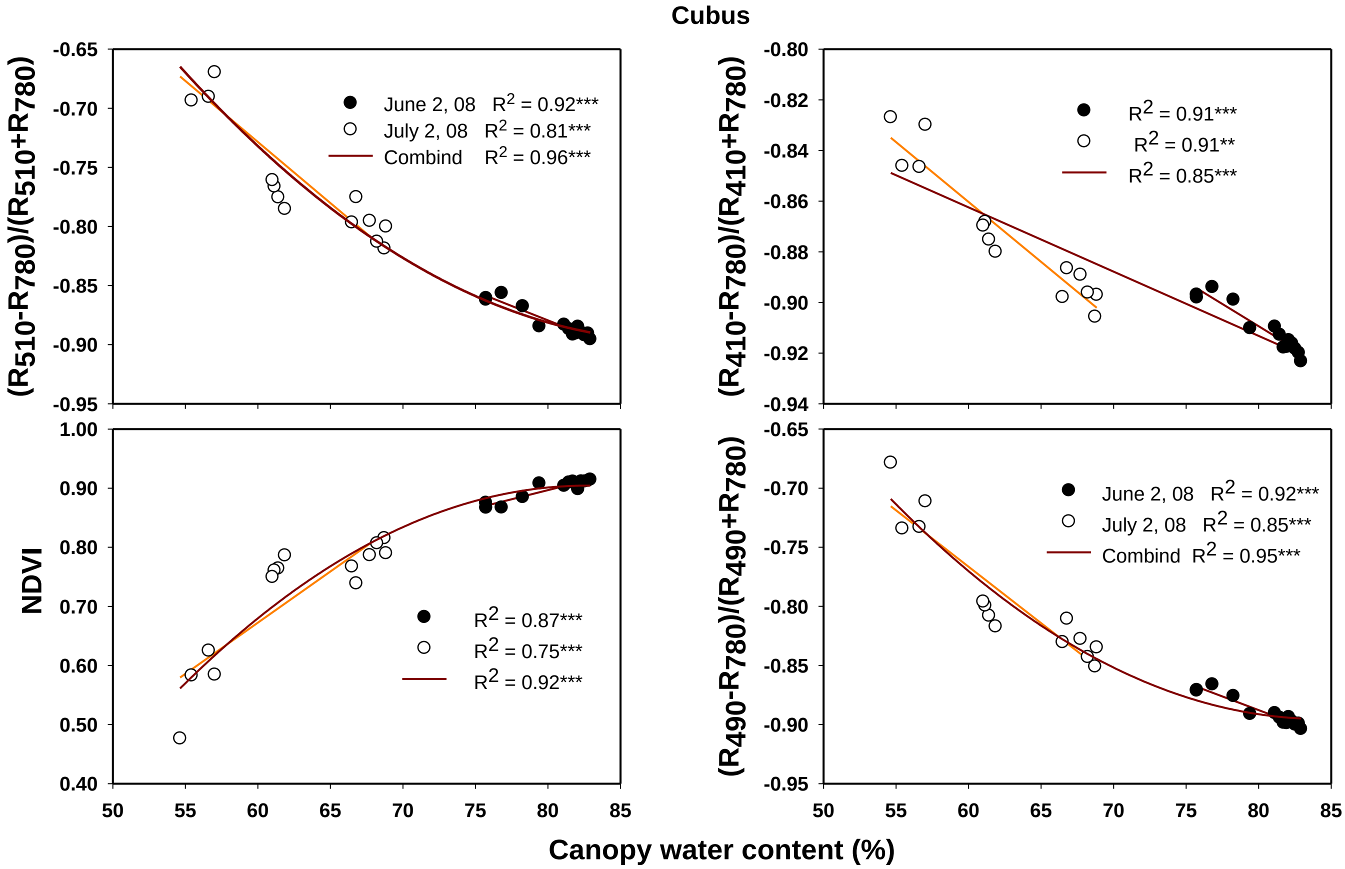

Figure 21. The relationship between canopy water content and four spectral indices for Cubus subjected to four watering regimes. Data were pooled across four watering regimes. Measurements were taken at two dates and the regressions over all were fitted.

Table 27. 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***	0.92***	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***

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

3.2.3.5 The relationship between canopy water mass and spectral indices of wheat cultivars subjected to four watering regimes in 2008

The coefficients of determination for the relationships between five spectral indices and canopy water mass of Cubus and Mulan at individual measurement and combined data for each cultivar are given in Table 28. The relationship between four indices and canopy mass of Cubus at individual measurements and combining data for each cultivar are shown in Figure 22. 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 ($R^2 \geq 0.53$; $P \leq 0.001$). 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 ($R^2 = 0.88$; $P \leq 0.001$).

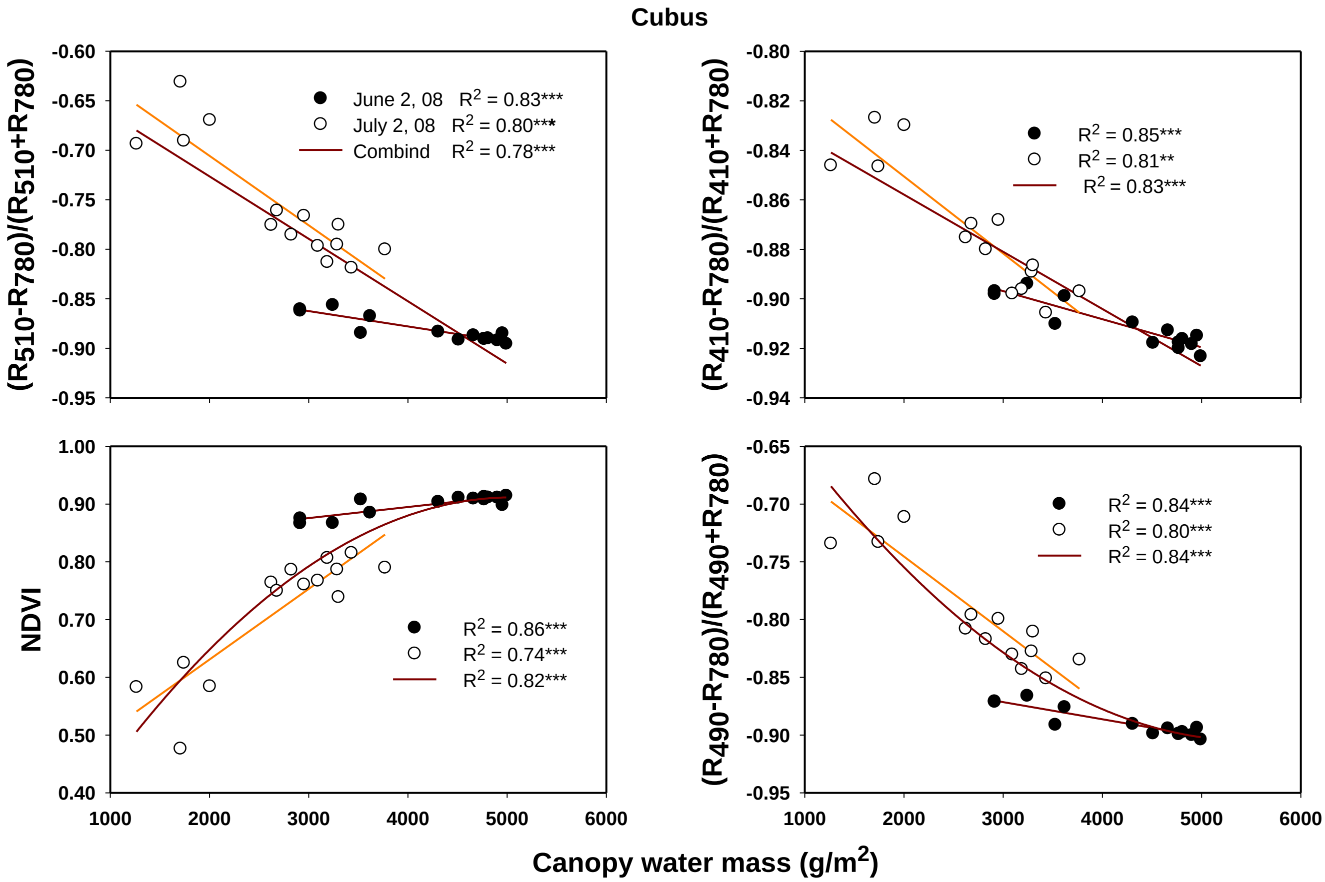

Figure 22. The relationship between canopy water mass and four spectral indices for Cubus subjected to four watering regimes. Data were pooled across four watering regimes. Measurements were taken at two dates and the regressions over all were fitted.

Table 28. 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***

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

3.2.3.6 The relationship between leaf water potential and spectral indices of two wheat cultivars subjected to four watering regimes in 2008

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. 23). 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.

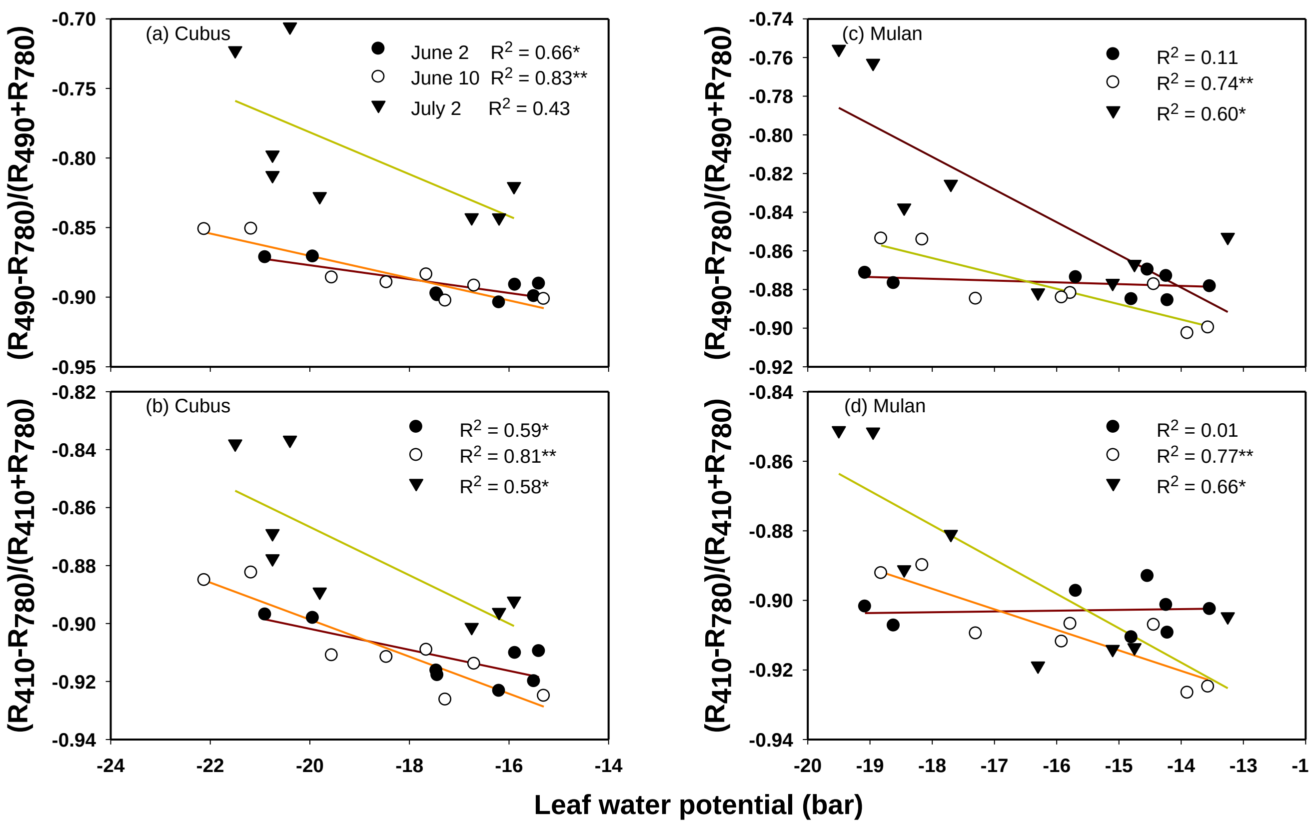

Figure 23. The relationship between leaf water potential and two spectral indices of (a & b) Cubus and (c & d) Mulan subjected to four watering regimes in 2008.

3.2.4 The stability of spectral reflectance indices to detect water content in winter wheat cultivars by combining data from two passive reflectance sensors

The data of spectral indices and water content of plant was combined throughout three years to study the stability of spectral indices to detect the water content of plants regardless of the effects of cultivars, growth stage and the types of passive sensors.

3.2.4.1 The relationship between canopy water content and three spectral indices of wheat cultivars throughout three years

The relationship between CWC and three spectral indices NDVI, R_{600}/R_{780} and $(R_{490} - R_{780})/((R_{490} + R_{780})$ were determined throughout three years at different growth stages of five cultivars (Fig. 24). NDVI and the $(R_{490} - R_{780})/(R_{490} + R_{780})$ increased with increasing CWC, whereas R_{600}/R_{780} tended to decrease. The relationships between CWC and NDVI, R_{600}/R_{780} and $(R_{490} - R_{780})/(R_{490} + R_{780})$ were uniformly strong, both for each individual cultivar ($R^2 \geq 0.86$***, $R^2 \geq 0.83$***, and $R^2 \geq 0.72$***, respectively) as well as for pooled values across all cultivars ($R^2 = 0.88$***, $R^2 = 0.84$***, and $R^2 = 0.67$***, respectively). $(R_{490} - R_{780})/(R_{490} + R_{780})$ was more affected by cultivars compared to NDVI and $R_{600}/R_{780.}$

3.2.4.2 The relationship between canopy water mass and spectral index $(R_{410} - R_{780})/(R_{410} + R_{780})$ of wheat cultivars throughout three years

The relationship between CWM and the spectral index $(R_{410} - R_{780})/(R_{410} + R_{780})$ was determined throughout three years at different growth stages of five cultivars (Fig. 25). Significant nonlinear negative relationships were found between CWM and $(R_{410} - R_{780})/(R_{410} + R_{780})$ both for each individual cultivar ($R^2 \geq 0.60$***) and across all cultivars pooled together ($R^2 = 0.60$***). $(R_{410} - R_{780})/(R_{410} + R_{780})$ showed differences between all cultivars and the relationship between this index and CWM was not the same across the cultivars evaluated.

