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Occurrence of repeated drought events: can repetitive stress situations and recovery from drought be traced with leaf reflectance?

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Abstract

*Within the last years a lot of effort has been made to improve irrigation efficiency and early drought stress detection by using various remote sensing techniques. In the present study two different species of wheat (*Triticum aestivum *and* Triticum durum*), cultivated in a growth chamber, were used to investigate the effects of drought occurring at different phenological stages. Plant physiological traits and spectral leaf reflectance were used to assess the potential of remote sensing techniques. Drought stress was applied either at flowering and/or at grain filling. Subsequently, a treatment following recovery after drought stress at flowering was set up. The effects of drought were traced by following the changes in plant physiological traits (i.e. photosynthetic rate, leaf conductance, relative and actual leaf water content) as well as in leaf reflectance. Drought resulted in a significant reduction of plant physiological traits and water relations, independently of the time of its occurrence. Rewatering plants after the stress period at flowering resulted in a recovery of plant physiological traits. Single leaf reflectance of plants subjected to drought increased over the entire range of the spectrum. However, five spectral regions with relatively high differences were observed: 520–530 nm, 570–590 nm, 690–710 nm, 1410–1470 nm and 1880–1940 nm. Additionally, three spectral indices were tested towards their applicability for tracing drought stress and subsequent recovery, yielding a reasonable relationship with measured leaf water content, photosynthetic rate and leaf nitrogen content.*

INTRODUCTION

Water scarcity is increasingly important in many parts of the world. Within the next centuries global climate change is expected to result in a long-term trend towards higher temperatures, greater evapotranspiration, and an increased incidence of drought in specific regions *(1, 2)*. Moreover, not only changes in the spatial but also in the temporal distribution patterns of precipitation and radiation are to be expected *(3)*; e.g.: in Europe higher precipitation levels are predicted for the winter half-year and drier periods for the summer half-year *(2)*.

Under conditions of drought stress, absorption of radiation by the leaf tends to decrease due to lower leaf water content. Although water absorbs most strongly in the wavelengths of the infrared region of the

TABLE 1

Summary of climatic conditions in the growth chamber.

	Spring	Summer
Temperature	07–14°C day / 06–12°C night	17–26°C day / 14–20°C night
Relative humidity	60–80% day / 75–90% night	50–70% day / 60–90% night
Light (1m above ground)	~ 700 µmol m^{-2} s^{-1}	~ 700 µmol m^{-2} s^{-1}
Day length	13.5 h	15.5 h

spectrum from approximately 1300 nm to 2500 nm *(4)*, some absorption also occurs at lower wavelengths. As water is lost from a leaf, reflectance increases and absorption decreases, primarily as a result of water's radiative properties *(5, 6)*. Even after accounting for the radiative characteristics of water, secondary effects occur. These include the influence of water content on absorption by other substances in the leaves, such as pigments. Also included as secondary are the effects of water content on wavelength-independent processes, particularly multiple reflections inside the leaf *(7)*.

Moreover, drought stress not only causes leaf water content to decline but also affects physiological processes (e.g. leaf conductance, photosynthetic rates, etc.). Furthermore, changes in pigment and nitrogen concentration of plant tissue will occur. For example, chlorophyll and RubisCO contents decline as the leaf remobilizes resources under stress conditions *(8)*. Chlorophyll and accessory pigments absorb strongly in the visible range *(9, 10)*. Carter and Knapp *(11)* described a consistent stress induced alteration of leaf reflectance at visible wavelengths (~400–720nm) since chlorophyll is the major absorber in the leaf and the metabolic disturbance brought about by stress alters leaf chlorophyll concentrations *(9)*. Leaf reflectance in the visible range of plants experiencing nutrient deficiency was also found to increase since nitrogen (and magnesium) is essential in the formation of chlorophyll. As leaves become more chlorotic, reflectance increases and the reflectance peak, normally centred at about 550 nm, broadens towards the red as absorption of incident light by chlorophyll decreases *(12)*. Plant responses to water deficit therefore include both biochemical and morphological changes that primarily lead to acclimation and later to functional damage and the loss of plant parts *(13)*.

A lot of effort has been made towards the use of spectral reflectance of leaves and canopies for stress detection in agricultural environments. While leaf reflectance is driven by the chemical composition of the leaves, the reflectance of a canopy is influenced by its geometry – the leaf area index, inclination and clumping of the leaves – as well as the reflectance of single leaves. In this study we only concentrate on the reflectance of leaves and not of the whole canopy.

The aim of the present study was, on the one hand, to evaluate the impact of drought stress on plant physiological traits and leaf reflectance of wheat (*Triticum aestivum* and *Triticum durum*) occurring at different phenological stages (at flowering and/or grain filling). On the other hand, the incidence of two consecutive drought events and recovery of plants after drought was investigated. The analysis of the effect of consecutive stress periods and recovery on changes in leaf reflectance has rarely been performed until now but might gain in importance considering the predicted increased frequency of drought events whereby plants could be exposed to drought repeatedly *(2, 14, 15, 16)*.

MATERIAL AND METHODS

1. Experimental Setup

Plants (*Triticum aestivum* L. cv. Xenos and *Triticum durum* L. cv. Floradur) were grown in 8 litre plastic pots *(7)*. Simulation of seasons in the growth chamber was based upon long-time observation of temperature and relative humidity (past 10 years; meteorological station: 16°29' eastern longitude and 48°15' northern latitude). Illumination of the growth chamber was accomplished by 54 lamp units consisting of a lamp (Powerstar HQI TS 250/NDL UVS, 250W, Osram, Germany) and an appropriate reflector (Osram, Germany) yielding a PPFD of ~1200 µmol m^{-2} s^{-1} in 1.5m above the ground. Detailed climatic conditions are summarized in Table 1.

