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High-Throughput Phenotyping for Drought Tolerance in Winter Wheat

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Zusammenfassung

Trockenheit stellt einen stark limitierenden Faktor des Pflanzenwachstums und der Pflanzenproduktion dar. Um die weltweite Nachfrage nach Lebensmitteln zu sichern, ist es notwendig die Trockenheitstoleranz zu erhöhen. Die Züchtung von trockenstresstoleranten Weizensorten ist äußerst komplex und wird durch variable Feldbedingungen zusätzlich erschwert. Hinzu kommt, dass die physiologischen Prozesse der Trockenstresstoleranz nicht ausreichend bekannt sind. Die Erfassung von Trockenstress ist sehr zeitaufwändig, personal- und kostenintensiv und in vielen Fällen werden besondere Messgeräte benötigt. Um schneller trockenstresstolerante Genotypen identifizieren zu können, wurden jüngst verschiedene Hochdurchsatz-Phänotypisierungs-Plattformen entwickelt. Diese liefern detaillierte und nicht-invasive Informationen über diverse Pflanzenparameter, wie bspw. den Pflanzenwasserstatus zur Bestimmung des Trockenstresses.

Im ersten Teil dieser Arbeit wurden spektrale Reflexionsmessungen, thermale Bildgebung und nicht-bildgebende Hochdurchsatzmessungen zur Schätzung des Pflanzenwasserstatus von zwanzig Weizensorten (*Triticum aestivum* L.) verglichen. In zweijährigen Feldstudien wurden alle Messungen unter Trockenstress- und Kontrollbedingungen verglichen. Thermometrische Messungen wiesen eine starke lineare Beziehung zu Trockenstressparametern (relativer Blattwassergehalt und Kohlenstoffisotopen-Diskriminierung von Blatt und Korn) auf, sowie zum Kornertrag unter Trockenstress. Des Weiteren erwies sich die thermometrische Messung als besonders geeignet für Hochdurchsatz-Messungen und ist der thermographischen Messung vorzuziehen. Zusätzlich wurden während des Ährenschiebens, der Blüte und der Kornfüllung fünf Wasser-Indices (WI und NWI 1-4) erfasst und spektrale Messungen im Bereich von 500 bis 1200 nm durchgeführt und mittels partial least square regression (PLSR) Modellen analysiert. Um Kornertrag und Trockenstressparameter vorhersagen zu können, wurden die Modelle kalibriert und kreuzvalidiert. Im Vergleich zu den Wasser-Indices, erhöhte der Einsatz von PLSR-Modellen die Vorhersagegüte des Kornertrags und der Trockenstressparameter. Die Ergebnisse zeigen, dass durch Präzisionsphänotypisierung die Einbindung von spektralen Informationen in Züchtungsprogrammen eine rasche und kostengünstige Phänotypisierung von Genotypen ermöglicht. Diese Annahme wird durch die Tatsache unterstützt, dass Kornertrag und die Nahinfrarot (NIR) basierten Indizes eine ähnliche Heritabilität aufwiesen.

Zusätzlich wurden im zweiten Teil dieser Arbeit, vier aktive und passive Sensoren, bestehend aus einem hyperspektralen passiven Sensor, einem aktiven Xenonsensor, dem LED-basierten Crop Circle- und GreenSeeker-Sensor, in ihrer Eignung destruktive und nicht-invasiv erhobene morphophysiologische Trockenstressparameter zu erfassen, bewertet. Im Vergleich der aktiven Sensoren zum Zeitpunkt des Ährenschiebens, der Blüte und der Kornfüllung wies der Crop Circle die signifikantesten und robustesten Beziehungen zu den Trockenstressparametern auf. Im Vergleich dazu zeigten zum Zeitpunkt der Blüte die fünf Wasser-Indices (WI und NWI 1-4), welche nur bei dem passiven Sensor zur Verfügung standen, die engsten Beziehungen zu den Trockenstressparametern und zum Kornertrag. Diese Ergebnisse unterstützen die Beobachtung, dass die Wasser-Indices geeignet sind, um in Züchtungsprogrammen integriert zu werden, um eine schnelle und günstige Identifizierung von trockenstresstoleranten Genotypen zu ermöglichen. Diese Aussage wird durch die Tatsache unterstützt, dass der Kornertrag und die Wasser-Indices eine vergleichbare Heritabilität aufwiesen.

Obwohl die Feldphänotypisierung grundlegend ist für eine erfolgreiche Züchtung, werden die meisten pflanzenphysiologischen Experimente unter kontrollierten Bedingungen in kleinen Gefäßen mit eingeschränktem Wurzelvolumen durchgeführt. Es ist bekannt, dass ein eingeschränktes Wurzelvolumen viele physiologische Prozesse beeinflusst. Daher wird im dritten Teil der folgenden Arbeit, die Übertragbarkeit auf Feldbedingungen in Frage gestellt. Im Rahmen dieser zweijährigen Untersuchungen wurde die Übertragbarkeit der erfassten Trockenstresstoleranz innerhalb zwei verschiedener Gefäßgrößen (6.6-L kleine Gefäß vs. 19.4-L Röhren) und sechs geprüfter Winterweizensorten auf Feldbedingungen evaluiert. Trockenstresstoleranz wurde mit den gleichen physiologischen Parametern erfasst, welche für die beiden vorangegangenen Hochdurchsatzphänotypisierungs-Versuche verwendet wurden. Alle gemessenen Parameter, mit Ausnahme des relativen Blattwassergehalts und des Kornertrags, unterschieden sich signifikant beim Vergleich zwischen kleinen Gefäßen und Röhren mit Feldbedingungen. Auf genotypischer Ebene zeigten sich Sorten, die sich unter Feldbedingungen als trockenstresstolerant erwiesen, als trockenstressanfällig, wenn sie unter kontrollierten Bedingungen in kleinen Gefäßen angezogen wurden. Das eingeschränkte Wurzelvolumen beeinträchtigte die Pflanzenphysiologie nicht nur unter Trockenstress, sondern auch unter optimal bewässerten Bedingungen. Im Gegensatz dazu erwiesen sich die Röhren als geeignet, trockenstresstolerante Genotypen zu identifizieren und wiesen eine potentielle Übertragbarkeit auf Feldbedingungen auf.

Summary

Drought is a major limiting factor of plant growth and production. To meet the worldwide increasing demand for food, it is necessary to create drought-tolerant wheat cultivars. Breeding drought-tolerant wheat cultivars is highly complex and challenging under variable field conditions, and there remains insufficient knowledge regarding physiological processes. Moreover, the determination of drought stress is laborious, time consuming, costly, and may partly require specialized equipment. To accelerate the identification of drought tolerant wheat cultivars, numerous high-throughput phenotyping platforms (HTPPs) have been developed to screen various cultivars by offering detailed, non-invasive information regarding various plant parameters to detect drought stress such as plant water status.

The first part of this study compares spectral reflectance, thermal imaging, and non-imaging high-throughput measurements to estimate the water statuses of twenty wheat (*Triticum aestivum* L.) cultivars. Measurements were conducted in a 2-year study, including a drought stress and a control environment under field conditions. Thermometric measurements showed a strong linear relationship to drought-related parameters (relative leaf water content and carbon-isotope discrimination of leaf and grain) and grain yield under drought stress, and demonstrated a high suitability for high-throughput measurements. Thermometry was revealed to be preferable to detect leaf temperature. Additionally, five water indices (WI and NWI 1 – 4) and spectral measurements from 500 to 1200 nm were determined for the heading, anthesis, and grain filling growth stages. Spectral measurements from 500 to 1200 nm were analyzed by partial least square regression (PLSR) models, which were calibrated and cross-validated for the prediction of grain yield and drought-related parameters. Overall, the PLSR models improved the prediction of grain yield and drought-related parameters, compared to the water indices. The results of this study indicate that precision phenotyping allows the integration of these traits in breeding programs to rapidly and cost-effectively phenotype drought-tolerant genotypes. This assumption is supported by the fact that grain yield and the near-infrared (NIR)-based indices showed the similar heritability under drought conditions.

Additionally, four passive and active reflectance sensors, including a hyperspectral passive sensor, an active flash sensor, the Crop Circle and the GreenSeeker, were evaluated to assess drought-related destructive and non-destructive morphophysiological parameters. A comparison of the active sensors at the heading, anthesis and grain-filling stages indicated that the Crop Circle provided the most significant and robust relationships with drought-related parameters. In comparison with the passive sensor, the five water and normalized water indices (WI and NWI 1 – 4), which are only provided by the passive sensor, showed the strongest relationships with the drought stress-related parameters and grain yield at anthesis. These results indicate that water indices are appropriate to be included in breeding programs to rapidly and cost-effectively identify drought-tolerant genotypes. This is supported by the fact that grain yield and the water indices showed the same heritability under drought conditions.

Besides the importance of field phenotyping for successful breeding, most plant physiological experiments are conducted under controlled conditions wherein plants are grown in rather small pots with a restricted rooting volume. Because a restricted root zone affects various physiological processes, it questions the extrapolation from pot to field conditions. This 2-year study aimed to evaluate the transferability of drought tolerance of winter wheat (six varieties tested) grown in two different pot sizes (6.6-L pots vs. 19.4-L tubes) to that grown under field conditions. Drought tolerance was assessed via the same key physiological parameters used for the high-throughput phenotyping experiments. Comparing the pot and tubes with the field conditions, all measured parameters significantly differed, except relative leaf water content and grain yield. At the genotypic level, the varieties considered to be tolerant to drought under field conditions appeared to be susceptible to drought when grown in the small pots. The limited rooting volume imposed by the pots strongly influences plant physiological processes not only under drought stress but also under well-watered conditions. In contrast, the tubes were found to be reliable for identifying drought-tolerant wheat varieties.

1. Introduction

1.1 Challenges in Plant Science in a Thirsty World

1.2 Section I: Detection of drought stress related traits and prediction of grain yield by spectral and thermal high - throughput measurements in winter wheat

The reduction of available water for agricultural production is already a serious issue in many parts of the world. (Elliott *et al.*, 2014). Global agricultural production is strongly affected by the increase in the frequency of drought periods, which leads to stagnation and decrease in agricultural yields. In contrast, the global demand for agricultural products, particularly corn, rice, and wheat, increases yearly (Pingali, 2007; Tilman *et al.*, 2011; Godfray, 2014). However, the area of productive arable land is decreasing due to water scarcity and groundwater salinization (Turner *et al.*, 2011). To meet food security requirements, the increase of crop yield on existing agricultural land is more sustainable than converting natural land to new farmland (Matson and Vitousek, 2006; Tilman *et al.*, 2011; Tscharntke *et al.*, 2012; Godfray, 2014). Wheat is one of the most extensively cultivated cereals globally, and plays a crucial role in the daily carbohydrate intake in most countries (Shiferaw *et al.*, 2013). A major challenge in a thirsty world is to create drought-tolerant wheat phenotypes (Campos *et al.*, 2006; Sinclair, 2011).

During the last few decades, numerous field experiments investigating drought stress have been conducted, focusing on plant responses and strategies to control water status under drought (Cornic and Massacci, 1996; Chaves *et al.*, 2003). Nevertheless, accomplishing drought-tolerant wheat cultivars has proven complex under highly variable field conditions, and there remains insufficient knowledge regarding physiological processes (Chaves *et al.*, 2003; Campos *et al.*, 2004; Boyer *et al.*, 2013). The breeding process, including the assessment of phenotypic traits, for new drought-tolerant wheat varieties remains hampered by laborious field work and costly laboratory analyses. During the last few decades, a number of methods to evaluate drought stress have been established, such as relative leaf water content (RLWC) (Slatyer, 1967), leaf surface temperature (Blum *et al.*, 1982; Reynolds *et al.*, 1994), and carbon isotope discrimination (CID) (Farquhar *et al.*, 1989; Condon *et al.*, 2004). However, all these methods share one or more shortcomings: the application of the methods may be laborious, time consuming, and costly, and may partly require specialized equipment. Breeders attempt to determine the phenotypes of large numbers of lines in a precise and expeditious way so as to identify the most promising progeny (Araus and Cairns, 2014). Consequently, a great demand to increase breeding efficiency exists.

To accelerate the breeding process, numerous high-throughput phenotyping platforms (HTPPs) have been developed (Schmidhalter *et al.*, 2001; Furbank and Tester, 2011). These platforms provide screening of various cultivars by offering detailed, non-invasive information regarding various plant to detect drought stress such as plant water status (Schmidhalter, 2005; Winterhalter *et al.*, 2011), leaf temperature (Fischer *et al.*, 1998; Rischbeck *et al.*, 2014), and yield level (Kipp *et al.*, 2014a). Under drought conditions, spectral measurements of canopy reflectance can be used to assess plant water status by light absorption of water at certain visible and near-infrared wavelengths due to a decrease in the absorption of radiation by the leaf at lower leaf water content (Penuelas *et al.*, 1997; Linke *et al.*, 2008). Moreover, measuring canopy reflectance allows the determination of additional information such as biomass and senescence by using the reflectance spectra. Hyperspectral passive sensors, using sunlight as the source of light, provide measurements of wavelengths in the visible (VIS; 400–700 nm), near-infrared (NIR; 700–1100 nm) and shortwave infrared (SIR; 1100–2500 nm) ranges, which allows the calculation of different vegetation indices (Hackl *et al.*, 2013). Several studies have proven that wavelengths in the NIR region reflect the plant water status (Babar *et al.*, 2006b; Gutierrez *et al.*, 2010; Rischbeck *et al.*, 2014; El-Hendawy *et al.*, 2015).

For this purpose, different water bands have recently been selected to identify significant indices (Penuelas *et al.*, 1993; Babar *et al.*, 2006c). A well-known index is the water index ($WI = R_{970}/R_{900}$) developed by Penuelas *et al.* (1993), which has turned out to be associated with RLWC in the case of drought stress (El-Shikha *et al.*, 2007). Based on the WI, two normalized water indices were developed by Babar *et al.* (2006c) ($NWI\text{-}1 = ([R_{970} - R_{900}]/[R_{970} + R_{900}])$ and $NWI\text{-}2 = ([R_{970} - R_{850}]/[R_{970} + R_{850}])$) to screen spring wheat genotypes under drought conditions.

In addition, Prasad *et al.* (2007) supplemented the NWI-3 ($NWI\text{-}3 = [R_{970} - R_{880}]/[R_{970} + R_{880}]$) and NWI-4 ($NWI\text{-}4 = [R_{970} - R_{920}]/[R_{970} + R_{920}]$) for screening grain yield of winter wheat genotypes affected by drought stress. Under drought conditions, these five water indices (WI and NWI-1–4) demonstrated a high potential for use as selection tools for grain yield in winter wheat (Prasad *et al.*, 2007; El-Hendawy *et al.*, 2015). Numerous indices for different wavelength regions exist; however, little validation under field and drought conditions has been conducted. It appears that there are as yet no publications that discuss the approach of using a broad range of wavelengths to assess the plant water status.

1.3 Section II: Evaluation of yield and drought using active and passive spectral sensing systems at the reproductive stage in wheat

Around the world, agriculture is challenged with an increased frequency of drought periods. An important issue is the reduction of available water for agricultural production, resulting in the stagnation and decrease of crop yields. Coincidentally, the global demand for agricultural products, especially corn, rice and wheat, increases every year (Pingali, 2007; Tilman *et al.*, 2011; Godfray, 2014). Wheat is one of the most extensively cultivated cereals that is often under abiotic stress (Cossani and Reynolds, 2012) and plays a crucial role regarding world food supplies (Shiferaw *et al.*, 2013). Against this background, in a thirsty world, it is an absolute necessity to create drought-tolerant wheat phenotypes (Campos *et al.*, 2006; Sinclair, 2011). Nonetheless, producing drought-tolerant wheat cultivars has proven complex under highly variable field conditions, and there is insufficient knowledge of physiological processes (Chaves *et al.*, 2003; Campos *et al.*, 2004; Boyer *et al.*, 2013).

Breeding new varieties for water-limited environments is still dominated by laborious field work and high priced laboratory analyses. In the last decades, a number of methods to evaluate drought stress have been established, such as the relative leaf water content (RLWC) (Slatyer, 1967), leaf surface temperature (Blum *et al.*, 1982; Reynolds *et al.*, 1994) and carbon isotope discrimination (CID) (Farquhar *et al.*, 1989; Condon *et al.*, 2004). However, in large-scale field evaluations, these methods are expensive in terms of time and financial resources and partly require special equipment. Spectral canopy reflectance indices can also be used to assess plant water status because they change in response to crop water content (Penuelas *et al.*, 1997; Stimson *et al.*, 2005). Consequently, there is a great demand to increase breeding efficiency to guarantee the phenotyping of high numbers of lines in an exact and expeditious way (Araus and

Cairns, 2014). In the last decades, numerous high-throughput phenotyping platforms (HTPPs) have been developed (Schmidhalter *et al.*, 2001; Furbank and Tester, 2011) to accelerate the breeding process by screening various cultivars; these platforms offer detailed and non-invasive information about diverse plant parameters to determine plant water status (Schmidhalter, 2005; Winterhalter *et al.*, 2011), leaf temperature (Rischbeck *et al.*, 2014), and crop yield (Kipp *et al.*, 2014a).

These HTPPs carry either passive or active spectral sensors or a combination of both (Mistele and Schmidhalter, 2008; Mistele and Schmidhalter, 2010; Erdle *et al.*, 2011; Rischbeck *et al.*, 2016), which can either be applied for scientific purposes or farm management. Passive sensor systems use sunlight as a source of light, whereas active sensor systems possess their own light-emitting units and therefore are independent of varying irradiation conditions and day and night (Hatfield *et al.*, 2008). Furthermore, active sensors are frequently used due to their easy handling and relatively low purchase costs, which is especially attractive for developing countries. However, active sensors are limited to specific wavelengths according to the type of light source (Jasper *et al.*, 2009; Erdle *et al.*, 2011).

Both sensor systems measure the reflection of a plant by converting the reflection signal into an electrical output. Hyperspectral passive sensors provide measurements of wavelengths in the visible (VIS; ~ 400 - 700 nm) and near-infrared (NIR; ~ 700 - 2500 nm) ranges, which allows the calculation of different vegetation indices (Hackl *et al.*, 2013). Therefore, spectral measurements from passive sensors can be applied to highly versatile conditions depending on the appropriate requirements (Hatfield *et al.*, 2008; Erdle *et al.*, 2011).

Nonetheless, both sensor systems provide similar indices for estimating various plant parameters. One of the most widely used indices is the normalized difference vegetation index ($NDVI = (R_{780}-R_{670})/(R_{780}+R_{670})$). The NDVI combines spectral information of the VIS and NIR regions and provides predictions of green biomass and photosynthetic capacity (Babar *et al.*, 2006b). Furthermore, previous research has shown that wavelengths in the NIR region are appropriate to detect plant water status (Babar *et al.*, 2006b; Gutierrez *et al.*, 2010; Rischbeck *et al.*, 2014; El-Hendawy *et al.*, 2015). One of these NIR-based indices is the water index ($WI = R_{970}/R_{900}$), developed by Penuelas *et al.* (1993). The WI has become an established index to detect RLWC under water-limited conditions. Based on the WI, Babar *et al.* (2006c) developed two normalized water indices ($NWI\text{-}1 = ([R_{970} - R_{900}]/[R_{970} + R_{900}])$ and $NWI - 2 = ([R_{970} - R_{850}]/[R_{970} + R_{850}])$) to screen spring wheat genotypes under drought conditions.

In addition, Prasad *et al.* (2007) added the NWI - 3 (NWI - 3 = $[R_{970} - R_{880}]/[R_{970} + R_{880}]$) and NWI - 4 (NWI - 4 = $[R_{970} - R_{920}]/[R_{970} + R_{920}]$) for screening grain yield of winter wheat genotypes affected by drought stress. These five water indices (WI and NWI - 1 – 4) demonstrated high potential for use as selection tools for grain yield in winter wheat under drought conditions (Prasad *et al.*, 2007; El-Hendawy *et al.*, 2015). One of the commercially available active sensors is the Crop Circle ACS-470® (Holland Scientific Inc., Lincoln, NE, USA), which is equipped with modulated polychromatic light emitting diodes (LEDs) as a source of light. The Crop Circle provides filters for 670, 730 and 760 nm to estimate the biomass and nitrogen status of various crops (Kipp *et al.*, 2014b).
In addition to the Crop Circle, the GreenSeeker (NTech Industries Inc., Ukiah, CA, USA) is also a widely used active sensor. The GreenSeeker includes two separate LEDs as sources of light and provides two fixed wavelengths at 774 nm and 656 nm to estimate green biomass and nitrogen supply in corn and wheat (Tremblay *et al.*, 2009; Li *et al.*, 2010; Shaver *et al.*, 2010). In recent years, the high potential of active and passive sensors in estimating agronomic and physiological traits has been shown in various studies. Nevertheless, passive and active sensors have rarely been compared, and only little information is available regarding how diverse stressors, such as drought stress, influence the sensors' performance.

1.4 Section III: Can we scale up (extrapolate) drought stress in winter wheat from pots to the field?

Worldwide, increasing drought periods are responsible for the serious reduction of water available for agricultural production, resulting in the stagnation and decrease of crop yields (Elliott *et al.*, 2014). The challenge over the next decades is to meet the yearly increasing demand for agricultural products, particularly for corn, rice, and wheat (Tilman *et al.*, 2011; Godfray, 2014). Nevertheless, because of water scarcity and groundwater salinization, the area of agricultural arable land is constantly decreasing (Turner *et al.*, 2011). Hence, to meet food security requirements, increasing crop yields on existing agricultural lands is necessary (Tilman *et al.*, 2011).

Wheat is one of the most extensively cultivated cereals that is often grown under abiotic stress (Cossani and Reynolds, 2012) and plays a crucial role regarding world food supplies (Shiferaw *et al.*, 2013). In a thirsty world, it is a great and inevitable challenge to create drought-tolerant wheat phenotypes (Campos *et al.*, 2006; Sinclair, 2011). Therefore, phenotypic and physiological drought stress experiments are essential. However, field experiments are influenced by a great variability in local environmental conditions such as soil heterogeneities, air temperature, humidity, and light intensity (Passioura, 2006).

Thus, most physiological experiments are conducted in small pots that offer limited root growth under controlled conditions, e.g., greenhouses or growth chambers. These controlled environments provide the advantage of consistent growth conditions, which particularly simplifies abiotic stress treatments, such as cold, heat, and drought stress. Furthermore, unlike in field experiments, where climatic conditions are harder to control, the pot experiments are conducted independent of the growth seasons, and they also provide rapid replication. Therefore, the number of published studies on drought stress physiology has greatly increased over the past decades (Ray and Sinclair, 1998; Passioura, 2006; Wu *et al.*, 2011). This begs the question of whether the use of small pots could be extrapolated to field conditions. Although many authors (Ray and Sinclair, 1998; Passioura, 2006; Wu *et al.*, 2011; Poorter *et al.*, 2012) have raised concerns about the transferability from pots to fields, the pot size itself appears to have received little consideration, and as noted by Poorter *et al.* (2012), it is regularly not reported in the materials and methods section of publications. Passioura (2006) discussed that owing to aberrant water relations, soil temperature, and soil structure, plant experiments conducted in small pots may always robustly extrapolate to field conditions.

Moreover, prior research has documented how a restricted rooting volume often influences the growth of plants and their various physiological processes (Liu and Latimer, 1995; Ismail and Davies, 1998; Hurley and Rowarth, 1999; Wu *et al.*, 2011; Poorter *et al.*, 2012). Nevertheless, breeders still often use pot experiments as a pre-selection tool to identify novel plant material. In particular, when breeding plants for drought tolerance, it is necessary to identify promising genotypes in an early breeding step. Further, as indicated by Passioura (2012), pot experiments investigating drought stress typically use pots which are not representative of actual field conditions; this can lead to a much faster consumption of available water, leading to its depletion over a matter of days instead of weeks or months. This crucial fact should be considered when selecting for drought-tolerant genotypes.

Despite numerous studies on the effect of pot size in general, reports on the transferability of drought tolerance of individual varieties from pots to the field are lacking. In this context, we have grown six wheat cultivars in two different pots sizes under controlled conditions as well as under field conditions in a 2-year study. Established plant physiological measurements, such as relative leaf water content (RLWC) (Slatyer, 1967), leaf surface temperature (LT) (Blum *et al.*, 1982; Reynolds *et al.*, 1994), carbon isotope discrimination (CID) (Farquhar *et al.*, 1989; Condon *et al.*, 2004), and grain yield were used to detect and quantify drought stress. Moreover, the normalized difference vegetation index (NDVI) was applied to evaluate differences in leaf spectral reflectance under conditions of drought stress.

2. Objectives

The objectives of this Ph.D. thesis are indicated separately for Section I to III.

The objectives of the **Section I** were (i) to test the hypothesis that it is possible to replace time-demanding and costly measurements with non-destructive assessments, (ii) to evaluate the performance of thermography and thermometry under drought stress conditions in the field, (iii) to determine the potential of spectral indices to assess plant water status in a high-throughput mode by identifying the most reliable relationships with drought-related traits (leaf temperature, RLWC, CID) and yield under drought conditions.

The purpose of **Section II** were therefore (i) to compare passive and active spectral sensor systems with respect to several indices and (ii) to determine the potential of spectral indices to assess plant water status in a high-throughput mode by identifying the most reliable relationships with drought-related traits (leaf temperature, RLWC, CID) ground cover and yield under drought conditions.

To evaluate the transferability of pot experiments to field conditions, the objectives of **Section III** were to test whether (i) wheat plants grown in tubes or pots differ in their response to drought stress, (ii) phenotypic and physiological measurements could be extrapolated to field conditions, and (iii) the drought stress response of different genotypes is influenced by pot size.

