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**Rye (*Secale cereale* L.): agronomic performance under drought and
methods of crop physiology to determine the drought tolerance of
winter rye.**

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Abbreviations

ANOVA	Analysis of variance
Ca	Calcium
C_i/C_a	Intercellular to atmospheric CO ₂ concentration ratio
CIMMYT	International Maize and Wheat Improvement Center
CO ₂	Carbon dioxide
CTD	Canopy temperature depression ($T_{\text{air}} - T_{\text{canopy}}$, in °C)
CT _{diff}	Canopy temperature difference ($T_{\text{canopy}} - T_{\text{air}}$, in °C)
DWD	German Weather Service
Δ	Carbon isotope discrimination rate (‰)
δ	Carbon isotope composition (‰)
DOY	Day of year
FAO	Food and Agricultural Organization of the United Nations
g_s	Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)
IPCC	Intergovernmental Panel on Climate Change
IR	Infrared
IRMS	Isotope ratio mass spectrometer
K	Potassium
LAI	Leaf area index ($\text{m}^2 \text{m}^{-2}$)
m_a	Mineral concentration (%)
Mg	Magnesium
PAR	Photosynthetic active radiation
PASW	Plant available soil water
r	correlation coefficient
Si	Silicon
T_{air}	Air temperature (°C)
T_{canopy}	Canopy temperature (°C)
TKW	Thousand kernel weight (g)
UAV	Unmanned aerial vehicle
VPD	Vapor pressure deficit (kPa)
WSC	Water-soluble carbohydrates
WUE	Water use efficiency

1. Summary

Winter rye (*Secale cereale* L.) is predominantly cultivated on light and sandy soils with a low water holding capacity and will therefore be especially affected by drought induced yield losses in Central and Eastern Europe in the future. Drought adaption through breeding is therefore an important task in order to adapt this crop to future climate conditions. In this context, the crop physiology methods canopy temperature depression ($CTD = T_{\text{air}} - T_{\text{canopy}}$) and carbon isotope discrimination (Δ) were examined for their suitability as selection criterion under drought on a small number of genotypes. Two sets of each 16 genotypes were therefore grown under different drought conditions in rain-out shelters and under well-watered conditions in the years 2011, 2012, and 2013. The CTD was determined several times during the growth period using two infrared (IR) thermometers and an IR camera. Δ -analyses were performed on mature flag leaves (Δ_L) and grains (Δ_G). Furthermore, ash content in mature flag leaves and grains, as well as mineral concentrations in mature flag leaves (Ca, K, Mg, and Si) were examined for their use as surrogates for the expensive and time-consuming Δ -analyses. In addition to the evaluation of possible selection criteria, the agronomic performance of rye in the different drought regimes was assessed: Grain-, straw-, and total aboveground biomass yields, the grain yield components spikes m^{-2} , kernels spike $^{-1}$, and thousand kernel weight (TKW), leaf area index (LAI), and phenological characteristics were examined.

Drought induced grain yield reductions ranged from 14 to 57%, whereas straw yield was generally lesser affected. The growth period was shortened by up to 12 days under drought conditions compared to optimal water supply. Grain yield was positively associated to straw yield, LAI, spikes m^{-2} , and kernels spike $^{-1}$ under water deficit. High number of grains per area land seemed to be especially important for high grain yields under drought. Furthermore, the results suggest a strong importance of pre-anthesis reserves for the reallocation of assimilates for grain filling under drought in rye.

Regarding the suitability of possible selection criteria, CTD was significantly positively related to grain yield under drought. Significant correlations between CTD and grain yield were, however, only observed when the measurements were carried out on days with optimal weather conditions. Optimal conditions turned out to be days with a clear sky, a solar irradiation $>700 \text{ W m}^{-2}$, an air temperature of at least 20°C , as well as wind speeds $<3 \text{ m s}^{-1}$. Furthermore, the results showed that also rather inexpensive IR instruments are suitable to assess the CTD. Regarding the carbon isotope discrimination, Δ_L was significantly positively related to grain yield under water deficit, but the correlation was weaker than between CTD and grain yield. Δ_G was not related to grain yield at all. Ash content and mineral concentrations were significantly related to grain yield under drought, but the correlations were quite inconsistent between the two experimental years. Because of the weak or missing relationship with grain yield, carbon isotope discrimination and its potential surrogates ash content and mineral concentration cannot be recommended for their use as selection criteria under German climate conditions at present. A general limitation of the present work was, however, the low genetic variability of the genotypes, which may have reduced the significance of the results. The results should therefore be validated with a more diverse set of genotypes. However, especially the CTD seemed to be a promising selection criterion which may help to develop drought tolerant rye genotypes, if this method can be successfully integrated into the breeding process.