For germination, 25 seeds of *T. aestivum* / *T. durum* were placed in each pot (7 replicates) and seedlings were thinned to 20 plants per pot. Nitrogen fertilization (2.11g N per pot; equivalent to 150kg N/ha) with Nitramoncal (27% N) was evenly split in three bits (before sowing, at stem elongation and at heading). P and K were supplied with Hortipray (NPK 0:52:34; 2.05g/pot, equivalent to 180kg K/ha). Prior to sowing the agricultural soil was additionally fertilized with »Flory Basisdünger 10®« (Euflor GmbH, Germany; trace elements). As cultural substrate, a 2:1 mixture of air-dried and sieved (<4mm) agricultural top soil (6.33kg; A-Horizon; chernozem) and quartz sand (3.17kg; 0.2-2.0 mm) was used.

Four different treatments were set up per species – one control treatment and three treatments exposed to drought at different times during ontogeny:

AC/DC: control plants of *T. aestivum* / *T. durum;* AF/DF: *T. aestivum* / *T. durum* exposed to drought stress at flowering; recovery after anthesis; AG/DG: *T. aestivum* / *T. durum* exposed to drought stress at grain filling; AFG/DFG: *T. aestivum* / *T. durum* exposed to drought stress at flowering and grain filling.

Soil humidity of control plants was consistently held at 20–23 vol% (AC/DC). Drought stress at flowering was imposed by halving water supply 10 days before the beginning of pollen shedding resulting in a soil humidity of ~10 vol% (TDR Trime, Imko Micromodultechnik GmbH, Germany) at flowering (AF/DF). After flowering, plants receiving a second stress at grain filling were allowed to recover for 8 days (water supply similar to control plants) before the second stress was imposed by halving water supply again (soil humidity during measuring period ~10 vol%; AFG/DFG). Plants receiving drought stress only at grain filling (AG/DG) were treated similar to control plants until after flowering. Drought stress was imposed at the same time as in plants of the treatment stressed twice.

2. Measurements

2.1. Physiological Measurements

Physiological and spectral measurements were made in the mid region of the youngest fully expanded leaves at three developmental stages: vegetative growth, flowering and grain filling.

Gas exchange measurements (A/C_i curves) were made using a CIRAS-I system (PP-Systems, U.K.) with an external air conditioning system. Leaves were placed in a cuvette of 2.5 cm^2, which was illuminated with a PPFD of 1000 $\mu mol\ m^{-2}\ s^{-1}$. Temperature of the leaf chamber was maintained at 20 °C, air flow was set to 300 ml min^{-1} and relative humidity of the incoming air was held at 45–55%. Light saturated photosynthetic rates (A_{sat}) refer to measurements at growth conditions under saturating light intensities (CO_2: 350–370 $\mu mol\ mol^{-1}$; light: 1000 $\mu mol\ m^{-2}\ s^{-1}$).

Actual leaf conductance (g_L) was measured with a steady state porometer (PMR-4, PP-Systems; U.K.). Data were collected separately for both upper (adaxial) and lower (abaxial) leaf surface.

Chlorophyll content (Chl_{tot}) of leaves was determined with a SPAD-502 hand held chlorophyll meter (Minolta, Japan; *(18)*). For the measurement of absolute chlorophyll content per unit leaf area [$\mu g\ cm^{-2}$] small leaf discs of known area were cut and transferred to 5 ml of *N,N*-Dimethylformamide. Samples were stored at –18°C until spectrophotometer readings of the eluate were taken (DU-7400, Beckman, USA; *(19)*). A calibration curve of SPAD readings versus absolute chlorophyll content was used to convert the SPAD readings into area based chlorophyll contents.

For calculation of the relative water content (RWC), leaf material was collected and fresh weight was immediately determined. Saturated weight was measured after placing the leaf discs in Petri dishes between wet filter paper for 24 hours (4 °C, dark). Dry weight was determined after drying leaf material to constant weight at 70 °C. Relative water content *(20)* was then calculated as:

$$RWC = ((\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})) * 100\,[\%]$$

and actual leaf water content was calculated as

$$AWC = ((\text{fresh weight} - \text{dry weight}) / (\text{fresh weight})) * 100\,[\%].$$

Plant material for measuring leaf nitrogen content ([N], expressed as percentage of dry matter) was dried to constant weight (70°C) and milled (Cyclotec® Sample Mill; Planetary Ball Mill, PM 4000, Retsch). An aliquot of 1–2 mg of each sample (pooled samples) was weighed into tin capsules and analysed by isotope ratio mass spectrometry (IRMS). A continuous-flow IRMS system, consisting of an elemental analyser (EA 1110, CE Instruments, Milan, Italy) which was interfaced to the IRMS (DeltaPLUS, Finnigan MAT, Germany) was used.

2.2. Spectral measurements

Leaf spectral reflectance was measured with a FieldSpec Pro FR in connection with a plant reflectance probe from Analytical Spectral Devices Inc., Boulder, CO. The radiometer operates in the spectral range from 350 to 2500 nm. In the 350 to 1000 nm range, the sampling interval is approximately 1.4 nm and the spectral resolution (full width at half maximum) is 3 nm. In the 1000 to 2500 nm range, the sampling interval is 2 nm and the spectral resolution is 10 to 12 nm. The reflectance probe is equipped with an internal light source and works with a bi-conical measurement geometry. The device was adapted for a sample area of 19 mm by 7 mm to be able to measure the reflectance of individual wheat leaves. The detector field of view subtends an angle of up to 25°, and its axis is inclined by an angle of 25° to the sample normal. Radiance measurements were performed on single leaves (youngest fully expanded) and on a Spectralon panel serving as a white reference. Reflectance values were obtained as ratios of leaf radiances and Spectralon radiances.