3. Materials and Methods

The field experiments were conducted at the Dürnast research station of the Technical University of Munich in southern Germany (11°41´60´´ E, 48°23´60´´ N) in a mobile rain-out shelter (Figure 1). In this region, the average annual precipitation is approximately 800 mm with an average annual temperature of 8°C. The major demand for water by the crops occurs from April to the end of July; during this period, the average precipitation is approximately 350 mm with an average temperature of 13.7°C. The soil is characterized as a calcaric cambisol consisting of silty loam.

Figure 1: Rain-out shelter at the research station Dürnast from the Technical University of Munich.

3.1 Experimental design of Section I and II

The experiment was conducted as a randomized block design consisting of four replicates arranged in six rows, in two seasons in 2014 and 2015 in a rain-out shelter (Figure 1). The experiment comprised two different environments: a drought stress environment, created by withholding precipitation, and a control environment, grown next to the shelter with optimal water supply. Winter wheat plants (*Triticum aestivum* L.) were grown under natural weather conditions. Specifically, when raining, the shelter closed automatically and prevented any water from reaching the plants. The experiment adopted a randomized block design consisting of four replicates of both environments. Twenty high-yielding wheat varieties (Table 1) were grown in individual plots, consisting of eight 1.7 m long rows spaced at 15 cm. The sowing density employed was 350 kernels m^{-2}. A total of 180 kg N ha^{-1} was applied as ammonium sulfate nitrate (ASS) at tillering (100 kg N ha^{-1}) and calcium ammonium nitrate (CAN) at stem elongation (80 kg N ha^{-1}). All other nutrients, including P, K, S, and micronutrients, were supplied in adequate quantities to the crops. Integrated pest management was applied and the plots were kept free of weeds.

Table 1: Winter Wheat cultivars grown in 2014 and 2015.

Cultivar	Usage
Akteur	Bread wheat
Anapolis	Fodder wheat
Colonia	Bread wheat
Elixer	Biscuit, fodder, malting wheat
Genius	Bread wheat
Hybery	Bread wheat
Hybred	Bread wheat
Hyfi	Bread wheat
Hyland	Bread wheat
Hylux	Bread wheat
Hystar	Bread wheat
Impression	Bread wheat
JB Asano	Bread wheat
Kometus	Bread wheat
Manager	Bread wheat
Mulan	Bread wheat
Patras	Bread wheat
Piko	Hybrid father line
SUR.99820	Hybrid mother line
Tobak	Bread wheat

3.2 Experimental design of Section III

The same six varieties were used for the field experiment and they were grown under controlled conditions in 2015 and 2016. The experimental design was a complete randomized block design with four replicates. To compare the different pot systems as used for testing their drought tolerance, the plants were grown in either 6.6 L pots (20 cm diameter × 21 cm depth) or in 19.4 L tubes (15 cm diameter × 110 cm depth) filled with sandy loam. Comparably sized pots and tubes are commonly used for crop physiology studies and breeding purposes. Both container systems described allow for the development of a crop canopy when pots and tubes are placed side by side at the same height. The nominal plant density was similar to that used in seeding rates for wheat field production in Germany. The seeds were selected for homogeneity; 18 were planted per pot and nine per tube. Following their establishment, the plants were thinned to 15 per plot and to six per tube. The air temperature ranged from 18°C to 22°C (day) and from 15°C to 17°C (night), with a relative air humidity of 60 %, a photoperiod of 12 h, and ~400 µmol m^{-2} s^{-1} PAR on average. All the pots were watered by hand and the drought stress treatment was created by withholding water during the reproductive stage, at the beginning of heading (BBCH 51). The difference size between the pot and tube containers led to different durations of drought stress (Table 2). A total of 120 kg N ha^{-1} was applied as combination of nitrate (15 %), phosphate (10 %), potassium oxide (15 %), and magnesium (2 %) (Hakaphos® blue) at the stages of tillering (80 kg N ha^{-1}) and stem elongation (40 kg N ha^{-1}).

Table 2: Number of plants and days without irrigation per pot system and wheat variety for 2015 and 2016. *C*, control; *DS*, drought stress.

Variety	Pot System	Number of Plants		Days without irrigation		2015	2016
		C	DS	2015	2016		
Anapolis	pot	15	15	5	6	X	X
	tube	6	6	18	20	X	X
Genius	pot	15	15	5	6		X
	tube	6	6	18	20		X
Patras	pot	15	15	5	6	X	X
	tube	6	6	18	20	X	X
Hyland	pot	15	15	5	6	X	X
	tube	6	6	18	20	X	X
Hylux	pot	15	15	5	6		X
	tube	6	6	18	20		X
Hystar	pot	15	15	5	6	X	X
	tube	6	6	18	20	X	X

3.2.1 Plant Material

Six winter wheat varieties (Hyland, Hylux, Hystar, Anapolis, Genius, and Patras) were chosen among 20 varieties because these six showed the lowest and highest drought tolerance responses in the field experiment. The varieties Hyland, Hylux, and Hystar are hybrids, bred by SAATEN-UNION, and deemed as drought tolerant. The other three varieties - Anapolis was provided by Hauptsaaten, Genius by SAATEN-UNION, and Patras by IG-Pflanzenzucht - are recognized as high yielding and are commonly grown in Germany.

3.3 Spectral measurements Section I

Spectral measurements were conducted parallel to RLWC, CID, and thermal measurements, using a passive spectrometer device enabling hyperspectral readings in a range of 400-1200 nm and a bandwidth of 3.3 nm (Mistele and Schmidhalter, 2010). Based on the provided range of wavelength the water indices WI and NWI 1-4 were calculated (Table 3). Two Zeiss MMS1 silicon diode array spectrometers are included in the passive spectrometer, which together measured canopy reflectance proximally in a circular field of view (FOV) of approximately 0.28 m^2 in the center of each plot, and recorded across the plot covering approximately 25 % of the entire plot area. In addition, solar radiation was detected as a reference signal using a second unit. The sensor device was mounted 1 m above the canopy in a nadir position on the mobile phenotyping platform PhenoTrac 4 developed by the Chair of Plant Nutrition, Technical University of Munich (http://www.pe.wzw.tum.de; Figure 2). For calibration, a grey standard was used before each measurement. Forty sensor readings per second were simultaneously recorded with GPS coordinates from the TRIMBLE RTK-GPS (real-time kinematic global positioning system; Trimble, Sunnyvale, CA, USA). For each plot, approximately 70 sensor readings were recorded and averaged. To match the sensor readings with GPS coordinates the mapping platform ArcGIS was used (Esri, CA, USA). All measurements were conducted simultaneously under cloudless sky at noon (12 to 2 pm) to provide optimal conditions for passive recording.

Figure 2: PhenoTrac4, carrying five passive and active spectral sensors.

Table 3: Water indices and corresponding abbreviations

Index	Index abbreviation
R_{900}/R_{970}	P_WI
$[R_{970}-R_{900}]/[R_{970}+R_{900}]$	P_NWI-1
$[R_{970}-R_{850}]/[R_{970}+R_{850}]$	P_NWI-2
$[R_{970}-R_{880}]/[R_{970}+R_{880}]$	P_NWI-3
$[R_{970}-R_{920}]/[R_{970}+R_{920}]$	P_NWI-4

3.4 Spectral measurements Section II

In parallel with RLWC, CID and thermal measurements, spectral measurements were conducted using a passive spectrometer device enabling hyperspectral readings in a range of 400 to 1200 nm and with a bandwidth of 3.3 nm (Mistele and Schmidhalter, 2010). The passive spectrometer included two Zeiss MMS1 silicon diode array spectrometers, which together measured canopy reflectance in a circular field of view (FOV) of approximately 0.28 m^2 in the center of each plot. Measurements were recorded across the plot, covering approximately ¼ of the whole plot area. Additionally, solar radiation was detected as a reference signal with a second unit. In addition to the passive sensor, three active devices, a commercially available GreenSeeker RT100® (NTech Industries, Inc., Ukiah, CA, USA), a Crop Circle ACS-470® (670, 730 and 760 nm, Holland Scientific, Inc., Lincoln, NE, USA) and an active flash sensor (AFS) similar to the N-Sensor ALS® (YARA International, ASA) but limited to a single sensor and a USB interface, were used. A light source flashing xenon light was included. This light source produced a spectral range of 650 to 1100 nm with ten flashes per second and a circular FOV of approximately 0.15 m^2.

The GreenSeeker included two LEDs, which detected the reflection in the VIS (656 nm, ~25 nm band width) and the NIR (774 nm, ~ 25 nm band width) spectral region. The FOV is a narrow strip with an approximate area of 0.009 m^2 at a height of 66-112 cm above the plant canopy (NTech Industries, Inc., Ukiah, CA, USA, 2007). The Crop Circle operates in a similar way to the GreenSeeker. An advantage of the Crop Circle is that it provides more flexibility in the selection of detected wavelengths due to a choice of interference filters.

For this study, filters for 670, 730, and 760 nm were selected. The FOV of the Crop Circle is an oval with an approximate area of 0.09 m^2. For both active sensors, the FOV runs perpendicular to the sowing direction. The sensor device was mounted 1 m above the canopy in a nadir position on the mobile phenotyping platform PhenoTrac 4 developed by the Chair of Plant Nutrition, Technical University of Munich (http://www.pe.wzw.tum.de; Figure 2). Hence, simultaneous high-throughput measurements for all plots were obtained. Sensor readings were simultaneously recorded with GPS coordinates from a TRIMBLE RTK-GPS (real-time kinematic global positioning system; Trimble, Sunnyvale, CA, USA). In each plot, approximately 70 sensor readings were recorded and averaged. All measurements were conducted under cloudless sky at noon. To illustrate the different reflectance intensities in the VIS and NIR ranges of all used sensor systems, ten indices were selected (Table 4). Because the active sensors are not always able to exactly detect the wavelengths of these indices, similar wavelengths and combinations were used to calculate ratios (Table 4) based on the six initial indices. In 2014, the active sensor Crop Circle was not available.

Table 4: Indices and wavelengths of four sensor systems and the corresponding abbreviations.

Sensor	Index	Index abbreviation
Passive	R_{900}/R_{970}	P_WI
	$[R_{970}-R_{900}]/[R_{970}+R_{900}]$	P_NWI-1
	$[R_{970}-R_{850}]/[R_{970}+R_{850}]$	P_NWI-2
	$[R_{970}-R_{880}]/[R_{970}+R_{880}]$	P_NWI-3
	$[R_{970}-R_{920}]/[R_{970}+R_{920}]$	P_NWI-4
	R_{760}/R_{670}	P_760/670
	R_{774}/R_{656}	P_774/656
	R_{760}/R_{730}	P_760/730
	R_{730}/R_{760}	P_730/760
	$[R_{780}-R_{670}]/[R_{780}+R_{670}]$	P_NDVI
Active flash sensor	R_{900}/R_{970}	ALS_WI
	R_{760}/R_{730}	ALS_760/730
	R_{730}/R_{760}	ALS_730/760
GreenSeeker	$[R_{774}-R_{656}]/[R_{774}+R_{656}]$	GS_NDVI
	R_{7740}/R_{656}	GS_774/656
Crop Circle	R_{730}/R_{670}	CC_730/670
	R_{760}/R_{730}	CC_760/730
	R_{760}/R_{670}	CC_760/670

3.5 Section I to III: Determination of leaf surface temperature

For the field experiments, the leaf surface temperature was determined at heading, anthesis and grain filling, using thermography and thermometry in Section I, thermometry in Section II. For the experiments under controlled conditions in Section III, the leaf surface temperature was determined at anthesis, using thermography. For thermometry measurements, two HEITRONICS KT15.83D infrared (IR) thermometers (Heitronics GmbH, Wiesbaden, Germany) were mounted opposed to each other on the Phenotrac 4 in a 45 ° angle and a FOV of 10 cm, possessing a spectral range of 8–14 µm and a temperature resolution of 0.06°C. The measurements were recorded in the central part of each plot moving across the entire length of the plot. Leaf surface temperature was determined by averaging the temperatures measured by both sensors. To conduct the thermography measurements, a hand-held IR thermal camera (Model T335; FLIR Systems, Wilsonville, OR, USA) was used.

The camera operates in a wavelength range of 7.5–13 µm with a thermal resolution of 0.05°C, and produces a spatial resolution of 320 × 240 pixels (Hackl *et al.*, 2012). The emissivity was set to 0.98, which differs slightly but negligibly from the emissivity of plant leaves (Jackson *et al.*, 1981; Hackl *et al.*, 2012). Thermal and red, green, blue (RGB) images were taken simultaneously from the center of each plot at a 45° angle to avoid soil influences (approximately 1.3 m above the ground).

The software FLIR QUICKREPORT 1.2 SP1 was used to export the temperature matrix of the thermal images. To separate the leaf surface temperature from the soil temperature, a LabVIEW-based software was used (National Instruments, Austin, TX, USA). The RGB image matrices were converted in lightness, chroma, hue (LCH) color space. To separate leaves from the soil, threshold settings of chroma and lightness of the LCH images were used. After separating the leaf surface from the background, the RGB image was matched with the thermal image (Figure 3) and the average leaf surface temperature was calculated.

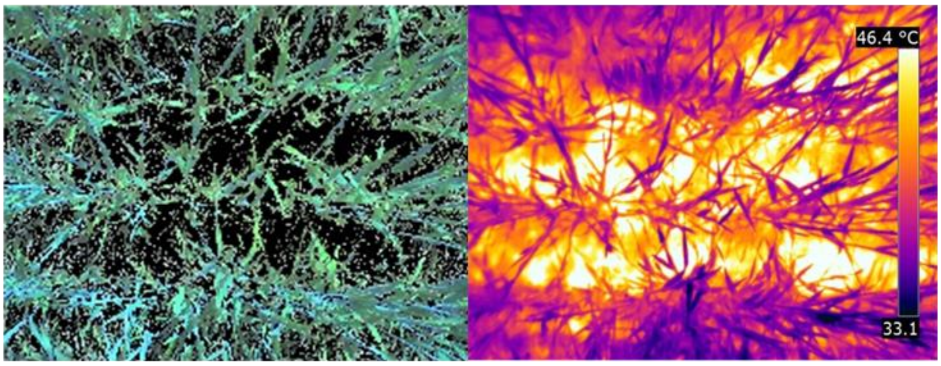

Figure 3: Red, green blue (RGB) picture of the field plot with selected plant from the soil (left side) and the appertaining thermal image (right side).

3.6 Section I to III: Relative leaf water content

The RLWC was determined in the field experiments on F-1 leaves at heading, anthesis and grain filling synchronously with spectral reflectance measurements for all environments. Five leaves per plot were collected, and the fresh weight (FW) was immediately documented. The bottom parts of the leaves were placed in distilled water contained in sample tubes for 16 h at 5°C in darkness, and the turgid weight (TW) was recorded (Hackl *et al.*, 2014). After 48 h at 60°C, the dry weight (DW) was measured. The same procedure was applied at anthesis for the

experiments under controlled conditions in Section III, except that, due to limited biomass, only three leaves were sampled instead of five.
The RLWC was calculated according to the following formula:

$$RLWC = \frac{(\mathrm{FW}-\mathrm{DW})}{(\mathrm{TW}-\mathrm{DW})} \times 100 \qquad (1)$$

3.7 Section I to III: Carbon isotope − discrimination

For the field experiments, the CID was determined for F-1 leaves at heading, anthesis and grain filling, and grains at maturity. For each plot, five leaves were sampled and dried at 60°C for 48 h. At maturity, grains of 15 plants were collected, ground to a fine powder, and dried at 60°C for 48 h. For the experiments under controlled conditions in Section III, leaves at anthesis were sampled.
The carbon isotope composition was measured using a mass spectrometer (Europe Scientific, Crewe, UK). The CID was calculated according to following formula:

$$CID(‰) = \frac{(\delta a - \delta p)}{(1 + \delta p)} x\ 1000, \qquad (2)$$

where δa = $\delta^{13}C$ of atmospheric CO_2 (−8‰) and δp = $\delta^{13}C$ of the sample (Farquhar *et al.*, 1989).

3.8 Section II: Ground cover measurements based on pixel analysis of RGB images

Images were captured using a Nikon D5100 reflex camera. To guarantee constant operational conditions, all images were captured under overcast conditions. The camera was manually held in a nadir position over the canopy at a height of 140 cm. In this position, approximately six rows of each plot were captured by the FOV of the camera. Digital image analyses of RGB (red, green and blue) images were conducted using ImageJ, a free, public domain Java image processing analysis program (Abramoff *et al.*, 2004). To differentiate green wheat pixels from brown soil pixels, thresholds for hue, saturation, and brightness were manually selected for each growth stage (Figure 4).

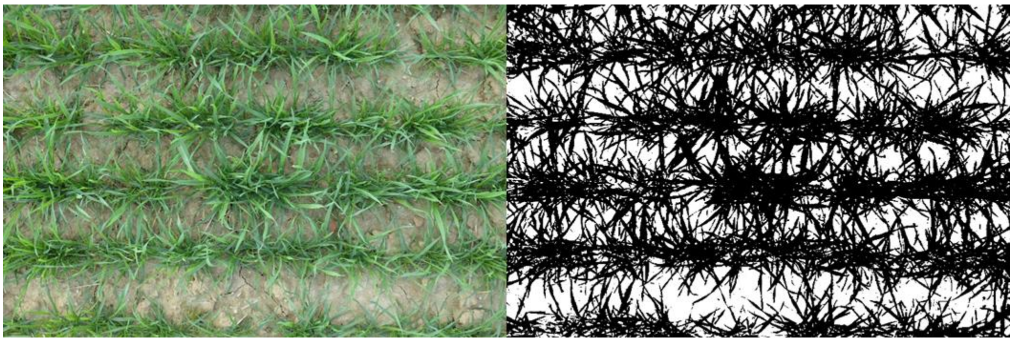

Figure 4: RGB picture (left side) and with ImageJ selected plants (right side).

3.9 Section III: Rooting Depth

Root distributions under drought stress were determined at the following profile depths in the field: 0–30, 30–60, 60–90, and 90–120 cm in the plot centers of the varieties Anapolis, Patras, Hyland, Hylux, and Hystar in 2015. No soil samples were taken from the variety Genius in 2015. The root sampling took place after the harvesting. Soil cores of 10 cm wide and 120 cm long were extracted (using a hydraulic soil corer). To separate the roots from the soil, the cores were first washed carefully in water, followed by drying at 60°C for 48 h, and then weighing. The same procedure was applied for all six varieties grown under the controlled conditions in the tubes, but at the slightly different depths: 0–20, 20–50, 50–80, and 80–110 cm. Due to the limited rooting depth in the 6.6 L pots, the root distribution for this treatment could not be analyzed.

3.10 Statistical analyses

3.10.1 Statistics with SPSS

SPSS 21 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene’s tests, as implemented in SPSS 21.0. To analyze the relationships between different drought stress parameters, a simple linear regression was calculated in **Section I** and **II**. Correlation coefficients and significance levels were determined in nominal alpha values of 0.05, 0.01, 0.001, and 0.0001. Since lateral water influx affected the northern border row and two plots in the western heading column in 2014, this data was not considered for further evaluation.

For **Section III**, Pearson's correlation was used to analyze the relationship between different drought-related parameters and grain yield. Correlation coefficients and significance levels were determined for nominal alpha values of 0.05, 0.01, 0.001, and 0.0001. Effects of the growing environment (up to three levels: field, tube, or pot) and wheat cultivar (six variety levels) on the plant physiological responses were tested with ANOVAs. Multiple pair-wise comparisons of means via Duncan's test were performed whenever an ANOVA indicated a significant difference ($P < 0.05$).

3.10.2 Section I: Multivariate data analysis

To calibrate and validate partial least square models, The Unscrambler X multivariate data analysis software version 10.3 (CAMO Software AS, Oslo, Norway) was used for Section I. Partial least square regression (PLSR) is a tool to select sensitive information from spectral reflectance for the entire range of wavelengths (400-1200 nm). A detailed description of PLSR can be found in (Esbensen *et al.*, 2002). To correct for light scattering, spectral data was normalized by log transformation. PLSR generates orthogonal latent variables across input variables (single wavebands) which are then used to predict the dependent variables RLWC, leaf temperature measured by a thermal camera (TFLIR), leaf temperature measured by infrared sensors (TIRS), carbon isotope discrimination of leaf (CIDL), carbon isotope discrimination of grain (CIDG), and yield. The dataset was randomly separated in subsets, using 2/3 of the observations for model calibration and 1/3 for model validation. The quality of calibration and validation is represented by coefficients of determination of calibration (R^2 Cal) and validation (R^2 Val), and root mean square error (RMSE) for calibration and validation.

3.10.3 Section I and II: Calculation of Heritability

3.10.3.1 Analysis within single treatments

Data were analyzed separately for each year. Within each treatment, data were analyzed using a linear model with the factors variety and replicate block. The significance of factors was determined using analysis of variance (ANOVA), and means were separated using Tukey's HSD test. The normality of distribution of the residuals was tested using the Shapiro-Wilk test. To calculate heritability, a model was fitted with both factors taken as random, using the lme4 package (Bates *et al.*, 2014), and heritability on a mean basis was calculated as Vg/(Vg + Vr/r), where Vg and Vr are the genotypic and residual variance components, respectively, and r is the number of replicate blocks (Holland *et al.*, 2003). All analyses were carried out using the R statistical package in R (R Core Team, 2016).

3.10.3.2 Analysis across treatments (within years)

To test for significant genotype–treatment interaction, a linear model with the factors variety, treatment, their interaction, and replicate block nested within treatments was fitted, and the significance was determined using ANOVA.

4. Results

4.1 Section I: Detection of drought stress related traits and prediction of grain yield by spectral and thermal high-throughput measurements in winter wheat

4.1.1 Influence of drought stress during heading, anthesis, and grain filling

To determine the development and level of drought stress, the traits RLWC, TFLIR, TIRS, CIDL, CIDG, and grain yield were measured during both experimental years at the growth stages heading, anthesis, and grain filling (Figure 5). During both experimental years and all three growth stages, drought stress led to a statistically significant decrease of RLWC, CIDL, CIDG, and grain yield, as well as a significant increase of leaf temperature compared with the control plants (Figure 5).

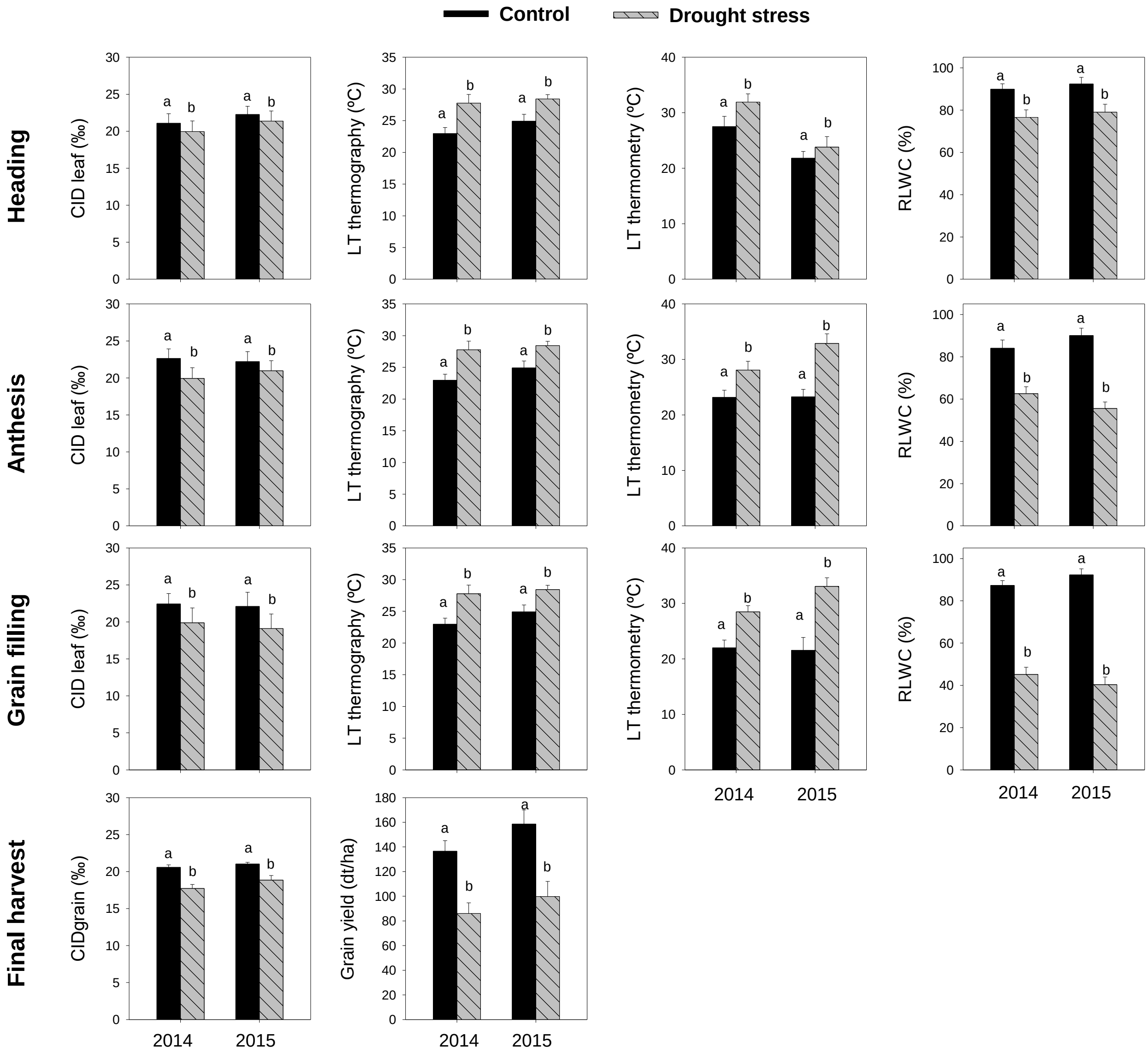

Figure 5: Means (±standard error (SE)) of carbon isotope discrimination (CID) of leaf and grain, leaf temperature (LT), relative leaf water content (RLWC), and yield at different growth stages during two experimental years. Different subscripts show significant difference (Alpha = 0.05).