2. Zusammenfassung

Winterroggen (*Secale cereale* L.) wird vorwiegend auf leichten und sandigen Böden mit niedriger Wasserhaltekapazität angebaut, und wird dadurch besonders von zukünftigen trockenstressinduzierten Ertragseinbußen in Mittel- und Osteuropa betroffen sein. Die Zucht auf Trockentoleranz ist daher eine wichtige Aufgabe, um Roggen an zukünftige Klimabedingungen anzupassen. In diesem Zusammenhang wurden die ertragsphysiologischen Methoden „Canopy temperature depression“ ($CTD = T_{Luft} - T_{Bestand}$) und Kohlenstoffisotopendiskriminierung (Δ) an einer kleinen Anzahl von Genotypen auf ihre Eignung als Selektionskriterium unter Trockenstress hin untersucht. 2 x 16 Genotypen wurden hierfür unter verschiedenen Trockenstressvarianten in Rain-out Shelters sowie unter optimal bewässerten Bedingungen in den Jahren 2011, 2012 und 2013 angebaut. Die CTD wurde im Laufe der Vegetationsperiode mehrmals mithilfe zweier Infrarot (IR) -Thermometer und einer Thermokamera gemessen. Δ -Analysen wurden an Fahnenblättern und Körnern durchgeführt. Desweiteren wurden der Aschegehalt in Fahnenblättern und Körnern sowie Einzelelementkonzentrationen im Fahnenblatt (Ca, K, Mg, Si) auf ihre Eignung als Surrogate für die teuren und aufwendigen Δ -Analysen geprüft. Ergänzend wurde die agronomische Leistung von Winterroggen unter verschiedenen Trockenstressvarianten untersucht: hierfür wurden Korn-, Stroh- und gesamter oberirdischer Biomassertrag, die Ertragskomponenten Ähren m^{-2} , Körner Ähre⁻¹ und Tausendkorngewicht (TKW), der Blattflächenindex (LAI) sowie phänologische Charakteristika untersucht.

Trockenstressinduzierte Kornertragsreduktionen lagen zwischen 14 und 57%, während der Strohertrag generell geringer reduziert wurde. Unter Trockenstress wurde die Vollreife im Vergleich zu optimal bewässerten Bedingungen um bis zu 12 Tagen früher erreicht. Der Kornertrag war unter Trockenstress positive mit Strohertrag, LAI, Ähren m^{-2} und Körner Ähre⁻¹ korreliert. Eine hohe Kornanzahl war besonders wichtig für einen hohen Kornertrag

unter Trockenstress. Ferner deuteten die Ergebnisse auf eine besondere Bedeutung vegetativer Reservepools als Assimilatquelle für die Kornfüllung hin.

Die CTD stand unter Trockenstress signifikant mit dem Kornertrag in Beziehung. Signifikante Korrelationen zwischen CTD und Kornertrag wurden jedoch ausschließlich an Tagen mit optimalen Wetterbedingungen gefunden. Optimale Bedingungen für die Messung der CTD fanden sich an Tagen mit einem wolkenlosen Himmel, einer Globalstrahlung $>700 \text{ W m}^{-2}$, einer Lufttemperatur $>20^\circ\text{C}$ sowie einer Windgeschwindigkeit $<3 \text{ m s}^{-1}$. Ferner zeigten die Ergebnisse, dass auch verhältnismäßig günstige IR-Geräte für die CTD-Messungen geeignet sind. Bei der Kohlenstoffisotopendiskriminierung war Δ_L signifikant positiv mit dem Kornertrag korreliert, die Korrelation war jedoch deutlich schwächer als die zwischen CTD und Kornertrag. Δ_G war nicht mit dem Kornertrag korreliert. Aschegehalten und Einzelelementkonzentrationen standen zwar mit dem Kornertrag in Beziehung, die Korrelationen schwankten innerhalb der Jahre jedoch stark. Aufgrund der schwachen, beziehungsweise fehlenden Korrelationen können die Kohlenstoffisotopendiskriminierung sowie deren Surrogate zum jetzigen Zeitpunkt nicht für den Einsatz als Selektionskriterium unter den vorliegenden Klimabedingungen empfohlen werden. Eine generelle Einschränkung dieser Arbeit war jedoch die geringe genetische Variabilität der untersuchten Genotypen, welche die Aussagekraft der Ergebnisse abgeschwächt haben könnte. Aufgrund dessen sollten die Ergebnisse mit einem vielfältigeren genetischen Material überprüft werden. Die CTD jedoch scheint geeignet zu sein, um als Selektionskriterium verwendet werden zu können. Diese Methode könnte die Entwicklung trockenoleranter Roggengenotypen beschleunigen, wenn sie erfolgreich in den Züchtungsprozess eingebunden werden kann.