Relative difference of reflectance spectra between stress and control treatments ($\Delta R/R$) was calculated as $[((R_{stress}-R_{control})/R_{control})*100;\ (\%)]$.

In addition, three spectral indices were calculated: photochemical reflectance index (PRI), an index for the estimation of relative water content (RWC_i) and an index for the estimation of the actual water content (AWC_i). The PRI is widely used for the estimation of photosynthetic radiation use efficiency. It was proposed according to the finding that the interconversion of xanthophyll cycle pigments in intact leaves can be detected as subtle changes in absorbance at 505–510 nm *(21)* or the reflectance at 531 nm *(22)*. The photochemical reflectance index (PRI), incorporating reflectance at 531 nm (xanthophyll cycle signal), was then defined as $[(R_{570}-R_{531}) / (R_{570}+R_{531})]$ in the attempt to establish a reflectance-based photosynthetic index *(23)*. Concerning the attempt to trace leaf water content (RWC and AWC) with spectral indices, a lot of effort has been made and a number of different indices have been developed for numerous crop species: among them the water index (WI; R_{900} / R_{970};

TABLE 2

Summary of physiological traits of *T. aestivum* and *T. durum*. Significance levels refer to the differences between control and stress treatments. n=5-30; n.s.: not significant, *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

		Triticum aestivum L.				*Triticum durum* L.			
		AC	AF	AG	AFG	DC	DF	DG	DFG
A_{sat}	vegetative	21.2				17.5			
	flowering	16.9	10.7***			15.6	9.9***		
	grain filling	13.8	12.2 n.s.	4.4***	6.9***	11.5	14.5**	3.9***	6.1***
g_L US	vegetative	185.0				224.3			
	flowering	453.9	81.2***			342.9	113.3***		
	grain filling	508.2	387.2***	56.9***	93.0***	382.5	370.7 n.s.	86.8***	125.0***
g_L LS	vegetative	84.0				87.4			
	flowering	164.1	18.4***			129.3	25.7***		
	grain filling	171.8	116.3**	15.3***	20.3***	142.5	101.1*	26.4***	48.1***
RWC	vegetative	86.7				91.3			
	flowering	83.8	74.0**			86.5	82.9 n.s.		
	grain filling	76.3	81.9*	57.1***	64.0***	81.8	82.0 n.s.	67.4***	74.2*
AWC	vegetative	81.2				83.1			
	flowering	72.2	68.8**			77.4	76.0 n.s.		
	grain filling	74.1	74.8 n.s.	68.3 n.s.	71.1**	77.8	78.0 n.s.	75.5**	76.0 n.s.
Chl_{tot}	vegetative	46.8				53.5			
	flowering	55.0	59.2***			55.7	53.4*		
	grain filling	48.3	50.3**	61.7***	55.6***	49.2	54.0***	52.1**	53.4***
Leaf[N]	vegetative	4.3				4.6			
	flowering	4.4	4.2**			4.2	3.8***		
	grain filling	2.4	2.3 n.s.	1.9**	2.0**	2.4	2.5 n.s.	2.1*	2.2 n.s.

Abbreviations: A: *T. aestivum*; D: *T. durum*; C: control; F: drought at flowering, plants were recovered at grain filling; G: drought stress at grain filling; FG: drought stress at flowering and grain filling. A_{sat}: [µmol m^{-2} s^{-1}], g_L: [mmol m^{-2} s^{-1}], RWC: [%], AWC [%], Chl_{tot}: [µg cm^{-2}]; Leaf[N]: leaf nitrogen content in % dry matter; US: upper leaf surface, LS: lower leaf surface

(24)), the water band index (WBI; R_{905} / R_{980}; *(25)*) and some other indices described by Yu *et al. (26)*. In the present study, for estimating RWC the ratio $RWC_i = R_{1483} / R_{1650}$ and for estimating AWC the ratio $AWC_i = R_{1121} / R_{1430}$ *(26)* were used.

2.3. Statistical Analysis

To test the level of significance between control plants and those of the stress treatments, data were subjected to a one-way analysis of variance (ANOVA; Systat 8, SPSS Inc.). For spectral measurements the mean of the five regions showing greatest differences between treatments was calculated (520–530nm, 570–590 nm, 690–710 nm, 1410-1470nm and 1880-1940nm) and used for statistics (ANOVA). All tests were made separately for the different species and phenological stages. Correlation analysis, testing the relationship between physiological parameters and spectral indices, was performed with Statgraphics Plus 5.0 software package (Statistical Graphics Inc.).

RESULTS

Physiological Measurements

Drought stress at flowering substantially reduced light saturated photosynthetic rates (A_{sat}) of both species (AF: –36%, DF: –37%; Table 2). Rewatering caused A_{sat} to re-