4.1.2 Phenotypic correlation of drought-stress parameters during heading, anthesis, and grain filling

Highly significant relationships between all measured parameters could be observed in the drought stress environment for both years and all three growth stages (Table 5). The phenotypic correlations for the physiological parameters, TIRS, CIDL, and CIDG provided strong relationships (r > 0.50) to yield for all growth stages. RLWC and TFLIR showed strong correlations at heading and anthesis. In general, correlations between NWI-3 and yield provided higher correlation values for all growth stages than the physiological parameters. Weaker correlations could be observed in the control environment for both years and all three growth stages. When comparing the growth stages heading, anthesis, and grain filling of both experimental years, measurements during anthesis provided the closest relationships to grain yield (Table 6).

Table 5: Correlations of drought related parameters, yield an NWI-3 in winter wheat in drought and control environments for heading, anthesis and grain filling.

		NWI-3											
		heading				anthesis				grain filling			
		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.
RLWC	DS	-0.35	**	-0.36	***	**-0.60**	****	**-0.50**	****	-0.37	**	-0.39	****
	C	0.23	ns	-0.26	ns	0.26	ns	0.08	ns	0.23	ns	-0.06	ns
TFLIR	DS	0.27	*	0.42	****	**0.74**	****	0.38	****	0.46	****	0.41	****
	C	0.26	ns	0.24	ns	-0.05	ns	-0.07	ns	0.26	ns	-0.16	ns
TIRS	DS	0.22	ns	**0.58**	****	**0.81**	****	**0.65**	****	**0.76**	****	**0.60**	****
	C	0.30	ns	0.21	ns	0.30	ns	0.11	ns	0.30	ns	-0.08	ns
CIDL	DS	-0.42	****	**-0.56**	****	**-0.81**	****	**-0.64**	****	**-0.74**	****	**-0.60**	****
	C	-0.26	ns	0.13	ns	-0.12	ns	-0.13	ns	-0.26	ns	-0.13	ns
CIDG	DS	-0.34	**	**-0.57**	****	**-0.82**	****	**-0.61**	****	**-0.78**	****	**-0.62**	****
	C	-0.23	ns	-0.16	ns	-0.28	ns	-0.23	ns	-0.23	ns	0.02	ns
yield	DS	**-0.58**	****	**-0.66**	****	**-0.90**	****	**-0.85**	****	**-0.85**	****	**-0.86**	****
	C	0.22	ns	0.17	ns	-0.23	**	-0.36	**	0.22	ns	-0.10	ns

RLWC relative leaf water content, *TFLIR* leaf temperature FLIR-Camera, *TIRS* leaf temperature IR-sensors, *CIDL* carbon isotope discrimination of leaf, *CIDG* carbon isotope discrimination of grain, *yield* grain yield.
[b] Treatments, drought stress (DS), control (C).
[c] r Correlation coefficient.
[d] Statistical significance as indicated by p-value *ns* non-significant: *p < 0.05, **p < 0.01, *** < 0.001, ****p < 0.0001.
Bold data display correlations > r = 0.50.

Table 6: Correlations of drought-related parameters in winter wheat under drought and control conditions for heading, anthesis, grain filling (results of 2014 are presented in lower diagonal; results of 2015 are presented in the upper diagonal)

heading

						2015							
		RLWC		TFLIR		TIRS		CIDL		CIDG		yield	
Trait[a]	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
RLWC	DS			-0.06	ns	-0.41	****	-0.33	***	-0.38	****	**0.50**	****
	C			-0.19	ns	-0.03	ns	0.00	ns	-0.01	ns	0.04	ns
TFLIR	DS	**-0.53**	****			0.03	ns	-0.27	*	-0.02	ns	-0.17	ns
	C	0.21	***			-0.11	ns	-0.09	ns	0.28	*	0.17	ns
TIRS	DS	-0.47	****	**0.50**	****			**-0.53**	****	**-0.60**	****	**-0.75**	****
	C	**0.50**	***	0.13	ns			-0.15	ns	-0.17	ns	0.04	ns
CIDL	DS	**0.52**	****	**-0.54**	****	**-0.54**	****			**0.55**	****	**0.73**	****
	C	0.01	ns	-0.13	ns	-0.13	ns			0.20	ns	-0.15	ns
CIDG	DS	**0.65**	****	**-0.64**	****	**-0.64**	****	**0.81**	****			**0.78**	****
	C	0.12	ns	-0.03	ns	-0.03	ns	0.26	ns			0.20	ns
yield	DS	**0.63**	****	**-0.67**	****	**-0.67**	****	**0.74**	****	**0.79**	****		
	C	0.03	ns	-0.00	ns	-0.00	ns	-0.15	ns	0.21	ns		
						2014							

anthesis

						2015							
		RLWC		TFLIR		TIRS		CIDL		CIDG		yield	
Trait	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
RLWC	DS			-0.63	****	-0.44	****	0.47	****	0.62	****	**0.58**	****
	C			0.02	ns	0.17	ns	-0.15	ns	0.06	ns	0.06	ns
TFLIR	DS	**-0.54**	****			-0.67	****	-0.59	***	**-0.71**	****	**-0.64**	****
	C	-0.05	ns			0.01	ns	-0.08	ns	-0.08	ns	0.17	ns
TIRS	DS	**-0.65**	****	**0.52**	****			-0.53	****	**-0.60**	****	**-0.74**	****
	C	0.07	ns	0.21	ns			-0.24	*	-0.44	***	-0.13	ns
CIDL	DS	**0.65**	****	**-0.67**	****	**-0.74**	****			**0.57**	****	**0.74**	****
	C	0.02	ns	0.24	ns	0.38	*			0.35	**	0.06	ns
CIDG	DS	**0.57**	****	**-0.73**	****	**-0.68**	****	**0.79**	****			**0.78**	****
	C	0.12	ns	0.16	ns	0.03	ns	0.31	*			0.20	ns
yield	DS	**0.59**	****	**-0.71**	****	**-0.80**	****	**0.85**	****	**0.79**	****		
	C	0.16	ns	0.19	ns	0.19	ns	0.09	ns	0.21	ns		
						2014							

grain filling

						2015							
		RLWC		TFLIR		TIRS		CIDL		CIDG		yield	
Trait	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
RLWC	DS			-0.44	****	-0.44	****	0.41	****	**0.54**	****	**0.45**	****
	C			0.06	ns	0.06	ns	-0.16	ns	0.06	ns	0.25	*
TFLIR	DS	**-0.54**	****			**-0.55**	****	-0.42	***	**-0.62**	****	**-0.50**	****
	C	-0.05	ns			0.05	ns	-0.13	ns	-0.10	ns	0.13	ns
TIRS	DS	-0.29	*	**0.35**	***			**-0.53**	****	**-0.60**	****	**-0.66**	****
	C	0.11	ns	0.17	ns			-0.34	*	-0.24	*	-0.10	ns
CIDL	DS	0.33	**	**-0.36**	***	**-0.67**	****			**0.56**	****	**0.70**	****
	C	0.08	ns	0.18	ns	0.31	*			0.25	*	0.05	ns
CIDG	DS	0.34	**	**-0.51**	****	**-0.62**	****	**0.71**	****			**0.76**	****
	C	0.04	ns	0.11	ns	0.08	ns	0.05	ns			0.19	ns
yield	DS	0.33	**	**-0.54**	****	**-0.69**	****	**0.82**	****	**0.86**	****		
	C	0.03	ns	0.13	ns	0.03	ns	0.21	ns	0.20	ns		
						2014							

[a] *RLWC* relative leaf water content, *LT* leaf temperature, *CIDL* carbon isotope discrimination of leaf, *CIDG* carbon isotope discrimination of grain, *GC* ground cover, *yield* grain yield. [b] Treatments, drought stress (DS), control (C). [c] r Correlation coefficient. [d] Statistical significance as indicated by p-value *ns* non-significant: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Bold data display correlations > r = 0.50.

4.1.3 Partial least square regression models

PLSR models for RLWC, TFLIR, TIRS, CIDL, CIDG, and yield were calculated for the growth stages heading, anthesis, and grain filling for each experimental year in the drought stress environment (Figure 6 and Table 7). The statistical information of the PLSR models indicated a good ability for predicting the measured drought stress parameters during anthesis, achieving an R^2 in the validation set varying from 0.56 to 0.87, depending on the parameter. Furthermore, the spectral dataset from 2014 was used to predict grain yield for 2015 (R^2 = 0.62) and *vice versa* (R^2 = 0.73) (Figure 7), indicating a good transferability of spectral information.

Table 7: Estimation of plot grain yield, relative leaf water content (RLWC), leaf temperature by thermography (TFLIR), leaf temperature by thermography (TIRS), and carbon isotope discrimination (CID) of leaves and grain by PLSR analysis of spectral information under drought conditions and three different growth stages.

		2014						2015					
				R^2		RMSE%[e]				R^2		RMSE%	
Trait	GS	n[a]	PC[b]	Cal[c]	Val[d]	Cal	Val	n	PC	Cal	Val	Cal	Val
Grain yield [dt/ha]	heading	78	6	0.69	0.62	21.62	24.55	78	6	0.60	0.45	20.03	23.49
	anthesis	78	3	0.81	0.79	16.58	17.74	78	5	0.87	0.85	10.75	11.94
	grain filling	78	5	0.64	0.59	22.91	25.08	78	3	0.79	0.76	14.45	15.96
RLWC [%]	heading	80	2	0.40	0.37	6.08	6.35	80	7	0.55	0.41	4.29	5.02
	anthesis	80	7	0.67	0.54	7.03	8.46	80	6	0.60	0.53	8.28	9.20
	grain filling	80	7	0.49	0.28	12.69	15.21	80	1	0.33	0.31	15.99	16.51
TFLIR [°C]	heading	80	4	0.53	0.43	3.67	4.10	80	6	0.54	0.44	3.69	4.19
	anthesis	80	5	0.72	0.63	3.81	4.45	80	2	0.61	0.57	3.20	3.30
	grain filling	80	5	0.49	0.38	2.72	3.05	80	2	0.38	0.34	3.84	4.04
TIRS [°C]	heading	80	5	0.57	0.46	7.21	8.08	80	6	0.50	0.33	10.16	11.80
	anthesis	80	1	0.61	0.59	5.12	5.30	79	6	0.80	0.76	3.28	3.74
	grain filling	80	1	0.58	0.56	4.25	4.35	80	6	0.64	0.52	3.30	3.90
CIDL [‰]	heading	80	5	0.51	0.43	2.76	3.06	80	1	0.30	0.27	3.08	3.18
	anthesis	80	1	0.74	0.63	2.65	3.26	80	7	0.73	0.65	3.24	3.77
	grain filling	80	1	0.59	0.58	3.41	3.51	80	2	0.38	0.36	2.72	2.83
CIDG [‰]	heading	80	5	0.58	0.51	4.00	4.40	79	6	0.43	0.26	5.47	5.99
	anthesis	80	6	0.75	0.67	3.27	3.78	79	2	0.56	0.54	2.99	3.10
	grain filling	80	6	0.74	0.64	3.33	3.95	79	7	0.74	0.68	3.78	4.34

[a] *n* Number of samples
[b] *PC* Principal components
[c] *Cal* Calibration
[d] *Val* Validation
[e] *RMSE* Root mean square error

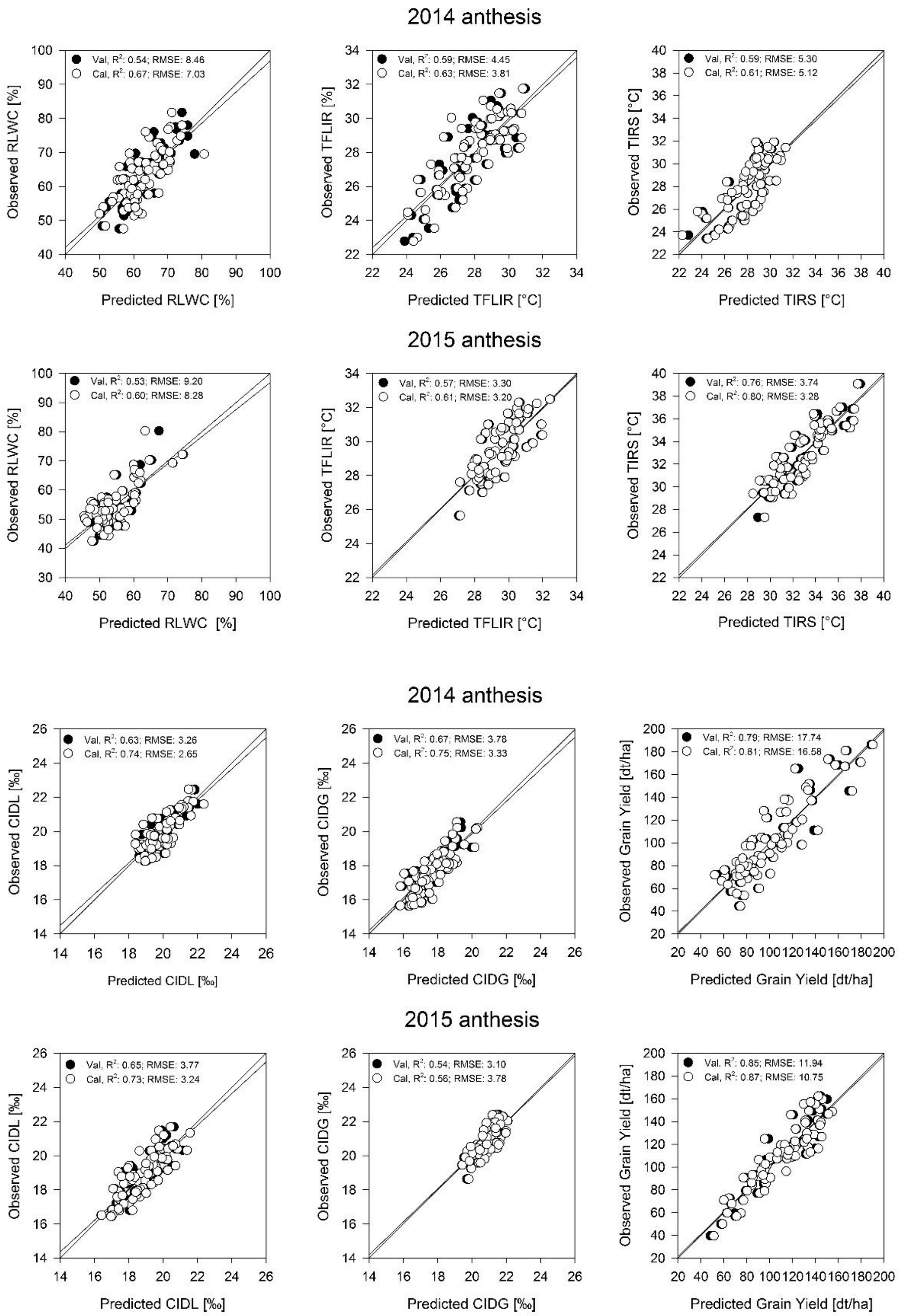

Figure 6: Prediction of relative leaf water content (RLWC), leaf temperature by thermography (TFLIR), leaf temperature by thermography (TIRS), and carbon isotope discrimination (CID) of leaves and grain and grain yield by PLSR of spectral information under drought conditions at anthesis. RMSE is displayed in %.

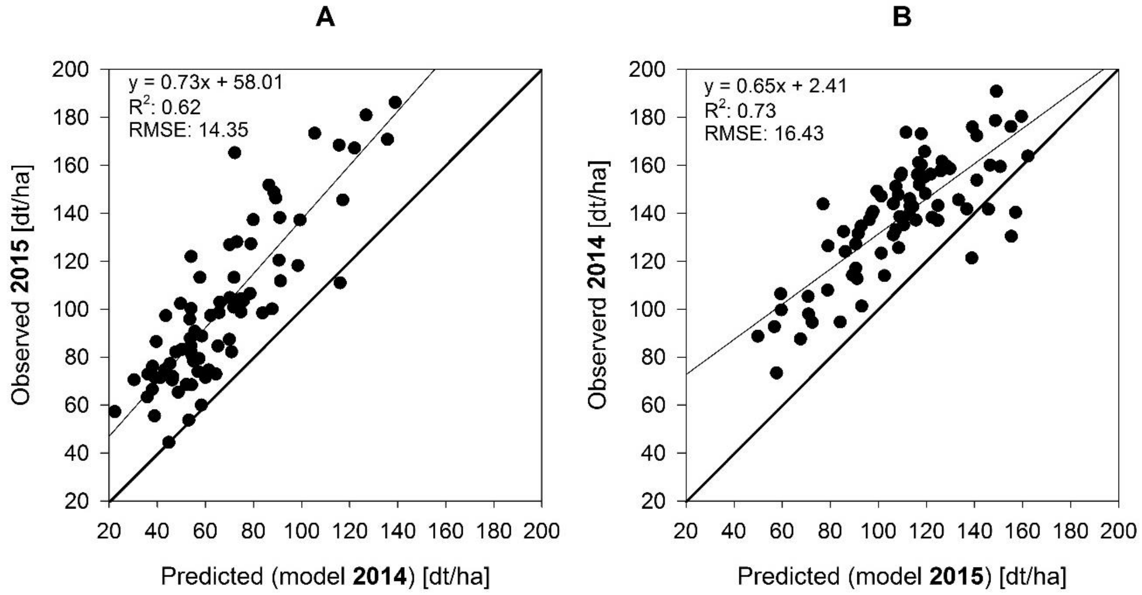

Figure 7: Observed yield under drought stress (X-axis) *versus* yield predicted by a model (based on spectral data of another year (Y-axis)) using wavelengths from 500 to 1200 nm in winter wheat. RMSE is displayed in %.

4.1.4 Heritability of drought-related parameters and spectral indices

Heritability of RLWC was moderate for both years under drought stress conditions (Table 8). During 2014, heritability was lower in the control environment compared to the drought environment. During 2015, under drought conditions, the genetic variance was estimated to be 0; hence, no heritability for RLWC could be calculated. The thermal measurements (TFLIR) showed moderate heritability under drought and control conditions for both experimental years. In contrast, for the thermal measurements, conducted by TIRS, the genetic variance was estimated to be 0 under control conditions in 2014 and under drought conditions in 2015. Furthermore, the heritability of the carbon isotope was strong during 2014 for both environments and moderate during 2015. The studied water indices had moderate to strong heritabilities that were on a comparable level of grain yield heritability under drought conditions, during 2015 and 2014 (Table 8). Overall, the heritability of grain yield was moderate to strong under drought and control conditions for both experimental years.

Table 8: Heritability of drought-related parameters and NWI-3 at anthesis under drought and control conditions.

Trait[a]	2014		2015	
	drought	control	drought	control
	h^{2b}	h^2	h^2	h^2
RLWC	0.66	0.32	0.57	0
TFLIR	0.19	0.57	0.61	0.53
TIRS	0.52	0	0	0.42
CIDL	0.65	0.82	0.28	0.42
CIDG	0.72	0.86	0.44	0.39
yield	0.62	0.74	0.61	0.83
NWI-3	0.54	0.35	0.49	0.75

[a] *RLWC* relative leaf water content (%), *TFLIR* leaf temperature FLIR-camera (°C), *TIRS* leaf temperature IR-sensors (°C), *CIDL* carbon isotope discrimination of leaf (‰), *CIDG* carbon isotope discrimination of grain (‰), *GC* ground cover (%), *yield* grain yield (dt/ha), *WI* water index, *NWI1-4* normalized water indices.
[b] *Heritability*

4.2 Section II: Evaluation of yield and drought using active and passive spectral sensing systems at the reproductive stage in wheat

4.2.1 Impact of drought stress on morphophysiological parameters

During both experimental years, and across the heading, anthesis and grain-filling stages, the drought-related parameters, i.e., RLWC, LT, CIDL, CIDG, GC and grain yield, were measured (Table 9). The induced drought stress led to a statistically significant impairment of all morphophysiological parameters of the winter wheat plants during the three growth stages and in both experimental years. A significant decrease in RLWC, CIDL, CIDG, GC and grain yield, as well as a significant increase in leaf temperature was observed compared with the control plants (Table 9).

Table 9: Means (±standard error (SE)) of grain yield, carbon isotope discrimination (CID) of leaf and grain, leaf temperature (LT), relative leaf water content (RLWC), and ground cover (GC) at different growth stages during two experimental years. Different subscripts show significant difference (Alpha = 0.05).

		2014				2015			
		Drought		Control		Drought		Control	
Trait	GS	Mean	SE[b]	Mean	SE	Mean	SE	Mean	SE
Grain yield [dt/ha]	heading								
	anthesis								
	grain filling	86.08[a]	8.57	136.53[b]	8.32	99.83[a]	12.25	158.50[b]	11.34
RLWC [%]	heading	76.69[a]	5.90	87.59[b]	4.14	79.02[a]	5.08	90.36[b]	3.15
	anthesis	62.55[a]	3.79	84.02 [b]	3.43	55.56[a]	3.29	90.22[b]	0.88
	grain filling	45.16[a]	8.02	87.28[b]	2.72	40.33[a]	7.95	90.70[b]	2.20
LT [°C]	heading	31.92[a]	2.45	27.50[b]	1.06	23.81[a]	2.48	21.82[b]	0.48
	anthesis	27.76[a]	1.75	22.97[b]	0.81	32.88[a]	0.95	23.26[b]	0.36
	grain filling	28.48[a]	1.93	21.99[b]	0.96	33.06[a]	1.84	21.75[b]	1.02
CIDL [‰]	heading	19.96[a]	0.81	21.37[b]	0.42	21.09[a]	0.77	22.27[b]	0.19
	anthesis	19.62[a]	0.51	22.64[b]	0.28	20.96[a]	0.36	22.21[b]	0.34
	grain filling	19.08[a]	1.03	22.42[b]	0.43	19.10[a]	0.67	21.82[b]	0.41
CIDG [‰]	heading								
	anthesis								
	grain filling	17.73[a]	0.53	20.59[b]	0.33	18.85[a]	0.62	21.03[b]	0.23
GC [%]	heading	59.84[a]	17.48	92.44[b]	8.47	66.43[a]	6.07	91.14[b]	5.35
	anthesis	55.18[a]	6.54	97.65[b]	0.84	60.68[a]	6.04	86.07[b]	5.09
	grain filling	53.83[a]	13.45	87.70[b]	1.02	46.44[a]	13.06	65.97[b]	2.07

4.2.2 Phenotypic correlation of drought-related parameters

Table 10 contains identical drought stress parameters as reported in table 6, however extended by GC as an indicator for biomass. Highly significant relationships were observed between all measured parameters for both experimental years and during the heading, anthesis and grain-filling stages (Table 10). All measured drought-related parameters exhibited strong phenotypic correlations ($r > 0.50$) with yield during all growth stages, but particularly at anthesis. The relative leaf water content showed the weakest relationship with all other measured parameters. In the control environment, no obvious relationships were observed in either year or in any of the growth stages. A comparison of the heading, anthesis, and grain-filling stages of both experimental years indicated that measurements during anthesis were most closely related to grain yield (Table 10).