3. General introduction

3.1 Rye

Rye (*Secale cereale* L.) is an important cereal crop in Central and Eastern Europe, which is almost exclusively cultivated as winter crop. Cultivated rye is an allogamous plant which derived from the wild species *S. montanum* and *S. vavilovii*. The primary center of origin of rye is today's Turkey, where the first rye cropping took place about 6000 years ago. Today, rye is grown on 5.6 million hectares worldwide (Schlegel, 2013). The cold tolerance and winter hardiness of rye contribute to its wide distribution in Central and Eastern Europe, where rye is cultivated on 4.8 million hectares. The most important countries for rye production are the Russian Federation, Poland, Germany, Belarus, and the Ukraine. These countries produce more than 75% of the worldwide rye (FAO, 2014). In Germany, rye is mainly used for livestock feeding (50%), baking (22.5%), and ethanol production (17.5%). In recent years, the use of rye for biogas production (currently 7.5%) is becoming more and more important (Roux et al. 2010, Blumtritt 2007). Rye has a higher yield potential than wheat on sandy, infertile, and poorly drained soils. It is, however, out-yielded by wheat on medium and high fertile soils (Schlegel, 2013). Therefore, rye is primarily cultivated on marginal soils with low fertility, on which other cereals can hardly be grown (Miedaner et al. 2012). Rye is recognized to be the most drought tolerant cereal crop because of its extensive and well branched root system, which takes up water very efficiently (Starzycki 1976). The root dry weight of rye exceeds that of wheat and triticale (Sheng and Hunt 1991). Furthermore, rye uses 20-30% less water per unit of dry matter than wheat (Starzycki, 1976). In experiments with the winter cereal crops barley (*Hordeum vulgare* L.), rye, triticale (*Triticosecale* Wittmack), and wheat (*Triticum aestivum* L.) Schittenhelm et al. (2014) found the lowest grain yield reduction in winter rye when the crops were solely dependent on residual winter soil moisture. Winter rye was also found to be only slightly negatively

influenced during a severe drought in multi location trials with eight crops in the Czech Republic (Hlavinka et al. 2009). However, despite the relative good drought tolerance of rye compared to other cereals when grown on the same soil, its cultivation on marginal soils makes it especially vulnerable to drought events. For example, the intense drought in the spring 2007 reduced the mean grain yield of winter rye in Germany by 16% compared to the mean of 2000-2009 (Statistisches Bundesamt 2014, DWD 2008). The grain yields of wheat, barley, and triticale were reduced by only 6 to 9% in the same year, most likely due to their cultivation on soils with higher fertility. For this reason, the development of drought tolerant rye cultivars is of great importance.

3.2 Drought stress

There are four main definitions of drought: meteorological, agricultural, hydrological, and socio-economic drought (Wilhite and Glantz 1985): Meteorological drought originates from a deficiency of precipitation over a certain period of time. Agricultural drought is a consequence of the meteorological drought resulting in a soil moisture deficit which leads to an insufficient water supply to crops. A hydrological drought is present when water reserves in aquifers, lakes, and reservoirs fall below the average. Socio-economic drought is defined as the practical consequences of the above-mentioned types of drought, affecting the supply and demand of economic goods and services. Hereinafter, drought is always referred to as agricultural drought. Drought is a worldwide problem in agriculture and recognized to be the most important abiotic stress (Spinoni et al. 2014). The region of Central and Eastern Europe is characterized by a humid climate and is not a typical drought region like the Mediterranean area, where drought events occur quite regularly (EEA 2009). However, droughts in Europe are not restricted to the Mediterranean area and can occur in all regions in any season (Lloyd-Hughes and Saunders, 2002). There were, for example, already some significant drought events during spring and summer in Central and Eastern Europe in the last years - possibly the

first signs of the ongoing climate change. The drought and heat wave in 2003, for example, significantly reduced the primary productivity in Europe by 30% (Ciais et al. 2005). In 2011, rainfall was only 40 – 80 % of the long term mean between January and May, leading to strong reductions in cereal grain yields (Statistisches Bundesamt 2014, DWD 2011). Although it is unclear whether the total annual precipitation in Central and Eastern Europe is increasing or decreasing in the next decades, it is very likely that the precipitation during the main growth phase in summer is decreasing. Together with the predicted temperature rise and the subsequent increasing evaporative demand, the intensity and frequency of drought events are predicted to further increase in this region during the next decades (IPCC, 2014).