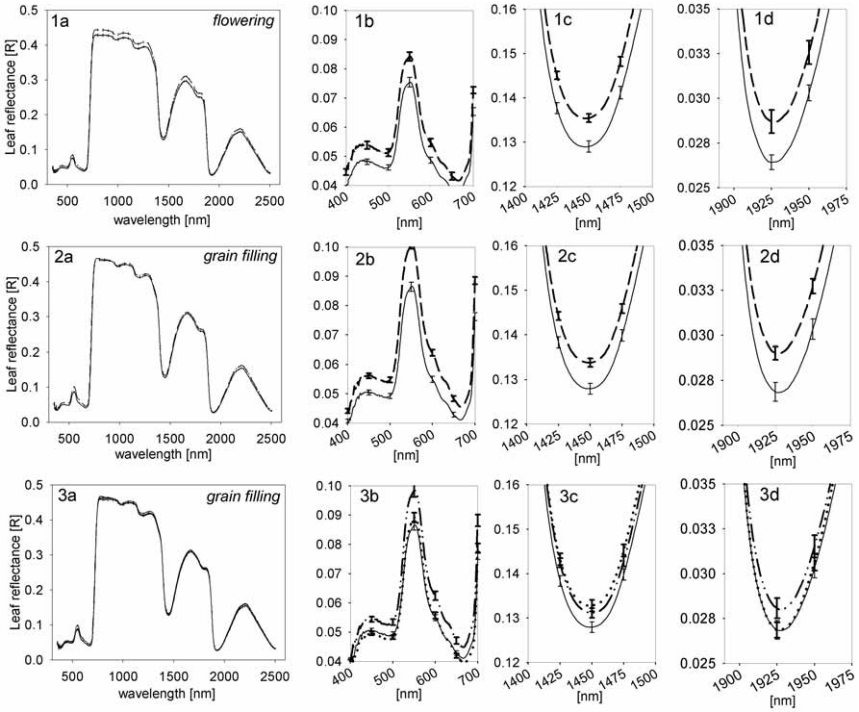

Figure 1. *Leaf reflectance of* T. aestivum. *Row 1a-d shows leaf reflectance of control plants (AC; ——) and drought stressed (AF; – – –) plants at flowering. Row 2a-d represents leaf spectra of control plants (AC; ——) and plants rewatered for 15 days (AF; – – –; recovery) at grain filling. Row 3a-d shows leaf reflectance of control plants (AC; ——) and plants stressed at grain filling either the first time (AG; · · · ·) or the second time (AFG; – ··· –). 1-3a shows the original leaf spectrum and 1-3/b-d show the regions of greatest differences between stress and control treatments in detail. Curves represent the mean of 20-30 leaf spectra ± standard error.*

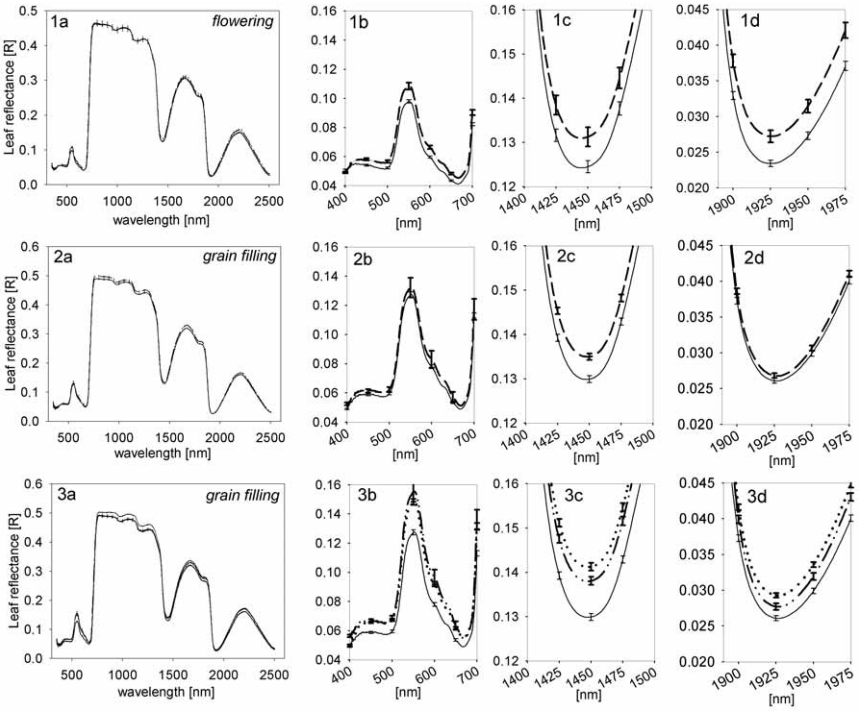

Figure 2. *Leaf reflectance of T. durum. Row 1a-d shows leaf reflectance of control plants (DC; ——) and drought stressed (DF; – – –) plants at flowering. Row 2a-d represents leaf spectra of control plants (DC; ——) and plants rewatered for 15 days (DF; – – –; recovery) at grain filling. Row 3a-d shows leaf reflectance of control plants (DC; ——) and plants stressed at grain filling either the first time (DG; · · · ·) or the second time (DFG; – ··· –). 1-3a shows the original leaf spectrum and 1-3/b-d show the regions of greatest differences between stress and control treatments in detail. Curves represent the mean of 20-30 leaf spectra ± standard error.*