Table 10: Correlations of drought-related parameters in winter wheat under drought and control conditions for heading, anthesis, grain filling (results of 2014 are presented in lower diagonal; results of 2015 are presented in the upper diagonal).

heading

									2015				
		RLWC		LT		CIDL		CIDG		GC		yield	
Trait[a]	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
RLWC	DS			-0.06	ns	-0.41	****	-0.33	***	0.38	**	**0.50**	****
	C			-0.19	ns	-0.03	ns	0.00	ns	0.11	ns	0.04	ns
LT	DS	**-0.53**	****			0.03	ns	-0.27	*	**-0.65**	****	-0.17	ns
	C	0.21	***			-0.11	ns	-0.09	ns	-0.01	ns	0.17	ns
CIDL	DS	-0.47	****	**0.50**	****			**-0.53**	****	**0.70**	****	**-0.75**	****
	C	**0.50**	***	0.13	ns			-0.15	ns	-0-17	ns	0.04	ns
CIDG	DS	**0.52**	****	**-0.54**	****	**-0.54**	****			**0.69**	****	**0.73**	****
	C	0.01	ns	-0.13	ns	-0.13	ns			0-03	ns	-0.15	ns
GC	DS	**0.65**	****	**-0.64**	****	**-0.64**	****	**0.81**	****			**0.78**	****
	C	0.12	ns	-0.03	ns	-0.03	ns	0.26	ns			0.20	ns
yield	DS	**0.63**	****	**-0.67**	****	**-0.67**	****	**0.74**	****	**0.51**	****		
	C	0.03	ns	-0.00	ns	-0.00	ns	-0.15	ns	0.03	ns		
			2014										

anthesis

									2015				
		RLWC		LT		CIDL		CIDG		GC		yield	
Trait	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
RLWC	DS			-0.63	****	-0.44	****	0.47	****	**0.50**	****	**0.58**	****
	C			0.02	ns	0.17	ns	-0.15	ns	0.19	ns	0.06	ns
LT	DS	**-0.54**	****			-0.67	****	-0.59	***	**-0.68**	****	**-0.64**	****
	C	-0.05	ns			0.01	ns	-0.08	ns	-0.23	*	0.17	ns
CIDL	DS	**-0.65**	****	**0.52**	****			-0.53	****	**0.70**	****	**-0.74**	****
	C	0.07	ns	0.21	ns			-0.24	*	0.05	ns	-0.13	ns
CIDG	DS	**0.65**	****	**-0.67**	****	**-0.74**	****			**0.66**	****	**0.74**	****
	C	0.02	ns	0.24	ns	0.38	*			0.16	ns	0.06	ns
GC	DS	**0.57**	****	**-0.73**	****	**-0.68**	****	**0.79**	****			**0.78**	****
	C	0.12	ns	0.16	ns	0.03	ns	0.31	*			0.20	ns
yield	DS	**0.59**	****	**-0.71**	****	**-0.80**	****	**0.85**	****	**0.93**	****		
	C	0.16	ns	0.19	ns	0.19	ns	0.09	ns	0.39	****		
			2014										

grain filling

									2015				
		RLWC		LT		CIDL		CIDG		GC		yield	
Trait	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
RLWC	DS			-0.44	****	-0.44	****	0.41	****	0.48	****	**0.45**	****
	C			0.06	ns	0.06	ns	-0.16	ns	-0.19	ns	0.25	*
LT	DS	**-0.54**	****			**-0.55**	****	-0.42	***	**-0.51**	****	**-0.50**	****
	C	-0.05	ns			0.05	ns	-0.13	ns	-0.16	ns	0.13	ns
CIDL	DS	-0.29	*	**0.35**	***			**-0.53**	****	**0.54**	****	**-0.66**	****
	C	0.11	ns	0.17	ns			-0.34	*	-0.22	*	-0.10	ns
CIDG	DS	0.33	**	**-0.36**	***	**-0.67**	****			**0.60**	****	**0.70**	****
	C	0.08	ns	0.18	ns	0.31	*			0.10	ns	0.05	ns
GC	DS	0.34	**	**-0.51**	****	**-0.62**	****	**0.71**	****			**0.76**	****
	C	0.04	ns	0.11	ns	0.08	ns	0.05	ns			0.19	ns
yield	DS	0.33	**	**-0.54**	****	**-0.69**	****	**0.82**	****	**0.86**	****		
	C	0.03	ns	0.13	ns	0.03	ns	0.21	ns	**0.59**	****		
			2014										

[a] *RLWC* relative leaf water content, *LT* leaf temperature, *CIDL* carbon isotope discrimination of leaf, *CIDG* carbon isotope discrimination of grain, *GC* ground cover, *yield* grain yield. [b] Treatments, drought stress (DS), control (C). [c] r Correlation coefficient. [d] Statistical significance as indicated by p-value, *ns* non-significant: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Bold data display correlations > r = 0.50.

4.2.3 Phenotypic correlation of drought-related parameters and spectral indices

Selected indices from the VIS and NIR region, originating from the passive and active sensors, have been validated with respect to their ability to estimate drought-related parameters such as RLWC, LT, CIDL, CIDG, GC, and grain yield. At the heading and grain-filling stages, both sensor systems demonstrated similar capabilities with respect to estimating drought-related parameters. However, at anthesis, the passive sensors showed stronger relationships to the measured parameters compared with the active sensors.

Furthermore, during anthesis and grain filling, the normalized water indices (NWI – 1 - 4), which could only be calculated using the broad wavelength range of the passive sensor, demonstrated similar or stronger relationships to the drought-related parameters, GC and grain yield compared with the other indices (Table 11). Across all three growth stages and both experimental years, the active sensors showed a slightly stronger relationship to RLWC than the passive sensor. When comparing the heading, anthesis, and grain-filling stages for both experimental years, measurements during anthesis and grain filling provided the closest relationships (Table 11). Measurements conducted by the passive sensor tended to be more stable for all three growth stages, especially during anthesis.

Table 11: Correlations of drought-related parameters, yield and selected indices of passive and active sensors in winter wheat in drought and control environments for heading, anthesis, grain filling.

heading

		RLWC				LT				CIDL				CIDG				GC				yield			
		´14		´15		´14		´15		´14		´15		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r	r	sig.	r	sig.	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
P_R$_{760}$/R$_{670}$	DS	0.60	****	0.54	****	-0.55	****	-0.89	****	0.62	****	0.64	****	0.72	****	0.72	****	0.31	*	0.73	****	0.74	****	0.85	****
	C	-0.01	ns	-0.11	ns	0.05	ns	-0.02	ns	0.07	ns	0.13	ns	0.20	ns	0.11	ns	-0.10	ns	0.29	**	-0.12	ns	-0.05	ns
P_R$_{774}$/R$_{656}$	DS	0.43	****	0.48	****	-0.50	****	-0.77	****	0.48	****	0.66	****	0.56	****	0.61	****	0.17	****	0.78	****	0.70	****	0.84	****
	C	0.31	*	-0.11	ns	-0.26	ns	0.08	ns	-0.08	ns	0.04	ns	0.07	ns	0.16	ns	-0.10	ns	0.21	ns	-0.18	ns	0.10	ns
P_R$_{760}$/R$_{730}$	DS	0.33	*	0.30	****	-0.54	****	-0.15	****	0.46	****	0.38	****	0.50	****	0.23	****	0.24	*	0.50	****	0.64	****	0.47	****
	C	0.29	ns	-0.07	ns	-0.16	ns	-0.23	*	0.06	ns	0.06	ns	0.05	ns	0.11	ns	0.01	ns	0.13	ns	-0.12	ns	0.00	ns
P_R$_{730}$/R$_{760}$	DS	-0.62	****	0.55	****	0.64	****	-0.89	****	-0.71	****	0.64	****	-0.78	****	0.73	****	-0.48	****	-0.48	****	-0.85	****	0.84	****
	C	0.01	ns	-0.11	ns	-0.15	ns	0.06	ns	-0.04	ns	0.14	ns	-0.19	ns	0.09	ns	0.13	ns	0.31	**	0.20	ns	-0.07	ns
P_NDVI	DS	0.64	****	0.52	****	-0.60	****	-0.87	****	0.71	****	0.67	****	0.79	****	0.68	****	0.53	****	0.77	****	0.80	****	0.84	****
	C	-0.05	ns	-0.09	ns	0.11	ns	-0.04	ns	0.04	ns	0.10	ns	0.25	ns	0.10	ns	-0.11	ns	0.27	**	-0.17	ns	-0.08	ns
P_WI	DS	-0.37	**	-0.35	****	0.58	****	0.21	ns	-0.57	****	-0.42	****	-0.58	****	-0.33	****	-0.21	ns	-0.54	****	-0.65	****	-0.56	****
	C	0.27	ns	0.22	ns	0.19	ns	0.30	ns	0.14	ns	0.26	ns	-0.17	ns	-0.05	ns	0.12	ns	0.03	ns	0.17	ns	0.05	ns
P_NWI-1	DS	-0.37	***	-0.35	**	0.58	****	0.21	ns	-0.57	****	-0.43	****	-0.58	****	-0.33	**	-0.21	ns	-0.21	ns	-0.65	****	-0.57	****
	C	-0.27	ns	0.22	ns	0.18	ns	0.31	ns	0.14	ns	-0.26	ns	-0.16	ns	0.21	ns	0.12	ns	0.19	ns	0.17	ns	0.22	ns
P_NWI-2	DS	-0.39	****	-0.32	**	0.57	****	0.17	ns	-0.57	****	-0.41	****	-0.57	****	-0.29	**	-0.21	ns	-0.20	ns	-0.63	****	-0.51	****
	C	-0.26	ns	0.23	ns	0.14	ns	0.31	ns	0.20	ns	-0.30	ns	-0.17	ns	-0.22	ns	0.13	ns	0.21	ns	0.16	ns	0.23	ns
P_NWI-3	DS	-0.36	***	-0.35	**	0.58	****	0.22	ns	-0.56	****	-0.42	****	-0.57	****	-0.34	**	-0.21	ns	-0.21	ns	-0.66	****	-0.58	****
	C	-0.26	ns	0.23	ns	0.21	ns	0.30	ns	0.13	ns	-0.26	ns	-0.16	ns	-0.23	ns	0.11	ns	0.20	ns	0.17	ns	0.22	ns
P_NWI-4	DS	-0.37	***	-0.33	**	0.57	****	0.18	ns	-0.56	****	-0.41	****	-0.58	****	-0.30	**	-0.20	ns	-0.21	ns	-0.64	****	-0.54	****
	C	-0.27	ns	0.21	ns	0.16	ns	0.31	*	0.17	ns	-0.29	ns	-0.16	ns	-0.22	ns	0.11	ns	0.20	ns	0.17	ns	0.23	ns
ALS_WI	DS	0.12	ns	0.42	****	-0.24	*	-0.71	****	0.24	*	0.46	*	0.23	**	0.53		0.24	*	0.61	****	0.27	ns	0.63	*
	C	0.39	*	-0.12	ns	0.44	**	-0.11	ns	-0.07	ns	0.10	ns	0.08	ns	-0.02	ns	0.05	ns	0.23	*	-0.04	ns	-0.03	ns
ALS_R$_{760}$/R$_{730}$	DS	0.65	****	0.51	****	-0.62	****	-0.79	****	0.69	****	0.52	****	0.79	****	0.58		0.44	****	0.65	****	0.80	****	0.72	****
	C	-0.03	ns	-0.12	ns	0.10	ns	-0.10	ns	0.10	ns	0.20	ns	0.33	*	-0.03	ns	-0.12	ns	0.25	*	0.08	ns	-0.06	ns
ALS_R$_{730}$/R$_{760}$	DS	-0.65	****	-0.50	****	0.63	****	0.79	****	-0.70	****	-0.53	****	-0.80	****	-0.55		-0.48	****	-0.65	****	-0.79	****	-0.70	****
	C	0.03	ns	0.12	ns	-0.10	ns	0.11	ns	-0.09	ns	-0.20	ns	-0.32	*	0.03	ns	0.12	ns	0.25	*	-0.05	ns	0.06	ns
CC_R$_{760}$/R$_{670}$	DS	NA		0.58	****	NA		-0.82	****	NA		0.57	****	NA		0.70	****	NA		0.68	****	NA		0.80	****
	C	NA		-0.17	ns	NA		0.01	ns	NA		0.19	ns	NA		0.02	ns	NA		0.22	*	NA		-0.11	ns
CC_R$_{760}$/R$_{730}$	DS	NA		0.52	****	NA		-0.85	****	NA		0.56	****	NA		0.66	****	NA		0.67	****	NA		0.78	****
	C	NA		-0.12	ns	NA		-0.00	ns	NA		0.19	ns	NA		0.05	ns	NA		0.10	ns	NA		0.04	ns
CC_R$_{730}$/R$_{760}$	DS	NA		0.60	****	NA		-0.80	****	NA		0.58	****	NA		0.70	****	NA		0.68	****	NA		0.80	****
	C	NA		-0.17	ns	NA		0.02	ns	NA		0.17	ns	NA		0.01	ns	NA		0.25	*	NA		-0.16	ns
GS_NDVI	DS	0.62	****	0.59	****	-0.61	****	-0.79	****	0.57	****	0.57	****	0.69	****	0.65	****	0.42	****	0.70	****	0.70	****	0.77	****
	C	-0.07	ns	-0.02	ns	0.10	ns	-0.01	ns	0.16	ns	0.22	ns	0.20	ns	-0.02	ns	0.04	ns	0.18	ns	-0.12	ns	-0.09	ns
GS_R$_{774}$/R$_{656}$	DS	0.61	****	0.58	****	-0.58	****	-0.77	****	0.56	****	0.55	****	0.67	****	0.66	****	0.35	*	0.68	****	0.63	****	0.78	****
	C	-0.03	ns	-0.02	ns	0.08	ns	-0.01	ns	0.20	ns	0.23	*	0.20	ns	-0.02	ns	0.07	ns	0.18	ns	-0.08	ns	-0.09	ns

		anthesis																							
		RLWC				LT				CIDL				CIDG				GC				yield			
		´14		´15		´14		´15		´14		´15		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
P_R$_{760}$/R$_{670}$	DS	0.55	****	0.47	****	-0.73	****	-0.77	****	0.70	****	0.65	****	0.79	****	0.61	****	0.83	****	0.83	****	0.51	****	0.84	****
	C	-0.27	ns	-0.18	ns	-0.29	*	-0.16	ns	0.10	ns	0.02	ns	0.23	ns	0.10	ns	-0.26	ns	0.14	ns	-0.16	ns	-0.16	ns
P_R$_{774}$/R$_{656}$	DS	0.51	****	0.42	****	-0.65	****	-0.74	****	0.63	****	0.62	****	0.71	****	0.61	****	0.75	****	0.77	****	0.76	****	0.80	****
	C	-0.19	ns	-0.11	ns	-0.43	**	-0.06	ns	0.10	ns	0.11	ns	0.24		-0.17	ns	-0.16	ns	0.08	ns	-0.09	ns	-0.10	ns
P_R$_{760}$/R$_{730}$	DS	0.54	****	0.45	****	-0.73	****	-0.64	****	0.71	****	0.64	****	0.76	****	0.52	****	0.84	****	0.81	****	0.84	****	0.82	****
	C	-0.22	ns	-0.15	ns	-0.38	*	-0.06	ns	0.10	ns	0.04	ns	0.15	ns	0.03	ns	-0.23	ns	0.17	ns	-0.19	ns	-0.27	*
P_R$_{730}$/R$_{760}$	DS	-0.53	****	0.44	****	0.77	****	-0.26	ns	-0.73	****	0.19	ns	-0.78	****	0.30	*	-0.87	****	0.16	ns	-0.85	****	0.20	ns
	C	0.24	ns	0.16	ns	0.30	*	0.14	ns	-0.12	ns	0.00	ns	-0.19	ns	-0.02	ns	0.22	s	-0.19	ns	0.20	ns	0.24	ns
P_NDVI	DS	0.54	****	0.42	****	-0.72	****	-0.74	****	0.69	****	0.63	****	0.76	****	0.59	****	0.82	****	0.81	****	0.81	****	0.82	****
	C	-0.25	ns	0.15	ns	-0.33	*	0.22	ns	0.10	ns	0.10	ns	0.26		0.10	ns	-0.21	ns	0.16	ns	0.13	ns	0.40	**
P_WI	DS	-0.60	****	-0.49	****	0.82	****	0.70	****	-0.80	****	-0.66	****	-0.82	****	-0.63	****	-0.85	****	0.81	****	-0.89	****	-0.86	****
	C	0.25	ns	0.10	ns	0.31	ns	0.10	ns	-0.11	ns	-0.13	ns	-0.28	ns	-0.22	ns	0.19	ns	-0.21	ns	-0.21	**	-0.37	**
P_NWI-1	DS	-0.60	****	-0.49	****	0.82	****	0.69	****	-0.80	****	-0.66	****	-0.82	****	-0.63	****	-0.85	****	-0.81	****	-0.89	****	-0.86	****
	C	0.25	ns	0.10	ns	0.31	ns	0.10	ns	-0.11	ns	-0.13	ns	-0.28	ns	-0.22	ns	0.19	ns	-0.21	ns	-0.21	**	-0.37	**
P_NWI-2	DS	-0.62	****	-0.50	****	0.80	****	0.74	****	-0.80	****	-0.66	****	-0.82	****	-0.65	****	-0.86	****	-0.82	****	-0.88	****	-0.88	****
	C	0.25	ns	0.10	ns	0.30	ns	0.11	ns	-0.14	ns	-0.11	ns	-0.28	ns	-0.21	ns	0.21	ns	-0.16	ns	-0.24	**	-0.36	**
P_NWI-3	DS	-0.60	****	-0.50	****	0.81	****	0.65	****	-0.81	****	-0.64	****	-0.82	****	-0.61	****	-0.85	****	-0.80	****	-0.90	****	-0.85	****
	C	0.26	ns	0.10	ns	0.30	ns	0.11	ns	-0.12	ns	-0.13	ns	-0.28	ns	-0.23	ns	0.20	ns	-0.21	ns	-0.23	**	-0.36	**
P_NWI-4	DS	-0.61	****	-0.49	****	0.81	****	0.68	****	-0.79	****	-0.64	****	-0.81	****	-0.62	****	-0.85	****	-0.82	****	-0.88	****	-0.87	****
	C	0.24	ns	0.09	ns	0.31	ns	0.10	ns	-0.14	ns	-0.12	ns	-0.28	ns	-0.21	ns	0.19	ns	-0.20	ns	-0.23	**	-0.37	**
ALS_WI	DS	0.53	****	-0.33	*	-0.50	****	-0.54	****	0.62	****	0.28	*	0.69	****	0.38		0.71	****	0.42	****	0.69	****	0.43	****
	C	-0.10	ns	-0.02	ns	-0.15	ns	-0.14	ns	0.14	ns	0.11	ns	0.34	*	-0.01	ns	0.22	ns	-0.18	ns	0.20	ns	0.15	ns
ALS_R$_{760}$/R$_{730}$	DS	0.56	****	0.32	*	-0.60	****	-0.70	****	0.63	****	0.51	****	0.71	****	0.55	****	0.81	****	0.70	****	0.71	****	0.73	****
	C	-0.23	ns	-0.06	ns	-0.16	ns	-0.04	ns	0.19	ns	0.18	ns	0.33	*	0.01	ns	-0.06	ns	-0.23	ns	0.08	ns	0.07	ns
ALS_R$_{730}$/R$_{760}$	DS	-0.57	****	0.52	****	-0.65	****	-0.22	ns	-0.66	****	-0.18	ns	-0.74	****	0.19	ns	-0.82	****	0.15	ns	-0.73	****	0.18	ns
	C	-0.24	ns	0.07	ns	-0.13	ns	-0.04	ns	-0.19	ns	-0.17	ns	-0.32	*	0.01	ns	0.08	ns	0.24	ns	-0.05	ns	0.07	ns
CC_R$_{760}$/R$_{670}$	DS	NA		0.52	****	NA		-0.78	****	NA		0.58	****	NA		0.56	****	NA		0.75	****	NA		0.75	****
	C	NA		0.01	ns	NA		-0.16	ns	NA		0.17	ns	NA		0.06	ns	NA		-0.18	ns	NA		0.23	ns
CC_R$_{760}$/R$_{730}$	DS	NA		0.49	****	NA		-0.76	****	NA		0.57	****	NA		0.59	****	NA		0.77	****	NA		0.78	****
	C	NA		-0.05	ns	NA		-0.20	ns	NA		0.09	ns	NA		0.10	ns	NA		-0.12	ns	NA		0.17	ns
CC_R$_{730}$/R$_{760}$	DS	NA		0.53	****	NA		-0.78	****	NA		0.58	****	NA		0.60	****	NA		0.74	****	NA		0.76	****
	C	NA		0.01	ns	NA		-0.12	ns	NA		0.17	ns	NA		0.05	ns	NA		-0.17	ns	NA		0.21	ns
GS_NDVI	DS	0.59	****	0.49	****	-0.64	****	-0.70	****	0.65	****	0.56	****	0.70	****	0.51	****	0.73	****	0.68	****	0.71	****	0.69	****
	C	0.29	ns	0.01	ns	-0.27	ns	0.02	ns	0.21	ns	0.06	ns	0.20	ns	0.01	ns	-0.29	ns	-0.23	ns	0.12	ns	0.27	*
GS_R$_{774}$/R$_{656}$	DS	0.58	****	0.52	****	-0.64	****	-0.71	****	0.63	****	0.55	****	0.69	****	0.51	****	0.70	****	0.67	****	0.72	****	0.68	****
	C	0.24	ns	-0.00	ns	-0.30	ns	-0.00	ns	0.23	ns	0.05	ns	0.20	ns	0.01	ns	-0.26	ns	-0.24	ns	0.08	ns	0.27	*

grain filling

		RLWC				LT				CIDL				CIDG				GC				yield			
		´14		´15		´14		´15		´14		´15		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.	r	sig.
P_R_{760}/R_{670}	DS	0.33	***	0.34	***	-0.71	****	-0.65	****	0.75	****	0.61	****	0.78	****	0.61	****	0.45	****	0.50	****	0.81	****	0.84	****
	C	-0.27	*	-0.26	*	-0.36	*	0.23	ns	0.17	ns	0.18	ns	0.23	ns	0.11	ns	-0.26	ns	-0.04	ns	-0.16	ns	-0.05	ns
P_R_{774}/R_{656}	DS	0.33	***	0.34	**	-0.71	****	-0.66	****	0.75	****	0.61	****	0.78	****	0.61	****	0.75	****	0.63	****	0.81	****	0.84	****
	C	-0.26	*	-0.25	*	-0.37	*	0.21	ns	0.18	ns	0.18	ns	0.23	ns	0.10	ns	-0.25	nsn	-0.20	ns	-0.16	ns	-0.05	ns
P_R_{760}/R_{730}	DS	0.36	***	0.34	***	-0.73	****	-0.67	****	0.76	****	0.59	****	0.77	****	0.58	****	0.79	****	0.63	****	0.85	****	0.81	****
	C	-0.23	*	-0.15	ns	-0.37	*	0.08	ns	0.21	Ns	0.17	ns	0.17	ns	0.11	ns	-0.23	ns	-0.08	ns	-0.17	ns	0.01	ns
P_R_{730}/R_{760}	DS	-0.34	***	-0.31	***	0.75	****	0.65	****	-0.76	****	-0.58	****	-0.78	****	-0.55	****	-0.81	****	-0.62	****	-0.85	****	-0.82	****
	C	0.24	*	-0.16	ns	0.33	*	0.08	ns	-0.21	ns	-0.17	ns	-0.19	ns	0.11	ns	0.22	ns	-0.10	ns	-0.19	ns	0.00	ns
P_NDVI	DS	0.27	**	0.35	***	-0.72	****	-0.66	****	0.76	****	0.58	****	0.79	****	0.62	****	0.77	****	0.47	****	0.80	****	0.84	****
	C	-0.28	**	-0.29	**	-0.22	*	0.21	*	0.16	ns	0.18	ns	0.25	ns	-0.02	ns	-0.24	ns	0.02	ns	-0.17	ns	-0.08	ns
P_WI	DS	-0.36	**	-0.38	****	0.76	****	0.60	****	-0.75	****	-0.61	****	-0.78	****	-0.63	****	0.47	****	-0.67	****	-0.84	****	-0.86	****
	C	0.22	ns	-0.01	ns	0.30	ns	-0.06	ns	-0.26	ns	-0.12	ns	-0.23	ns	0.01	ns	0.19	ns	0.02	ns	0.22	ns	-0.09	ns
P_NWI-1	DS	-0.36	**	-0.38	**	0.76	****	0.60	****	-0.74	****	-0.61	****	-0.78	****	-0.63	****	0.47	****	-0.69	****	-0.84	****	-0.86	****
	C	0.22	ns	-0.01	ns	0.31	ns	-0.06	ns	-0.26	ns	-0.11	ns	-0.22	ns	0.01	ns	0.19	ns	0.02	ns	0.22	ns	-0.10	ns
P_NWI-2	DS	-0.35	**	-0.37	****	0.75	****	0.64	****	-0.75	****	-0.61	****	-0.80	****	-0.65	****	0.46	****	-0.65	****	-0.82	****	-0.87	****
	C	0.23	ns	-0.05	ns	0.31	ns	-0.05	ns	-0.29	ns	-0.14	ns	-0.22	ns	0.02	ns	0.20	ns	0.03	ns	0.23	ns	-0.10	ns
P_NWI-3	DS	-0.37	**	-0.39	****	0.76	****	0.60	****	-0.74	****	-0.60	****	-0.78	****	-0.62	****	0.48	****	-0.70	****	-0.85	****	-0.86	****
	C	0.23	ns	-0.06	ns	0.30	ns	-0.08	ns	-0.26	ns	-0.13	ns	-0.23	ns	0.02	ns	0.18	ns	0.02	ns	0.22	ns	-0.10	ns
P_NWI-4	DS	-0.35	**	-0.38	**	0.76	****	0.62	****	-0.74	****	-0.61	****	-0.78	****	-0.64	****	0.45	****	-0.68	****	-0.83	****	-0.87	****
	C	0.21	ns	-0.06	ns	0.31	ns	-0.06	ns	-0.29	ns	-0.14	ns	-0.22	ns	0.02	ns	0.20	ns	0.02	ns	0.23	ns	-0.11	ns
ALS_WI	DS	0.34	**	0.33	**	-0.45	****	-0.57	****	0.60	****	0.48	****	0.68	****	0.54	****	0.59	****	0.36	**	0.69	****	0.64	****
	C	-0.11	ns	-0.09	ns	-0.14	ns	0.02	ns	0.13	ns	0.07	ns	0.34	*	-0.02	ns	0.20	ns	-0.07	ns	0.21	ns	-0.03	ns
ALS_R_{760}/R_{730}	DS	0.35	***	0.34	***	-0.62	****	-0.67	****	0.76	****	0.55	****	0.79	****	0.51	****	0.73	****	0.41	****	0.80	****	0.72	****
	C	-0.23	ns	-0.07	ns	-0.30	***	0.08	ns	0.20	ns	0.07	ns	0.33	*	-0.03	ns	-0.08	ns	-0.09	ns	0.08	ns	-0.06	ns
ALS_R_{730}/R_{760}	DS	-0.32	**	-0.31	**	0.63	****	0.65	****	0.76	****	-0.54	****	-0.80	****	-0.50	****	-0.74	****	-0.38	****	-0.80	****	-0.71	****
	C	0.24	ns	0.09	ns	0.25	*	-0.08	ns	-0.20	ns	-0.07	ns	-0.32	*	0.03	ns	0.10	ns	0.09	ns	-0.05	ns	0.06	ns
CC_R_{760}/R_{670}	DS	NA		0.48	***	NA		-0.74	****	NA		0.56	****	NA		0.70	****	NA		0.49	****	NA		0.72	****
	C	NA		-0.12	ns	NA		0.27	*	NA		0.12	ns	NA		0.02	ns	NA		-0.13	ns	NA		-0.11	ns
CC_R_{760}/R_{730}	DS	NA		0.39	***	NA		-0.71	****	NA		0.53	****	NA		0.66	****	NA		0.45	****	NA		0.78	****
	C	NA		-0.13	ns	NA		0.18	ns	NA		0.08	ns	NA		0.05	ns	NA		-0.11	ns	NA		0.04	ns
CC_R_{730}/R_{760}	DS	NA		0.48	***	NA		-0.74	****	NA		0.57	****	NA		0.70	****	NA		0.49	****	NA		0.71	****
	C	NA		-0.11	ns	NA		0.27	*	NA		0.11	ns	NA		0.01	ns	NA		-0.11	ns	NA		-0.16	ns
GS_NDVI	DS	0.29	**	0.38	***	-0.62	****	-0.73	****	0.67	****	0.55	****	0.69	****	0.65	****	0.69	****	0.47	****	0.70	****	0.77	****
	C	-0.29	ns	-0.01	ns	-0.32	*	0.09	ns	0.08	ns	0.02	ns	0.20	ns	-0.02	ns	-0.27	ns	0.03	ns	-0.12	ns	-0.09	ns
GS_R_{774}/R_{656}	DS	0.37	**	0.43	***	-0.59	****	-0.73	****	0.68	****	0.54	****	0.67	****	0.54	****	0.64	****	0.48	****	0.70	****	0.78	****
	C	-0.24	ns	-0.01	ns	-0.40	*	0.09	ns	0.07	ns	0.03	ns	0.20	ns	-0.02	ns	0.25	ns	0.03	ns	-0.08	ns	-0.09	ns

[a] *P* passive sensor, *WI* water index, *NWI* normalized water index, *ALS* active flash light, *CC* Crop Circle, *GS* GreenSeeker; [b] *T* trait, [c] correlation coefficient, [d] statistical significance as indicated by p-value *ns* non-significant: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, *NA* not ascertained, passive indices are stronger than active; water indices are stronger than all other indices; active indices are stronger than passive

4.2.4 Heritability of drought-related parameters and spectral indices

In the drought environment, heritability for RLWC was moderate for both years (Table 12). During 2014, heritability was lower in the control compared with the drought environment. During 2015, under drought conditions, the genetic variance was estimated to be 0; hence, no heritability for RLWC could be calculated. Leaf temperature measurements, conducted by IR-sensors, showed moderate heritability under drought conditions in 2014 and under control conditions in 2015. Moreover, the heritability of the carbon isotope discrimination was strong during 2014 for both environments and was moderate during 2015. Grain yield demonstrated a strong heritability under drought and control conditions for both experimental years. The studied water indices had moderate to strong heritabilities that were comparable with grain yield heritability under drought conditions during 2015 and 2014 (Table 12). The vegetation indices, determined by the passive sensor, demonstrated moderate heritabilities under drought conditions. Vegetation indices determined by the active sensors showed moderate heritabilities (ALS and GreenSeeker devices) and strong heritability (Crop Circle). For all active sensors, in most cases, the genetic correlation was estimated to be 0 in the control environment.