3.3 Effect of drought stress on cereal crops

The effects of drought on plants range from molecular to morphological levels during all phenological stages (Farooq et al. 2009). One of the first reactions of plants to drought is the closing of stomata (Condon et al. 1990), which results in reduced photosynthesis rates and carbon assimilation (Cornic and Massacci 1996). Stomatal closure decreases the transpiration rate, causing an increase in canopy temperature (Jones and Leinonen, 2003) and limited uptake of minerals via the transpiration stream (Masle et al. 1992). Furthermore, the lower carbon assimilation under drought diminishes cell division and expansion, which leads to reduced plant growth (Barnabás et al. 2008). Water deficit consequently causes a reduction in aboveground biomass (Estrada-Campuzano et al. 2012) and LAI (Breda 2003), as well as a faster leaf senescence (Hafsi et al. 2007). Furthermore, the phenological development is accelerated under drought, causing an earlier flowering and a shorter grain filling period (Foulkes et al. 2007, Gooding et al. 2003). All mentioned and further drought effects result in reduced grain yields in cereal crops in the end because grain yield is the final consequence of all previous reactions to water deficit. Reported drought-induced yield reductions of different cereals species cover a wide range. For winter rye, reported yield decreases under drought

range from 24% (rainfed conditions, Hübner et al. 2013) to 60% (residual soil moisture only, Schittenhelm et al. 2013). For winter wheat grown under rainfed conditions and severe drought in Serbia, grain yield decreases of 8 and 38% were found by Dodig et al. (2008). For winter wheat grown under rainfed conditions in Italy, Guinta et al. (1993) found a decrease of 25-54% compared to a well-watered control. The grain yield reduction is, however, dependent on the timing, intensity, and duration of the water deficit because the different development stages show different sensitivities to water deficit (Cattivelli et al. 2008). Grain yield can be analyzed in terms of the yield components spikes m^{-2} , kernels spike $^{-1}$, and thousand kernel weight (TKW). Drought during early development stages prior to anthesis affects grain yield through reduced spike number and reduced number of kernels per spike, leading to a smaller number of kernels per area land (Dolferus et al. 2011). Drought during grain filling, on the other hand, will reduce the duration of the grain filling period, leading to a reduced kernel weight (Gooding et al. 2003). Grain number is generally considered to be the main determinant for changes in grain yield while the grain weight has only a minor influence on final grain yield (Slafer et al. 2014, Estrada-Campuzano et al. 2012, Chmielewski and Köhn 2000).

3.5 Drought tolerance and drought adaption mechanisms

Drought tolerance of crop plants must be defined in terms of yield in relation to limited water supply (Passioura 1996), such as the ability to grow, flower, and display economic yield under suboptimal water supply (Farooq 2009). A crop which produces more yield under suboptimal water supply compared to another by means of the different adaption mechanisms can be considered to be relatively more drought tolerant. No single mechanism can explain the drought tolerance of a crop alone; drought tolerance is always an interaction of different adaption mechanisms. The different mechanisms related to drought tolerance are often categorized into drought escape, dehydration avoidance, and dehydration tolerance (Blum,

2005). Drought escape can be attained by synchronizing the crop cycle with water availability. An earlier anthesis was found to increase grain yields in Mediterranean environments because of the resulting lower drought stress level and lower temperatures during the earlier grain filling period (Loss and Siddique 1994). Dehydration avoidance can be achieved by reduced water loss, for example, through stomatal control of transpiration and/or a deeper and larger root system, which allows a better access to water. Different root traits were, for example, recognized to play a major role in drought adaption of wheat (Kirkegaard et al. 2007, Manschadi et al. 2006). Dehydration tolerance is defined as the maintenance of physiological functions when the plant is already dehydrated (Blum 2005). An example is osmotic adjustment, which is the active accumulation of solutes in response to water deficit. This lowers the osmotic potential and attracts water into the cell and maintains its turgor (Moinuddin et al. 2005). Osmotic adjustment is an important adaption mechanism under water deficit in many crop species (Ludlow and Muchow, 1990). All above mentioned and further adaption mechanisms diminish the negative effects of drought stress, but they represent a tradeoff between plant survival and yield.

3.6 Secondary traits for drought tolerance improvement

Cereal breeding is at present primarily based on direct selection for grain yield (Araus et al. 2002). This approach is, however, not optimal for the selection of drought tolerant genotypes, as grain yield is characterized by a low heritability and a high genotype x environment interaction (Jackson et al. 1996). As a promising alternative, the use of secondary traits has often been suggested (Balota et al. 2008, Araus et al. 2002, Reynolds et al. 1994). Secondary traits, which are defined as plant characteristics beside grain yield, can give further information about how yield changes under drought and may, therefore, help to improve yields under water limited conditions. According to Monneveux and Ribaut (2006), a secondary trait should be genetically associated with grain yield under drought, genetically

variable, highly heritable, and easy, inexpensive, and rapid to assess. However, the most crucial factor is that the trait must be related to grain yield (Araus et al. 2002). Reynolds et al. (2005) suggested four groups of secondary traits which are related to increased productivity under drought in wheat: (1) traits related to pre-anthesis growth (e.g. early vigor, stem carbohydrate reserves), (2) traits related to access to water (canopy temperature depression, carbon isotope discrimination, relative water content, osmotic adjustment), (3) traits related to water use efficiency (carbon isotope discrimination, harvest index), and (4) traits related to photo-protection (anti-oxidants, leaf anatomy). Many of these suggested secondary traits have been examined in recent years, such as stem carbohydrate reserves (Zhang et al. 2013), canopy temperature depression (Lopez et al. 2012, Balota et al. 2008), carbon isotope discrimination (Monneveux et al. 2005, Araus et al. 2001), relative water content (Larbi and Mekliche, 2004), and osmotic adjustment (Moinuddin et al. 2005).