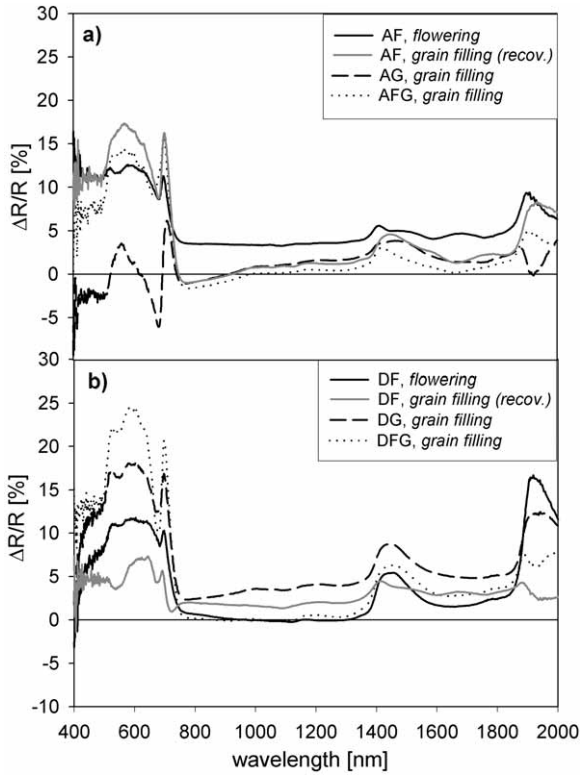

Figure 3. *Relative difference (ΔR/R) between reflectance of stressed and control plants of a)* T. aestivum *and b)* T. durum. *Differences were calculated as [($R_{stress} - R_{control}$)/ $R_{control}$]*100 (%). Legend: AF. DF: differences between control and plants stressed at flowering. measured at flowering; AF. DF (recov.): differences between control and plants stressed at flowering. measured at grain filling after being rewatered for 15 days; AG. DG: differences between control and plants stressed at grain filling. measured at grain filling; AFG. DFG: differences between control and plants stressed at flowering and grain filling. measured at grain filling; n=20-30.*

cover to nearly control values until grain filling in *T. aestivum* (–11%). In *T. durum*, values at grain filling even exceeded those of control plants (+25%). At grain filling however, in both species, reductions were more pronounced in plants receiving drought stress only at grain filling (AG: –68%, DG: –66%) than in those already stressed at flowering (AFG: –50%, DFG: –47%).

Regarding leaf conductance (g_L), *T. aestivum* was more susceptible to drought than *T. durum* independently of phenology (Table 2). Rewatering plants after drought stress at flowering restored g_L on the upper surface in *T. durum*. In *T. aestivum*, however, values remained somewhat below values of control plants. Drought at grain filling more strongly affected g_L in AG/DG compared to AFG/DFG.

Relative water content (RWC) of plants stressed at flowering was reduced (AF: –12%, DF: –4%; Table 2). At grain filling, RWC of formerly stressed plants was equal to or even exceeded values of control plants (AF: +7%, DF: +0.3%). Drought at grain filling resulted in an even stronger reduction of RWC than at flowering (average: A: –21%, D: –14%). In both species, RWC of plants stressed only at grain filling was lower than that of plants already stressed at flowering.

Actual leaf water content (AWC) was also reduced significantly under drought (Table 2). In contrast to RWC the changes of AWC in the course of phenology were more pronounced which is due to the fact that the AWC only represents the water content as percentage of fresh weight whereas the RWC represents the actual water content given with respect to a standard measure (leaves under conditions of water saturation).

Drought at flowering caused an increase of total chlorophyll content (Chl_{tot}; µg cm^{-2}) in AF (+11%) and a decrease in DF (–4%; Table 2). Rewatering resulted in higher Chl_{tot} contents at grain filling (AF: +4%, DF: +10%). Those plants subjected to drought at grain filling, either the first or the second time, also showed higher Chl_{tot} values compared to control plants (AG: +28%, DG: +6% and AFG: +15%, DFG: +8%, respectively).

Leaf nitrogen content (leaf [N], in % of dry matter) was reduced in plants subjected to drought at flowering (AF: –6%, DF: –11%; Table 2). At grain filling, leaf [N] from formerly stressed plants was still lower in *T. aestivum* (–5%) but higher in *T. durum* (+8%) when compared to control plants of either species. Plants subjected to drought stress during grain filling showed a reduction in leaf [N]. However, the reductions were more pronounced in plants stressed only at grain filling (AG: –20%, DG: –11%; AFG: –16%, DFG: –7%).

Spectral Measurements

Subjecting plants to drought stress, either at flowering or at grain filling, resulted in a general increase of single leaf reflectance (R; Figure 1–3). In both species, five spectral regions with relatively high differences were observed: 520–530 nm, 570–590 nm, 690–710 nm, 1410–1470 nm and 1880–1940 nm (Figure 3). Drought at flowering increased R in these spectral regions by up to +12%, +12%, +10%, +5% and +9% in *T. aestivum* and by up to +11%, +12%, +9%, +5% and +15% in *T. durum* (Table 3). Although rewatering plants after the stress period at flowering resulted in a recovery of plant physiological traits and water relations (see above) the effects observed on leaf R were different. Changes in leaf reflectance (ΔR/R, %) between control plants and formerly stressed plants of *T. aestivum* in the range of 520–530 nm, 570–590 nm and 690-710 nm were even greater after recovery than during the stress period (520–530 nm: +15%, 570–590 nm: +17%, 690–710 nm: +15%; Table 3). However, the differences at 1410–1470 nm and 1880-1940 nm decreased during recovery. In contrast, ΔR/R of *T. durum* decreased during recovery within the entire range of the spectrum. The greatest decrease in ΔR/R was observed in the 1880-1940 nm range.

At grain filling, *T. aestivum* stressed solely at grain filling (AG) showed the smallest increase of R in comparison to control plants which was surprising since the

TABLE 3

Summary of relative difference (%) for physiological parameters and spectral regions of greatest difference. All data refer to the differences between plants subjected to drought stress either at flowering and/or grain filling and control plants. Bold values highlight the differences between control plants and recovered plants (measured at grain filling). Relative difference (%) was calculated as [(stress – control)/ (control)*100]. Significance levels refer to the differences between stress treatments or recovered plants and control. n=5-30; n.s.: not significant, *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