Table 12: Heritability of drought-related parameters and spectral reflectance indices at anthesis under drought and control conditions.

Trait[a]	2014		2015	
	drought	control	drought	control
	h^{2b}	h^2	h^2	h^2
RLWC	0.66	0.32	0.57	0
LT	0.52	0	0	0.42
CIDL	0.65	0.82	0.28	0.42
CIDG	0.72	0.86	0.44	0.39
GC	0.23	0	0	0.62
yield	0.62	0.74	0.61	0.83
P_WI	0.53	0.24	0.60	0.74
P_NWI-1	0.52	0.24	0.61	0.74
P_NWI-2	0.54	0.19	0.46	0.80
P_NWI-3	0.54	0.35	0.49	0.75
P_NWI-4	0.54	0.13	0.43	0.78
P_760/670	0.41	0	0.45	0
P_774/656	0.34	0	0.31	0
P_760/730	0.22	0	0.49	0
P_730/760	0.19	0	0.18	0
P_NDVI	0.42	0	0.16	0.54
ALS_WI	0.78	0	0.14	0
ALS_760/730	0.45	0.48	0.41	0
ALS_730/760	0.35	0.48	0.67	0
GS_NDVI	0.58	0	0.11	0.54
GS_774/656	0.70	0	017	0.54
CC_730/670	NA	NA	0.75	0.23
CC_760/730	NA	NA	0.66	0.41
CC_760/670	NA	NA	0.61	0.11

[a] *RLWC* relative leaf water content (%), *LT* leaf temperature FLIR-camera (C°), *LT* leaf temperature IR-sensors (C°), *CIDL* carbon isotope discrimination of leaf (‰), *CIDG* carbon isotope discrimination of grain (‰), *GC* ground cover (%), *yield* grain yield (dt/ha), *WI* water index, *NWI1-4* normalized water indices, *P* passive, *ALS* active flash light, *GS* GreenSeeker, *CC* Crop Circle.
[b] *Heritability*
NA not ascertained

4.3 Section III: Can we scale up (extrapolate) drought stress in winter wheat from pots to the field?

4.3.1 Impact of drought on drought-related parameters under controlled and field conditions

In this 2-year experiment, drought stress was induced on six selected wheat varieties under field and controlled conditions, the latter including two different pot systems. For all the environments and varieties the drought-related parameters of plants, i.e., RLWC, LT, CID, NDVI, and grain yield, were measured at the reproductive stage (Table 13). The induced drought stress led to a statistically significant impairment of all five morphophysiological parameters on the winter wheat plants. A significant decrease in the RLWC, CID, NDVI, and grain yield, as well as a significant increase in LT, was observed relative to the control plants for each tested variety.

Table 13: Average values from the two growth cycles (field: 2014/2015, pot: 2015/2016) under drought and control conditions for grain yield, RLWC, LT, CID, and NDVI of four winter wheat varieties. Phenotypic correlations (Pearson correlation coefficient, *r*) of all the traits with grain yield and roots (field: 90–120 cm; tubes 80–110 cm) under drought are shown (corr. yield) with the significant correlations indicated: * $P > 0.05$; ** $P > 0.005$; *** $P > 0.001$. Means followed by a different letter within rows are significantly different ($P < 0.05$) according to the l.s.d. test.

		Anapolis		Genius		Patras		Hyland		Hylux		Hystar		corr. yield	corr. roots
Trait	Env.^{A}	DS	C	DS	C	DS	C	DS	C	DS	C	DS	C	DS	DS
Grain yield [dt/ha]	Field	104.22^{a}	151.11^{a}	103.37^{a}	152.01^{a}	93.26^{b}	154.00^{a}	114.94^{a}	151.00^{a}	109.87^{a}	173.76^{a}	120.00^{b}	133.55^{b}		0.78
	Tube	8.57^{abc}	12.50^{b}	6.80^{c}	10.90^{a,b}	6.94^{c}	10.82^{a,b}	10.54^{a}	12.26^{b}	7.48^{cb}	9.87^{a}	9.45^{ab}	11.89^{ab}		0.54
Grain yield [g/plant]	Pot	3.31a^{a}	4.44^{a}	3.47a^{a}	4.84^{a}	3.11^{bc}	4.58^{a}	2.30^{c}	4.30^{a}	2.55^{bc}	4.58^{a}	2.28^{c}	4.40^{a}		
RLWC [%]	Field	55.43^{a}	89.21^{a}	55.95^{a}	91.47^{a,b}	54.20^{b}	92.30^{a,b}	58.71^{a}	90.30^{a,b}	57.66^{a}	93.34^{a,b}	62.16^{a}	89.62^{b}	0.46**	0.57
	Tube	44.98^{c}	94.59^{a,b}	40.73^{c}	95.15^{b}	46.45^{d}	81.96^{a}	48.63^{a}	94.21^{a,b}	48.74^{b}	97.18^{a,b}	52.66^{ab}	95.80^{ab}	0.48**	0.81
	Pot	53.77^{a}	95.56^{a,c}	56.14^{a}	96.22^{a,c}	42.26^{a}	95.05^{c}	44.37^{b}	94.80^{a,c}	41.38^{b}	91.73^{a}	43.03^{b}	94.30^{b}	0.43**	
LT [°C]	Field	28.83^{a}	24.15^{a}	31.41^{a}	27.20^{a}	30.56^{a}	26.75^{a}	28.94^{a}	24.40^{a}	31.19^{a}	26.76^{a}	29.11^{a}	24.25^{a}	-0.50***	-0.18
	Tube	21.23^{ab}	19.74^{a}	21.77^{b}	20.27^{a}	21.42^{ab}	19.54^{a}	20.60^{a}	19.68^{a}	21.01^{ab}	20.38^{a}	20.62^{a}	19.60^{a}	-0.80***	-0.70
	Pot	22.65^{a}	21.33^{a}	23.31^{a}	21.59^{a}	22.95^{a}	21.03^{a}	22.85^{a}	21.03^{a}	23.29^{a}	21.48^{a}	22.77^{a}	21.17^{a}	-0.59***	
CID [‰]	Field	18.16^{abc}	20.55^{a}	17.79^{cb}	20.62^{ab}	17.05^{c}	20.73^{abc}	18.93^{ab}	21.18^{bc}	19.61^{a}	21.28^{c}	18.89^{ab}	20.93^{abc}	0.74***	0.97**
	Tube	19.00^{b}	22.19^{abc}	20.59^{ab}	24.18^{abc}	18.80^{b}	21.20^{ab}	20.73^{ab}	21.70^{abc}	22.84^{a}	24.67^{ac}	20.91^{ab}	22.12^{abc}	0.78***	0.51
	Pot	19.88^{ab}	21.37^{a}	21.60^{a}	22.78^{a}	19.40^{ab}	21.22^{a}	17.89^{bc}	20.97^{a}	19.69^{ab}	22.76^{a}	17.45^{b}	21.34^{a}	0.77 ***	
NDVI	Field	0.45^{a}	0.67^{a}	0.55^{a}	0.62^{a}	0.53^{a}	0.63^{a}	0.56^{a}	0.64^{a}	0.51^{a}	0.65^{a}	0.53^{a}	0.67^{a}	0.68***	0.28
	Tube	0.57^{ab}	0.63^{ab}	0.53^{a}	0.63^{b}	0.56^{b}	0.61^{a}	0.58^{ab}	0.63^{ab}	0.59^{ab}	0.64^{ab}	0.52^{ab}	0.60^{ab}	-0.04	0.23
	Pot	0.33^{ab}	0.41^{b}	0.32^{a}	0.38^{b}	0.31^{ab}	0.41^{b}	0.34^{b}	0.40^{b}	0.34^{ab}	0.33^{b}	0.28^{b}	0.40^{a}	0.24	

4.3.2 Impact of different environments on plant performance under drought stress and well-watered conditions

Six selected wheat varieties were grown under controlled conditions in small pots and large tubes. Additionally, the same varieties were grown under field conditions. Irrespective of the environment, drought was induced during the reproductive stage at the onset of anthesis. Except for grain yield, all the measured parameters showed a significant difference among the two pot systems and the field under drought conditions (Table 14). Under controlled conditions, the growth environment also had a significant impact on the drought-related parameters (except for grain yield and RLWC). The influence of the growth environment was clearly demonstrated by calculating the differences in the CID and LT values between the tubes and pots under the control conditions (Figure 8). Apart from Patras, the varieties showed a reduced CID in the small pots. Furthermore, for each level of variety and growth environment, the differences between control and the drought plant response data were calculated for RLWC, LT, CID, and NDVI (Figure 9). The growth environment demonstrated a great impact on the measured drought-related parameters. Those varieties which showed a high tolerance for drought under field conditions showed a low tolerance in small pots under controlled conditions, and vice versa (Figure 9 B, C). Additionally, the ranking of the individual varieties was affected by the growing environment (Table 15).

Table 14: Mean value comparison indicated separately for each growth environment and measured parameter over two experimental years (field. 2014/2015; pot and tubes: 2015/2016). *DS* drought stress, *C* control, *RLWC* relative leaf water content, *LT* leaf temperature, *CID* carbon isotope discrimination, *NDVI* normalized difference vegetation index. Different letters indicate significant differences at $P \leq 0.05$.

	Environment		Standardized yield			RLWC			LT			CID			NDVI		
DS	field	Mean	0.83			57.37					29.59		18.30			0.51	
		SE[a]	*0.22*			*3.05*					*0.69*		*0.55*			*0.22*	
	tube	Mean	0.85				47.50		21.05			20.81			0.56		
		SE	*0.26*				*2.74*		*0.58*			*0.71*			*0.29*		
	pot	Mean	0.77				47.44			22.90			19.73				0.32
		SE	*0.25*				*2.05*			*0.69*			*0.84*				*0.24*
C	field	Mean	1.18			90.60					25.14		22.41			0.59	
		SE	*0.13*			*2.33*					*0.55*		*0.54*			*0.11*	
	tube	Mean	1.15			92.54			19.77			23.89			0.62		
		SE	*0.13*			*1.27*			*0.53*			*1.03*			*0.14*		
	pot	Mean	1.23			93.73				21.21			22.45				0.39
		SE	*0.16*			*2.12*				*0.66*			*1.44*				*0.10*
			a	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**

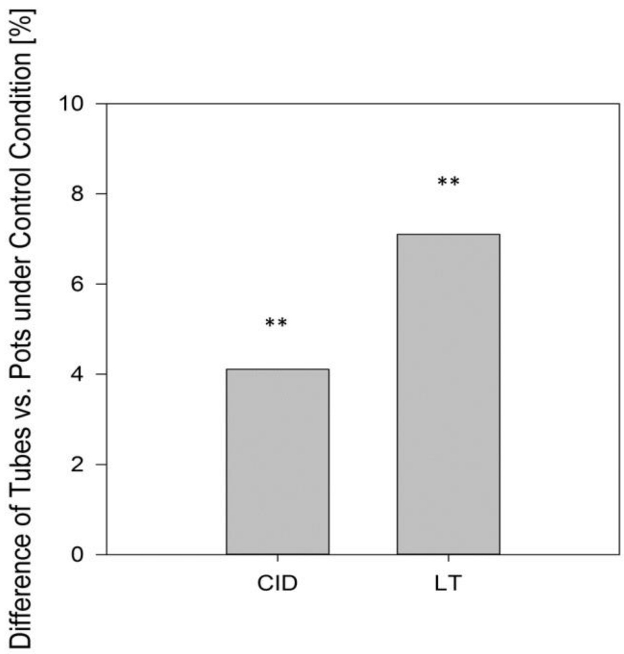

Figure 8: Difference of CID and LT values between pots and tubes under control conditions.
** P > 0.005.

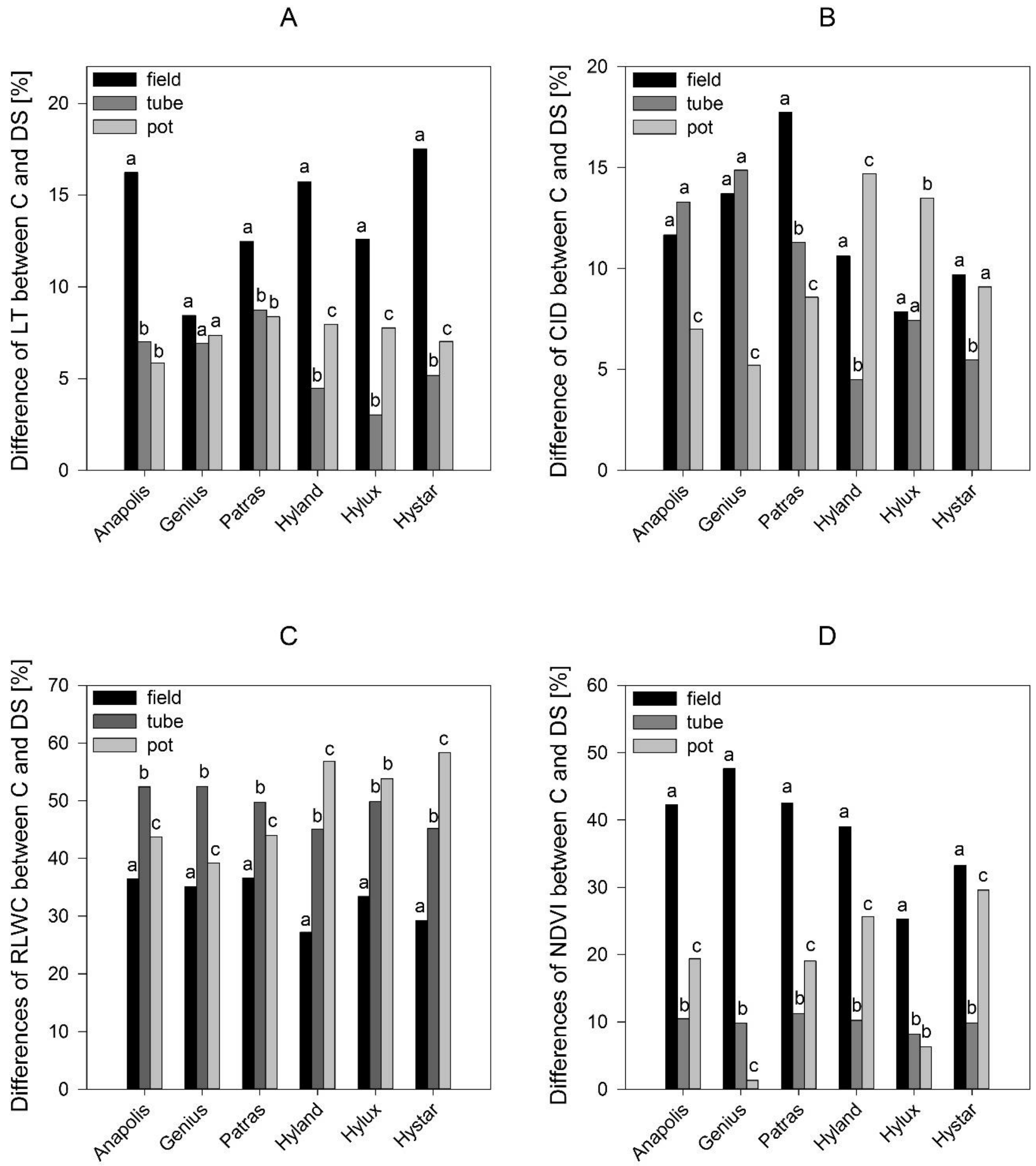

Figure 9: Differences between control (C) and drought stress (DS) treatments for each wheat variety, for drought-related parameters LT, CID, RLWC and NDVI for the growth environments field, pot and tube over two experimental years.

Table 15: Statistical ranking of varieties under drought stress for yield, RLWC, LT, CID and NDVI for the growth environments field, tube and pot. Different letters within columns are significantly different ($P < 0.05$) according to Duncan's. test.

Ranking	field	tube	pot
yield			
1	Hystar^{a}	Hyland^{a}	Genius^{a}
2	Hyland^{a}	Hystar^{ab}	Anapolis^{a}
3	Hylux^{a}	Anapolis^{abc}	Patras^{ab}
4	Anapolis^{a}	Hylux^{bc}	Hylux^{bc}
5	Genius^{a}	Patras^{c}	Hyland^{c}
6	Patras^{b}	Genius^{c}	Hystar^{c}
RLWC			
1	Hyland^{a}	Hyland^{a}	Genius^{a}
2	Hystar^{a}	Hystar^{ab}	Patras^{a}
3	Hylux^{a}	Hylux^{b}	Anapolis^{a}
4	Genius^{a}	Anapolis^{c}	Hylux^{b}
5	Anapolis^{a}	Genius^{c}	Hyland^{b}
6	Patras^{b}	Patras^{d}	Hystar^{b}
LT			
1	Anapolis^{a}	Hyland^{a}	Anapolis^{a}
2	Hyland^{a}	Hystar^{a}	Hystar^{a}
3	Hystar^{a}	Hylux^{ab}	Hyland^{a}
4	Genius^{a}	Anapolis^{ab}	Patras^{a}
5	Patras^{a}	Patras^{ab}	Hylux^{a}
6	Hylux^{a}	Genius^{b}	Genius^{a}
CID			
1	Hylux^{a}	Hylux^{a}	Genius^{a}
2	Hyland^{ab}	Hystar^{ab}	Anapolis^{ab}
3	Hystar^{ab}	Hyland^{ab}	Hylux^{abc}
4	Anapolis^{abc}	Genius^{ab}	Patras^{abc}
5	Genius^{bc}	Anapolis^{b}	Hyland^{bc}
6	Patras^{c}	Patras^{b}	Hystar^{c}
NDVI			
1	Hylux^{a}	Genius^{a}	Genius^{a}
2	Hystar^{a}	Hylux^{ab}	Hylux^{ab}
3	Genius^{a}	Anapolis^{ab}	Anapolis^{ab}
4	Patras^{a}	Hystar^{ab}	Patras^{ab}
5	Anapolis^{a}	Hyland^{ab}	Hyland^{b}
6	Hyland^{a}	Patras^{b}	Hystar^{b}

4.3.3 Impact of different environments on plant performance under drought stress and well-watered conditions

To compare the yield performance of the different wheat varieties in different growth environments under drought stress, their standardized yields, over 2 years within each treatment, was calculated (Figure 10). Under the field conditions, the hybrids Hyland, Hylux, and Hystar showed an above-average yield compared to Anapolis, Genius, and Patras under drought stress. By contrast, when grown in the small pots, the hybrids had a below average yields when compared to the other varieties. However, the yield of varieties grown in the tubes was similar to that in the field. Under well-watered conditions, a similar trend could be observed (Figure 10).

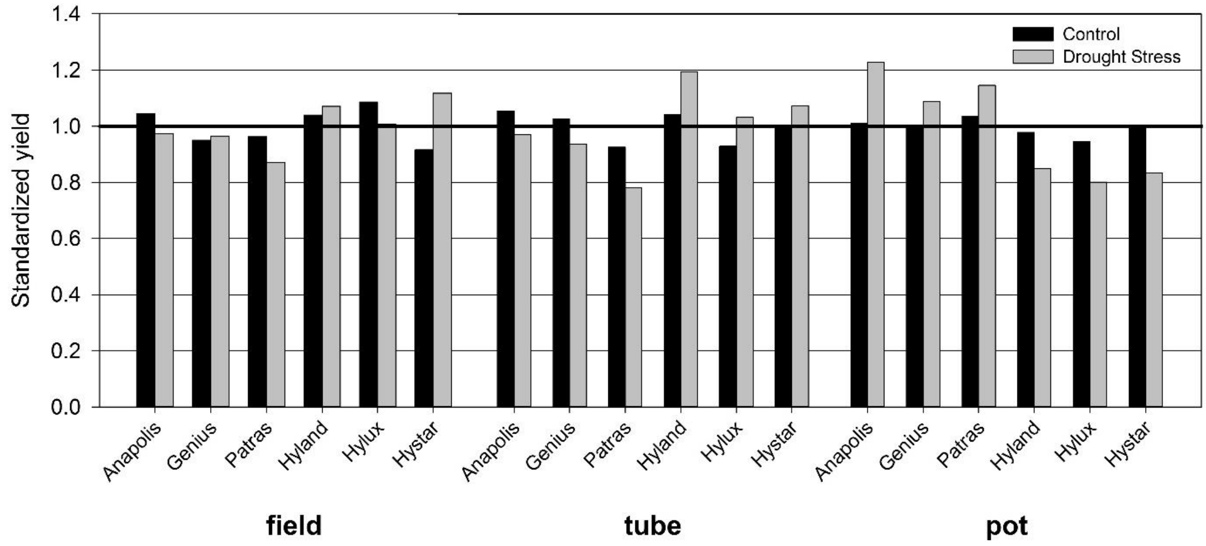

Figure 10: Standardized yield means, standardized over of two years, within each treatment (control, drought) for each wheat variety.

4.3.4 Rooting depth under drought conditions in field plots and tubes

Root mass at depth is recognized as a main factor contributing to the drought tolerance of plants. In this context, and to identify possible drought tolerance mechanisms, the focus of the root distribution analyses was on the deepest layers of the soil core: 90–120 cm in the field and 80–110 cm in the tubes. Irrespective of the growth environment, the varieties Anapolis, Genius, and Patras had a significantly lower root mass at these depths than did the hybrids Hyland, Hylux, and Hystar (Figure 11). Root DW at the deepest part of the soil core was negatively associated with LT whereas it positively associated with RLWC, NDVI, CID, and yield (Table 13). Figures showing the results for the depths of 0–20, 20–50, and 50–80 cm in soil cores from tubes can be found in the supplemental material (Supplemental Table B. 1).