Canopy temperature depression ($CTD = T_{\text{air}} - T_{\text{canopy}}$)¹ and carbon isotope discrimination (Δ) are related to access to water. Both traits are recognized as indicators for plant water status and were suggested as selection criteria for cereal grain yield under dry conditions (Reynolds et al. 2006, Rebetzke et al. 2002). During photosynthetic gas exchange, C₃-plants discriminate against the heavier and less abundant ¹³C isotope in favour of the lighter and more abundant ¹²C isotope, which leads to a depletion of ¹³C in plant matter (Farquhar et al. 1989). Carbon isotope discrimination, therefore, provides information about the transpiration efficiency during the whole growth period of the sample tissue (Farquhar and Richards, 1984). The CTD allows the contact-free and non-destructive detection of changes in plant water status. Stomatal closing affects the transpiration rate and reduces transpiration cooling, which increases the canopy temperature. The canopy temperature can then be detected with infrared

¹The difference between canopy and air temperature can be expressed as canopy temperature depression ($CTD = T_{\text{air}} - T_{\text{canopy}}$) or canopy temperature difference ($CT_{\text{diff}} = T_{\text{canopy}} - T_{\text{air}}$). Both expressions are commonly used in literature, and they are only distinguished in the sign of the difference. The CTD is used in this work.

thermometry and photometry (Jones and Leinonen, 2003). Genotypes with high CTD (low canopy temperature) and/or high Δ under drought would, therefore, be recognized as relatively drought tolerant, because they can maintain a higher plant water status than genotypes with low CTD and/or Δ . Both CTD and Δ have been examined on a wide range of plant species and in different regions: The CTD has been used in experiments with wheat (Balota et al. 2007, Fischer et al. 1998), maize (Irmak et al. 2000), rice (Takai et al. 2010), cotton (Cohen et al. 2005), sorghum (O'Shaughnessy et al. 2012) and peanut (Balota et al. 2012). Similarly, Δ has been examined in wheat (Zhu et al. 2008, Monneveux et al. 2004, Merah et al. 2001), barley (Chen et al. 2012, Voltas et al. 1998), maize (Caberea-Bosquet et al. 2009), sugar beet (Bloch et al. 2006), groundnut (Rajabi et al. 2009), and grassland species (Tsialtas et al. 2002). The mentioned studies were predominantly examined under arid and semi-arid climate conditions, for example in Mexico (Gutierrez et al. 2010), Texas, USA (Balota et al. 2007), Spain (Royo et al. 2002), Southern France (Merah et al. 2001), and South Australia (Condon et al. 1990). The CTD was mostly positively related to grain yield under drought stress measured under arid and semiarid conditions (Balota et al. 2007, Rashid et al. 1999). The relationship between Δ and grain yield, however, was strongly dependent on location, crop species, and type and age of the examined plant organ. The correlations ranged from positive (Monneveux et al. 2005, Merah et al. 2001) to negative (Condon and Hall 1997), and some authors could not observe a relationship between Δ and grain yield (Hafsi et al. 2007).

3.7 Aims / Objectives

The first part of this thesis (sections 4 and 5) deals with the suitability of CTD and Δ for the selection of drought tolerant winter rye genotypes in Germany. Information is scarce about the suitability of both methods in a temperate climate because previous experiments were almost exclusively carried out in arid and semiarid regions. Furthermore, neither CTD nor Δ

have so far been examined on rye. Beside their suitability as selection criterion, further objectives for the CTD were the evaluation of optimal weather conditions for the measurements as well as a comparison of three IR measurement devices. Further objectives for Δ were the evaluation of ash content and single mineral concentrations as possible surrogates for the rather expensive and time consuming Δ -analyses.

The second part of this thesis (section 6) focuses on the effects of different drought events on the agronomic performance of winter rye as there is hardly any information available on this topic. The effect of timing, intensity, and duration of drought stress on yield, yield components, and further morphological characteristics were studied.

4. Suitability of canopy temperature depression in a temperate climate with drought-stressed winter rye, determined with three infrared measurement devices.

Kottmann, L., Schittenhelm, S., Wittich, K.P., and Wilde P., 2013. Suitability of canopy temperature depression in a temperate climate with drought-stressed winter rye, determined with three infrared measurement devices. *Journal of Agronomy and Crop Science* 199, 385-394.