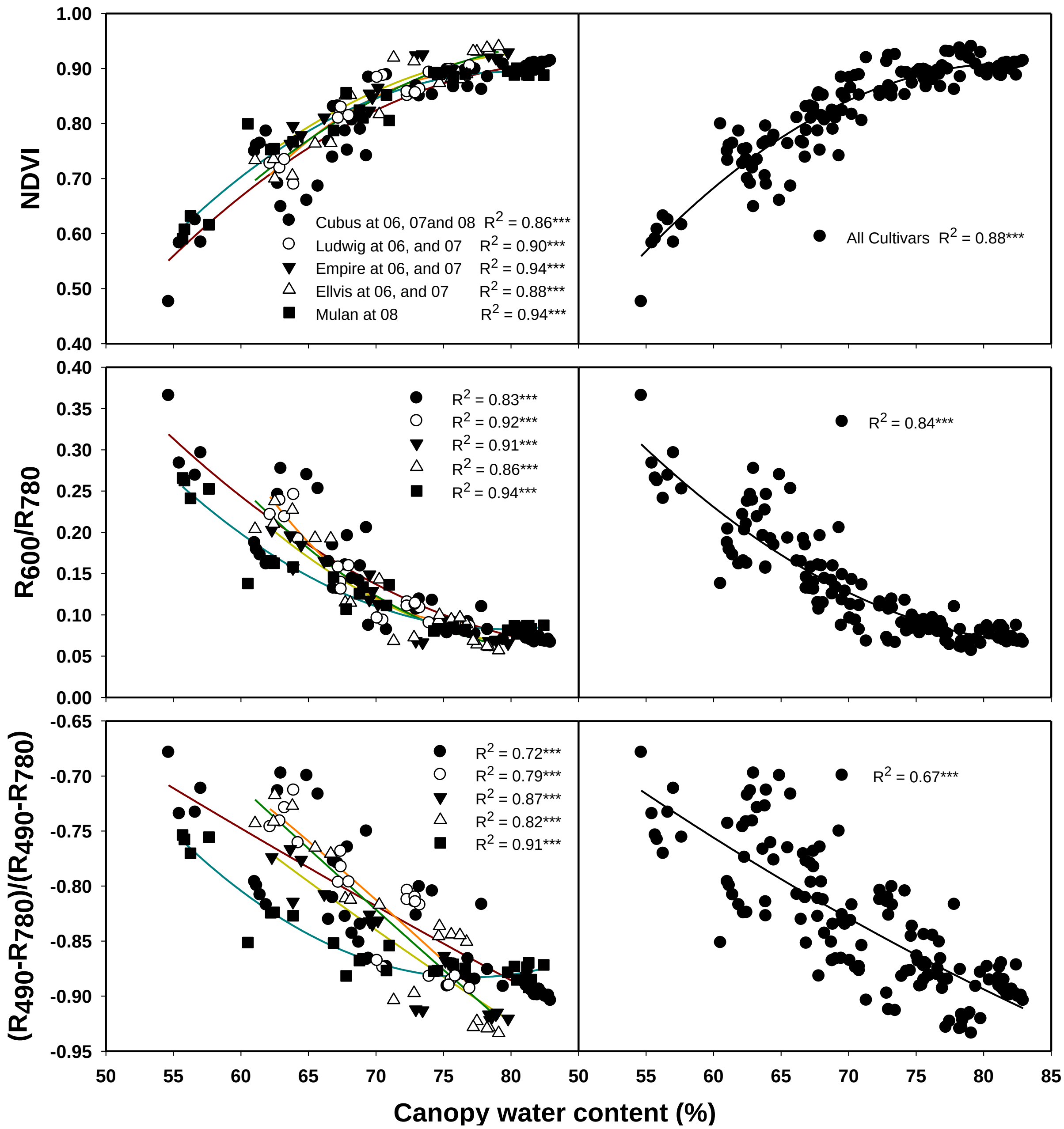

Figure 24. The relationship between canopy water content and three spectral indices of five cultivars. Data were pooled across four watering regimes and presented for each individual cultivar (left) and for all cultivars together (right).

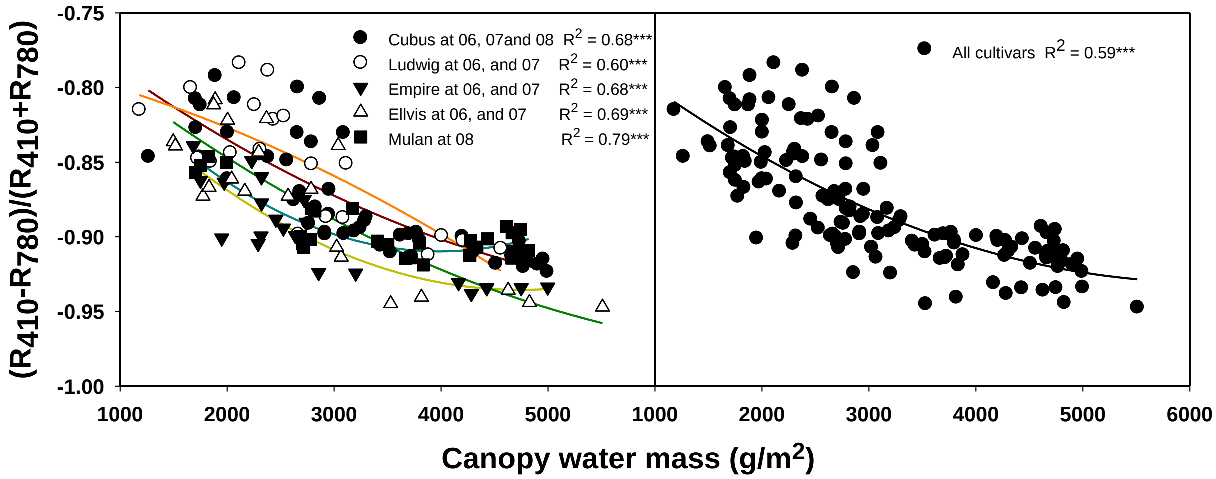

Figure 25. The relationship between canopy water mass and three $(R_{410} - R_{780})/(R_{410} + R_{780})$ of five cultivars. Data were pooled across four watering regimes and presented for each individual cultivar (left) and for all cultivars together (right).

3.2.5 Near infrared temperature measurements and physiological parameters of winter wheat in 2005, 2006 and 2007

3.2.5.1 The relationship between leaf water potential and canopy temperature of wheat cultivars subjected to four watering regimes throughout three years

The coefficients of determination for the relationship between leaf water potential and canopy temperature of all cultivars are given in Table 29 for 2006 and depicted in Figures 26 and 27 for 2005 and 2007**.** The leaf water potential was negatively related to canopy temperature for all individual measurements and for the combined data for both each cultivar and all cultivars. A significant relationship ($R^2 > 0.90$, $P \leq 0.05$) or ($R^2 > 0.38$, $P \leq 0.05$) to the LWP could be shown with canopy temperature at the individual measurement dates or across all measurements in 2005, 2006 and 2007. More than half of the R^2-values for the individual measurements showed $R^2 >$ 0.55 and not all of the relationships were significant. Leaf water potential showed stronger relationships with canopy temperature in 2005 ($R^2 > 0.69$, $P \leq 0.001$) and 2007 ($R^2 > 0.65$, $P \leq$

0.001) when the data were combined for both each cultivar and all cultivars compared to those in 2006 ($R^2 > 0.25$, $P \leq 0.05$).

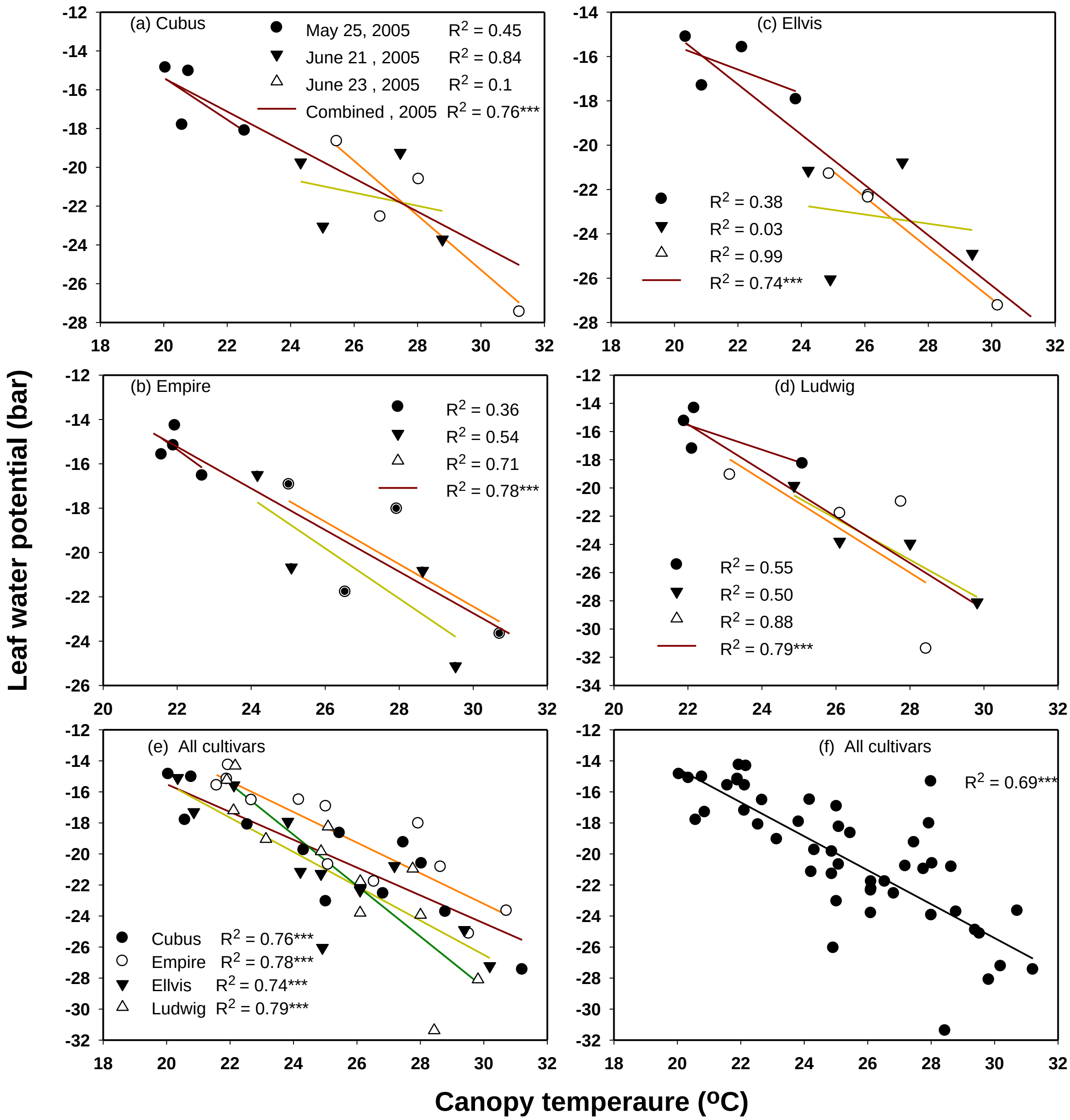

Figure 26. The relationship between canopy temperature and leaf water potential of four cultivars (a) Cubus, (b) Empire, (c) Ellvis, (d) Ludwig at individual measurement and across all measurement times in 2005, as well as by combining data for each and all cultivars (e and f).

Table 29. Coefficients of determination between leaf water potential and canopy temperature of four wheat cultivars subjected to four watering regimes at individual measurements and across all measurement times in 2006, as well as combining data for each and all cultivars.

Cultivars	Dates	Min and max canopy temperatures	Min and max leaf water potentials	R^2
	June 12, 2006	20.9 - 22.5	(-16.9) - (-12.9)	0.51
Cubus	June 21, 2006	25.5 - 27.7	(-19.9) - (-14.7)	0.94*
	July 11, 2006	25.3 - 29.1	(-20.1) - (-12.7)	0.00
	Combined dates	20.9 - 29.1	(-20.1) - (-12.7)	0.10
	June 12, 2006	21.2 - 24.4	(-15.7) - (-12.4)	0.14
Empire	June 21, 2006	24.7 - 29.3	(-16.7) - (-14.2)	0.88
	July 11, 2006	25.2 - 29.4	(-19.7) - (-13.4)	0.75
	Combined dates	21.2 - 29.4	(-19.7) - (-12.4)	0.38*
	June 12, 2006	20.8 - 24.2	(-17.4) - (-12.7)	0.41
Ellvis	June 21, 2006	24.7 - 29.3	(-18.2) - (-14.6)	0.96*
	July 11, 2006	25.5 - 29.9	(-20.3) - (-14.4)	0.41
	Combined dates	20.8 - 29.9	(-20.3) - (-12.7)	0.30
	June 12, 2006	21.6 - 24.5	(-15.2) - (-12.0)	0.42
Ludwig	June 21, 2006	24.9 - 28.2	(-18.0) - (-14.0)	0.95*
	July 11, 2006	25.5 - 29.7	(-20.1) - (-16.8)	0.75
	Combined dates	21.6 - 29.7	(-20.1) - (-12.0)	0.73***
All cultivars	Combined dates	20.8 - 29.9	(-20.3) - (-12.0)	0.25***

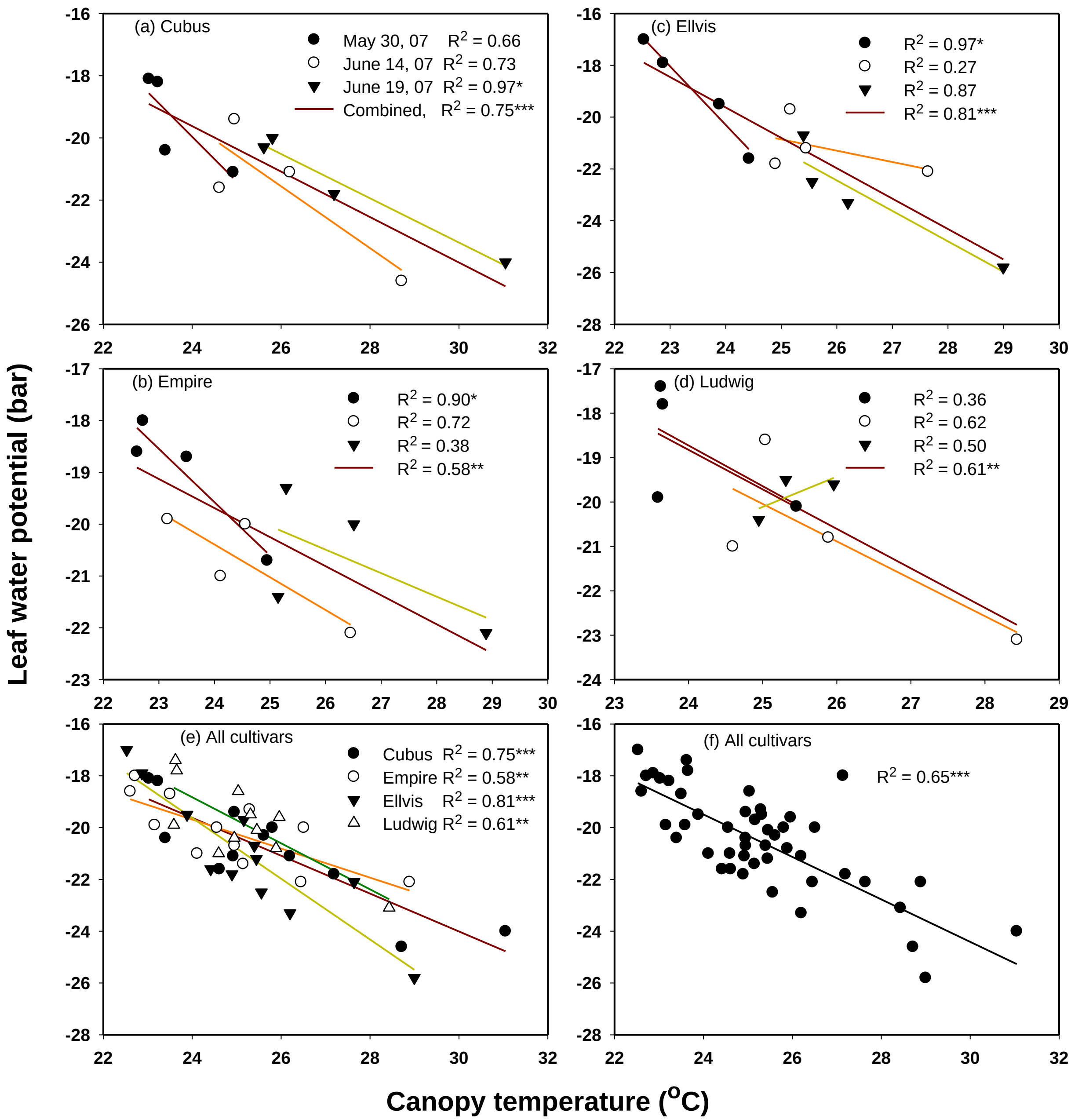

Figure 27. The relationship between canopy temperature and leaf water potential of four cultivars (a) Cubus, (b) Empire, (c) Ellvis, (d) Ludwig at individual measurement and across all measurement times in 2007, as well as by combining data for each and all cultivars (e and f).