		Triticum aestivum L.			*Triticum durum* L.		
		AF/AC	AG/AC	AFG/AC	DF/DC	DG/DC	DFG/DC
RWC	flowering	–12%**			–4% n.s.		
	grain filling	**+7%***	–25%***	–16%***	**+0,3%** n.s.	–18%***	–16%*
AWC	flowering	–5%**			–2% n.s.		
	grain filling	**+1%** n.s.	–8% n.s.	–4%**	**+0,3%** n.s.	–3%**	–2% n.s.
Chl_{tot}	flowering	+11%***			–4%*		
	grain filling	**+4%****	+28%***	+15%***	**+10%*****	+6%**	+15%***
Leaf[N]	flowering	–6%**			–11%***		
	grain filling	**–5%** n.s.**	–20%**	–16%**	**+8%** n.s.**	–11%*	–16% n.s.
$R_{520-530}$	flowering	+12%***			+11%***		
	grain filling	**+15%*****	+0,2% n.s.	+13%***	**+4%** n.s.**	+17%***	+22%***
$R_{560-590}$	flowering	+12%***			+12%***		
	grain filling	**+17%*****	+3% n.s.	+14%***	**+5%** n.s.**	+18%***	+24%***
$R_{690-710}$	flowering	+10%***			+9%***		
	grain filling	**+15%*****	+2% n.s.	+14%***	**+5%** n.s.**	+4%***	+19%***
$R_{1410-1470}$	flowering	+5%***			+5%*		
	grain filling	**+4%*****	+4%**	+3%*	**+4%*****	+9%***	+6%***
$R_{1880-1940}$	flowering	+9%***			+15%***		
	grain filling	**+7%*****	+1% n.s.	+4%*	**+3%***	+12%***	+7%***

Abbreviations: see Table 2, R: leaf reflectance.

changes in physiological traits were greatest (Table 2). In *T. durum*, however, ΔR/R between stressed plants and control was greater than that observed in plants stressed at flowering. (The only exception gave the wavelength range of 1880–1940 nm where the differences were smaller at grain filling compared to flowering.)

Drought not only caused leaf R in the near infrared region (NIR) to increase but also in the visible range of the spectrum. Here, the changes were even greater than in the NIR independently of the occurrence of drought in phenology. Of all stress treatments and stress periods in phenology, the greatest ΔR/R in the visible range was observed at grain filling in the treatment stressed twice (second stress period; Table 3).

Spectral indices for estimating leaf RWC (RWC_i) and AWC (AWC_i) as well as photochemical reflectance index (PRI) were calculated to follow RWC and AWC as well as A_{sat} and leaf[N] in the course of phenology (Figure 4, 5; Table 4). In both species, RWC_i was less correlated with the measured values (*T. aestivum*: $r^2=0.079$, *T. durum*: $r^2=0.467$) than AWC_i was (*T. aestivum*: $r^2=0.715$, *T. durum*: $r^2=0.953$). Tracing the measured values of RWC using RWC_i was neither possible in *T. aestivum* nor in *T. durum* (Figure 4). Using AWC_i it appeared possible to follow the trend of measured AWC in both *T. aestivum* and *T. durum*, during phenology but only for control plants. At grain filling, the difference in the AWC estimated from leaf R in *T. aestivum* between recovered and

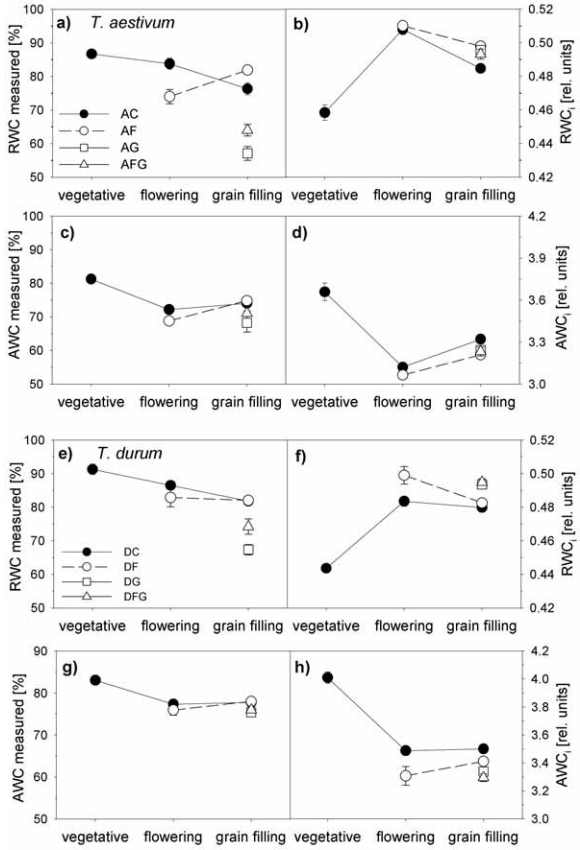

Figure 4. *Comparison of the phenological course of measured and estimated RWC and AWC. a-d) T. aestivum and e-h)* T. durum. *Legend: A:* T. aestivum*; D:* T. durum*; C: control. F: drought at flowering. recovered at grain filling. G: drought at grain filling. FG: drought at flowering and grain filling. n=6 for measured RWC and AWC. n=20-30 for estimated RWC (RWC$_i$) and AWC (AWC$_i$). Errors represent standard error.*