DROUGHT STRESS

Suitability of Canopy Temperature Depression in a Temperate Climate with Drought-Stressed Winter Rye, Determined with Three Infrared Measurement Devices

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Abstract

Canopy temperature has been recognised as an indicator of crop water status and may thus be a useful secondary trait in selecting for yield under dry conditions. The aim of this study was to test the suitability of canopy temperature depression ($CTD = T_{\text{air}} - T_{\text{canopy}}$) in a temperate climate with winter rye, by means of three infrared (IR) temperature measuring devices. In the years 2011 and 2012, 16 winter rye genotypes were examined under drought stress conditions in a rainout shelter and under well-watered conditions. In each year, the CTD was determined several times during the growth period using two IR thermometers and an IR camera. By means of CTD, it was possible to detect drought stress and to differentiate between water regimes. The three measurement devices showed comparable results, despite greatly different costs. Under drought-stress conditions, a significant positive correlation between grain yield and CTD was found on most measurement dates in 2011 and on some dates in 2012. When the CTD was pooled across water regimes, a significant positive correlation between grain yield and CTD was obtained on every measurement date. However, as genotypic differences for CTD were non-existent, the correlations are less meaningful. The missing genotypic differences for CTD were rather caused by the limited genetic variability of the genotypes used in this study, than by climatic conditions. Due to this limitation, we were not able to make a concluding statement about the CTD in a temperate climate, although the results are quite promising and indicate that the CTD can potentially be used in a temperate climate.

Introduction

Drought is one of the most yield-limiting environmental stresses worldwide. Because of the ongoing increase in temperature and the increasing evaporative demand of the atmosphere, droughts are expected to occur more frequently in the future, even in Central Europe (Acamo et al. 2007). Winter rye (*Secale cereale* L.) is typically grown in Central and Eastern Europe especially on sandy soils with low water-holding capacity (Miedaner et al. 2012). Therefore, rye could be greatly affected by drought stress-induced yield losses due to climate change, although this crop is recognised as drought-tolerant comparatively to other cereals. One aspect of the tolerance to drought is the highly

developed root system that takes up water very efficiently (Starzycki 1976).

Because water evaporation is an energy-demanding process, increased plant transpiration causes a decrease in the plant surface temperature (Maes and Steppe 2012). When drought stress occurs, plants close their stomata to avoid transpiration water losses. The resulting decrease in transpiration cooling causes an increase in canopy temperature (Jones and Leinonen 2003). Therefore, it is possible to detect changes in stomatal conductance contact-free and non-destructively through measuring canopy temperature, which has been recognised as an indicator of plant water status for a long time (Jackson et al. 1981, Idso 1982). The difference between air and canopy temperature

is often referred to as canopy temperature depression ($CTD = T_{\text{air}} - T_{\text{canopy}}$) or canopy temperature difference ($CT_{\text{diff}} = T_{\text{canopy}} - T_{\text{air}}$). Both CTD and CT_{diff} are commonly used in the literature. The two expressions are only distinguished in the sign of the difference. The canopy temperature has been used in drought (Rashid et al. 1999, Lopes et al. 2012, Yan et al. 2012) and heat stress experiments (Reynolds et al. 1994, Amani et al. 1996, Ayeneh et al. 2002) as well as for irrigation scheduling (Lobo et al. 2004, Gontia and Tiwari 2008, Alchanatis et al. 2010). A wide range of plant species has been studied: among others wheat (Amani et al. 1995, Fischer et al. 1998, Balota et al. 2007), maize (Irmak et al. 2000), rice (Sadler et al. 2002, Takai et al. 2010, Yan et al. 2012), cotton (Cohen et al. 2005, Alchanatis et al. 2010, Padhi et al. 2012) and sorghum (O'Shaughnessy et al. 2012).

For wheat, several authors found significant genotypic effects on CTD and a significant relationship between CTD and grain yield. Balota et al. (2007) reported a significant influence of genotype on CTD, and a significant positive correlation between CTD and grain yield under dry land and irrigated conditions. Similar results were found by Blum et al. (1989) and Rashid et al. (1999). All the above-mentioned studies were conducted under semi-arid conditions. Other studies, for example Royo et al. (2002), could not detect many significant genotypic effects or strong significant relationships between CTD and grain yield. Because of the contrasting results, the suitability of the CTD as a predictive parameter should be determined for individual environments and climate zones as it is highly dependent on climatic conditions.

To our knowledge, the canopy temperature has never been used to assess the plant water status of rye in temperate climates. The objectives of this study were (i) to test the method of CTD in a temperate climate with winter rye, (ii) to compare three IR devices for canopy temperature measurement and (iii) to examine the suitability of CTD as a method for phenotyping in plant breeding.