3.2.5.2 The relationships between canopy water content and canopy temperature of wheat cultivars subjected to four watering regimes throughout three years

The relationships between canopy water content and canopy temperature in three different year are shown in Figurers 28 a, b & c. The effect of water stress on wheat induced decreases in CWC and increases in CT. Canopy water content shows a significant relationship with CT at most of the measurements dates in 2005 ($R^2 > 0.61$, $P \leq 0.05$), in 2006 ($R^2 > 0.64$, $P \leq 0.05$) and in 2007 ($R^2 > 0.68$, $P \leq 0.05$). It was not possible to fit one single regression curve across all measurements.

3.2.5.3 The relationship between canopy water mass and canopy temperature of wheat cultivars subjected to four watering regimes throughout three years

The relationships between canopy water mass and canopy temperature in three different years are shown in Figures 29 a, b & c. Canopy water mass showed a negative association with CT, but at most of the measurements dates non-significant relationships in 2005 and 2006 were found. On the other hand, canopy water mass had a significant relationship with CT in 2007. The coefficients of determination for the significant relationship between CWM and CT varied between 0.61* to 0.94*** over three years measurements. The relation was affected by day and cultivars.

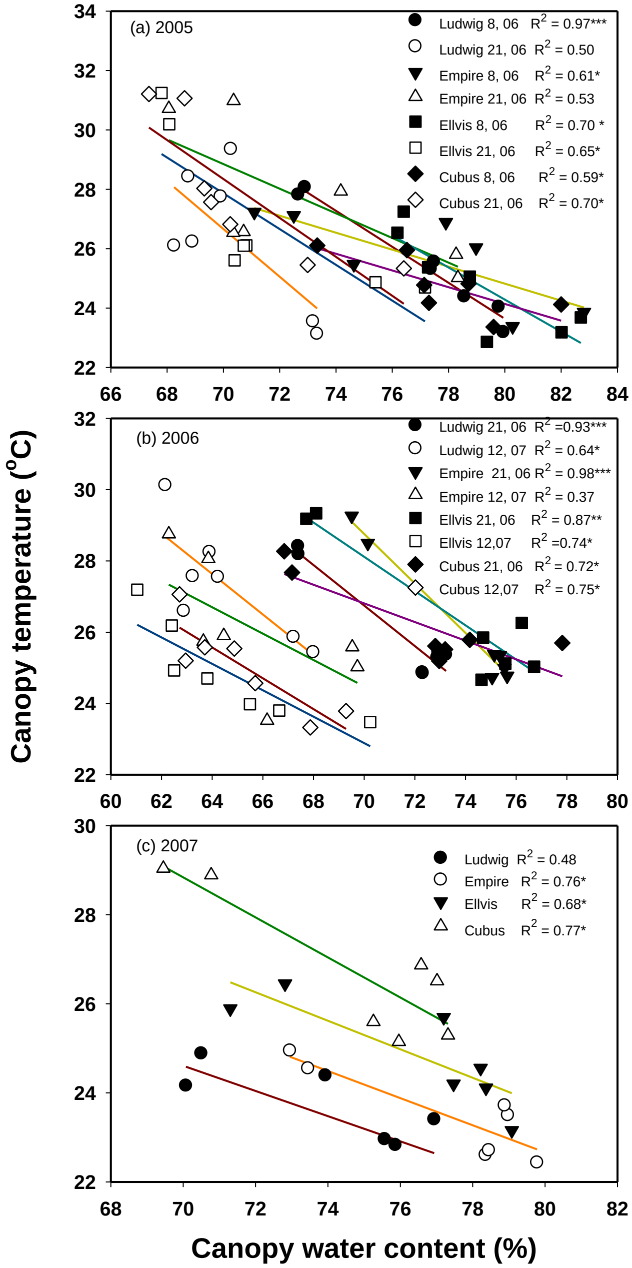

Figure 28. The relationship between canopy temperature and canopy water content of four cultivars at individual measurements in (a) 2005, (b) 2006 and (c) 2007 subjected to four watering regimes.

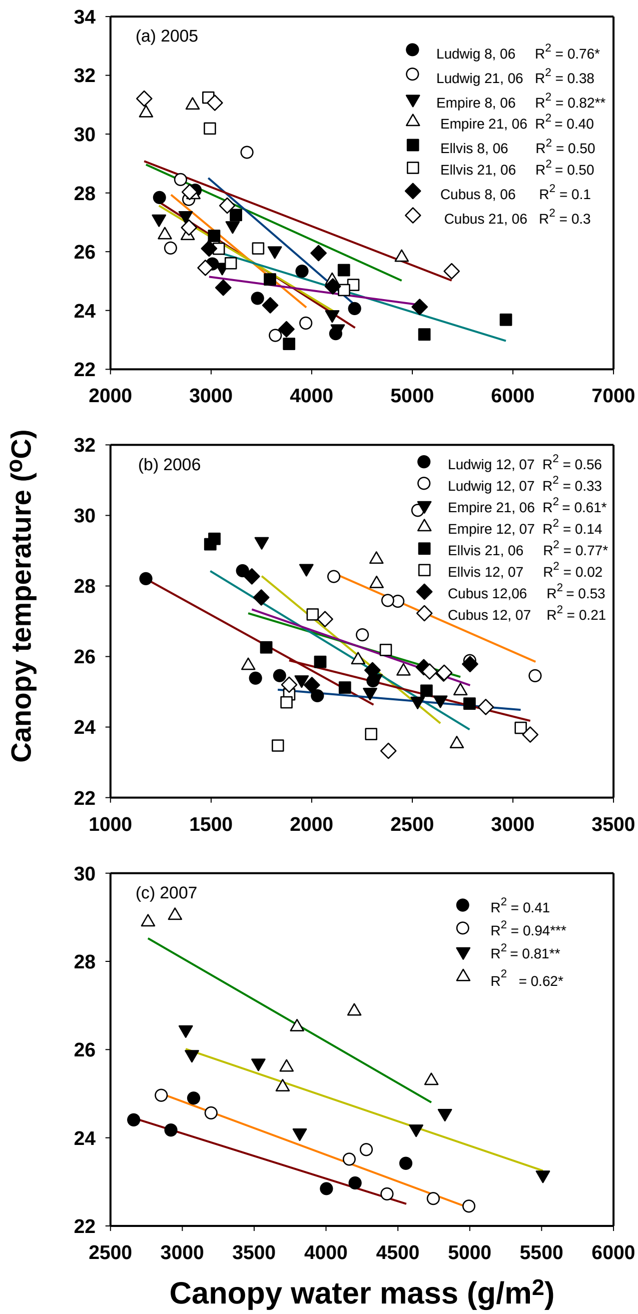

Figure 29. The relationship between canopy temperature and canopy water mass of four cultivars at individual measurements in (a) 2005, (b) 2006 and (c) 2007 subjected to four watering regimes.

3.3 Darkroom experiments

3.3.1 Influence of six water treatments on leaf water potential, relative water content, leaf water content and canopy water content of wheat and maize

The changes in leaf water potential (LWP), relative water content (RWC), leaf water content (LWC) and canopy water content (CWC) of wheat and maize were determined under six water treatments (Table 30). The LWP, RWC, LWC, and CWC of wheat and maize varied with water stress levels and the lowest values were generally observed at the highest stress level of six water treatments of wheat but they were not constant with maize. The LWP, RWC, LWC, and CWC of wheat decreased more markedly at increasing stress level but the difference in maize was rather small. The change in leaf water potential of wheat was more pronounced between the stress levels than for the other parameters. Significant differences in LWP, RWC, LWC, and CWC due to changing in stress levels, although not generally, were observed for wheat and maize. No significant changes were observed in LWC of maize between six water treatments. The sensitivity of maize to drought stress was less than that in wheat. Standard deviations of LWC and CWC in wheat and maize were much lower compare to the standard deviations of LWP and RWC.

3.3.2 Influence of six water treatments on the leaf growth of wheat and maize

The changes in leaf elongation of wheat under six water treatments from July 15 to July 20, 07.2009 were 20.5, 20.8, 19.3, 17.1, 15.6, and 11.9 cm for control, stress 1, stress 2, stress 3, stress 4, and stress 5 treatments, respectively (Table 31). The data for maize were 16.3, 17.3, 15.9, 15.5, 14.4 and 14.2 cm (Table 32). Both crops wheat and maize showed reductions in leaf growth particularly with high stress levels. Leaf growth of wheat decreased more markedly under higher stress than that of maize.

Table 30. The leaf water potential (bar), relative water content (%), leaf water content (%) and canopy water content (%) of wheat and maize as affected by six water treatments. The mean difference was significant at the 0.05 level. SD indicates standard deviations. Values with the same letter are not statistically different ($P \leq 0.05$).

Water regimes	Physiological parameters							
	LWP	SD	RWC	SD	LWC	SD	CWC	SD
				wheat				
Control	-15.3 a	4.4	87.9 a	2.6	0.79 a	0.1	0.82 a	0.0
Stress 1	-22.3 b	2.6	65.7 b	4.2	0.76 ab	0.0	0.78 b	0.0
Stress 2	-25.0 bc	1.8	60.4 bc	4.6	0.76 ab	0.0	0.76 bc	0.0
Stress 3	-27.8 cd	1.3	60.5 bc	2.3	0.76 ab	0.0	0.73 d	0.0
Stress 4	-28.7 d	2.7	58.5 c	4.3	0.75 b	0.0	0.74 cd	0.0
Stress 5	-38.6 e	2.7	54.8 c	3.6	0.71 c	0.0	0.72 d	0.0
				Maize				
Control	-8.3 a	0.5	94.6 a	2.7	0.83 a	0.0	0.87 a	0.0
Stress 1	-9.3 b	1.1	92.2 ab	1.9	0.83 a	0.0	0.86 b	0.0
Stress 2	-10.3 b	1.3	87.9 c	2.5	0.83 a	0.0	0.84 c	0.0
Stress 3	-11.4 c	0.6	88.4 bc	1.3	0.82 a	0.0	0.84 c	0.0
Stress 4	-11.9 c	0.7	90.7 bc	2.0	0.82 a	0.0	0.85 c	0.0
Stress 5	-11.4 c	1.0	89.3 bc	3.5	0.82 a	0.0	0.84 c	0.0

Table 31. Influence of six water treatments throughout five days on leaf growth of wheat. Measured values at each treatment for one day are determined from the average value obtained from eight leaves. SD indicates standard deviation.

Dates	Control Length cm	SD	Stress 1 Length cm	SD	Stress 2 Length cm	SD	Stress 3 Length cm	SD	Stress 4 Length cm	SD	Stress 5 Length cm	SD
July, 15	20.3	1.7	19.5	1.1	20.7	1.8	20.7	1.2	17.3	1.6	18.9	1.6
July, 16	25.8	1.6	25.1	1.2	26.1	3.8	26.4	1.1	22.8	1.8	23.5	1.8
July, 17	29.8	2.3	30.6	1.2	31.8	3.8	32.0	0.9	28.3	1.9	26.9	2.0
July, 18	35.7	1.5	35.5	1.2	36.6	3.2	36.6	0.9	32.1	2.0	29.6	2.5
July, 19	38.6	1.5	38.6	1.1	39.3	3.0	37.8	0.8	32.9	2.0	30.8	2.7
July, 20	40.8	1.5	40.3	1.2	39.9	2.6	37.8	0.8	32.9	2.0	30.8	2.7

Table 32. Influence of six water treatments throughout five days on the leaf growth of maize. Measured values at each treatment for one day are determined from the average value obtained from eight leaves. SD indicates standard deviation.

Dates	Control Length cm	SD	Stress 1 Length cm	SD	Stress 2 Length cm	SD	Stress 3 Length cm	SD	Stress 4 Length cm	SD	Stress 5 Length cm	SD
July, 15	12.2	0.6	12.2	0.4	11.6	0.8	11.5	0.8	11.7	0.7	11.7	0.6
July, 16	14.4	0.7	15.4	0.2	14.0	0.9	14.0	0.8	14.5	0.9	15.0	0.6
July, 17	16.5	0.7	17.6	0.2	16.3	1.0	16.0	0.8	16.2	0.8	16.9	0.8
July, 18	20.7	0.7	21.5	0.5	20.3	1.3	20.1	1.0	19.9	1.0	20.1	0.8
July, 19	24.2	0.8	25.0	0.6	23.6	1.5	23.2	1.2	22.5	0.9	22.9	1.2
July, 20	28.5	0.9	29.4	0.8	27.5	1.6	27.0	1.6	26.1	1.2	25.9	1.6

3.3.3 Interrelationships between seven physiological parameters of water status in wheat and maize

The interrelationships between seven indicators of water stress of wheat and maize is shown in Table 33. Soil water content was significantly related to six physiological parameters of wheat. But in maize, the parameter soil water content was only related to relative water content, canopy water content and aerial biomass. Leaf water potential of wheat was more closely related to drought stress in wheat than to all other parameters (LWP > RWC > AB > CWC > LWC > DW). There were more significant relationships observed for all physiological parameters of wheat than those in maize. No significant relationships existed between LWP, RWC and LWC in maize, however, some relationships existed between physiological parameters of the leaf and the plant canopy. Leaf water potential was related to aerial biomass and dry weight, as well as the relative water content was related to the canopy water content of maize and the leaf water content was related to the dry weight

Table 33. Interrelationship between leaf water potential, relative water content, leaf water content, canopy water content, dry weight, aerial biomass and soil water content for wheat and maize under six water regimes. Values for coefficients of determination (R^2-values) are indicated.