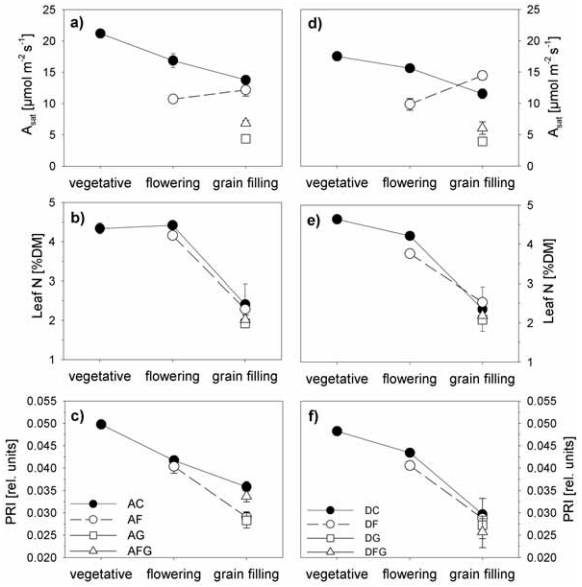

Figure 5. *Comparison of the phenological course of light saturated photosynthetic rates (A$_{sat}$). leaf nitrogen content (leaf [N]) and photochemical reflectance index (PRI). a-c)* T. aestivum *and d-f)* T. durum. *Legend: A:* T. aestivum*; D:* T. durum*; C: control. F: drought at flowering. recovered at grain filling. G: drought at grain filling. FG: drought at flowering and grain filling. n=3-12 for A$_{sat}$ and leaf [N]. n=20-30 for PRI. Errors represent standard error.*

TABLE 4

Correlation statistics for the relationship between physiological parameters (RWC, AWC, A_{sat} and leaf [N]) and spectral indices (RWC_i, AWC_i and PRI). In addition to the correlation coefficient (r^2) and the significance level (p), the slope and intercept of the linear equation are given.

	r^2	p	slope	intercept
Triticum aestivum L.				
RWC	0.079	0.542	-4.526	0.526
AWC	0.715	0.017	0.037	0.564
A_{sat}	0.679	0.023	1.096	0.024
leaf [N]	0.774	0.009	5.774	0.019
Triticum durum L.				
RWC	0.467	0.091	-1.611	0.613
AWC	0.953	0.000	0.094	-3.848
A_{sat}	0.514	0.070	1.293	0.020
leaf [N]	0.986	0.000	8.499	8.551

control plants give the impression of an even greater reduction than during the stress period at flowering itself, although the measurements of AWC reveal full recovery. In *T. durum* the differences decreased during recovery. However, values of AWC_i remained below control plants despite the complete recovery becoming obvious from measured values (Figure 4; see also ΔR/R, Figure 3).

PRI correlated quite well with A_{sat} but even better with leaf [N] in both species (Table 4). Tracing phenological changes in A_{sat} and leaf [N] using PRI did not give good results for plants subjected to drought stress at any time in ontogeny. Better results for this correlation were only obtained for control plants. Therefore, neither recovery of plants after drought at flowering nor the extent of change in A_{sat} and leaf [N] due to drought could be estimated appropriately.

4. DISCUSSION

Drought stress significantly influenced plant physiological traits independently of the time of its application in phenology. The lowering of the actual leaf conductance (g_L), as observed during all stress periods in the present study, is one of the first processes occurring under decreased soil water availability providing a higher water use efficiency to the plant *(27, 28, 29)*. Moreover, as reviewed by Cornic *(30)*, stomatal closure is mainly responsible for the decline in net photosynthetic rate of C_3 leaves subjected to moderate drought stress. However, at a certain stage of stress, internal CO_2 concentration (C_i)

frequently increases, indicating the predominance of non-stomatal limitations to photosynthesis *(31, 32, 33)*. Reductions of light saturated photosynthetic rates (A_{sat}) in the present experiment were mainly due to stomata limitation since a significantly lower C_i was found (data not shown).

In the present study, drought stress resulted in higher leaf reflectance (R) over the entire spectrum both in *T. aestivum* and in *T. durum,* a response also found elsewhere *(c.f. 34, 35, 26)*. However, five regions with relatively high differences were observed: 520–530 nm, 570–590 nm, 690–710 nm, 1410–1470 nm and 1880–1940 nm (Figure 1–3).

Rewatering plants after the stress period at flowering allowed them to restore their physiological traits until grain filling (15 days rewatered). RWC of recovered plants even exceeded that of control in *T. aestivum* (+7%) and was restored to control level in *T. durum* (+0.3%). Therewith, A_{sat} also recovered. Only g_L of plants from both species remained somewhat lower than that of control plants. However, the results from leaf R did not follow this trend. In *T. aestivum*, ΔR/R within the range of 1410–1470 nm and 1880–1940 nm remained nearly as high as during the stress period at flowering despite the 7% higher RWC of recovered plants. Though in *T. durum* a reduction of ΔR/R was found, leaf R still remained above that of control plants. Within the visible range of leaf spectra ΔR/R in *T. aestivum* even increased during recovery compared to the actual stress period. In *T. durum* ΔR/R within the visible range decreased during recovery but R still remained above that of control plants as already observed for the near infrared region. The results of the present study therefore indicate that quantifying the extent of change for either leaf water content or Chl_{tot} and leaf [N] from changes in leaf R is problematic. Especially recovery from drought could not be traced using leaf R since the differences between formerly stressed plants and control plants remained high as observed in *T. aestivum* or decreased only slightly as in *T. durum* but in neither of the species investigated leaf R returned to control level despite the complete recovery of physiological traits.

The reason for the enduring differences in leaf R between fully recovered plants and control plants remains rather unclear and information on leaf R during recovery of plants after a stress period is rare in literature. However, it is assumed that secondary effects following drought stress might be involved. Drought can affect the cell structure and biochemistry *(e.g.: 36, 37, 38, 39)* and is further known to influence the morphology of the leaf surface by means of changes in the content and/or composition of epicuticular waxes *(40, 41, 42, 43)* or the occurrence of hairs *(42)*. Moreover, drought has the potential to accelerate ontogenetic development *(44, 45)*. Such alterations of leaf morphology and/or biochemical composition could not only have influenced leaf R after recovery but also have attributed to (or might be the reason for) the unexpectedly great differences in leaf R observed in plants subjected to a second stress period at grain filling. This result contrasts again with the observations of physiological traits since those were more affected by drought in AG and DG compared to AFG and DFG at grain filling. The less pronounced reaction of physiological traits to a second drought period is attributed to some preconditioning of plants already exposed to drought at flowering and/or the higher amount of green biomass (transpiring surface) of plants from the treatment stressed solely at grain filling. Plants of the treatment stressed twice (AFG and DFG) were watered optimally for eight days after drought at flowering before water supply was halved again. Leaf osmotic potential remained below (more negative) that of control plants during these days providing a better initial situation concerning osmotic adjustment (data not shown) for plants already experiencing a first drought period at flowering.