Materials and Methods

Field experiments

The experiments were conducted with winter rye in the years 2011 and 2012 on the experimental field (52.30 N, 10.44 E, 80 m above sea level) of the Julius Kühn-Institute near Braunschweig, Germany. The soil was a Haplic Luvisol (FAO 1997) with an available water capacity of 120 mm and a water table 10 m below ground. The sizes of the plots were 5.6–7.2 m² and the seedling density was 230 seeds m⁻². Nitrogen was applied as 130 kg ha⁻¹ calcium ammonium sulphate in both years, split into 60 kg N ha⁻¹ at the beginning of vegetation and 70 kg N ha⁻¹ at the beginning of

stem elongation. Growth regulators were used to avoid lodging. Fungicides and pesticides were applied to avoid any plant diseases.

The experimental design was a 4 × 4 alpha-lattice with two replications. Plants were grown under drought-stressed and well-watered conditions. Drought stress was produced by growing the rye plants under a foil tunnel covered with a 200- μm polythene foil that was mounted at the beginning of stem elongation (DOY 103 in 2011 and DOY 95 in 2012). Plants received no rain or irrigation from that date until harvest (DOY 201 in 2011 and DOY 205 in 2012). Only the field-stored soil moisture was accessible. Therefore, the plant available soil water (PASW) decreased constantly during the course of the vegetation period. To attain good ventilation in the foil tunnel, the front and the sides of the foil tunnel were open. The control plots under field conditions were supplementary irrigated by a drip irrigation system to keep the field capacity between 60 % and 80 %.

Plant material

A total of 16 winter rye (*S. cereale* L.) genotypes were used in this experiment: three parental inbred lines (Lo115-N, Lo90-N and Lo117-N) and 12 F₂ :₄ lines selected from the two respective biparental populations F1 (Lo115-N × Lo90-N) and F1 (Lo115-N × Lo117-N). All these lines were outcrossed to the same cytoplasmic male sterile tester. These genotypes were used because of their diverse grain yield performance under drought stress in previous breeding trials. For example, the parental line Lo115 showed the best performance among the three parental lines under low rainfall (Hübner et al. 2013). Additionally, the hybrid cultivar Palazzo was used as a reference, which showed comparable grain yield and CTD performance to the 15 other genotypes. All plant materials were provided by KWS Lochow GmbH (Bergen, Germany).

Canopy temperature depression

The CTD is the difference between air and canopy temperature ($CTD = T_{\text{a}} - T_{\text{c}}$), and it takes a positive value when the canopy is cooler than the air. The canopy temperature was measured with the hand-held infrared thermometers (IRTs) Raynger MX (IRT-R; Raytek Corporation, Santa Cruz, CA, USA) and KT 17 (IRT-H; Heitronics, Wiesbaden, Germany), and the infrared (IR) camera ThermaCam PM675 (IRC; FLIR Systems, Wilsonville, OR, USA). Emissivity (ϵ) was set to 0.98 for all devices. Further technical details about the devices are given in Table 1. A comparison among all three measurement devices was made before the beginning of the field experiments to estimate their bias to each other. The comparison took place in the laboratory

Table 1 Specifications of the infrared devices

Device	Accuracy (°C)	Spectral range (μm)	Field of view
Raynger MX (IRT-R)	± 0.75	8–14	6°
Heitronics KT17 (IRT-H)	± 0.35	8–14	30°
FLIR ThermaCAM (IRC)	± 2 (absolute) ± 0.1 (relative)	7.5–13	24° × 18°

to exclude wind effects and to ensure constant environmental radiation conditions. It was found that, with respect to IRT-H, the IRC measured a higher surface temperature by 0.2 Celsius (°C) while the IRT-R device provided a lower one by 1 °C. Outside the laboratory, however, after setting the real (sky-affected) environmental temperature in the IRC, the radiation signal from the target surface provided a higher temperature by up to 2 °C compared to IRT-H. The canopy temperature measurements in the field started when the canopy was closed and no soil was visible. The measurements were performed around noon on eight days each year except for the drought stress treatment in 2011, where CTD was measured six times because the plants were completely senescent at the last two measurement dates. The IRTs were held, with the sun in the back, 50 cm above the canopy at an angle of 30° to the horizon. Each four series of measurements was taken per plot and averaged. With the IR camera, one picture of two plots was taken with a resolution of 320 × 260 pixels and analysed with the 'ThermaCAM Researcher' software (FLIR Systems). Sampling of all plots took about 1 h with the IRTs and 30 min with the IRC.

Grain yield

The plants were harvested at maturity. In 2011, the whole plots were hand-harvested. In 2012, 0.5 m² of the plots were harvested by hand and the rest of the plots by a Nursery Master plot combine (Wintersteiger, Ried, Austria). The hand-harvested plants were separated into ears and stems. The ears were threshed and winnowed. Grain samples were oven-dried to a constant weight at 105 °C for 24 h. Grain yield was calculated on the basis of 0 % water content in t ha⁻¹.