Cultivars	Physiological parameters					
	LWP & LWC	LWP & RWC	LWP & CWC	LWP & AB	LWP & DW	LWP & SWC
Wheat	0.93**	0.98**	0.96**	0.96**	0.88**	0.98**
Maize	0.77*	0.36	0.62	0.98**	0.83**	0.54
	LWC & RWC	LWC & CWC	LWC & AB	LWC & DW	LWC & SWC	RWC & CWC
Wheat	0.96**	0.67*	0.73*	0.73*	0.81*	0.92*
Maize	0.04	0.48	0.6	0.70*	0.13	0.91*
	RWC & AB	RWC & DW	RWC & SWC	CWC & AB	CWC & DW	CWC & SWC
Wheat	0.92*	0.84*	0.96**	0.98**	0.92*	0.90**
Maize	0.45	0.13	0.70*	0.66	0.29	0.69*
	AB & DW	AB & SWC	DW & SWC			
Wheat	1.00***	0.92**	0.75*			
Maize	0.86*	0.70*	0.43			

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

3.3.4 The effect of six water treatments on spectral reflectance in the visible, near infrared and middle infrared regions for wheat and maize at the leaf and canopy level

The effect of six water treatments on spectral reflectance in the visible, the near infrared and the middle infrared regions is shown in Fig. 30. The effect of water stress on the reflectance regions was clear at the canopy level in wheat and maize (Fig. 30 a, c). As water content in the leaf or the plant decreased by the water stress level in wheat, the reflectance tended to increase throughout most of wavelengths in the visible, near infrared and middle infrared regions (Fig. 30). On the other hand, in maize there was a change in the reflectance of the canopy, but sometimes, it did not follow the water stress level, because the plants were subjected to low water stress and the change in CWC between the six water regimes was only 3%. A change in reflectance at the canopy level of maize was obviously only observed between the control and higher stress level treatments in the visible and the middle infrared regions (Fig. 30). The change in reflectance at the leaf level of maize was not clearly different between the six water treatments. No change was observed in the red edge at the canopy and leaf level of wheat and maize.

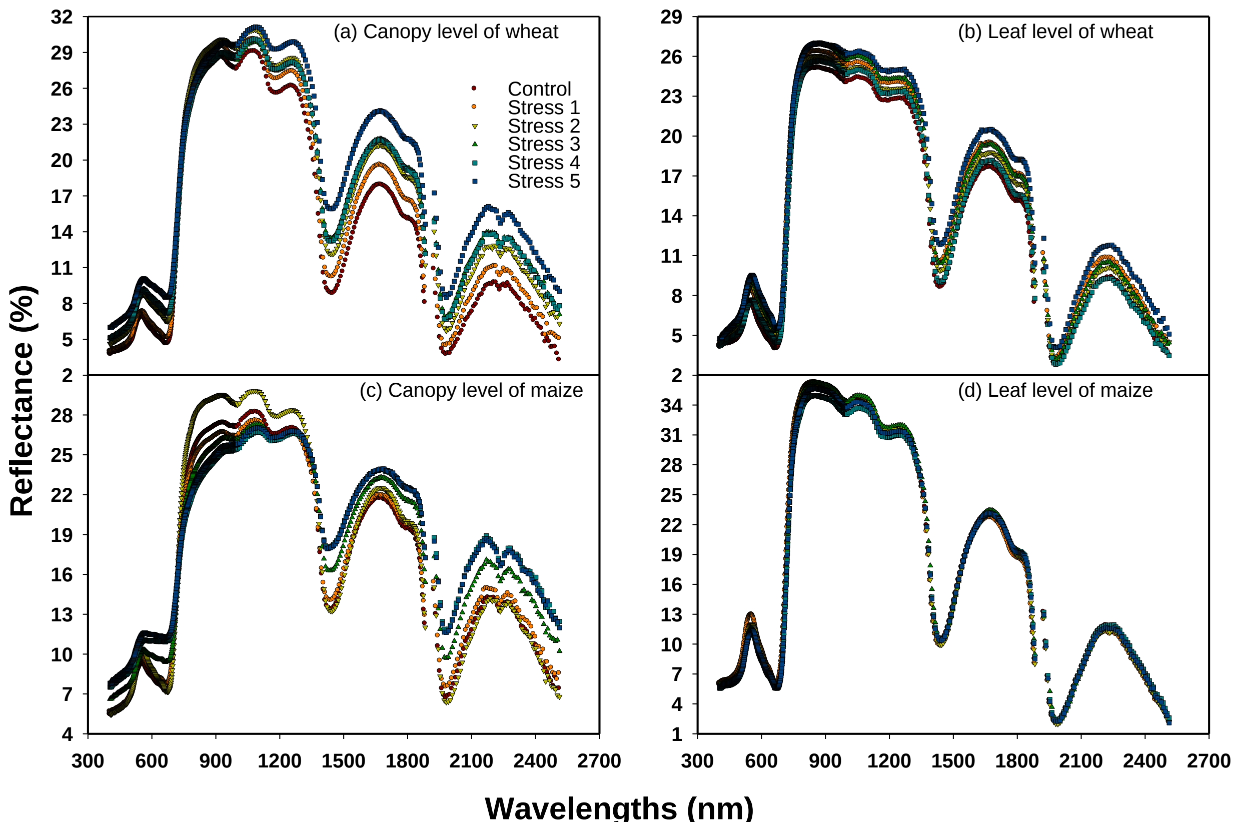

Figure 30. Change in spectral reflectance (%) of wheat at (a) the canopy level and (b) leaf level as well as for maize at (c) the canopy level and (d) the leaf level with plants being subjected to six water regimes.

3.3.5 The relationships between reflectance bands and water status of wheat and maize

Figures 31 a, b, c & d. shows the coefficients of determination of the linear relationship between spectral reflectance bands in the visible, the near infrared and the middle infrared regions and LWP, RWC, LWC and CWC at the canopy and the leaf level of wheat and maize. A statistically significant relationship (R^2- values) $R^2 \geq 0.65$* to the LWP, RWC, LWC, and CWC at both canopy and leaf level of wheat and maize could be shown at most reflectance bands in Fig. 31. There was a more pronounced significant relationship between spectral reflectance and water status in the plant at canopy level than within the leaf level of wheat and maize. Spectral reflectance at canopy level of wheat had stronger relationships with LWP and CWC than with

RWC and LWC. The LWP, RWC, LWC and CWC at canopy level of wheat were significantly related to spectral reflectance at the range of 410 - 733 nm and the coefficients of determination varied between (0.88** to 0.95**), (0.65* to 0.74*), (0.68* to 0.79*) and (0.85** to 0.93**), respectively. As well with the spectral reflectance range at 1147 nm - 2510 nm, the coefficients of determination varied between (0.87*** to 0.97***), (0.67* to 0.82**), (0.66* to 0.88**) and (0.86** to 0.97***), respectively. At the leaf level of wheat, LWP had significant relationships with spectral reflectance at the visible and near infrared regions. On the other hand, LWC was significantly related to spectral reflectance in the near infrared and middle infrared regions. The highest coefficients of determination for the relationship between LWP and LWC with spectral reflectance band were found at the wavelengths of 663 nm (R^2 = 0.75*) and 2007 nm (R^2 = 0.72*). The relative water content showed a non-significant relationship with all spectral bands but it reflected relatively close values for the coefficients of determination increasing to more than 0.6 at the visible region. Spectral reflectance at the canopy level of maize had stronger relationships with LWP and LWC than with RWC and CWC. There was a more pronounced significant relationship between spectral reflectance and LWP at the canopy level of maize at the visible and the middle infrared regions. On other hand, LWC had a more pronounced significant relationship with spectral reflectance band in the visible, the near infrared and the middle infrared regions. The highest coefficients of determination for LWP and LWC with spectral reflectance were found at wavelengths of 1668 nm (R^2 = 0.92**) and at 2500 nm (R^2 = 0.88**), respectively. Canopy water content had a significant relationship with spectral reflectance R_{1350} (R^2 = 0.68*) as well as RWC had a significant relationship with spectral reflectance R_{718} (R^2 = 0.67*). At the leaf level of maize, there was only a significant relationship between LWP and R_{411} (R^2 = 0.67*). The leaf water content was related to spectral reflectance at some wavelengths at near and middle infrared regions and the highest coefficients of determination were found at the wavelength 1868 nm ($R^2 = 0.78$*).

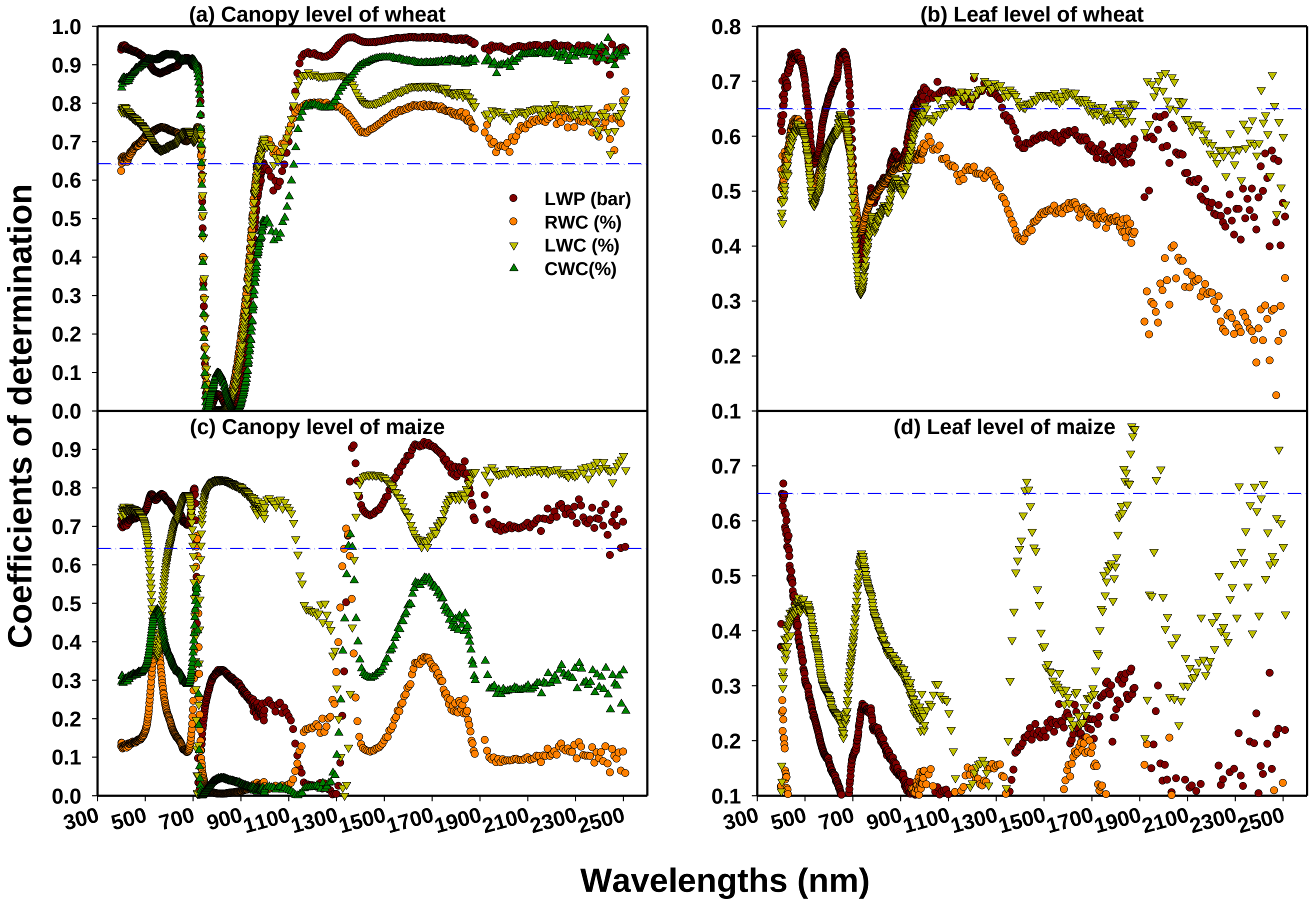

Figure 31. Coefficients of determination of the relationship between LWP, RWC, LWC, CWC and the reflectance bands of wheat at (a) canopy level and (b) leaf level as well as with maize at (c) the canopy level and (d) leaf level subjected to six water regimes. The values of the coefficients of determination above the dash-dot line are significant ($R^2 \geq 65$).

3.3.6 The relationships between spectral indices and water status of wheat and maize

Relationships between the LWP, RWC, LWC, and CWC and several spectral indices of wheat and maize at canopy and leaf level are shown in Fig. 32 and in Table 34.

A statistically significant relationship (R^2- values) $R^2 \geq 0.89$**, $R^2 \geq 0.91$**, $R^2 \geq 0.68$*, and $R^2 \geq 0.94$** to the LWP, RWC, LWC and CWC, respectively, at the canopy level of wheat as well as, at the leaf level of wheat ($R^2 \geq 0.70$*, $R^2 \geq 0.66$* and $R^2 \geq 0.66$*) to LWP, RWC, and LWC, respectively, could be shown for all spectral indices in Table 34. There was a more pronounced significant relationship between spectral reflectance and the water status in the plant at the canopy level than within the leaf level of wheat. Spectral reflectance at the canopy level of wheat had stronger relationships with LWP, CWC and RWC than with LWC.

In maize, a statistically significant relationship $R^2 \geq 0.79$* and $R^2 \geq 0.74$* to the LWP and LWC, respectively, at the canopy level could be shown for all spectral indices in Table 35. There was no significant relationship between spectral indices and four physiological parameters of maize at leaf level (Table 35).