The differences observed in ΔR/R during recovery between *T. aestivum* and *T. durum* show that no general prediction can be made concerning the potential to trace recovery from a stress situation with leaf reflectance. Apparently, different species and even cultivars respond inconsistently to drought stress with respect to their spectral signature. Especially the cultivar of *T. aestivum* used in this study (cv. Xenos) appears not promising for tracing recovery with leaf reflectance. In *T. durum* (cv. Floradur) ΔR/R decreased during recovery within the entire range of the spectrum but the greatest decrease in ΔR/R occurred in the 1880–1940 nm range. Since this spectral range falls into the main atmospheric water bands it is unsuitable for remote sensing by satellite or airplane. However, to test an eventual potential for short distance remote sensing/ precision farming, we performed simulations of the transmittance in these wavelength ranges using the code LOWTRAN 7 *(46)* assuming the worst case scenario (99% air humidity). Results showed that at distances below 100 m the transmittance was larger than 50% in the wavelength range 1410–1470 nm. At 1880–1940 nm transmittance became larger than 50% only at distances below 15 m. This shows a potential for a short distance (below 100 m) remote sensing mainly in the wavelength range 1410–1470 nm. This remote sensing application would however at least require an accurate determination of the distance between sensor and canopy, an artificial radiation source (since the solar radiation is already totally absorbed) and an accurate determination of air humidity (to apply a correction to the measured transmittance). Other aspects like sensor sensitivity, characteristics of the radiation source, requirements regarding the accuracy of the sensor to determine plant optical path etc… would be needed to be investigated within the scope of a future study.

In contrast to changes in leaf R within the range of 1410–1470 nm and 1880–1940 nm, which can be attributed mainly to differences in leaf water content, the changes within the visible range are not well defined with respect to a certain stressor. As already described by Carter *(47)* an increased reflectance at visible wavelengths (400–700 nm) is the most consistent response to stress within the 400–2500 nm range. The often made assumption that the chlorophyll content of leaves was propor-

tional to moisture content *(e.g. 48)* may be correct for some species but cannot be generalized to all ecosystems. Variations in chlorophyll content can be caused by water stress but also by phenological status of the plant, atmospheric pollution, nutrient deficiency, toxicity, plant disease, and radiation stress *(39, 49)*. These findings are supported by the results from the present study where different trends for RWC, Chl_{tot} and leaf [N] were found. Due to these adverse effects of leaf [N] (decrease) and Chl_{tot} (increase) an interpretation of the increased leaf R is difficult. At least the specific cause of these differences remains uncertain. However, the increased Chl_{tot} content found might result from leaf shrinkage leading to a seemingly higher chlorophyll content per unit leaf area ($\mu g\ cm^{-2}$).

Finally, three spectral indices (RWC_i, AWC_i and PRI) were tested towards their ability in estimating biophysical parameters (RWC, AWC, A_{sat} and leaf [N]; Table 4). Concerning the estimation of leaf water content a better correlation was found for AWC. Unfortunately, the AWC is the less meaningful parameter since it only gives the water content as percentage of fresh weight which might vary greatly between species, phenology and environmental conditions *(39)*. The RWC, however, represents the actual leaf water content with respect to a standard measure (leaves under conditions of water saturation; (*39*) and is therefore the more appropriate indicator of plant water status. Moreover, following changes in biophysical parameters using these indices was not possible due to the different extent of changes in leaf R compared to physiological traits under drought stress at different phenological stages. From these results it is concluded that a good relationship between spectral indices and biophysical parameters does not necessarily lead to an appropriate estimation of biophysical parameters at a given phenological state and/or physiological status.

5. CONCLUSION

Drought stress occurring at different phenological stages increased leaf R throughout the whole spectrum. Unfortunately, the degree to which plant physiological traits and water relations changed could not be quantified by the extent of change in leaf R, at least when drought occurred at different phenological stages. The main concern of the present study, however, was to test the ability of leaf reflectance to follow recovery of physiological traits after a stress period which may be of essential importance when considering the occurrence of repeated drought events. Distinguishing between a currently occurring stress situation and an already passed one could become crucial in context with the application of spectral measurements in the field to trace stress situations and to make recommendations on fertilization or irrigation. Unfortunately, recovery from drought stress could not be traced by leaf R since the differences between formerly stressed plants and control plants remained either high as observed in *T. aestivum* or decreased only slightly as in *T. durum*. In neither species leaf R returned to control level despite the complete recovery of physiological traits. These results, however, also indicate that rather big differences between different species might occur and further investigations using different species with different leaf morphology and anatomy would be needed.

Estimating leaf water content (RWC and AWC) as well as Chl_{tot} and leaf [N] from reflectance measurements gave good correlations. For tracing changes in physiological parameters during phenology and stress periods, however, the use of these indices was not promising due to false estimation of stress situations and recovery (Figure 4, 5). An appropriate estimation appeared, if at all, only possible in unstressed control plants. A good correlation between spectral indices and physiological parameters alone is therefore not necessarily sufficient for estimating physiological parameters from leaf spectra appropriately.

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