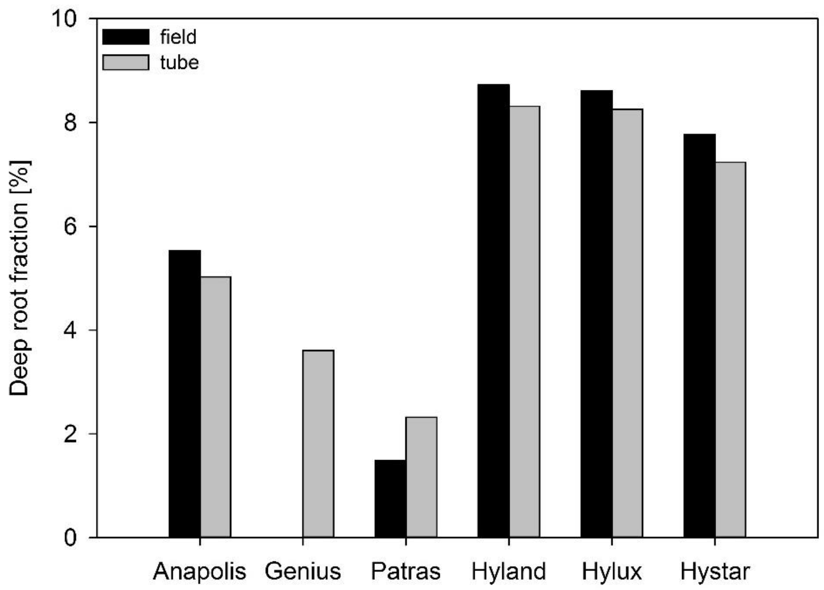

Figure 11: Deep root fraction for each wheat variety under drought stress of field grown (90-120 cm) wheat and wheat grown in tubes (80-110 cm) under controlled conditions.

5. Discussion

5.1 Section I: Detection of drought stress related traits and prediction of grain yield by spectral and thermal high-throughput measurements in winter wheat

5.1.1 A comparison of thermography and thermometry to measure leaf temperature

During recent years, leaf temperature measurements by thermography or IR-thermometry have become well-established and globally accepted methods to quantify drought stress levels in different crops (Idso *et al.*, 1981; Siddique *et al.*, 2000; Möller *et al.*, 2007; Jones *et al.*, 2009; Hackl *et al.*, 2012; Rebetzke *et al.*, 2013). Nevertheless, our study ascertained decisive differences in the quality of the resulting data depending on the measurement method (thermography or thermometry) and specific growth stage under field conditions. To the best of our knowledge, the present study is the first field study to examine the differences between thermometric or thermographic leaf temperature measurements. Several studies have been published in recent years indicating that whole-canopy temperature measurements are preferable compared to point measurements (Grant *et al.*, 2007; Hackl *et al.*, 2012).

In our study, during the earlier growth stage (heading), the results of the two methods varied by up to 5°C, independent of the experimental year and whether the measurements were conducted under drought stress or in the control environment (Table 6). Measurements became closer when taken during anthesis or grain filling, which perhaps occurs due to a higher stress level. As reported by Möller *et al.* (2007) and Wang *et al.* (2010), an advantage of thermography is that the opportunity for further processing is provided.

A LabVIEW-based software was therefore developed to select the background soil (very hot) from the plants to minimize soil influences by overlapping RGB and thermal images. On the other hand, images for each single plot are required and need to be processed further in the Laboratory. For one image, the entire process to the final results requires approximately 3 min. This processing is time consuming and does not fulfill the requirements for high throughput.

Therefore, the future transfer to high-throughput phenotyping remains to be demonstrated. In contrast, the IR-sensors carried by the Phenotrac 4 offer data collection in high-throughput mode, also providing simultaneously recorded spectral measurements. As reported by Munns *et al.* (2010) and Costa *et al.* (2013), the accuracies of thermal imaging measurements are highly affected by environmental variability (e.g., light intensity, air temperature, relative air humidity, and wind speed). Therefore, encumbered by a relatively long measuring time, one possible reason for differences between thermography and thermometry could be that temperature measurements implemented by thermography are more susceptible to environmental variability.

Besides, differences between thermography and thermometry probably originate from different viewing angles, and thus, varying soil influences (Rodriguez *et al.*, 2005; Möller *et al.*, 2007; Jones *et al.*, 2009; Blum, 2011; Hackl *et al.*, 2012). Within this context, it is surprising that thermometric measurements provide closer relationships, although due to the drought stress, the ground cover was reduced to 60% soil coverage (unpublished data). When comparing thermography and thermometry, we show in Table 6 that leaf temperature, as measured by thermometry, is more capable of describing the physiological status of a crop at heading, anthesis, and grain filling, as leaf temperature is related to not only the final grain yield but also stress parameters such as RLWC, CIDL, and CIDG, considering the two experimental years. Moreover, in 2014, heritability of leaf temperature, measured by thermometry, was higher under drought conditions than leaf temperature, measured by thermography (Table 8).

Previous research on wheat under drought stress has documented relationships between leaf temperature and grain yield (Blum *et al.*, 1989; Rebetzke *et al.*, 2013). The results obtained by Rattey *et al.* (2011); Rebetzke *et al.* (2013) demonstrated that measurements taken at post-anthesis show much stronger associations to grain yield and leaf temperature than measurements taken at pre-anthesis. In contrast to these studies, our results indicated that measurements taken at pre-anthesis or at anthesis provided much stronger relationships to grain yield than measurements taken post-anthesis (Table 6). Nevertheless, the earlier during plant growth development that grain yield predictions are made, the earlier breeders can reach a decision regarding further breeding steps, which accelerates the breeding process. Based on these results, it can be concluded that in this case, thermometry is the preferable method to detect leaf temperature. Thermometry provides a rapid and easy determination of leaf temperature of a high number of plots, and offers good relationships to grain yield and drought related parameters (Table 6).

5.1.2 Correlations among leaf temperature and NIR-based indices of broad range wavelengths (500-1200 nm)

Previous research has shown that leaf temperature, which is sensed remotely using an infrared thermometer or sensed by a hand held thermal camera, provides close relationships to grain yield of wheat cultivars (Reynolds *et al.*, 1994; Fischer *et al.*, 1998). Leaf temperature increases when stomata are closed to avoid dehydration due to water evaporation from the surface of leaves. The assessment of canopy water status is based on strong absorption by water at wavelengths in the NIR region (Gutierrez *et al.*, 2010). Five NIR-based indices (WI and NWI-1–4) were developed using the information from spectral reflection at 850 nm, 880 nm, 900 nm, 920 nm, and 970 nm wavelengths, respectively, whereby 970 nm is a water absorption band in which sensitivity depends on the water content at the canopy level (Bull, 1991; Babar *et al.*, 2006a). This assumption is based on the hypothesis that the NIR radiation at 970 nm penetrates deeper in to the canopy, which probably estimates water content in a more precise way than other indices (Babar *et al.*, 2006a; Gutierrez *et al.*, 2010).

Babar *et al.* (2006a) showed positive relationships between canopy temperature and NIR-based indices at the growth stages heading and grain filling. Our results are consistent with these findings and show also highly significant relationships between leaf temperature and NWI-3 (see other indices Supplemental Table 1-3) at heading, anthesis, and grain filling (Table 7). These highly significant relationships indicate that genotypes with higher leaf water content, as represented by lower NIR-based indices values, had lower leaf temperatures. This assumption is supported by significant negative correlations between leaf temperature and relative leaf water content (Table 6). However, leaf temperature as measured by thermometry provides stronger correlations than measurements by thermography over two years.

Measurements conducted at anthesis showed highest correlations for both experimental years; therefore, anthesis provides a preferable growth stage to determine leaf temperature by NWI-3. As reported by Gutiérrez-Rodríguez *et al.* (2004) and Babar *et al.* (2006a), WI, from which the NWI-3 is derived, could be used as an alternative to leaf temperature measurements. These findings agree with our results over a broad range of genotypes and two experimental years. Moreover, in addition to the NIR-based indices, the current study (as far as the authors are aware), is the first which includes, by using a PLSR, the broad range of spectral information from 500 to 1200 nm wavelengths to estimate leaf temperature. Including all spectral information provides an improved relationship to leaf temperature of up to 20% (Table 7).

Separate year models were fit since the information of both years was statistically different. This information is in line with previous notion of Hackl *et al.* (2013) and Hackl *et al.* (2012) where the influence of seasons on the spectral assessment of wheat plants was addressed, grown in pots and containers under saline conditions, and was compared to several other papers. For future breeding purposes, the combination of spectral information and thermometric measurements can assist in selecting genotypes with lower leaf temperatures, and thus, higher leaf water content and therefore higher productivity under drought stress conditions. These results are supported by a strong heritability (h^2 = 0.52) of thermometry and NIR-based indices (h^2 = 0.49–0.54) under drought conditions (Table 8).

5.1.3 The potential of water indices and broad range wavelengths to screen water status parameters

Numerous studies have shown that different morphophysiological parameters can be measured and estimated simultaneously in a nondestructive and rapid way, providing that these parameters demonstrate a significant correlation with spectral information of the plant at different wavelengths under drought stress (Araus *et al.*, 2001; Babar *et al.*, 2006b; Erdle *et al.*, 2011; Erdle *et al.*, 2013; Kipp *et al.*, 2014a; Kipp *et al.*, 2014b). The focus of recent research on spectral detection of plant water status has been on the range of wavelengths from 500 to 1200 nm and the five NIR-based water indices, WI and NWI-1–4. These indices compare the energy absorbed by water at 970 nm and different reference wavelengths of 850, 880, 900, and 920 nm, which do not display absorption by water (Penuelas *et al.*, 1997; Prasad *et al.*, 2007). As NWI-3 provided similar results as WI, NWI - 1–2 and NWI-4, the NWI-3 was chosen as a representative for the selected NIR-based water indices. In the work of Penuelas *et al.* (1993) WI was proven to assess canopy water status and to monitor changes in relative leaf water, leaf temperature, and stomatal conductance.

Our results have reconfirmed these findings, considering that the highest correlations were obtained at anthesis (Table 5). The drought stress parameter RLWC is an adjuvant indicator of plant water status under drought stress (Slatyer, 1967; Chaves *et al.*, 2003). Although Eitel *et al.* (2006) showed a weak relationship with WI and RLWC and Bandyopadhyay *et al.* (2014) showed a low correlation of RLWC and NWI-3. A drought stress-based field study by Gutierrez *et al.* (2010) found significant correlations of wheat genotypes between these water indices and RLWC across booting, anthesis, and grain filling, but not at individual growth stages. However, we have succeeded in detecting significant correlations at individual growth stages between the NIR-based water indices and RLWC (Table 5, Supplemental Table A. 1-3).

Measurements conducted at anthesis showed the highest correlations, at heading and grain filling relationships were weaker but still significant. Furthermore, correlations became much stronger when using the entire range of spectral information from 500 to 1200 nm. Using the broad range of wavelengths provides a combination of wavelengths proven to describe the water content in wheat (visible range: 500–700 nm (Graeff and Claupein, 2007) and near-infrared: 700–1200 nm (Penuelas *et al.*, 1993; Gutierrez *et al.*, 2010)). Almost every previous study working on the spectral reflections of plants under drought stress focused on single indices. In our study, the combination of a broad range of wavelengths by using a PLSR led to significantly stronger relationships to RLWC than by using NWI-3, and therefore provided more consistent information regarding plant water status.

Moreover, measurements of CID of leaves and grain were conducted. This parameter is a well-known indicator for water use efficiency and can provide an indirect determination of the effective water used by the plants (Araus *et al.*, 2002; Condon *et al.*, 2002; Blum, 2009). Only the study by Lobos *et al.* (2014) has presented work engaging with the relationship of CID and plant reflectance measured for wheat at the middle of grain filling. In the study by Lobos *et al.* (2014), the NIR-based index NWI-3 showed no relationship to CID under severe drought stress, but did to CID under mild drought stress.

However, our results provide strong relationships of NWI-3 and CID for both leaves and grain under drought stress conditions, which were comparable to the severe stress of the mentioned study. Additionally, significant associations of CID and the other NIR-based indices could be detected (Supplemental Table A. 1-4). As already has been observed for RLWC, the strongest associations could be found at anthesis as well as at grain filling. While the inclusion of a broad range of wavelengths led to significant stronger relationships for RLWC, in the case of CID of leaves and grain, the relationships to spectral information were maintained at a comparably high level. Correlations between the NIR-based indices and CID as well as between CID and the PLSR model showed comparable relationships of leaves and grain. Therefore, measurements of leaves should be preferred because leaves can be harvested concurrently with the spectral measurement.

When examining the heritability of RLWC and CID leaf/grain, a moderate to strong heritability (h^2 = 0.28–0.72) under drought stress could be observed (Table 8). However, it should be considered that measuring RLWC is highly time consuming, and as mentioned by Lopes *et al.*

(2014), costly analyses are required for the measurements of CID of leaves and grain, which will limit their suitability in breeding programs. The results of the current study indicated that a prediction by NIR-based parameters or with a broad range of wavelengths allows the integration of these traits in breeding programs to select drought-tolerant genotypes in a rapid and cost-effective manner. However, measurements taken at anthesis provided the strongest predictions of RLWC and CID, and the inclusion of a broad range of wavelengths offers stronger correlations than the single NIR-based water index. Nevertheless, it should be mentioned that the NIR-based indices have been proven for robust results in various studies (Babar *et al.*, 2006b; Babar *et al.*, 2006c; Prasad *et al.*, 2007; Gutierrez *et al.*, 2010) and different environments. A contour plot analysis, which tested all dual wavelength ratios of all measured parameters, was used to detect further suitable indices (data not shown). However, no combination of wavelengths could be detected that provided better estimations of the measured parameters than the applied water indices. Using a PLSR-model enables the inclusion of spectral information from visible and near infrared wavelengths; therefore, biomass information and from the NIR range and therefore for water status information. Additionally, a PLSR-model provides the possibility of an individual adjustment which needs to be tested in different environments.

5.1.4 Correlations between spectral reflectance indices and grain yield and the prediction of grain yield based spectral reflectance

Grain yield represents the entire life of a plant; therefore, grain yield reflects the level of stress that the plants have been exposed to. In both experimental years, grain yield was reduced by approximately 60 % due to the impact of drought stress (Figure 5). Babar *et al.* (2006c) has demonstrated a strong association between NIR-based indices and grain yield in wheat. In the present study, the correlations between spectral indices (WI and NWI-1–4) and grain yield of wheat showed the strongest relationships in comparison with the measured drought-related traits, whereas the drought-related traits demonstrated weaker associations to grain yield. Besides, for all indices, a significant negative relationship at heading, anthesis, and grain filling under drought conditions could be detected (Table 5, Supplemental Table A. 1-3). This relationship is based on increased water content in the plant, which decreased the reflectance of the water band (970 nm); consequently, negative correlations were obtained between these indices and grain yield (Babar *et al.*, 2006c).

The study of Tucker (1979) indicated a superiority of normalized indices over a ratio index under drought stress conditions by removing influences caused by external factors such as solar altitude, exposure to light, and soil influences. Although in our study, normalizing the water indices (NWI-1–4) did not significantly improve the relationship. The maximum correlation coefficient could be observed at anthesis and grain filling for all NIR-based indices with a range from −0.85** to −0.90**, indicating the efficiency of NIR-based indices for selecting drought-tolerant genotypes for grain yield production. These results are consistent with the findings of Prasad *et al.* (2007) and Gutierrez *et al.* (2010).

Furthermore, this predication is supported by the fact that the heritability of grain yield and NIR-based indices is on the same level in the drought environment (Table 8). Hence, as already mentioned by Gizaw *et al*. (2016), indirect selection for secondary traits is a preferred selection approach when secondary traits have comparable heritability with the target traits. This applies in particular when the secondary trait is simple to measure, with a low budget, and in a high-throughput way, which is provided by NWI-3. However, as reported, in the present study, for the water status parameters such as leaf temperature, RLWC, and CID, the inclusion by PLSR of a broad range of wavelengths led to significantly (up to 10%) stronger relationships to grain yield (Table 7). Due to the fact that the wavelengths from 500 to 1200 nm cannot be treated as a single trait, the calculation of heritability could not be accomplished.

Nevertheless, it is assumed that the PLSR of the wavelengths is also adequate for indirect selection as a secondary trait. This assumption is based on the fact that the wavelengths, from which NWI-3 is composed of, are components of the broad range that is used. Furthermore, as already mentioned, the PLSR analysis demonstrated a significantly stronger association to grain yield than the NIR-based indices and therefore promises to provide a high heritability. In addition, we used the spectral information from 500 to 1200 nm and twenty wheat cultivars to develop a model for grain yield prediction. Using a model from 2014 provides a grain yield prediction of 62 % for the year 2015 (Figure 6) and *vice versa* using a model from 2015 led to a grain yield prediction of 73 % for 2014. In conclusion, by using a model for individual years, a strong grain yield prediction could be accomplished. On the other hand, using a model including several years could result in weaker prediction. This assumption needs to be validated in the near future.

5.2 Section II: Evaluation of yield and drought using active and passive spectral sensing systems at the reproductive stage in wheat

5.2.1 Correlations between drought-related parameters

The assessment of plant water status provides information about the actual stress level under drought conditions. Measuring relative leaf water content is a well-proven, direct indicator of the actual plant water status (Slatyer, 1967; Chaves *et al.*, 2003). In the present study, a decrease in RLWC in response to increasing drought stress was observed during the heading, anthesis and grain-filling stages (Table 9). Another approach for assessing plant water status is measuring leaf temperature. Measurements obtained using IR-sensors provide information on plant transpiration as the main contributor to reduce leaf temperature (Monneveux *et al.*, 2012). This assumption was supported by significant negative correlations between RLWC and LT during all three growth stages (Table 10). Specifically, a low RLWC indicates a reduced transpiration rate as a water-saving strategy, which results in higher leaf temperatures. A lower transpiration rate leads to warmer leaves and lower stomatal conductance; both of these factors decrease net photosynthesis and crop duration (Monneveux *et al.*, 2012). Carbon isotope discrimination integrates stomatal conductance and photosynthesis capacity to transpiration over the life time of the organ being measured (Richards *et al.*, 2010) and is considered as an indirect indicator of plant water status (Farquhar *et al.*, 1989; Acevedo, 1993). For both experimental years, grain CID demonstrated strong linear relationships with grain yield; this finding agrees with the results reported from the studies conducted by Lopes and Reynolds (2010) and Araus *et al.* (2008). Moreover, leaf CID decreased with increasing drought stress (Table 10), agreeing with the results of Wang *et al.* (2016).

At anthesis, leaf and grain CID exhibited strong positive relationships with RLWC and strong negative relationships with leaf temperature for both experimental years (Table 10). Thus, the assumption can be made that measurements of CID can be substituted with indirect measurements, such as leaf temperature measured using IR-sensors. This type of indirect measurement can be easily applied, is rapid and has low costs. This is important since measurements of CID are associated with relatively high costs and the need for mass spectrometer facilities (Araus *et al.*, 1997; Lobos *et al.*, 2014). Furthermore, Monneveux *et al.* (2012) showed significant associations between leaf temperature and grain yield under drought conditions when measurements were conducted pre-anthesis and during grain filling. By contrast, our study demonstrated the strongest relationships with grain yield at anthesis for both experimental years (Table 10).

Moreover, Monneveux *et al.* (2012) stated that under drought conditions, a relatively lower leaf temperature indicates a high capacity for taking up soil water to maintain a constant plant water status. During both experimental years, the ground cover showed strong relationships with the leaf temperature, RLWC and CID of the leaves and grains at anthesis (Table 10). Similar results were observed during grain filling, except for RLWC, which can be explained by a decrease in cell water due to progressive senescence. Briefly, in our study, low leaf temperatures, low CID and high RLWC were associated with higher ground cover. This leads to the supposition that more extensive ground cover helps to conserve soil moisture at the beginning of the growing season and is associated with relatively high net photosynthesis and cooler canopies. The digital ground cover approach offers several advantages over other measurement tools. To determine ground cover, no special equipment is needed, i.e., a commercial, affordable digital camera and free or inexpensive digitizing software (e.g., ImageJ: https://imagej.nih.gov/ij/) are sufficient. Among the three growth stages, the most significant and robust relationships were observed during anthesis, which represents the preferable growth stage to estimate drought stress. For this reason, heritability of all drought-related parameters was calculated at anthesis (Table 12). The drought stress parameter RLWC showed a moderate heritability ($h^2 = 0.57 – 0.66$) in the drought environment. Furthermore, leaf and grain CID showed moderate to high heritability under drought stress ($h^2 = 0.28 – 0.72$), which supports the observations of Rebetzke *et al.* (2008). The genetic variation of LT and GC in the control environment in 2014 and in the drought environment in 2015 was estimated to be 0; hence, heritability could not be calculated. As reported by Rebetzke *et al.* (2013), changes in cloud cover and wind speed and direction can potentially influence differences in leaf temperature among genotypes, which can negatively affect the calculation of heritability.

Moreover, genotype × environment interactions and within-site variability contribute to larger sampling variance (Rebetzke *et al.*, 2002; Richards *et al.*, 2002). To our knowledge, this may the first study that provides a comprehensive comparison of a broad range of destructive and non-destructive morphophysiological parameters regarding their suitability to characterize drought stress under field conditions. In conclusion, measuring RLWC and carbon isotope discrimination of leaves and grains provided a good estimation of grain yield under drought stress at anthesis and also indicated high heritabilities. The main drawback of this approach is that the determination of both parameters is highly time consuming, error prone due to small sample sizes, and in the case of CID, associated with high financial expenses. By contrast, leaf temperature measurements using IR-sensors and the determination of ground cover provide an easy, low priced, and rapid measurement tool that is applicable to large field-scale experiments.

5.2.2 Comparison of active and passive sensors with respect to the prediction of drought-related parameters and grain yield

In the last years, numerous studies have shown that different drought-related morphophysiological parameters can be measured and estimated simultaneously in a nondestructive and rapid way, providing that these parameters demonstrate a significant correlation with spectral information of the plant at different wavelengths under drought stress (Araus *et al.*, 2001; Babar *et al.*, 2006b; Erdle *et al.*, 2011; Erdle *et al.*, 2013; Kipp *et al.*, 2014a; Kipp *et al.*, 2014b). For this purpose, several sensor systems are available, which are mainly classified as active and passive sensors. In the last decade, the potential of different active and passive sensor systems to assess agronomic and physiological parameters has been evaluated. However, the sensing principles have rarely been compared, and only limited information is available; therefore, further understanding is required. In this study, four reflectance sensors, including three active sensors (GreenSeeker, Crop Circle, ALS) and one passive, bi-directional hyperspectral sensor, which were all mounted on the mobile phenotyping platform Phenotrac 4 (Figure 2), were tested under drought conditions and over two experimental years. All applied indices from all four sensor systems were significantly correlated with the morphophysiological parameters RWLC, LT, leaf and grain CID, GC and yield under drought stress (Table 11). At heading, all sensor systems, independent of the light source, provided comparable correlations with the measured parameters except for the drought stress parameter RLWC. RLWC is an adjuvant indicator of plant water status under drought stress (Slatyer, 1967; Chaves *et al.*, 2003). The active sensors tended to yield slightly stronger relationships with RLWC compared with the passive sensor (Table 11). In addition, the vegetation indices R_{760} / R_{730} and NDVI showed strong relationships with LT and GC for both experimental years. This fact indicates that these indices primarily detect the actual biomass, which was relatively high at heading due to moderate drought stress and was therefore associated with lower leaf temperatures and higher ground cover. Furthermore, the five NIR-based water indices (WI, NWI 1 - 4) showed similar relationships to the measured parameters, compared with the other applied indices, indicating that drought stress was not yet intensive enough to influence the plant reflectance in this range of wavelengths (800 ~ 900 nm). As a consequence of withholding precipitation, drought stress reached a severe level at anthesis (Table 9).

It is noticeable that at anthesis under severe drought stress, the passive sensor appeared to have an advantage over the active sensor systems, demonstrated by the stronger relationships with the measured parameters. This could be explained by differences in the sensor-dependent field of view (FOV). The passive sensor provides a larger FOV; thus, due to reduced ground cover of 55 % (Table 9), the measured reflectance better reflected the actual drought conditions. Furthermore, it could be argued that the penetration depth of artificial light is lower compared with natural light, which is used by the active sensors. This assumption is supported by Jasper *et al.* (2009), Winterhalter *et al.* (2013) and Elsayed *et al.* (2015), who mentioned that the artificial light source of active sensors penetrates less deeply into crop canopies compared with solar radiation. An exception was observed for RWLC, as during the heading stage, the spectral estimation of this drought-related parameter was slightly better with the use of active sensors in both experimental years (Table 11), based on a comparison with the same indices. The first assumption was that RLWC is dependent on existing biomass and therefore, the active sensors could have had an advantage due to reduced spectral penetration, which is associated with reduced soil influence. However, this assumption could not be confirmed as the spectral indices of the passive sensor were more strongly related to GC than the active indices. This may be based on the fact that the measured plant reflectance of the active sensors mainly integrates the upper leaf levels, which represent the actual water status, especially under prolonged drought stress. The passive sensor includes spectral information on the whole plant, which could, due to increasing senescence, negatively influence the relationship with RLWC. Although the study of Bandyopadhyay *et al.* (2014) showed low correlations between RLWC and NWI 1 - 4, the five NIR-based water indices showed significant relationships with RLWC, which were on the same level as those observed for the active sensors. Moreover, the water indices exhibited highly significant relationships with LT and leaf and grain CID. Compared with the other applied indices, the water indices tended to have stronger relationships with most of the measured parameters. The NIR-based water indices compare the energy absorbed by water at 970 nm and different reference wavelengths of 850, 880, 900, and 920 nm, which do not indicate absorption by water (Penuelas *et al.*, 1997; Prasad *et al.*, 2007) and therefore are especially suited to detect plant water status.