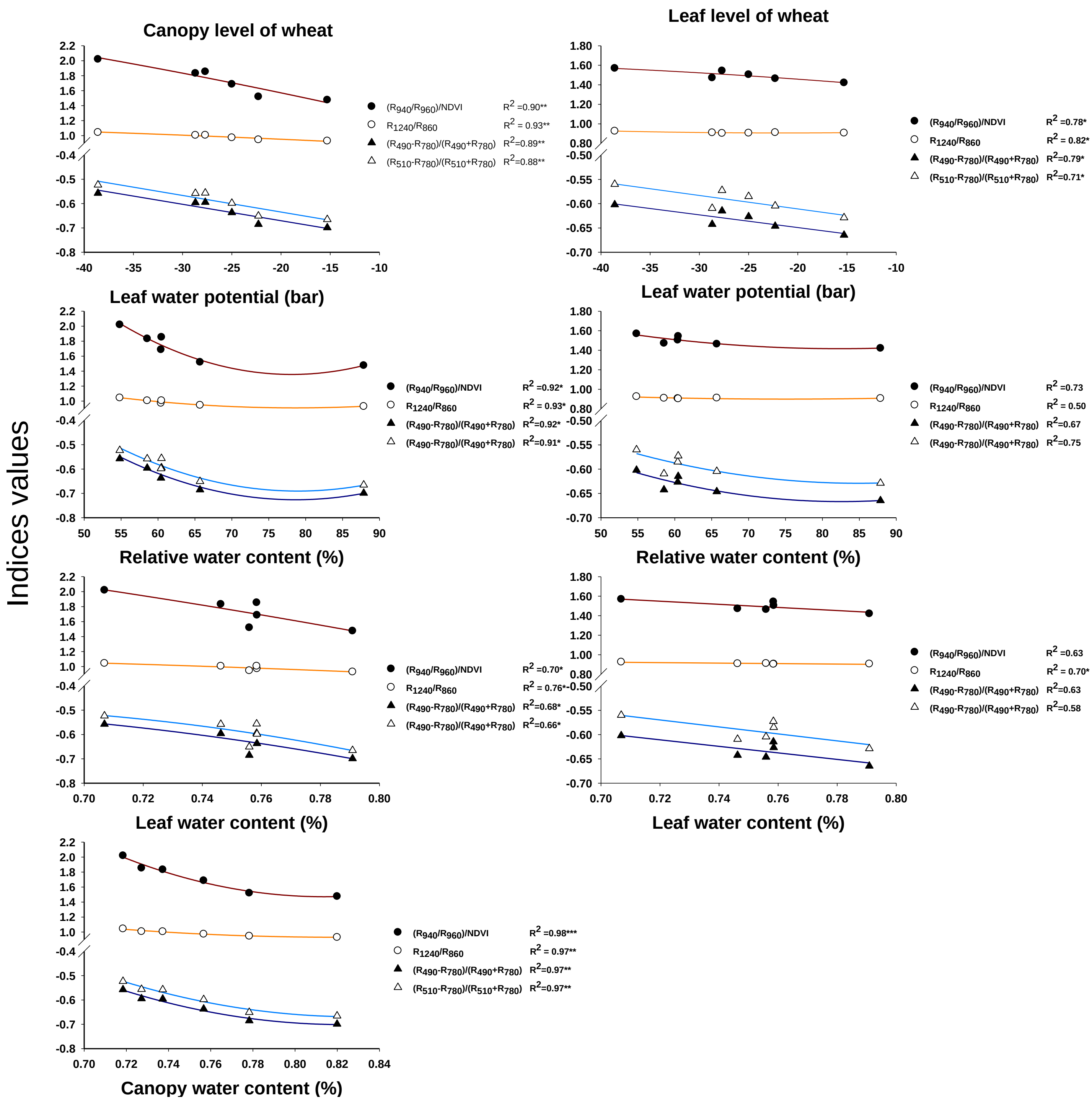

Figure 32 Relationship between four spectral indices with leaf water potential (bar), relative water content (%), leaf water content and canopy water content at the canopy and leaf level of water subjected to six water treatments.

Table 34. Relationship between spectral indices with leaf water potential (bar), relative water content (%), leaf water content (%) and canopy water content (%) at canopy and leaf level of wheat. Coefficients of determination (R^2-values) are indicated.

Spectral indices	Spectral measurements at canopy level				Spectral measurements at leaf level		
	LWP (bar)	RWC (%)	LWC (%)	CWC (%)	LWP (bar)	RWC (%)	LWC (%)
NDVI	0.89**	0.92*	0.68*	0.98**	0.80*	0.66*	0.66*
R_{600}/R_{780}	0.87**	0.90*	0.63	0.97**	0.70*	0.61	0.62
R_{940}/R_{960}	0.92**	0.95**	0.76*	0.94*	0.60	0.18	0.73*
(R_{940}/R_{960})/NDVI	0.90**	0.92*	0.70*	0.98**	0.77*	0.63	0.63
$(R_{410}-R_{780})/(R_{410}+R_{780})$	0.90**	0.93*	0.71*	0.97**	0.57	0.4	0.37
$(R_{490}-R_{780})/(R_{490}+R_{780})$	0.89**	0.92*	0.68*	0.97**	0.78*	0.65	0.64
$(R_{510}-R_{780})/(R_{510}+R_{780})$	0.88**	0.91*	0.64	0.97**	0.70*	0.6	0.59
R_{1240}/R_{860}	0.93**	0.93**	0.76*	0.97**	0.52	0.49	0.70*
$(R_{860}-R_{1650})/(R_{860}+R_{1650})$	0.94**	0.94**	0.77*	0.98**	0.51	0.34	0.68*

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

Table 35. Relationship between spectral indices with leaf water potential (bar), relative water content (%), leaf water content (%) and canopy water content (%) at canopy and leaf level of wheat. Coefficients of determination (R^2-values) are indicated.

Spectral indices	Spectral measurements at canopy level				Spectral measurements at leaf level		
	LWP (bar)	RWC (%)	LWC (%)	CWC (%)	LWP (bar)	RWC (%)	LWC (%)
NDVI	0.80*	0.06	0.83**	0.23	0.00	0.02	0.05
R_{600}/R_{780}	0.66*	0.08	0.77*	0.24	0.1	0.02	0.17
R_{940}/R_{960}	0.82*	0.00	0.90**	0.11	0.04	0.04	0.11
(R_{940}/R_{960})/NDVI	0.80*	0.07	0.75*	0.21	0.02	0.01	0.06
$(R_{410}-R_{780})/(R_{410}+R_{780})$	0.80*	0.06	0.82**	0.21	0.61	0.70*	0.08
$(R_{490}-R_{780})/(R_{490}+R_{780})$	0.80*	0.07	0.81**	0.22	0.38	0.07	0.39
$(R_{510}-R_{780})/(R_{510}+R_{780})$	0.79*	0.01	0.79*	0.23	0.28	0.06	0.37
R_{1240}/R_{860}	0.78*	0.01	0.86**	0.14	0.33	0.01	0.63
$(R_{860}-R_{1650})/(R_{860}+R_{1650})$	0.80*	0.04	0.89**	0.19	0.35	0.02	0.55

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

3.3.7 Influence of adaxial and abaxial leaf measurements on spectral reflectance in wheat and maize

In wheat leaves, reflectance increased with increasing stress level at the adaxial leaf side (Fig. 33a). In the abaixal leaf side the reflectance increased similar to the adaxial leaf.side (Fig. 33a). There was not a clear difference in spectral reflectance at any wavelengths in both adaxial and abaxial leaf sides of wheat (Fig. 33b), similar results were found between the adaxial and abaxial leaf sides of maize.

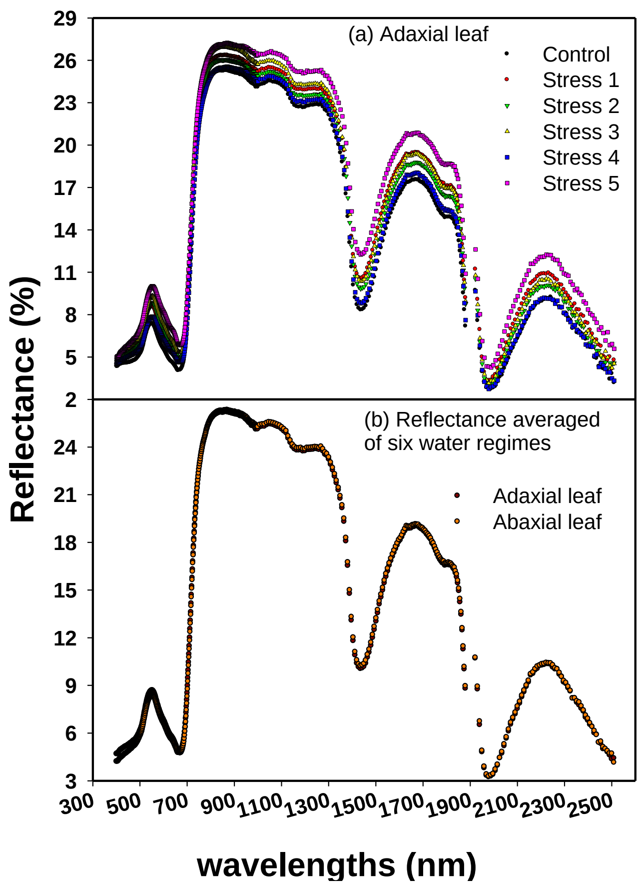

Figure 33. Relationship between (a) the wavelengths and the reflectance (%) of the adaxial side of the wheat leaf under six water regimes as well as with (b) reflectance averaged over adaxial and abaxial leaves and six water regimes.

4 DISCUSSION

4.1 Experiments under controlled growth chamber conditions

Changes in irradiance through diurnal light affect the water status in plants. After the onset of illumination of maize in a growth chamber, a rapid transpiration-induced decrease in LWP was observed (Schmidhalter et al. 1998a). This result is in good agreement with our results obtained for wheat and maize (Tables 9 and 10), where LWP decreased with progressively higher light intensities. By contrast, LWC generally showed a less marked decrease with increasing light intensities, a result that is in agreement with other observations.

Minimal changes in LWC were attempted within this work, to possibly find out whether spectral relationships to LWP can be established independent of significant changes in LWC. We tested two scenarios, non-stressed diurnal decreases in LWP and we investigated spectral relationships of moderately/mildly drought stressed wheat and maize plants, respectively. The focus was on the non-stressed plants. High LWP were indeed aimed at to obtain only small decreases in LWC. This was attempted to test the initial hypothesis whether changes in LWP can be detected spectrally. Such a relationship is inherently part of pressure / volume curves or LWC/LWP relationship representing the initial steep decline in LWP that is accompanied by only small decreases in LWC. Attempting to attain largely decreased negative water potentials would unavoidably result in significant structural changes of leaves making it difficult to ascertain whether changes in LWP can truly be ascertained or just represent colinear changes to other properties.

On the whole, LWP was influenced more greatly by changes in light intensity than was LWC, which showed only small changes if any (< 2.8 %; Table 11). Even so, decreases in LWP were only between -3.6 to -5.4 bar. The ratios of the maximum changes observed for LWP and LWC were generally higher than 1.7 and ranged up to 7.6 (Table 11), independent of whether the plants were well-watered or drought stressed. The minimal changes in both plant water status indicators can be traced to the experimental protocol, which induced transpiration-induced water losses for no longer than three hours after switching on the light in the growth chamber. This situation was to be expected at least for the well-watered treatments, where small decreases in LWC will be accompanied by more marked decreases in LWP. Interestingly, this trend was not accentuated greatly in those treatments that previously subjected the plants to mild drought stress. However, LWP and LWC were clearly decreased in both wheat and maize in these treatments.

Half of the experimental treatments showed no significant relationship between LWC and LWP. However, small changes in LWC of less than 1.5 % might be insufficient to demonstrate a significant relationship to decreases in LWP even though they are to be anticipated. Even so, our experimental protocol group deserves further attention because it enables a more rigorous assessment as to whether or not LWP can be ascertained spectrally when freed from the influence of accompanying (significant) changes in LWC. Indeed, the advantage of taking spectral measurements at the canopy level under constant environmental conditions in a growth chamber is that the relationship between plant water status and spectral indices is not affected by any external conditions such as atmospheric noise. In addition, the variation of spectral reflectance caused by soil background can be assumed to be constant and so should not disturb reflectance values from the densely grown plants because they covered the sensed area nearly fully in wheat and fairly little influence was expected in maize. Thus, we can reasonably assume that most of the variations in the values of the spectral index derive from variation in the plant canopy properties.

Our assessment of reflectance indices as a method to measure the water status in wheat demonstrated that strong relationships exist between the new spectral index (R_{940}/R_{960})/NDVI and LWP under both well-watered and drought conditions over a range of varying light intensities. Significant relationships for all other reflectance indices were generally restricted to the well-watered treatment (Table 12). Thus, it appears advantageous to combine information from both the visible and infrared regions (as (R_{940}/R_{960})/NDVI does) to measure water status characteristics such as LWP in drought-stressed wheat. This is confirmed by the findings of Peñuelas and Inoue (1999) that the reflectance index WI/NDVI is strongly correlated with relative water content (RWC).

We also found the index NDVI on its own to be related to LWP of wheat under well-watered conditions. This result agrees with findings obtained by Ruthenkolk et al. (2001), but their relationship was probably influenced by changes in biomass status as well. The index NDVI is also 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, however, both LAI and chlorophyll content likely changed only minimally if at all, indicating that a more direct relationship between NDVI and LWP might exist.

Results from the literature for the remaining spectral indices are mixed and the assessment of their potential for measuring plant water status is complicated by the use of different plant species and experimental conditions. For instance, consistent relationships seemingly independent of changes in plant biomass status between the index R_{1689}/R_{1657} and LWP of cotton and between each of R_{900}/R_{970}, NDVI, $(R_{900} - R_{680})/(R_{900} + R_{680})$ and LWP of lichen *Umbilicaria hirsuta* as well as between the normalized water index $(R_{970} - R_{880})/(R_{970}+R_{880})$ and LWP of wheat have been reported (e.g., Kakani et al. 2007; Gloser and Gloser 2007, Gutierrez et al. 2010). By contrast, no consistent relationship between PRI and the water potential of olive at different times of the same day was observed and PRI was strongly affected by canopy structure and background (Suárez et al. 2008). Similarly, LWP in mandarin and peach derived from image analysis in the red (580-680 nm) and green (490-580 nm) spectral channels also did not exhibit constant relationships on different days (Kriston-Vizi et al., 2008). Weak relationships between the LWP of *Populus deltoides* x *Populus nigra* and the water index (WI) or the red edge inflection point (REIP) were found at both the leaf and canopy levels (Eitel et al., 2006). Finally, Stimson et al. (2005) found a critical link between plant physiological characteristics tied to water stress and associated spectral signatures for two extensive co-occurring conifer species. They found significant relationships between the water potential of *Pinus edulis* and the normalized difference water index NDWI ($R^2 = 0.49$), at 970 nm ($R^2 = 0.44$), with NDVI ($R^2 = 0.35$), and with the red edge ($R^2 = 0.34$); however, no significant relationships were observed for LWP in *Juniperus monosperma*. The spectral index R_{1000}/R_{1100} includes two wavelengths from the near infrared region and reflectance changes will depend on the change in the internal leaf structure and the ratio between the intercellular air spaces and water.

Using a sensor with limited spectral range was advocated by previous experimentation being conducted under controlled or field conditions. In previous experiments (Ruthenkolk et al., 2001) indeed SWIR regions delivered favorable relationships, but also VIS/NIR based indices turned out to be usefully related to LWP. In carrying out a similar experiment with a full range spectrometer under controlled conditions it was noticed that when investigating only mild stress or looking to changes resulting from the diurnal change of LWP of non-stressed plants that the sensitivity of the sensing system was weakened due to increased signal-noise ratios, particularly in the SWIR region. Synchronously observing rapid changes in LWP caused by incrementally increasing the light intensity was hampered by the referencing procedure. This prompted to develop a bi-directional sensor measuring simultaneously incident and reflected light. The range however was limited to the 1700 nm range (Winterhalter et al., 2011; Mistele and Schmidhalter, 2008). Seen the higher signal noise ratio observed within the second (InGaAs) sensing unit measuring from 1100 to 1700 nm, we preferred to use only the limited range up to 1100 nm. This was supported by own results with the extended range that at least as good if not better results could be obtained from the VIS and NIR range as compared to the SWIR range. Findings from literature allow arriving at both conclusions, that both spectral ranges at the VIS/NIR or NIR/SWIR or SWIR are useful. In a rather comprehensive investigation done recently we have clearly identified the best relationships were clearly indentified in the VIS/NIR range (Winterhalter et al., 2011).

In these experiments, the LWP of wheat was significantly related to LWC for only one measurement cycle (Fig. 10a), whereas the new spectral index (R_{940}/R_{960})/NDVI was consistently related to LWP at all measurement dates, indicating that the relation between LWP and (R_{940}/R_{960})/NDVI appears to be independent of LWC. Moreover, any confounding influence of variation in biomass can be assumed to be negligible because the spectral measurements were taken over extremely short timeframes. In this, (R_{940}/R_{960})/NDVI shows a distinct advantage over many previously reported spectral reflectance indices that often failed to indicate significant relationships with LWP.

The situation is not as clear cut in maize. Again, LWP was related to LWC for only a single measurement cycle (Fig. 10b), but new spectral indices such as R_{940}/R_{960} were significantly related to LWP for only two measurement cycles at most and usually not at all (Table 13). Thus, the evidence for a relationship between LWP and the new spectral indices independent of detectable changes in water content is not as strong as for wheat.

The results show that changes in water potential can reliably be detected using spectral measurements in well-watered and drought-stressed wheat, with consistent and significant relationships between LWP and selected spectral indices being obtained with or without significant changes in LWC. However, it is cautioned that the exact relationships did differ with the date of measurement or the stress induced. Moreover, the choice of index is also of importance, particularly for maize. Of the many indices examined herein (R_{940}/R_{960})/NDVI performed the best for detecting LWP in wheat and R_{940}/R_{960} for maize under well-watered conditions. The relationships observed were less consistent in maize than in wheat, with significant relationships being demonstrated in two out of three experiments. Water potentials of wheat, particularly of the mildly drought stressed treatment, decreased much more as compared to maize. Whereas LWP of moderately stressed wheat plants decreased to -16 bar, non-stressed plants indicated values of about -9 bar, whereas LWP of mildly stressed maize dropped to values close to -7 bar, with non-stressed plants attaining values of -5 bar. The decreased sensitivity of the spectral indices to account for changes in LWP of maize, but also in LWC, can probably be explained by the rather small decreases in plant properties. It is worth noting, that previous experiments of field-grown maize showed that severely stressed maize plants decreased LWP throughout a full diurnal course to -15 bar only, and differences between stressed and non-stressed leaves were minimal (Camp, 1996). Seen that LWP can decrease much more markedly in wheat than in maize, this may also point out a reduced sensitivity in detecting changes in LWP by spectral assessments.

Overall, the results indicate that it is unlikely that a single consistent relationship between any spectral index and LWP across the plant development will be established. Even though this is shown here for a short growing period only, this is strongly supported by complementary data obtained across plant development (S El-Sayed, B Mistele and U Schmidhalter, unpublished data). From our experiences we can conclude that by combining data over time, even though good relationships occasionally may be obtained, a non-continuous behavior frequently becomes obvious by carefully inspecting combined time- or growth-stage specific data sets. Coincidental parallel developments in plant properties may lead to more globalized relationships, seen for example that drought stress may decrease biomass, aerial water content, but also lead to accompanying decreases in LWC and LWP. A note of caution is supported by recent spectral assessments of the biomass and nitrogen status of wheat and also the water status of maize indicating a lack of continuous associations over time and indicating a need to establish time-specific or growth-stage specific relationships (Mistele and Schmidhalter, 2008b, 2010; Reusch et al., 2010; Winterhalter et al., 2011). At the same time, the results indicate the possibility that time-consuming destructive pressure chamber measurements might be replaced in part by rapid, non-destructive spectrometric measurements. Doing so would require a reduced calibration data set obtained from pressure chamber measurements that will need to be augmented with spectral assessments of the water status. However, once this hurdle is overcome, this technology may open an avenue for fast, high-throughput assessments of LWP, which would simultaneously be useful for screening large numbers of plants (e.g., in breeding) as well as being equally important for management related actions. Testing the full potential of spectral methods will require further investigations, particularly under field conditions with variable environmental conditions where the rapid changes occurring throughout the course of the day will place a strong demand on developing efficient methods. This, in turn, would enable placing the well established pressure chamber method from the hand of the physiologist into, for example, those of the plant breeder. Finally, further work should also investigate whether or not relationships between plant water status and spectral indices are genotype-specific and should also be extended to the lower LWP values to investigate whether continuous associations to spectral indices potentially exist or not.

4.2 Field experiments

4.2.1 Laser-induced chlorophyll fluorescence measurements

In this study, high-throughput active laser sensing was found to present some major advantages, given that LICF measurements could be performed in a short time with a mobile metal carrier-mounted fluorescence sensor and could be done under field ambient conditions from 3 m above the plant canopy.

The physiological parameters CWC, CWM, AB, and LWP, CT and fluorescence intensities at 690 nm and 730 nm of the Ludwig cultivar were more affected by water stress than those of the other cultivars (Table 14).

Our results show that the closest relationships of the fluorescence intensity at 690 and 730 nm and the biomass index to canopy water content existed within the individual cultivars with $R^2 \geq 0.82^{***}$, as well as in the combined data of the four cultivars ($R^2 \geq 0.71^{***}$, Fig. 13). 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. Canopy water mass and aerial biomass were both significantly related to the fluorescence intensities at 690 and 730 nm and the biomass index within the individual cultivars and across all cultivars. The leaf water potential was more consistently related to the fluorescence intensity at 690 nm than to the fluorescence index or the biomass index.

The fluorescence intensity decreased with increasing canopy water content, canopy water mass, and aerial biomass, 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. Chlorophyll fluorescence indicates the chlorophyll activity and competes with heat dissipation. This will lead to a corresponding change in the fluorescence yield.

The biomass index was more closely related to canopy water content than to aerial biomass. The biomass index is related to the frequency with which a green plant was hit by the laser beam, and it was expected to be the best indicator to measure aerial biomass. The measurements were taken from the shooting stage until the ripening stage, and the biomass index values were decreased by the stress level. Our results agree with findings by Bredemeier and Schmidhalter (2003), who found that the decrease of the biomass index at BBCH 65 is probably related to the higher contribution of spikes and senescent leaves to the total signal detected at this development stage. The relationship between canopy water mass and biomass index was described by a polynomial function. The highest canopy water mass values were between approximately 4400 to 6000 g m^{-2}, whereas the biomass index seemed to remain constant (Fig 14).

The fluorescence ratio F690/F730 was not found to be a good indicator of water stress in wheat. Changes in the fluorescence ratio F690/F730 may primarily be influenced by changes in chlorophyll content (Günther et al., 1994; Gitelson et al., 1998; Buschmann, 2007). However, under the investigated experimental conditions, no significant differences in chlorophyll meter readings (SPAD values) were found between the treatments (Table 18). It may be that the fluorescence intensity was affected by different factors, such as leaf structure, photosynthetic activity, and the leaves' optical properties, as a result of the effects of water stress. Alternatively, if both fluorescence intensities changed in a stable manner due to water stress treatments, the fluorescence ratio would not be affected. Hypothetically, this can be explained because with increasing chlorophyll content, the 690 nm fluorescence band is decreased by re-absorption at 690 nm by photosynthetic pigments, whereas the fluorescence at 730 nm is much less affected, and this result leads to a shift in the ratio (Koizumi et al., 1988).

Previous studies found that the fluorescence ratio F690/F730 was a good indicator to detect chlorophyll and nitrogen contents as well (Bredemeier and Schmidhalter, 2005, Schächtl et al., 2005; Buschmann, 2007). Dahn et al. (1992) showed that the fluorescence ratio F690/F735 of water-stressed maize plants did not differ significantly from the values of control plants and that there was approximately 10% difference compared to non-stressed leaves.

The fluorescence intensities at 690 nm and 730 nm and the biomass index had stronger negative relationships with canopy temperature than with the fluorescence ratio F690/F730 (Table 17) for individual measurements in each cultivar. It was not possible to fit one single regression curve to all measurements because the relationships were affected by the sampling date (ambient temperature and radiation condition). The measurements were taken in short time to prevent the effects of changes in air temperature and global radiation on the fluorescence intensities at 690 nm and 730 nm. A temperature dependence of the F690/F730 fluorescence ratio has been described for different plant species by Agati et al. (1996), Agati et al. (2000), Bredemeier and Schmidhalter (2003), and Thoren et al. (2010), who all indicated a linear increase in the ratio from 5°C to 25°C. The latter authors found, that the fluorescence ratio F690/F730 was temperature-independent above 23°C, remaining constant above this temperature. No significant relationship between the F690/F730 ratio and the canopy temperature was found in our investigation, in which canopy temperatures reached up to 32.2°C.

The data clearly showed that under field conditions, the fluorescence intensities at 690 and 730 nm as well as the biomass index proved to be more sensitive to drought stress in wheat than the fluorescence ratio F690/F730.

4.2.2 Spectral reflectance measurements in 2006, 2007 and 2008

Two passive reflectance sensors present some major advantages given that spectral reflectance measurements were taken in short time by using mobile metal carrier-mounted sensors (high throughput technique). The technique can be used to measure under field conditions from large distances about 1.4 or 3 m above the plants. The sensor was connected with a portable computer and GPS to provide continuous measurements of water status in winter wheat cultivars.

Spectral measurements made on the field are often disturbed by different illumination conditions or soil back ground (Reusch, 1997; Read et al., 2002). To minimize these disturbing effects, the reflected wavelengths were used to create special vegetation indices and furthermore the passive reflectance sensor was linked to a four optic in one light fiber to create an optical mixed signal from four fields of view at different directions in the years 2006 and 2007. A signal was calculated as the average of the values of the four optics, so it may be nearly constant at any solar zenith angle. In addition, a passive sensor was developed to measure canopy reflectance and sun reflectance under the same conditions, either sunny or cloudy during the experiments in 2008.

The results demonstrate that five indices such as NDVI, $(R_{510} - R_{780})/(R_{510} + R_{780})$ $(R_{410} - R_{780})/(R_{410} + R_{780})$ and $(R_{490} - R_{780})/(R_{490} + R_{780})$ and R_{600}/R_{780} which were derived from visible and infrared regions, are apparently useful for describing drought stress of wheat canopies by using CWC regardless of growth stage, measurement time and environmental factors at individual measurements and across all measurements with two passive sensors as shown in Tables 22 & 27.

The advantage of these indices is that they had strong relationships with individual measurements and across all 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. In addition, Behrens et al. (2006) found that there were weak relationships between REIP, SRWI, $R_{850,}$ and R_{810}/R_{560} and plant water content of barley and oilseed rape across all measurements. Therefore theses indices are not only useful for an irrigation schedule when data are combined across all measurements in one year, but would also be useful for evaluating the phenotype for breeding purposes with individual measurements.

Several studies have examined the relationship between water content and spectral indices depending on water absorption bands (1200, 1450, 1950 and 2250) (Stimson et al., 2005; Wu et al., 2009), although the research for an optimal water band to measure water content has been elusive. The longer wavelengths, such as the 1450 and 1900 nm water absorption bands are poor indicators for estimating plant water content due to the low incoming solar energy and the high level of interference by atmospheric water vapor (Sims and Gamon, 2003). On the other hand several studies suggest that the ideal wavelengths for predicting water content are wavelengths with a weak absorption in the near infrared that allow the radiation to penetrate far into canopies, providing a suitable dynamic range (Peñuelas et al., 1993; Sims and Gamon, 2003). However, infrared reflected radiation is affected greatly by plant architecture, vegetation density and leaf structure, thus influencing estimation uncertainty (Elachi, 1987).

The 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.

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 at individual measurements and across all measurements for each cultivar throughout one year as shown in Tables 22 & 27. But the relation may depend on the LAI. Also, NDVI and

R_{600}/R_{780} showed strong relationships with CWC for each cultivar ($R^2 \geq 0.86$***, and $R^2 \geq 0.83$***, respectively) as well as when values were pooled across all cultivars throughout three years (Fig. 24). Therefore, NDVI and R_{600}/R_{780} is revealed to be a good indicator for irrigation schedule. The index $(R_{490} - R_{780})/(R_{490} + R_{780})$ also presented good relationships with CWC of individual cultivars. Compared to NDVI and R_{600}/R_{780} this index was affected by the cultivars. The index $(R_{490} - R_{780})/(R_{490} + R_{780})$ revealed to be a good indicator to distinguish between cultivars for breeding purposes (Fig. 24).

The combinations between violet, blue, green, red, and near infrared regions are able to detect canopy water content of winter wheat cultivars. Canopy water content had more stable relationships with spectral indices in different years than had the canopy water mass.

The canopy water mass had better relationships with four indices NDVI, $(R_{510} - R_{780})/(R_{510} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, $(R_{410} - R_{780})/(R_{410} + R_{780})$ at individual measurements and across all measurements in 2008 than in 2006 and 2007. The relationship between CWM and spectral indices was probably affected by the plant density. The index $(R_{410} - R_{780})/ (R_{410} + R_{780})$ which was derived from violet and infrared regions, seemed to be more stable to detect CWM in different years with only little influence by the plant density compared to other indices (Figs. 17 & 22). The index $(R_{410} - R_{780})/ (R_{410} + R_{780})$ is probably a good indicator to estimate CWM and it is possible to fit a single linear regression for all evaluations of each cultivar in three years. This index may be useful to distinguish between cultivars for breeding purposes, but is not suitable for irrigation scheduling.

The leaf water potential underlies rapid temporal fluctuation as a function of environmental conditions (Jensen et al., 1990). For this reason, we selected one replicate to measure LWP from each treatment for each cultivar at midday. The results show that changes in leaf water potential can reliably be detected by using spectral measurements under field condition. LWP was related to four spectral indices ((R_{1240}/R_{860}, $(R_{840} - R_{1650})/(R_{840} + R_{1650})$, $(R_{410} - R_{780})/(R_{410} + R_{780})$, and $(R_{490} - R_{780})/(R_{490} + R_{780})$) at most of the individual measurements throughout three years. It should be pointed out that the exact relationships did differ with the date of measurement (Figs. 18 & 23). The indices R_{1240}/R_{860} and $(R_{840} - R_{1650})/(R_{840} + R_{1650})$ showed good relationships with LWP across all measurements for each and all cultivars (Table 24 and Figs. 18 a, b, d, e, f & h). It was possible to fit a single linear regression for all evaluations together for all cultivars across a broad range of values of LWP (-12 to -24.6 bar). But it is not clear if this relationship between two indices and LWP is independent of water content or biomass, because the spectral measurements and LWP were measured in different growth stages. As well as infrared reflection is affected greatly by plant architecture, vegetation density, leaf structure and dry matter thus increasing the estimation uncertainty (Elachi, 1987; Grant, 1993).

The results for this study are in agreement with those reported in other papers such as Kakani et al. (2006) found that a strong exponential relationship between leaf water potential and a simple reflectance ratio R_{1689}/R_{1657} (R^2 = 0.68***) in pot grown cotton. In contrast Eitel et al. (2006) found the best relationship with the maximum normalized water index which, was derived from the SWIR region (1300 - 2500 nm). It was strongly correlated with RWC and EWT and weekly correlated with LWP in

Populus deltoids x *populus nigra (OP-367)* at the leaf and canopy level under controlled conditions.