The detection of leaf temperature is another indicator to quantify the drought stress level (Jones *et al.*, 2009; Hackl *et al.*, 2012; Rebetzke *et al.*, 2013). In a study conducted by Babar *et al.* (2006a), positive relationships between canopy temperature and NIR-based indices at heading and grain filling were detected. Our findings reinforce these results and also show highly significant relationships between leaf temperature and the NIR-based indices at anthesis (Table 11). Furthermore, measurements of leaf and grain CID were conducted (Table 9). Measurements of CID are well-accepted as an indicator of water use efficiency (Araus *et al.*, 2002; Blum, 2009). Only the study conducted by Lobos *et al.* (2014) reported on the relationship between CID and spectral indices for wheat during the middle of grain filling. In contrast to our findings, the study conducted by Lobos *et al.* (2014) showed no relationship between the NIR-based index and NWI-3. Further, our results indicate strong relationships between NWI-3 and CID for both leaves and grain under drought stress conditions for both experimental years (Table 11). In addition to the five water-based indices, the NDVI, which is associated with green biomass (Prasad *et al.*, 2007), exhibited a good relationship with LT, leaf and grain CID and GC for both the active and passive sensors (Table 11). This indicates that green biomass contributes greatly to these relationships; however, the hypothesis is that the NIR at 970 nm penetrates deeper into the canopy, which probably estimates water content in a more precise way than other indices (Babar *et al.*, 2006a; Gutierrez *et al.*, 2010). Therewith, the poorer relationships of the other indices from the active sensors as well as from the passive sensor could be explained. At grain filling, no explicit differentiation between the active and passive sensors could be observed (Table 11). The relationships between the evaluated indices (regardless of active or passive system) and RLWC were relatively weak, which is presumably associated with drought-induced premature senescence. By contrast, in 2015, the active sensors yielded a stronger relationship with RLWC compared with the passive sensor, albeit on a low relationship level. Furthermore, as already observed at anthesis, the five NIR-based indices tended to provide more robust spectral information compared with the selected indices. Briefly, in our study, the passive sensor yielded closer relationships with the measured destructive and non-destructive morphophysiological parameters compared to the active sensors. A comparison among the active sensors indicated that the Crop Circle yielded the most robust relationships. These findings support the results of Elsayed *et al.* (2015), who also made a comparison of different active sensors when measuring drought-stressed barley plants. In addition to the given indices of the active sensors and the equivalent indices and NIR-based indices of the passive sensor, a contour plot analysis, which tested all dual wavelength ratios of all measured parameters, was used to detect further suitable indices (data not shown). However, no combination of wavelengths could be detected that provided better estimations of the measured parameters than the applied water indices. However, as already shown by Erdle *et al.* (2011), the passive sensor proved to be more flexible to evaluate further indices due to the extended spectral range.

Grain yield represents the entire life of a plant and reflects the level of stress to which the plants have been exposed. In both experimental years, grain yield was reduced by approximately 60 % due to the impact of drought stress (Table 9). During the three growth stages and both experimental years, highly significant relationships between spectral information and grain yield were detected, and the strongest correlations were observed at anthesis. However, during all growth stages, the indices of the passive sensor demonstrated up to approximately 20 % stronger relationships with grain yield compared to the indices of the active sensors. Moreover, the five water indices (WI, NWI - 1 - 4) consistently exhibited higher correlations with grain yield under drought conditions compared to the widely used indices (NDVI, R_{760}/R_{730}, R_{730}/R_{760}, etc.; see Table 11).

These findings are consistent with the results of Prasad *et al.* (2007). The maximum correlation coefficient was observed at anthesis and grain filling for all five NIR-based indices, with a range from −0.85 to −0.90, indicating the efficiency of NIR-based indices for selecting drought-tolerant genotypes for grain yield production. The heritabilities of grain yield ($h^2 = 0.62$) and the water indices ($h^2 = 0.52$ to 0.61) were on the same level in the drought environment, over both experimental years, which supports the mentioned prediction (Table 12). The heritability of the other applied active and passive indices ranged from 0.11 to 0.78 under drought stress; however, these indices did not provide estimates of the drought-related parameters and grain yield that were as reliable as those provided by the water indices. Prasad *et al.* (2007) reported that the water indices NWI- 1 - 4 tended to explain more of the variability in grain yield when mean data, averaged over growth stages and years, were used. However, we succeeded in detecting highly significant relationships at individual growth stages, whereby all five water indices predicted grain yield under drought conditions.

Indirect selection of secondary traits is a preferred selection approach when these traits have comparable heritability with the target traits (Gizaw *et al.*, 2016). As reported by Jackson (2001), this applies especially when the secondary trait is easy to determine, is low priced, and is ascertainable in a high-throughput way. All of these requirements are fulfilled by the five NIR-based indices in our study. Furthermore, these indices demonstrated strong correlations with grain yield, and high heritabilities were observed for these indices. This could facilitate rapid measurements of a large number of plots used by breeders and farmers and could reduce the cost of individual measurements.

5.3 Section III: Can we scale up (extrapolate) drought stress in winter wheat from pots to the field?

5.3.1 Impact of growth environment on drought-related parameters

Under field conditions, many grain yield-determining processes occur over a long period of time; for example, the extraction of water from the deeper soil layers (Passioura, 2012). In most laboratories, small pots are often used to accelerate the throughput of experiments and to enhance the number of replicates (and thus statistical power). The limited soil volume in pots can lead to plant dehydration within days, whereas this process in the field takes place slowly, often over weeks (Passioura, 2012). However, under controlled conditions it is very difficult to fully imitate all the field-like conditions. This situation makes it particularly difficult to examine drought-induced changes in plant physiology due to the fact that the commonly used pots usually are much smaller than the plant available soil volume under field conditions (Poorter *et al.*, 2012). Therefore, due to a reduced total water holding capacity and therewith faster drying of soil, small pots could have a negative impact on the water status of plants growing in them (Tschaplinski and Blake, 1985). Furthermore, Passioura (2006) mentioned that the pot height —as a parameter that influences water relations and soil structure—may also have a significant effect on the structure and physiology of roots. These concerns illustrate how numerous factors jointly influence the results of pot experiments investigating drought stress responses by plants.

Lately, there is ongoing discussion about the transferability of pot experiments under controlled conditions to field conditions. Although many studies have examined the impact of pot size on plant physiological drought-related parameters, almost no explicit recommendation is yet given as to which size ought to be provided by a pot to enable a robust extrapolation to field conditions. This absence is not surprising upon realizing that most studies only compare different pot sizes without an appropriate corresponding field experiment. To help fill this gap, 2 years of field experimentation were conducted in parallel to experiments that used conventional pots vs. deep-rooting tubes under controlled conditions. Importantly, both sets of experiments addressed the same drought-related parameters: RLWC, LT, CID, and NDVI, as well as reproductive output (grain yield).

The RLWC is an indicator of plant water status, in that it is associated with cell volume which reflects the balance between the water supply and transpiration rate (Sinclair and Ludlow, 1985). Pot size seems to have no effect on RLWC, as no significant differences were observed between the pot and tubes (Table 14). Only the field-grown plants showed a significantly higher RLWC, but this was probably due to the different growth conditions. These results are consistent with those of Ronchi *et al.* (2006) who reported that pot size did not necessarily affects leaf water potential, a parameter closely related to RLWC (Lafitte, 2002).

Furthermore, assessing LT is a well-proven, non-destructive and indirect indicator of the actual water status of a plant (Jones *et al.*, 2009; Hackl *et al.*, 2012; Rebetzke *et al.*, 2013). Comparing all three growing environments (pot, tube, and field), the LT showed significant differences under drought stress (Table 13). In this case, however, we should say that the large difference between the two pot systems and the field likely resulted from higher ambient air temperatures under natural conditions. Nonetheless, although both the pots and tubes were grown under the same controlled temperature conditions, a significant difference in LT between them could be observed under drought stress (Table 14). As reported by Myburgh and Conradie (1996) and Wang *et al.* (2001), a limited soil volume could cause a reduction in the transpiration rate, which is a main contributor to leaf temperature (Monneveux *et al.*, 2012). This interpretation is supported by our results: when grown under well-watered conditions, the wheat plants in the pots also had significantly higher LTs than those in the tubes (Table 14).

Moreover, similar results could be observed when measuring CID (Table 14). This is known as an indirect indicator for water use efficiency as it provides an indirect determination of the water status in plants (Araus *et al.*, 2002; Blum, 2009). As noted by Poorter *et al.* (2012), a restricted rooting volume could cause a reduction in photosynthesis, which is associated with a reduced CID. In contrast to the controlled conditions of the greenhouse, multiple stressors, besides that of drought stress, such as heat or wind, probably occur under field conditions. Hence, the CID values under field conditions normally differ from those obtained under controlled conditions, and so they are not directly comparable. Nevertheless, as already observed for LT, the smaller pot size affects the CID also under well-watered conditions. This indicates that pot size exerts a strong negative influence on photosynthesis via its associated rooting volume.

Besides the above physiological parameters, a hand-held spectral sensor measured the NDVI, which is one of the most widely used indices to determine green biomass and photosynthetic capacity (Babar *et al.*, 2006b). Significant differences were found in NDVI among the three growing environments (Table 14); noteworthy is how the NDVI of pots was substantially reduced when compared with that of the tube and field-grown plants. Since drought stress is associated with premature leaf senescence, the restricted rooting volume in the pots probably hastened its progress leading to lost green biomass. In two recent studies, when grown under saline conditions, the wheat plants in large containers showed stronger associations with the spectral indices than did those plants grown in pots, which may be linked to differences in senescence (Hackl *et al.* (2013); Hu *et al.* (2016).

However, for all measured parameters except RLWC, significant differences were observed between pots and tubes and the field. Hence, given obvious impact of pot size on these various physiological processes, significant differences for grain yield were expected, too. Although the standardized yields of the pots were lower than those of the tubes and under field conditions, these differences were not statistically significant (Table 14). Furthermore, for all three growing environments, we found strong correlations of yield with both LT and CID, and also between yield and NDVI under field conditions (Table 13). The low correlation of NDVI with the pot and tube yields probably arose from the relatively low amount of biomass in the pot systems—as compared to field conditions—affecting the spectral measurements. Briefly, for all measured drought-related parameters (except grain yield) significant differences between the growth environments were found. Pots, with their limited rooting volume, seem to exert the greatest negative influence on plant physiological processes, not only under drought stress but also under well-watered conditions. Thus, conventional pots do not seem to offer a reliable and appropriate system to guarantee extrapolation to field conditions.

5.3.2 Impact of growth environment on genotypic drought tolerance

To accelerate the breeding process of drought-tolerant wheat cultivars, a seasonally-independent pre-screening of promising genotypes under controlled conditions could be an adjuvant method. With reason, crop breeders take little notice of greenhouse or growth chamber experiments, unless the extrapolation of specific traits has been demonstrated under field conditions (Passioura, 2012). To better meet this purpose, improving on the transferability of genotypic drought tolerance to field conditions is warranted.

Many studies have examined the impact of pot size on various plant physiological processes. Yet very few experiments investigate the effect of different pot sizes on yield-driving genotypic characteristics such as drought tolerance. To generate comparable data across the three growth environments, the percent differences between the control and drought stress treatments for LT, CID, RLWC and NDVI for each cultivar were explored (Figure 9). In general, it is conspicuous that the cultivars that showed a higher drought tolerance under field conditions, or under controlled conditions in the tubes, seemed more susceptible to drought in the small pots (Figure 9). This observation is especially pronounced for CID and RLWC responses, for which the cultivars Hyland, Hylux, and Hystar were seemingly more drought tolerant under field conditions. However, the very same cultivars were apparently drought susceptible when they were grown in the small pots. Moreover, pot size not only affected the plant responses under drought stress but also the whole plant physiology. As shown in Figure 8, the restricted rooting volume in the small pots led to a decrease in CID of c. 4 % and an increase in LT of c. 7 %, even under well-watered conditions, when compared with the tubes. This negative impact on plant physiology may be caused by chemical signals resulting from root restriction, even in absence of any drought stress (Liu and Latimer, 1995; Ismail and Davies, 1998; Hurley and Rowarth, 1999).

Consequently, the choice of pot size likely has a substantial effect on the genotypic evaluation of drought tolerance, which can lead to a misinterpretation of plant performance. In a recent study by Bourgault *et al.* (2016), similar observations to ours were made concerning pot size. Their study suggested that, once beyond the tillering stage, pot size can influence the ranking of genotypes for all treatments that impacts growth. In their early growth stages, plants are not affected by pot size given their relatively small root expansion; but later, having grown older, the effect of pot size becomes more pronounced, even in medium-sized pots (Poorter *et al.*, 2012).

Our present study confirms that pot size affects the ranking of genotypes for all the considered plant response parameters. In particular, the genotype rankings of RLWC, CID, and yield were strongly affected by the pot size used (Table 15). The varieties Hyland, Hystar and Hylux showed the highest RLWC and CID values when subjected to drought stress both under field conditions and in the tubes under controlled conditions, whereas in the small pots the rankings were reversed. Furthermore, yield represents the accumulated fitness outcome of diverse interacting factors, such as drought stress, that influence a plant throughout its life, and therefore represents the entire life of a plant. Ultimately, for breeders the yield is the parameter that counts the most. To provide sound evidence concerning yield performance, it is necessary that plants complete their full life cycle. In this time their root growth increases, as does their above ground biomass. Under field conditions, root growth is optimized as soil conditions are not restricted; but as mentioned by Bourgault *et al.* (2016), pot-grown varieties that show a higher root-to-shoot ratio will be restricted earlier in root growth, and therefore in plant physiology, than others, this could overshadow the response to a drought stress treatment.

Like for RLWC and CID, the varieties Hystar, Hyland, and Hylux provided the highest grain yields under drought stress in the field, as well as in the tubes (Table 15). The yields over 2 years – which were standardized for the control and drought stress conditions separately – also illustrates how the varieties that had above-average yields under drought stress conditions in the field likewise had it in the tubes (Figure 10). By contrast, and counter-intuitively, the varieties having the lowest yields under drought in the field gave the highest yield in the small tubes. Moreover, the high-yielding varieties, when grown with an optimal water supply, did not automatically provide high yields when under drought stress. These results clearly indicate that pot size affects general drought response of wheat in addition to the individual genotype response. We caution that pot-based rankings of cultivars for yield performance may lead to incorrect conclusions and applications.

To test the hypothesis that restricted root growth mainly determines plant physiology, and hence drought tolerance, we examined root distributions in the field- and in the tube-grown plants (Figure 11). Especially evident were the deep root fractions, contributing primarily to access deeper water resources. Strong correlations between the deep root fraction and CID under field conditions were found, indicating that deep rooting helped the plants to maintain photosynthetic activity under drought stress (Table 13). Moreover, the contribution of the deep root fraction agrees with the previously discussed rankings for the physiological parameters and grain yield. Specifically, these results suggest that genotypic drought tolerance is mainly based on the capability to develop a distinctive root system, one able to reach water reservoirs held in deeper soil layers. This interpretation is supported by Reynolds *et al.* (2007), who described how wheat plants that allocated more root mass to access the deeper soil profiles, also increased the ability to extract moisture from those depths. Consequently, plants grown in small pots face a restricted rooting volume under drought stress; they cannot benefit from deep rooting and hence they are not able to physiologically achieve drought tolerance.

Put briefly, rooting depth is the main contributor to genotypic drought tolerance. A restricted rooting volume can cause a potentially erroneous evaluation of drought tolerance of individual genotypes that cannot be extrapolated to field conditions.

6. Conclusions

6.1 Section I: Detection of drought stress related traits and prediction of grain yield by spectral and thermal high-throughput measurements in winter wheat

Thermometry was found to be the preferable method to detect leaf temperature under drought stress conditions. Thermometry provides a fast and easy application and shows good relationships to grain yield and drought-related parameters. In addition, the NIR-based index NWI-3 showed strong associations to drought stress-related parameters (leaf temperature, RLWC, CID) and explained a high proportion of the variability of grain yield. Furthermore, the NIR-based indices showed the same heritability as grain yield under drought stress; therefore, they can be used for indirect selection for grain yield productivity. Although the integration of a broad range of wavelengths by a PLSR model led to stronger predictions of all measured parameters, the NIR-based indices have been proven to deliver robust results in various environments. The investigations of the present study indicate that a prediction by NIR-based indices or with a broad range of wavelengths allows the integration of these traits in breeding programs to select drought-tolerant genotypes in a rapid and cost-effective manner.

6.2 Section II: Evaluation of yield and drought using active and passive spectral sensing systems at the reproductive stage in wheat

Assessing plant water status RLWC and leaf and grain CID is associated with highly time-consuming measurements and costly analysis. In contrast, measurements of leaf temperature using IR-sensors and the determination of ground cover using digital cameras provide a rapid and easy approach to determine drought stress under field conditions, showing good relationships with grain yield and drought-related parameters. Moreover, at anthesis, spectral measurements using active or passive sensors demonstrated significant relationships with the measured destructive and non-destructive parameters, whereas the passive sensor tended to yield more robust estimations of the drought-related parameters. However, an exception to this was the parameter RLWC; the active sensors tended to yield a slightly stronger relationship with RLWC compared with the passive sensor. The NIR-based water indices (WI, NWI- 1 – 4) demonstrated strong associations with the drought stress-related parameters (leaf temperature, RLWC, CID) and explained a high proportion of the variability in grain yield. Furthermore, in the current study, the NIR-based indices were proven to be useful for indirect selection for grain yield. This was indicated by the fact that they exhibited the same heritability. In addition, the active sensors systems were more flexible in terms of light and diurnal effects. However, the investigations of the present study indicate that to select drought-tolerant genotypes in a rapid and cost-effective manner, and therefore to accelerate breeding progress, future investigations will require broad-range spectral information to optimize the phenotyping of specific plant traits under drought conditions. The passive spectrometer provided the development of novel indices, which might be further transferred into active sensors.

6.3 Section III: Can we scale up (extrapolate) drought stress in winter wheat from pots to the field?

The limited rooting volume of the pots seems to have a pronounced negative influence on plant physiological processes under drought stress as well as under well-watered conditions. The experiments confirm rooting depth as perhaps the main contributor for genotypic drought tolerance. Therefore, a restricted rooting volume may cause a potentially erroneous evaluation of the drought tolerance of individual genotypes. As such, an extrapolation from small pots to the field is not recommended. However, our study demonstrates that by using a soil volume that approximates field conditions, like that provided by large tubes, field-like conditions can be simulated in controlled environments, such as in greenhouses or growth chambers. The tubes allow for reliable and seasonally-independent phenotyping under controlled conditions, which if adopted, could greatly accelerate the pre-breeding processes to identify much needed drought-tolerant wheat cultivars in the near future.

A.Supplemental Tables Section I

Table A. 1: Correlations of drought-related parameters, yield and NWI-1 in winter wheat in drought and control environments for heading, anthesis, grain filling.

		NWI-1											
		heading				anthesis				grain filling			
		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.
RLWC	DS	-0.37	***	-0.35	**	**-0.60**	****	-0.49	****	-0.36	**	-0.38	****
	C	-0.27	ns	0.22	ns	0.25	ns	0.10	ns	0.22	ns	-0.01	ns
TFLIR	DS	0.43	****	0.30	**	**0.74**	****	0.41	****	0.44	****	0.41	****
	C	0.25	ns	0.27	ns	-0.05	ns	-0.05	ns	0.26	ns	-0.15	ns
TIRS	DS	**0.58**	****	0.21	ns	**0.82**	****	**0.69**	****	**0.76**	****	**0.60**	****
	C	0.18	ns	0.31	ns	0.31	ns	0.10	ns	0.31	ns	-0.06	ns
CIDL	DS	**-0.57**	****	-0.43	****	**-0.80**	****	**-0.66**	****	**-0.74**	****	**-0.61**	****
	C	0.14	ns	-0.26	ns	-0.11	ns	-0.13	ns	-0.26	ns	-0.11	ns
CIDG	DS	**-0.58**	****	-0.33	**	**-0.82**	****	**-0.63**	****	**-0.78**	****	**-0.63**	****
	C	-0.16	ns	-0.23	ns	-0.28	ns	-0.22	ns	-0.22	ns	0.01	ns
yield	DS	**-0.65**	****	**-0.57**	****	**-0.89**	****	**-0.86**	****	**-0.84**	****	**-0.86**	****
	C	0.17	ns	0.22	ns	-0.21	ns	-0.37	**	0.22	ns	-0.10	ns

RLWC relative leaf water content, *TFLIR* leaf temperature FLIR-Camera, *TIRS* leaf temperature IR-sensors, *CIDL* carbon isotope discrimination of leaf, *CIDG* carbon isotope discrimination of grain, *yield* grain yield.

[b] Treatments, drought stress (DS), control (C).

[c] r Correlation coefficient.

[d] Statistical significance as indicated by p-value ns non significant: *p < 0.05, **p < 0.01, *** < 0.001, ****p < 0.0001.

Bold data display correlations > r=0.50.

Table A. 2: Correlations of drought-related parameters, yield and NWI-2 in winter wheat in drought and control environments for heading, anthesis, grain filling.

		NWI-2											
		heading				anthesis				grain filling			
		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.
RLWC	DS	-0.39	****	**-0.50**	****	**-0.62**	****	**-0.50**	****	-0.35	**	-0.37	**
	C	-0.26	ns	0.10	ns	0.25	ns	0.10	ns	0.23	ns	-0.05	ns
TFLIR	DS	0.44	****	0.46	****	**0.76**	****	0.46	****	0.41	****	0.44	****
	C	0.27	ns	0.02	ns	-0.04	ns	0.02	ns	0.30	ns	-0.16	ns
TIRS	DS	**0.57**	****	**0.74**	****	**0.80**	****	**0.74**	****	**0.75**	****	**0.64**	****
	C	0.14	ns	0.11	ns	0.30	ns	0.11	ns	0.31	ns	-0.05	ns
CIDL	DS	**-0.57**	****	**-0.66**	****	**-0.80**	****	**-0.66**	****	**-0.75**	****	**-0.61**	****
	C	0.20	ns	-0.11	ns	-0.14	ns	-0.11	ns	-0.29	ns	-0.14	ns
CIDG	DS	**-0.57**	****	**-0.65**	****	**-0.82**	****	**-0.65**	****	**-0.80**	****	**-0.65**	****
	C	-0.17	ns	-0.21	ns	-0.28	ns	-0.21	ns	-0.22	ns	0.02	ns
yield	DS	**-0.63**	****	**-0.88**	****	**-0.88**	****	**-0.88**	****	**-0.82**	****	**-0.87**	****
	C	0.16	ns	-0.36	**	-0.24	**	-0.36	**	0.23	ns	-0.10	ns

RLWC relative leaf water content, *TFLIR* leaf temperature FLIR-Camera, *TIRS* leaf temperature IR-sensors, *CIDL* carbon isotope discrimination of leaf, *CIDG* carbon isotope discrimination of grain, *yield* grain yield.

[b] Treatments, drought stress (DS), control (C).

[c] r Correlation coefficient.

[d] Statistical significance as indicated by p-value ns non significant: *p < 0.05, **p < 0.01, *** < 0.001, ****p < 0.0001.

Bold data display correlations > r=0.50.

Table A. 3: Correlations of drought-related parameters, yield and NWI-3 in winter wheat in drought and control environments for heading, anthesis, grain filling.

		NWI-4											
		heading				anthesis				grain filling			
		´14		´15		´14		´15		´14		´15	
Indices[a]	T[b]	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.	r[c]	sig.[d]	r	sig.
RLWC	DS	-0.37	***	-0.33	**	**-0.61**	****	-0.49	****	-0.35	**	-0.38	**
	C	-0.27	ns	0.21	ns	0.24	ns	0.09	ns	0.21	ns	-0.06	ns
TFLIR	DS	0.43	****	0.33	**	**0.74**	****	0.41	****	0.43	****	0.43	****
	C	0.25	ns	0.29	ns	-0.05	ns	-0.04	ns	0.29	ns	-0.14	ns
TIRS	DS	**0.57**	****	0.18	ns	**0.81**	****	**0.68**	****	**0.76**	****	**0.62**	****
	C	0.16	ns	0.31	*	0.31	ns	0.10	ns	0.31	ns	-0.06	ns
CIDL	DS	**-0.56**	****	-0.41	****	**-0.79**	****	**-0.64**	****	**-0.74**	****	**-0.61**	****
	C	0.17	ns	-0.29	ns	-0.14	ns	-0.12	ns	-0.29	ns	-0.14	ns
CIDG	DS	**-0.58**	****	-0.30	**	**-0.81**	****	**-0.62**	****	**-0.78**	****	**-0.64**	****
	C	-0.16	ns	-0.22	ns	-0.28	ns	-0.21	ns	-0.22	ns	0.02	ns
yield	DS	**-0.64**	****	-0.54	****	**-0.88**	****	**-0.87**	****	**-0.83**	****	**-0.87**	****
	C	0.17	ns	0.23	ns	-0.23	**	-0.37	**	0.23	ns	-0.11	ns

RLWC relative leaf water content, *TFLIR* leaf temperature FLIR-Camera, *TIRS* leaf temperature IR-sensors, *CIDL* carbon isotope discrimination of leaf, *CIDG* carbon isotope discrimination of grain, *yield* grain yield.

[b] Treatments, drought stress (DS), control (C).

[c] r Correlation coefficient.

[d] Statistical significance as indicated by p-value ns non significant: *p < 0.05, **p < 0.01, *** < 0.001, ****p < 0.0001.

Bold data display correlations > r=0.50.

Table A. 4: Heritability of drought-related parameters and spectral reflectance indices at anthesis under drought and control conditions.