4.2.3 Canopy temperature measurements in 2005, 2006 and 2007

In this study, a near infrared temperature sensor presented some major advantages given that canopy temperature measurements were taken in a short time by using a mobile metal carrier to mount a sensor (high throughput technique). The sensor was connected to a portable computer and GPS antenna to provide continuous measurements of water status in winter wheat cultivars.

The canopy temperature measurements were taken at midday with a measurement angle of 45^{o} above the plants to minimize or eliminate effects by environmental factors. Blum et al. (1982) and Turner et al. (1986) found that the near infrared temperature measurements can be affected by the soil surface temperature and the zenith angle of the sun in oblique views

Our results show that LWP, CWC and CWM decreased with increasing canopy temperature as a result of increased water stress, which agree with the results found by Blum et al. (1982). They found that midday temperatures of fully developed plant canopies were correlated with leaf water potential across various wheat strains. Lower canopy temperatures were indicative of higher leaf water potentials and the variation of leaf temperature of different cultivars, as measured across replicated plots, ranged within 1-2 ^{o}C.

The stressed plants showed higher leaf water potential and canopy temperature than controlled plants as indicated in Figurers 26 & 27 and Table 29. These results are in agreement with Kumar and Tripathi (1991) who found that stressed wheat plants had consistently lower LWP and higher canopy temperature than irrigated plants. The maximum difference between the irrigated and stressed plants was noticed during milk stage increasing to 9.0 bars in LWP and 3.8 ^{o}C in canopy temperature between 1330-1400 h. In addition, Jensen et al. (1990) found that canopy temperature differences between water stressed and fully irrigated crops up to 6 ^{o}C were measured under conditions of high evaporative demand whereas under conditions of low evaporative demand canopy temperature differences between water stressed and fully irrigated crops approached zero even at severe crop water stress.

The canopy temperature is related to LWP at most of the individual measurements over all three years. There was better relationship between CT and LWP when combining data for each cultivar and all cultivars in 2005 and 2007 (Figs 26 & 27) than in 2006 (Table 29). The relationship between CT and LWP in 2006 was affected by the day. These results are in agreement with Jensen et al. (1990) and Jones et al. (1990), who reported that the LWP and CT were a function of environmental conditions. In addition canopy temperature has been expected to be a useful physiological parameter to screen genotypes for tolerance to water stress and for yield potential, but it is strongly influenced by environmental conditions (Blum et al., 1989; Rashid et al., 1999; Richards et al., 2002). Our results are in agreement with other studies such as Cohen et al. (2004), who found good relationships between LWP and leaf temperature for cotton in two different months (July, R^2 = 0.73 and August R^2 = 0.87). Kumar and Tripathi (1991) found that significant negative linear correlations existed between LWP of wheat and CT (R= -0.89). In additional, Gutierrez et al. (2010) found that there was a strong relationship between canopy temperature and leaf water potential of the investigated cultivars across a broad range of values (-2.0 to - 4.0 MPa).

The canopy temperature had better relationships with CWC than with CWM for each cultivar at most of the measurements over all three years (Figs 28 &29). The relation was affected by the day and the cultivars. It was not possible to fit a single linear or quadratic regression for all evaluations of all cultivars.

There was a negative relationship between canopy water content and canopy temperature and our results are in agreement with Jiang et al. (2009) who found that canopy and ambient temperature differentials were negatively correlated with the leaf relative water content of six cultivars of perennial ryegrass (R = -0.77 to -0.78).

Field infrared thermometers have to be calibrated before measuring because the relationship differ at given days and may be this is due to that CT not only depends on changes in leaf water potential or plant water content but also on air temperature, relative air humidity, light intensity and wind speed (Cai and Kang, 1997; Wen-zhong et al., 2007).

4.3 Darkroom experiments

Leaf growth seems to be a good indicator of drought stress in wheat and maize. Both crops showed reductions in leaf elongation particularly in the strong water stress treatment (Tables 31 and 32). These findings are in good agreements with previous studies by Hu et al. (2006) who found, that there was a significant reduction in the length of wheat leaves under drought stress. Similarly, Schmidhalter et al. (1998 a & b) found that leaf elongation of maize decreased before changes in leaf water relation of non-growing zones of leaf blades were detected. The decreases in leaf elongation could be observed at very early stages of soil drying and before transpiration decreased.

Water status of wheat is more influenced by water stress than in maize (Table 30). The differences between the maximum and minimum values of LWP, RWC, LWC, and CWC of wheat were -22.7 bar, 0.33%, 0.09 % and 0.1%, respectively and in maize -3.6 bar, 0.06%, 0.01% and 0.03%, respectively. Both LWP and RWC were more influenced by water stress treatments in wheat and maize than the other parameters. The results are in agreement with Kakani et al. (2007), who reported, that the LWP is an important indicator of plant water status and it has been demonstrated that irrigation scheduling based on LWP is superior to other methods. Furthermore Slatyer (1967) reported that leaf water potential is the standard parameter while leaf relative water content is often used as a substitute. In addition, Liu and Stützel (2002) found that the relative water content was strongly related to leaf water potential of four genotypes of the vegetable amaranth ($R^2 \geq 0.97$) under drought stress. The reduction in the values of LWP and RWC was more pronounced than within LWC and CWC of wheat and maize (Tables 30). Maybe this is due that LWP and RWC can reflect the stress in plants in a short time. On the other hand LWC and CWC probably reflect the stress in plants in the longer time frame.

Results from the literature for the remaining spectral indices and spectral bands are mixed and the assessment of their potential for measuring plant water status is complicated by the use of different plant species and experimental conditions (Reusch, 1997; Serrano et al., 2000; Read et al., 2002; Fensholt and Sandholt, 2003; Pu et al., 2003; Sims and Gamon, 2003; Stimson et al., 2005). Indeed, the advantage of taking spectral measurements at the leaf and canopy level in a darkroom is that the relationship between the plant water status and spectral indices is not affected by any external conditions such as atmospheric noise. In addition, the variation of spectral reflectance is related to the leaves or the plants. We have used black sheet slit diaphragms to prevent background reflectance at the leaf level as well as at the canopy level the zenith angle of the optic was set to 60^{o}.

Spectral reflectance measurements at the canopy level are of greater interest to cover a large area of the plant at the short time and it is easy to acquire timely information over larger areas with high frequency (Danson and Bowyer, 2004). On the other hand spectral reflectance measurements at the leaf level are not practical from the breeders' perspective because it is difficult to cover large areas of plants in a short time. But they provide insights about changes in leaf spectral properties accompanied by changes in plant water status according to Eitel et al. (2006).

Our experimental protocol group deserves further attention because it enabled a more rigorous assessment of the water status can be ascertained spectrally when the plants were under low water stress in maize, because several studies suggest that water stress has to be well developed in order to be detectable by spectral reflectance (Carter, 1991; Cohen, 1991; Penuelas et al., 1993, 1997; Pu et al., 2003; Stimson et al., 2005). In this study, there were small changes in the LWP and LWC of maize under six water treatments (Table 30). But there were strong relationships between LWP or LWC and spectral bands (Fig. 31c) and spectral indices at the canopy level (Table 35). Thus, the relationship between LWP or LWC and spectral reflectance can be detected under low water stress in maize.

The relative water content of wheat changed from 54.8 to 87.95% and for maize from 87.9 to 94.6% and RWC of wheat was related to all spectral indices (Table 34) and spectral reflectance at canopy level (Fig. 31a). These findings are further supported by Hoffer and Johannsen (1969), who reported that in order to detect an increase in reflectance due to water deficiency in corn leaves between 500 and 2300 nm, RWC has to decrease below 66 %. Considering that wilting in corn occurs at RWC < 80% level. Seelig et al. (2008) found that the spectral indices R_{1300}/R_{1450} was strongly related to RWC of *Spathiphyllum Lynise* leaves across a broad range of values (10% to 98%). In addition, Eitel et al. (2006) found that the normalized differential water index (NDWI) was strongly related to RWC (R^2 = 0.94) of *Populus deltoides* x *Populus nigra* across a broad range (24 % to 100%) but there was a weak relationship between the maximum differential water index MDWI and RWC (R^2 = 0.05) across the investigated range 85% to 100%.

Leaf water potential and canopy water content of wheat showed stronger relationships with spectral bands in all reflectance regions at the canopy level than the other parameters. The coefficient of determinations for LWP and CWC varied between

0.87** to 0.97*** and from 0.85* to 0.97***, respectively (Table 34). The relationship between LWP and spectral reflectance depended on the change in CWC.

Our results agree with the finding by e.g., Kakani et al. (2007), Gloser and Gloser (2007) and Gutierrez et al. (2010) for the relationship between LWP and spectral indices. Our results agree also with the findings of several other studies evaluating relationships between canopy water content with spectral indices such as the normalized difference water index $NDWI_{1640}$ and the normalized difference water index $NDWI_{2130}$ (Yonghong et al., 2007), the NDVI , simple ratio (R_{810}/R_{560}) and the red edge inflection point (REIP) (Behrens et al., 2006).

This study showed that the observed spectral indices and of maize at the canopy level were generally more related to LWC than to LWP (Table 35). The reason for this could be that the LWC provides information solely about the water content within leaves. In contrast, LWP also gives information about the water content of other plant parts, like the stem and root system, which is not directly seen by the spectrometer (Eitel et al., 2006; Kozlowski et al., 1991). In contrast to wheat, LWP was more related to spectral indices than LWC. The relationship between LWP and spectral indices possibly was not independent of the change in CWC or biomass.

Water stress slightly affected the spectral reflectance of single leaves of wheat and maize (Figs. 30b and 30d), while there were a strong effects at the canopy level of wheat and maize (Figs. 30aand 30c). This was found in the entire spectral range from visible to the near infrared and middle infrared. This could be a result of a variety of broader scale factors, including variations in leaf area representing the water status from different layers of leaves rather than from the leaf level.

It is widely recognized that the adaxial and abaxial leaf surfaces can differ markedly in stomata density (Tari, 2003). Carter (1991) found that reflectance of the abaxial leaf surface tended to be greater than for the adaxial surface, particularly in the 400 - 700 nm and 1300 - 2500 nm regions. This result is in contrast with our results, because there were no differences in reflectance between both surfaces of wheat and maize leaves (Fig. 33b).

Our data clearly show that spectral reflectance measurements seem to provide good indicators to detect water status. Not only by measuring LWP, LWC, RWC and CWC in wheat at the canopy level when the plants were under high stress, but also in maize by measuring LWP and LWC, when the plants were under low water stress.

5 FINAL DISCUSSION

Classical measurements for estimating the water status of plants using oven drying or pressure chambers are tedious and time-consuming. In the field, frequent changes in environmental conditions may further influence the measurements and thus require fast measurements (Peñuelas et al., 1997; Winterhalter et al., 2011). Therefore, it is very important to develop an effective evaluation approach for detecting water status in plants, which should be reliable, quick, easy, practical, and economic. In this study, different high throughput sensing methods such as passive reflectance sensing, laser induced-chlorophyll fluorescence sensing and near infrared temperature sensing were used to detect water status in wheat and maize either under control conditions or under a mobile rain-out shelter.

5.1 High throughput sensing methods and leaf water potential

Our studies showed the possibility of using proximal/remotely sensed data to detect the leaf water potential of wheat and maize applying spectral reflectance indices, fluorescence indices and canopy temperature.

The two newly developed indices R_{940}/R_{960} and (R_{940}/R_{960})/NDVI were strongly related to LWP of wheat or maize under controlled conditions in a growth chamber without changes in biomass and in darkroom experiments with changes in biomass (Tables 12, 13, 34 and 35), but they were poorly related to LWP under field conditions. The two indices seem to be sensitive to changes in environmental conditions and the ability to validate them under field conditions is difficult. However, probably they may be used under green house conditions.

The new index $(R_{410} - R_{780})/(R_{410} + R_{780})$, which is derived from violet and near infrared regions, seems to be a good indicator to detect LWP of wheat under control conditions as well as under field conditions (Tables 34 & 24 and Figs 23 b & d).

For the previous three indices close relationships were found with LWP either under controlled conditions or under field conditions, however, they were strongly influenced by the date of measurement. Thus, global spectral relationships measuring LWP probably cannot be established across plant development. Even so, spectrometric measurements need to be supplemented by a reduced calibration data set from pressure chamber measurements. On the other hand, two indices R_{1240}/R_{860} and $(R_{840} - R_{860})/(R_{840} + R_{860})$ were related to LWP and the relation could be established across all measurements for each cultivar ($R^2 \geq 0.56$***) and all cultivars ($R^2 \geq 0.57$***) in 2006 and 2007 as depicted in Table 24.

Only few studies have yet documented the possibility of spectral indices to estimate LWP under different experimental conditions and cultivars. For instance, consistent relationships seemingly independent of changes in plant biomass status between (R_{1689}/R_{1657}) and LWP of cotton and between each of (R_{900}/R_{970}), NDVI $(R_{900} - R_{680})/(R_{900} + R_{680})$ and LWP of lichen *Umbilicaria hirsuta* as well as between the normalized water index $(R_{970} - R_{880})/(R_{970} + R_{880})$ (e.g., Kakani et al., 2007; Gloser and Gloser, 2007) have been demonstrated.

Fluorescence intensity at 690 nm proved also to a sensitive indicator for LWP in comparison to other fluorescence indices and the biomass index across all measurements for each cultivar ($R^2 \geq 0.64$***) and all cultivars ($R^2 \geq 0.44$***) in 2005. Our results are in agreement with Schmuck et al. (1991) found that both the variable chlorophyll fluorescence at 690 nm and 730 nm as well as the mean lifetime of the laser pulse (which indicates a faster energy transfer in water stressed plants at the leaf level) are good indicators of water potential in maize.

In addition, canopy temperature presented good relationships with LWP either in 2005 or 2007 across all measurements for each cultivar ($R^2 \geq 0.58$***) and all cultivars ($R^2 \geq 0.65$***) as shown in Figuers 26 & 27, but weak relations were found across all measurements in 2006 (Table 29). This is probably due to the canopy temperature not only being dependent on the changes in leaf water potential but also on air temperature, air humidity, light intensity and wind speed (Cai et al., 1997; Wen-zhong et al., 2007). Our results are in agreement with Cohen et al. (2004), who found good relationships between LWP and leaf temperature in cotton. In addition, Gutierrez et al. (2010) found that there was a strong relationship between canopy temperature and leaf water potential of cultivars across a broad range of values (-2.0 to - 4.0 MPa).

5.2 High throughput sensing methods and canopy water content

In several experiments under temperate field conditions as well as in one experiment under greenhouse conditions, canopy water content of wheat was the best indicator to detect water status as a function of proximal measurements compared to other parameters. Five spectral indices (NDVI, $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, $(R_{510} - R_{780})/(R_{510} + R_{780})$ and R_{600}/R_{780}), two fluorescence intensities at 690 nm and 730 nm, the biomass index and canopy temperature had strong relationships with canopy water content. All five spectral indices presented good relationships with canopy water content not only under controlled conditions ($R^2 \geq 0.97$***) but also under field conditions ($R^2 \geq 0.57$*) (Tables 22, 27 & 34). Generally, canopy water content was highly related to all spectral indices at the individual measurements and also highly related across all measurements for each cultivar in one year (Tables 22 & 27). Only the spectral index $(R_{410} - R_{780})/(R_{410} + R_{780})$ tended to produce similar relationships at specific days, with influences by the day (Figs 16 & 21). The strongest relationship between $(R_{510} - R_{780})/(R_{510} + R_{780})$ and canopy water content of wheat was recorded in 2008 under field conditions (R^2 = 0.96***) (Table 27). The results showed that NDVI and R_{600}/R_{780} are good indicator for detecting canopy water content of wheat across all measurements in three years ($R^2 \geq 0.84$***).

Generally, the fluorescence intensity at 690 nm and 730 nm as well as the biomass index proved to be more sensitive to drought stress in wheat than the fluorescence ratio F690/F730 under field conditions. The fluorescence intensity at 690 nm and 730 nm as well as the biomass index presented good relationship with canopy water content across all measurements for each cultivar ($R^2 \geq 0.83$***), and also across all measurements for all cultivars ($R^2 \geq 0.71$***) (Fig 13).

Canopy temperature had a good relationship with CWC ($R^2 \geq 0.59$*) for each of the investigated cultivars for most of the measurements over three years (Fig 28), but the relation was affected by day and cultivar.

Over all proximal remote sensing methods, global spectral and fluorescence relationships measuring CWC can be established across plant development. On the other hand, near infrared temperature measuring CWC can be used to compare between treatments or cultivars as specific days.

5.3 High throughput sensing methods and canopy water mass

The relationships between canopy water mass or absolute water content and five spectral indices (NDVI, $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, $(R_{510} - R_{780})/(R_{510} + R_{780})$, and R_{600}/R_{780}, two fluorescence intensities at 690 nm and 730nm, the biomass index and canopy temperature were tested under field conditions in three years. Five spectral indices showed stronger relationships with CWM in 2008 than in 2006 and 2007 (Figs 23 & 28). This may be due to CWM being affected by the density of the plants. The index $(R_{410} - R_{780})/(R_{410} + R_{780})$ which was derived from violet and infrared regions, seemed to be more stable to detect CWM in different years compared to other indices (Figs. 17 & 23)

The fluorescence intensity at 690 nm and 730 nm as well as the biomass index did not only present good relationship with canopy water mass across all measurements for each cultivar ($R^2 \geq 0.51$***), but also across all measurements for all cultivars ($R^2 \geq 0.64$***) (Fig 14).

Canopy temperature showed non-significant relationships with CWM for most of the measurements particularly in 2005 and 2006 (Fig. 29).

Over all proximal remote sensing methods which were used in the present study, global spectral and fluorescence relationships measuring CWM can be established across plant development.

5.4 Advantages of the investigated techniques under field conditions

Passive reflectance sensing, active laser sensing and near infrared temperature sensing present some major advantages given that all measurements could be taken in a short time by using a mobile metal carrier (high throughput technique) and could be used to measure from rather large distances about 1.3 to 3 m from the plant canopy.

The passive reflectance sensing consisted system used of four optics, which were positioned on the edges of a metal frame with a zenith angle of 50^o, so always one optic was facing the sun exposed side while another one was directed to the shadow side of the plants. Therefore, spectral reflectance measurements could be taken independently of viewing direction and solar zenith and azimuth angles. Another positive effect of the four optics was the increased amount of biomass in the field of view decreasing usually reflectance from soil background. In contrast other researchers reported clearly influenced canopy reflectance data from soil background (Borge and Mortensen, 2002).

For measuring in the nadir, scientists often use a spectralon reflectance standard to the sun radiation instead of simultaneous measurements. There is always a time shift between sun radiation measurements and canopy reflectance measurements. If the radiation conditions are not totally stables, it may result in an error within the measurements (Duggin and Cunia, 1983; Major et al., 2003). Therefore, in 2008 a passive reflectance sensing system was developed to measure canopy reflectance and sun reflectance within 15 sec.

Finally, the advantage of the active laser sensor was that, it could measure the fluorescence signals from sparse plant stands without any effects from the soil. In addition, it is expected that the laser sensor can be used in nearly all weather conditions, even at low light conditions or during the night.

5.5 Limitations

- A limitation of the passive reflectance sensor is the need of sufficient sun light because the passive sensor system has no own light source.
- For measuring in the nadir, spectral reflectance of canopies is always a mixed signal of soil reflectance and canopy reflectance and it is not possible to differentiate between both.
- Weather conditions in Germany are unstable and rapidly changing within short periods. Therefore, it is difficult to apply spectral measurements for extended periods throughout a day.
- Laser induced-chlorophyll fluorescence sensing should be used in short time at constant air temperature and incident light angles, because the fluorescence intensities at 690 nm and 730 nm are affected by air temperature and the sun angle.
- Leaf water potential is the most important physiological parameter to detect drought stress in plants, but the detection of LWP has limitations under temperate conditions.
- Spectral detected of canopy water mass in wheat is affected by the density of the plants per area.
- It reveled to be difficult to apply drought stress in maize under temperate conditions, for this reason, spectral measurements were taken under controlled growth chamber conditions.
- Cloudy or windy conditions should be avoided, which has an immediate effect on canopy temperature measurements.

SUMMARY

Available water is one of the most limiting factors in crop production and exposure of plants to drought leads to noticeable decreases in leaf water potential and water content with a concurrent increase in leaf temperature. The present study investigated the potential of high throughput sensing methods such as spectral reflectance measurements, laser induced-chlorophyll fluorescence measurements and near infrared temperature measurements to detect drought stress in plants by measuring leaf water potential, plant water content and canopy temperature. Validations were performed under controlled conditions (growth chamber and darkroom) to assess leaf water potential, leaf water content, canopy water content and relative leaf water content and under field conditions to assess leaf water potential leaf, canopy water content, canopy water mass and aerial biomass. To test the ability of spectral measurements to detect changes in leaf water potential without changes in biomass and only small changes in leaf water content of wheat and maize, the measurements were conducted under controlled conditions. To study the stability of spectral reflectance measurements, fluorescence emission and near infrared temperature measurements to detect water status in wheat under field conditions, another series of experiments were carried out under a mobile rain-out shelter to induce controlled water stress in plants.

The results obtained showed that significant relationships (R^2-values 0.74-0.92) between leaf water potential and new spectral indices ((R_{940}/R_{960})/NDVI; R_{940}/R_{960}) were detected with or without significant changes in leaf water content of both wheat and maize under controlled conditions. The exact relationships found, however, were strongly influenced by the date of measurement or water stress induced. Thus, global spectral

relationships measuring leaf water potential can probably not 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 diverse lines.

The results of spectral measurements under controlled and field conditions showed that five spectral indices (NDVI, $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, $(R_{510} - R_{780})/(R_{510} + R_{780})$ and R_{600}/R_{780}) revealed to be good indicators to detect canopy water content of wheat at individual measurements and across all measurements for each cultivar in different years and with coefficients of determinations varying between 0.59* to 0.98***. As well as the NDVI and the index R_{600}/R_{780} seemed to be good indicators to detect canopy water content of wheat across all measurements for all cultivars over three years of investigations ($R^2 \geq 0.84$***).

The results of laser-induced chlorophyll fluorescence measurements under field conditions showed that the canopy water content was closely related to chlorophyll fluorescence at 690 nm, 730 nm and to the biomass index for each of the four investigated cultivars with R^2- values ≥ 0.83***, $R^2 \geq 0.84$***, and $R^2 \geq 0.82$***, respectively, and averaged over all cultivars the coefficients of determinations were $R^2 = 0.71$***, $R^2 = 0.74$***, and $R^2 = 0.74$***, respectively. Cultivar-specific relationships to canopy temperature were within $R^2 = 0.62$* to 0.97***. Fluorescence intensity at 690 nm revealed to be a good indicator of leaf water potential for each of the cultivars, Ludwig, Ellvis, Empire and Cubus with $R^2 = 0.69$***, $R^2 = 0.66$***, $R^2 = 0.64$***, and $R^2 = 0.69$***, respectively. This work shows the possibility to detect drought stress by laser-induced chlorophyll fluorescence measurements in wheat by measuring fluorescence intensities at 690 nm, 730 nm or the newly developed biomass index revealing to be better indicators of drought stress than the fluorescence ratio F690/F730.

In addition, canopy temperature showed a good relationship to the canopy water content ($R^2 \geq 0.59$*) for each cultivar at most of the measurements over three years. But the relationship was affected by the day and cultivars. Canopy temperature assessments presented good relationships to leaf water potential either in 2005 or 2007 across all measurements for each cultivar ($R^2 \geq 0.58$***) and all cultivars ($R^2 \geq 0.65$***), but weak relations were found across all measurements in 2007.

Over all our results under temperate field conditions, canopy water content of plants was the most suitable physiological parameter to detect drought stress by proximal sensing methods using spectral reflectance and laser induced chlorophyll fluorescence measurements. Therefore, time-consuming destructive methods could be replaced by rapid, non-destructive methods. These technologies may open an avenue for fast, high-throughput assessments of water status in plants, which would simultaneously be useful for screening large numbers of plants (e.g., in breeding) as well as being equally important for management related actions.

ZUSAMMENFASSUNG

Verfügbares Wasser ist einer der wichtigsten limitierenden Faktoren in der Pflanzenproduktion. Die Einwirkung von Trockenheit auf Pflanzen führt zu einem messbaren Absinken des Blattwasserpotentials und des Wassergehalts bei gleichzeitiger Zunahme der Blatttemperatur. Die vorliegende Studie untersucht das Potential von Hochdurchsatzmessungen, wie der Messung der spektralen Reflexion, der Laser induzierten Chlorophyllfluoreszenz und Messungen der Infrarostrahlung zur Wiedergabe von Trockenstress-indikatoren wie dem Blattwasserpotential, dem Pflanzenwassergehalt und der Bestandestemperatur. Validierungen dieser Methoden wurden unter kontrollierten und unter Feldbedingungen durchgeführt. Eine Zielsetzung war es, die Fähigkeit spektraler Messungen zur Wiedergabe des Blattwasserpotentials bei konstanter Biomasse und geringen Veränderungen des Pflanzenwassergehalts unter kontrollierten Bedingungen nachzuweisen. Um die Stabilität von Fluoreszenz-, Infrarot- und spektralen Reflexionsmessungen unter Feldbedingungen festzustellen, wurden zahlreiche Experimente in einer Rain-Out-Shelter Anlage zur Stresssimulation von Trockenstress durchgeführt.

Signifikante Korrelationen (R^2-Werte 0.74-0.92) zwischen Blattwasserpotential und neu entwickelten spektralen Indizes ((R_{940}/R_{960})/NDVI; R_{940}/R_{960}) bei signifikanten oder nicht signifikanten Korrelationen der Indizes mit dem Blattwassergehalt von Weizen und Mais konnten unter kontrollierten Bedingungen nachgewiesen werden. Die exakten funktionalen Zusammenhänge wurden jedoch stark vom Tag der Messung oder dem Grad an Trockenstress beeinflusst. Daher konnten keine, während der gesamten Vegetationsperiode der Kultur gültigen Vegetationsindizes ermittelt werden. Dennoch

können spektrometrische Messungen, kalibriert anhand einer reduzierten Anzahl von Messungen des Blattwasserpotentials mit der Druckkammer, als schnelle und exakte Methode zur Prüfung einer großen Anzahl von Zuchtlinien verwendet werden.

Die Ergebnisse spektraler Messungen unter kontrollierten und Feldbedingungen zeigen, dass fünf spektrale Indizes (NDVI, $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, $(R_{510} - R_{780})/(R_{510} + R_{780})$ und R_{600}/R_{780}) gute Indikatoren des Bestandeswassergehalts von Weizen darstellen. Hohe Bestimmtheitsmaße von 0.59* bis 0.98*** ergaben sich sowohl bei individuellen Messungen als auch bei Regressionen über alle Messungen für verschiedene Sorten und Jahre. Bei einer Regression über alle Messungen, Sorten und drei Versuchsjahre erwiesen sich der NDVI und der Index R_{600}/R_{780} ($R^2 \geq 0.84$***) als die besten Indikatoren für den Bestandeswassergehalt von Weizen.

Die Ergebnisse der laserinduzierten Chlorophyllfluoreszenz unter Feldbedingungen zeigen, dass der Bestandeswassergehalt bei einer sortenspezifischen Regression eng mit der Chlorophyllfluoreszenz bei 690 nm ($R^2 \geq 0.83$***) und 730 nm ($R^2 \geq 0.84$***) und mit dem Biomasseindex ($R^2 \geq 0.82$***) korreliert. Bei einer Kalibrierung, die alle 4 Sorten einschließt, liegen die Bestimmtheitsmaße für die Fluoreszenz bei 690 nm bei 0.71***, für die Fluoreszenz bei 730 nm bei 0.74*** und für den Biomasseindex bei 0.74***. Sortenspezifische Regressionen der Bestandestemperatur mit der Chlorophyllfluoreszenz ergeben Bestimmtheitsmaße von 0.62* bis 0.97***. Die Fluoreszenzintensität bei 690 nm erwies sich als guter Indikator für das Blattwasserpotential für jede der Sorten Ludwig ($R^2 = 0.69$***), Ellvis ($R^2 = 0.66$***), Empire ($R^2 = 0.64$***) und Cubus ($R^2 = 0.69$***). Diese Arbeit zeigt die

Möglichkeit auf, Trockenstress in Weizen anhand der Chlorophyllfluoreszenz bei 690 nm, 730 nm und dem neu entwickelten Biomasseindex zu erkennen. Diese drei Methoden erwiesen sich als bessere Indikatoren für Trockenstress als der Fluoreszenzindex F690/F730.

Die Bestandestemperatur weist bei jeder einzelnen Sorte und einem Großteil der Experimente aus den drei Versuchsjahren eine gute Korrelation mit dem Bestandeswassergehalt auf ($R^2 \geq 0.59^*$). Der funktionelle Zusammenhang erwies sich aber als vom Tag der Messung und der Sorte beeinflusst. Die Bestandestemperatur wies ebenfalls eine gute Korrelation mit dem Blattwasserpotential bei einer Regression für einzelne Sorten über alle Messungen im Jahr 2006 bzw. 2007 auf. Im Jahr 2008 war der Zusammenhang bei einer Regression über alle Messungen jedoch gering.

Als Zusammenfassung der Ergebnisse aller Feldversuche unter gemäßigten Klimabedingungen können die basierend auf Reflexionsmessungen ermittelten spektralen Indizes und die laserinduzierte Chlorphyllfluoreszenz als beste Methoden zur indirekten Ermittlung des physiologischen Trockenstressindikators Bestandeswassergehalt bezeichnet werden. Mit ihrer Hilfe können zeitaufwändige destruktive Methoden durch schnelle nicht-destruktive Methoden zur Ermittlung des Wasserstatus ersetzt werden. Diese Technologien eröffnen die Möglichkeit einer effizienten Hochdurchsatzmessung des Wasserstatus von Pflanzen. Diese Methoden können sich für die Prüfung einer großen Anzahl von Pflanzen in der Züchtung, als auch für Anwendungen im Pflanzenbau wie der Bewässerungssteuerung als nützlich erweisen.

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