Trait[a]	2014		2015	
	drought	control	drought	control
	h^{2b}	h^2	h^2	h^2
RLWC	0.66	0.32	0.57	0
TFLIR	0.19	0.57	0.61	0.53
TIRS	0.52	0	0	0.42
CIDL	0.65	0.82	0.28	0.42
CIDG	0.72	0.86	0.44	0.39
yield	0.62	0.74	0.61	0.83
WI	0.53	0.24	0.60	0.74
NWI-1	0.52	0.24	0.61	0.74
NWI-2	0.54	0.19	0.46	0.80
NWI-3	0.54	0.35	0.49	0.75
NWI-4	0.54	0.13	0.43	0.78

[a] *RLWC* relative leaf water content (%), *TFLIR* leaf temperature FLIR-camera (C°), *TIRS* leaf temperature IR-sensors (C°), *CIDL* carbon isotope discrimination of leaf (‰), *CIDG* carbon isotope discrimination of grain (‰), *GC* ground cover (%), *yield* grain yield (dt/ha), *WI* water index, *NWI1-4* normalized water indices.
[b] *Heritability*

B. Supplemental Tables Section III

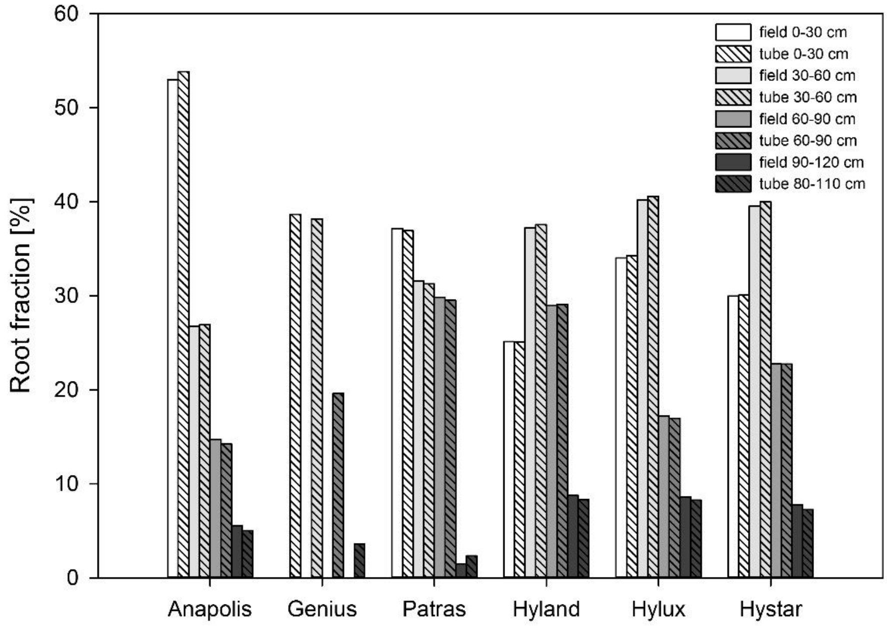

Table B. 1: Root fraction for each variety under drought stress of field grown wheat and wheat grown in tubes under controlled conditions.

References

Abramoff, M, Magalhaes, P & Ram, S**.** 2004. Image processing with ImageJ. Biophotonics Int 2004; 11 (7): 36–42. Biophotonics international 11 **7**: 36-42.

Acevedo, E**.** 1993.Potential of carbon isotope discrimination as a selection criterion in barley breeding. In: Stable isotopes and plant carbon-water relations,399-417 San Diego, CA: Academic Press.

Araus, J, Amaro, T, Zuhair, Y & Nachit, M**.** 1997. Effect of leaf structure and water status on carbon isotope discrimination in field-grown durum wheat. Plant, Cell & Environment 20 **12**: 1484-1494.

Araus, J, Casadesus, J & Bort, J**.** 2001.Recent tools for the screening of physiological traits determining yield. Application of Physiology in Wheat Breeding. In: Application of Physiology in Wheat Breeding,59-77 Mexico, D.F.: CIMMYT.

Araus, J, Slafer, G, Reynolds, M & Royo, C**.** 2002. Plant breeding and drought in C3 cereals: what should we breed for? Annals of Botany 89 **7**: 925-940.

Araus, JL & Cairns, JE**.** 2014. Field high-throughput phenotyping: the new crop breeding frontier. Trends in Plant Science 19 **1**: 52-61.

Araus, JL, Slafer, GA, Royo, C & Dolores Serret, M**.** 2008. Breeding for Yield Potential and Stress Adaptation in Cereals. Critical Reviews in Plant Sciences 27 **6**: 377-412.

Babar, M, Reynolds, M, Van Ginkel, M, Klatt, A, Raun, W & Stone, M**.** 2006a. Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. Crop Science 46 **3**: 1046-1057.

Babar, M, Van Ginkel, M, Klatt, A, Prasad, B & Reynolds, M**.** 2006b. The potential of using spectral reflectance indices to estimate yield in wheat grown under reduced irrigation. Euphytica 150 **1-2**: 155-172.

Babar, MA, Reynolds, MP, van Ginkel, M, Klatt, AR, Raun, WR & Stone, ML**.** 2006c. Spectral Reflectance Indices as a Potential Indirect Selection Criteria for Wheat Yield under Irrigation Crop Science 46 **2**: 578-588.

Bandyopadhyay, K, Pradhan, S, Sahoo, R, Singh, R, Gupta, V, Joshi, D & Sutradhar, A**.** 2014. Characterization of water stress and prediction of yield of wheat using spectral indices under varied water and nitrogen management practices. Agricultural Water Management 146: 115-123.

Bates, D, Mächler, M, Bolker, B & Walker, S**.** 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.

Blum, A**.** 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Research 112 **2**: 119-123.

Blum, A. 2011. Plant water relations, plant stress and plant production. Springer.

Blum, A, Mayer, J & Gozlan, G**.** 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Research 5: 137-146.

Blum, A, Shpiler, L, Golan, G & Mayer, J**.** 1989. Yield stability and canopy temperature of wheat genotypes under drought-stress. Field Crops Research 22 **4**: 289-296.

Bourgault, M, James, AT & Dreccer, MF**.** 2016. Pot size matters revisited: does container size affect the response to elevated CO2 and our ability to detect genotypic variability in this response in wheat? Functional Plant Biology 44 **1**: 52-61.

Boyer, J, Byrne, P, Cassman, K, Cooper, M, Delmer, D, Greene, T, et al. 2013. The US drought of 2012 in perspective: a call to action. Global Food Security 2 **3**: 139-143.

Bull, CR**.** 1991. Wavelength selection for near-infrared reflectance moisture meters. Journal of Agricultural Engineering Research 49: 113-125.

Campos, H, Cooper, M, Edmeades, G, Loffler, C, Schussler, J & Ibanez, M**.** 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the US corn belt. Maydica 51 **2**: 369.

Campos, H, Cooper, M, Habben, J, Edmeades, G & Schussler, J**.** 2004. Improving drought tolerance in maize: a view from industry. Field Crops Research 90 **1**: 19-34.

Chaves, MM, Maroco, JP & Pereira, JS**.** 2003. Understanding plant responses to drought—from genes to the whole plant. Functional Plant Biology 30 **3**: 239-264.

Condon, AG, Richards, R, Rebetzke, G & Farquhar, G**.** 2002. Improving intrinsic water-use efficiency and crop yield. Crop Science 42 **1**: 122-131.

Condon, AG, Richards, RA, Rebetzke, GJ & Farquhar, GD**.** 2004. Breeding for high water-use efficiency. Journal of Experimental Botany 55 **407**: 2447-2460.

Cornic, G & Massacci, A**.** 1996.Leaf photosynthesis under drought stress. In: Advances in Photosynthesis; Photosynthesis and the environment, Vol. 5,347-366.

Cossani, CM & Reynolds, MP**.** 2012. Physiological traits for improving heat tolerance in wheat. Plant Physiology 160 **4**: 1710-1718.

Costa, JM, Grant, OM & Chaves, MM**.** 2013. Thermography to explore plant–environment interactions. Journal of Experimental Botany 64 **13**: 3937-3949.

Eitel, JU, Gessler, PE, Smith, AM & Robberecht, R**.** 2006. Suitability of existing and novel spectral indices to remotely detect water stress in Populus spp. Forest Ecology and Management 229 **1**: 170-182.

El-Hendawy, S, Al-Suhaibani, N, Salem, AEA, Ur Rehman, S & Schmidhalter, U**.** 2015. Spectral reflectance indices as a rapid and nondestructive phenotyping tool for estimating different morphophysiological traits of contrasting spring wheat germplasms under arid conditions. Turkish Journal of Agriculture and Forestry 39 **4**: 572-587.

El-Shikha, D, Waller, P, Hunsaker, D, Clarke, T & Barnes, E**.** 2007. Ground-based remote sensing for assessing water and nitrogen status of broccoli. Agricultural Water Management 92 **3**: 183-193.

Elliott, J, Deryng, D, Müller, C, Frieler, K, Konzmann, M, Gerten, D, et al. 2014. Constraints and potentials of future irrigation water availability on agricultural production under climate change. Proceedings of the National Academy of Sciences 111 **9**: 3239-3244.

Elsayed, S, Rischbeck, P & Schmidhalter, U**.** 2015. Comparing the performance of active and passive reflectance sensors to assess the normalized relative canopy temperature and grain yield of drought-stressed barley cultivars. Field Crops Research 177: 148-160.

Erdle, K, Mistele, B & Schmidhalter, U**.** 2011. Comparison of active and passive spectral sensors in discriminating biomass parameters and nitrogen status in wheat cultivars. Field Crops Research 124 **1**: 74-84.

Erdle, K, Mistele, B & Schmidhalter, U**.** 2013. Spectral high-throughput assessments of phenotypic differences in biomass and nitrogen partitioning during grain filling of wheat under high yielding Western European conditions. Field Crops Research 141: 16-26.

Esbensen, KH, Guyot, D, Westad, F & Houmoller, LP. 2002. Multivariate data analysis: in practice: an introduction to multivariate data analysis and experimental design. CAMO.

Farquhar, GD, Ehleringer, JR & Hubick, KT**.** 1989. Carbon isotope discrimination and photosynthesis. Annual review of plant biology 40 **1**: 503-537.

Fischer, R, Rees, D, Sayre, K, Lu, Z-M, Condon, A & Saavedra, AL**.** 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Science 38 **6**: 1467-1475.

Furbank, RT & Tester, M**.** 2011. Phenomics – technologies to relieve the phenotyping bottleneck. Trends in Plant Science 16 **12**: 635-644.

Gizaw, SA, Garland-Campbell, K & Carter, AH**.** 2016. Use of spectral reflectance for indirect selection of yield potential and stability in Pacific Northwest winter wheat. Field Crops Research.

Godfray, HCJ**.** 2014. The challenge of feeding 9-10 billion people equitably and sustainably. Journal of Agricultural Science 152: S2-S8.

Graeff, S & Claupein, W**.** 2007. Identification and discrimination of water stress in wheat leaves (Triticum aestivum L.) by means of reflectance measurements. Irrigation science 26 **1**: 61-70.

Grant, OM, Tronina, Ł, Jones, HG & Chaves, MM**.** 2007. Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. Journal of Experimental Botany 58 **4**: 815-825.

Gutiérrez-Rodríguez, M, Reynolds, MP, Escalante-Estrada, JA & Rodríguez-González, MT**.** 2004. Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. Australian Journal of Agricultural Research 55 **11**: 1139-1147.

Gutierrez, M, Reynolds, MP & Klatt, AR**.** 2010. Association of water spectral indices with plant and soil water relations in contrasting wheat genotypes. Journal of Experimental Botany 61 **12**: 3291-3303.

Hackl, H, Baresel, JP, Mistele, B, Hu, Y & Schmidhalter, U**.** 2012. A Comparison of Plant Temperatures as Measured by Thermal Imaging and Infrared Thermometry. Journal of Agronomy and Crop Science 198 **6**: 415-429.

Hackl, H, Hu, Y & Schmidhalter, U**.** 2014. Evaluating growth platforms and stress scenarios to assess the salt tolerance of wheat plants. Functional Plant Biology 41 **8**: 860-873.

Hackl, H, Mistele, B, Hu, Y & Schmidhalter, U**.** 2013. Spectral assessments of wheat plants grown in pots and containers under saline conditions. Functional Plant Biology 40 **4**: 409-424.

Hatfield, J, Gitelson, AA, Schepers, JS & Walthall, C**.** 2008. Application of spectral remote sensing for agronomic decisions. Agronomy Journal 100 **Supplement_3**: S-117-S-131.

Holland, JB, Nyquist, WE & Cervantes-Martínez, CT**.** 2003. Estimating and interpreting heritability for plant breeding: an update. Plant breeding reviews 22: 9-112.

Hu, Y, Hackl, H & Schmidhalter, U**.** 2016. Comparative performance of spectral and thermographic properties of plants and physiological traits for phenotyping salinity tolerance of wheat cultivars under simulated field conditions. Functional Plant Biology 44 **1**: 134-142.

Hurley, MB & Rowarth, JS**.** 1999. Resistance to root growth and changes in the concentrations of ABA within the root and xylem sap during root-restriction stress. Journal of Experimental Botany 50 **335**: 799-804.

Idso, S, Reginato, R, Jackson, R & Pinter Jr, P**.** 1981. Measuring yield-reducing plant water potential depressions in wheat by infrared thermometry. Irrigation science 2 **4**: 205-212.

Ismail, MR & Davies, W**.** 1998. Root restriction affects leaf growth and stomatal response: the role of xylem sap ABA. Scientia Horticulturae 74 **4**: 257-268.

Jackson, P**.** 2001.Direction of physiological research in breeding: Issues from a breeding perspective. In: Application of physiology in wheat breeding,11-16 Mexico, D.F.: CIMMYT.

Jackson, RD, Idso, S, Reginato, R & Pinter Jr, P**.** 1981. Canopy temperature as a crop water stress indicator. Water Resources Research 17 **4**: 133-1138.

Jasper, J, Reusch, S & Link, A**.** 2009. Active sensing of the N status of wheat using optimized wavelength combination: impact of seed rate, variety and growth stage. Precision Agriculture 9: 23-30.

Jones, HG, Serraj, R, Loveys, BR, Xiong, L, Wheaton, A & Price, AH**.** 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Functional Plant Biology 36 **11**: 978-989.

Kipp, S, Mistele, B & Schmidhalter, U**.** 2014a. Identification of stay-green and early senescence phenotypes in high-yielding winter wheat, and their relationship to grain yield and grain protein concentration using high-throughput phenotyping techniques. Functional Plant Biology 41 **3**: 227-235.

Kipp, S, Mistele, B & Schmidhalter, U**.** 2014b. The performance of active spectral reflectance sensors as influenced by measuring distance, device temperature and light intensity. Computers and Electronics in Agriculture 100: 24-33.

Lafitte, R**.** 2002. Relationship between leaf relative water content during reproductive stage water deficit and grain formation in rice. Field Crops Research 76 **2-3**: 165-174.

Li, F, Miao, YX, Chen, XP, Zhang, HL, Jia, LL & Bareth, G**.** 2010. Estimating Winter Wheat Biomass and Nitrogen Status Using an Active Crop Sensor. Intelligent Automation and Soft Computing 16 **6**: 1221-1230.

Linke, R, Richter, K, Haumann, J, Schneider, W & Weihs, P**.** 2008. Occurrence of repeated drought events: can repetitive stress situations and recovery from drought be traced with leaf reflectance? Periodicum biologorum 110 **3**: 219-229.

Liu, A & Latimer, JG**.** 1995. Water relations and abscisic acid levels of watermelon as affected by rooting volume restriction. Journal of Experimental Botany 46 **8**: 1011-1015.

Lobos, GA, Matus, I, Rodriguez, A, Romero-Bravo, S, Araus, JL & del Pozo, A**.** 2014. Wheat genotypic variability in grain yield and carbon isotope discrimination under Mediterranean conditions assessed by spectral reflectance. Journal of Integrative Plant Biology 56 **5**: 470-479.

Lopes, MS, Rebetzke, GJ & Reynolds, M**.** 2014. Integration of phenotyping and genetic platforms for a better understanding of wheat performance under drought. Journal of Experimental Botany 65 **21**: 6167-6177.

Lopes, MS & Reynolds, MP**.** 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Functional Plant Biology 37 **2**: 147-156.

Matson, PA & Vitousek, PM**.** 2006. Agricultural intensification: Will land spared from farming be land spared for nature? Conservation Biology 20 **3**: 709-710.

Mistele, B & Schmidhalter, U**.** 2008. Estimating the nitrogen nutrition index using spectral canopy reflectance measurements. European Journal of Agronomy 29 **4**: 184-190.

Mistele, B & Schmidhalter, U**.** 2010. Tractor-Based Quadrilateral Spectral Reflectance Measurements to Detect Biomass and Total Aerial Nitrogen in Winter Wheat Agron. J. 102 **2**: 499-506.

Möller, M, Alchanatis, V, Cohen, Y, Meron, M, Tsipris, J, Naor, A, et al. 2007. Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. Journal of Experimental Botany 58 **4**: 827-838.

Monneveux, P, Jing, R & Misra, SC**.** 2012. Phenotyping for drought adaptation in wheat using physiological traits. 3 **429**: 126-137.

Munns, R, James, RA, Sirault, XRR, Furbank, RT & Jones, HG**.** 2010. New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. Journal of Experimental Botany 61 **13**: 3499-3507.

Myburgh, P & Conradie, JVZW**.** 1996. Effect of soil depth on growth and water consumption of young Vitis vinifera L. cv. Pinot noir. S. Afr. J. Enol. Vitic 17 **2**: 53.

Passioura, J**.** 2012. Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Functional Plant Biology 39 **11**: 851-859.

Passioura, JB**.** 2006. Viewpoint: The perils of pot experiments. Functional Plant Biology 33 **12**: 1075-1079.

Penuelas, J, Filella, I, Biel, C, Serrano, L & Save, R**.** 1993. The reflectance at the 950–970 nm region as an indicator of plant water status. International journal of remote sensing 14 **10**: 1887-1905.

Penuelas, J, Isla, R, Filella, I & Araus, JL**.** 1997. Visible and near-infrared reflectance assessment of salinity effects on barley. Crop Science 37 **1**: 198-202.

Pingali, P**.** 2007. Westernization of Asian diets and the transformation of food systems: Implications for research and policy. Food Policy 32 **3**: 281-298.

Poorter, H, Bühler, J, van Dusschoten, D, Climent, J & Postma, JA**.** 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology 39 **11**: 839-850.

Prasad, B, Carver, BF, Stone, ML, Babar, MA, Raun, WR & Klatt, AR**.** 2007. Potential Use of Spectral Reflectance Indices as a Selection Tool for Grain Yield in Winter Wheat under Great Plains Conditions Crop Sci. 47 **4**: 1426-1440.

R Core Team, R**.** 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015. URL h ttp. www. R-project. org.

Rattey, A, Shorter, R & Chapman, S**.** 2011. Evaluation of CIMMYT conventional and synthetic spring wheat germplasm in rainfed sub-tropical environments. II. Grain yield components and physiological traits. Field Crops Research 124 **2**: 195-204.

Ray, JD & Sinclair, TR**.** 1998. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. Journal of Experimental Botany 49 **325**: 1381-1386.

Rebetzke, G, Condon, AG, Richards, R & Farquhar, G**.** 2002. Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science 42 **3**: 739-745.

Rebetzke, GJ, Condon, AG, Farquhar, GD, Appels, R & Richards, RA**.** 2008. Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. Theoretical and Applied Genetics 118 **1**: 123-137.

Rebetzke, GJ, Rattey, AR, Farquhar, GD, Richards, RA & Condon, ATG**.** 2013. Genomic regions for canopy temperature and their genetic association with stomatal conductance and grain yield in wheat. Functional Plant Biology 40 **1**: 14-33.

Reynolds, M, Balota, M, Delgado, M, Amani, I & Fischer, R**.** 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Functional Plant Biology 21 **6**: 717-730.

Reynolds, M, Dreccer, F & Trethowan, R**.** 2007. Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58 **2**: 177-186.

Richards, R, Rebetzke, G, Condon, A & Van Herwaarden, A**.** 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Science 42 **1**: 111-121.

Richards, RA, Rebetzke, GJ, Watt, M, Condon, AT, Spielmeyer, W & Dolferus, R**.** 2010. Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. Functional Plant Biology 37 **2**: 85-97.

Rischbeck, P, Baresel, P, Elsayed, S, Mistele, B & Schmidhalter, U**.** 2014. Development of a diurnal dehydration index for spring barley phenotyping. Functional Plant Biology 41 **12**.

Rischbeck, P, Elsayed, S, Mistele, B, Barmeier, G, Heil, K & Schmidhalter, U**.** 2016. Data fusion of spectral, thermal and canopy height parameters for improved yield prediction of drought stressed spring barley. European Journal of Agronomy 78: 44-59.

Rodriguez, D, Sadras, V, Christensen, LK & Belford, R**.** 2005. Spatial assessment of the physiological status of wheat crops as affected by water and nitrogen supply using infrared thermal imagery. Crop and Pasture Science 56 **9**: 983-993.

Ronchi, CP, DaMatta, FM, Batista, KD, Moraes, GA, Loureiro, ME & Ducatti, C**.** 2006. Growth and photosynthetic down-regulation in Coffea arabica in response to restricted root volume. Functional Plant Biology 33 **11**: 1013-1023.

Schmidhalter, U. 2005. Sensing soil and plant properties by non-destructive measurements. Proceedings of the International Conference on Maize Adaption to Marginal Environments. 25th Anniversary of the Cooperation between Kasetsart University and Swiss Federal Institute of Technology, Nakhon Ratchasima, Thailand: 6-9.

Schmidhalter, U, Glas, J, Heigl, R, Manhart, R, Wiesent, S, Gutser, R & Neudecker, E. 2001. Application and testing of a crop scanning instrument–field experiments with reduced crop width, tall maize plants and monitoring of cereal yield. Proceedings of the International Conference on Precision Agriculture., 3rd, Montpellier, France: 18-20.

Shaver, T, Khosla, R & Westfall, D**.** 2010. Evaluation of two ground-based active crop canopy sensors in maize: growth stage, row spacing, and sensor movement speed. Soil Science Society of America Journal 74 **6**: 2101-2108.

Shiferaw, B, Smale, M, Braun, H-J, Duveiller, E, Reynolds, M & Muricho, G**.** 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security 5 **3**: 291-317.

Siddique, M, Hamid, A & Islam, M**.** 2000. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica 41.

Sinclair, T & Ludlow, M**.** 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. Functional Plant Biology 12 **3**: 213-217.

Sinclair, TR**.** 2011. Challenges in breeding for yield increase for drought. Trends in Plant Science 16 **6**: 289-293.

Slatyer, RO. 1967. Plant-water relationships Experimental Botany: An International Series of Monographs. University of Minnesota: Academic Press.

Stimson, HC, Breshears, DD, Ustin, SL & Kefauver, SC**.** 2005. Spectral sensing of foliar water conditions in two co-occurring conifer species: Pinus edulis and Juniperus monosperma. Remote Sensing of Environment 96 **1**: 108-118.

Tilman, D, Balzer, C, Hill, J & Befort, BL**.** 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences 108 **50**: 20260-20264.

Tremblay, N, Wang, Z, Ma, B-L, Belec, C & Vigneault, P**.** 2009. A comparison of crop data measured by two commercial sensors for variable-rate nitrogen application. Precision Agriculture 10 **2**: 145-161.

Tschaplinski, TJ & Blake, TJ**.** 1985. Effects of root restriction on growth correlations, water relations and senescence of alder seedlings. Physiologia Plantarum 64 **2**: 167-176.

Tscharntke, T, Clough, Y, Wanger, TC, Jackson, L, Motzke, I, Perfecto, I, et al. 2012. Global food security, biodiversity conservation and the future of agricultural intensification. Biological Conservation 151 **1**: 53-59.

Tucker, CJ**.** 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sensing of Environment 8 **2**: 127-150.

Turner, NC, Li, F-M, Xiong, Y-C & Siddique, KH**.** 2011. Climate change and agricultural ecosystem management in dry areas. Crop and Pasture Science 62 **6**: i-ii.

Wang, S, Okamoto, G, Hirano, K, Lu, J & Zhang, C**.** 2001. Effects of restricted rooting volume on vine growth and berry development of Kyoho grapevines. American journal of Enology and viticulture 52 **3**: 248-253.

Wang, XZ, Yang, WP, Wheaton, A, Cooley, N & Moran, B**.** 2010. Automated canopy temperature estimation via infrared thermography: A first step towards automated plant water stress monitoring. Computers and Electronics in Agriculture 73 **1**: 74-83.

Wang, Y, Zhang, X, Zhang, X, Shao, L, Chen, S & Liu, X**.** 2016. Soil Water Regime Affecting Correlation of Carbon Isotope Discrimination with Yield and Water-Use Efficiency of Winter Wheat. Crop Science 56: 760-772.

Winterhalter, L, Mistele, B, Jampatong, S & Schmidhalter, U**.** 2011. High throughput phenotyping of canopy water mass and canopy temperature in well-watered and drought stressed tropical maize hybrids in the vegetative stage. European Journal of Agronomy 35 **1**: 22-32.

Winterhalter, L, Mistele, B & Schmidhalter, U**.** 2013. Evaluation of active and passive sensor systems in the field to phenotype maize hybrids with high-throughput. Field Crops Research 154: 236-245.

Wu, Y, Huang, M & Warrington, DN**.** 2011. Growth and transpiration of maize and winter wheat in response to water deficits in pots and plots. Environmental and Experimental Botany 71 **1**: 65-71.

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