Climate conditions

Air temperature, solar irradiance, relative humidity and wind speed were recorded at 2 m height with an iMETOS weather station (Pessl Instruments, Weiz, Austria), located near the experiments and outside the foil tunnel. The PASW was calculated with the agrometeorological advisory system 'Agrowetter' from the German Weather Service (DWD 2013), and the values were periodically validated by

gravimetric soil sampling. During the CTD measurements, dry- and wet-bulb air temperature and relative humidity were additionally recorded in between the measured plots in and outside the foil tunnel at 2 m height with PT100 sensors within a psychrometer (self-developed by DWD, Braunschweig, Germany). The psychrometer was connected to a Combilog 1020 datalogger (Theodor Friedrichs, Schenefeld, Germany).

Statistics

Analyses of variance were carried out with the GLIMMIX procedure of SAS (SAS Institute, Cary, NC, USA). Individual years were analysed separately. Genotype and water regime were considered as fixed effects and replication as a random effect. For the CTD, analysis was performed separately for each IR device and for every measurement date. Differences between the three IR devices were analysed via a separate ANOVA. Correlation coefficients were calculated with the CORR procedure of SAS. Graphs were created with Sigmaplot 12 (Systat Software Inc., Chicago, IL, USA).

Results

Climatic conditions and grain yield

Under drought-stressed conditions, a total of 131 mm in 2011 and 112 mm in 2012 were accessible to the rye crops as PASW from April to July. Under well-watered condition, PASW amounted 464 mm in 2011 and 519 mm in 2012. The proportion of additional irrigation water was 196 and 162 mm in 2011 and 2012, respectively. The PASW in the drought stress treatment decreased progressively from stem elongation to maturity (Fig. 1), leading to an increasing level of stress during the vegetation period. The air was warmer and drier in 2011 compared to 2012 (Table 2), but the level of drought stress was nearly the same in both years as drought stress reduced the grain yield in both years by 57 % to 4.19 t ha⁻¹ in 2011 and to 4.58 t ha⁻¹ in 2012. The mean grain yield under well-watered conditions was 9.48 t ha⁻¹ in 2011 and 10.6 t ha⁻¹ in 2012. In both years, the effect of water regime on grain yield was significant ($P < 0.001$), and in 2012, grain yield showed also significant genotypic variation ($P < 0.05$). In neither of the 2 years, the genotype × water regime interaction was significant.

Effects of water supply on CTD

In each year, the seasonal mean CTD showed significantly lower values under drought stress than under well-watered conditions (Fig. 2). The differentiation between the treatments was higher in 2011 than in 2012 for all IR devices.

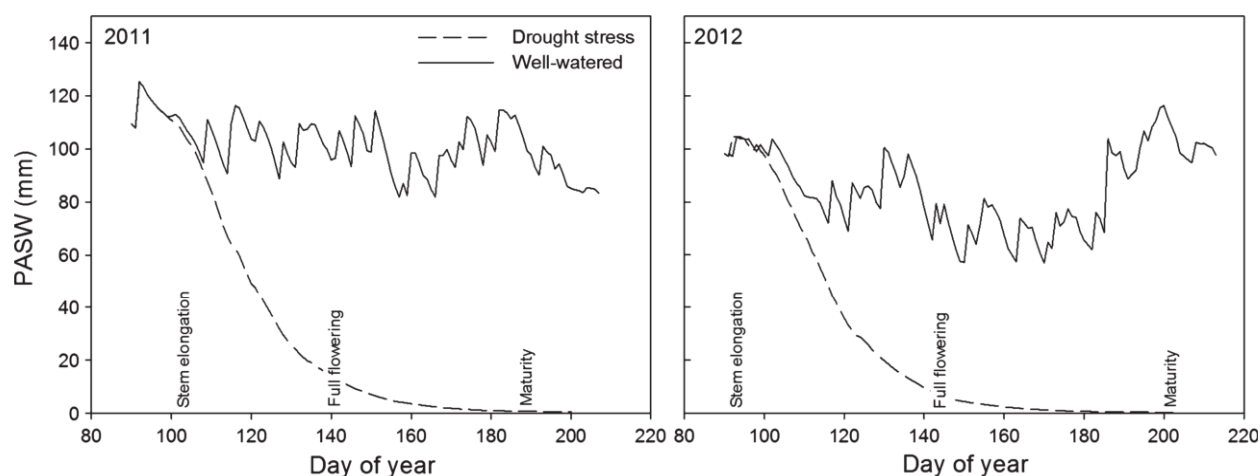


Fig. 1 Course of the plant available soil water under drought-stressed and well-watered conditions in the cropping seasons 2011 and 2012. The dates of stem elongation, full flowering and maturity refer to the drought-stressed plants.

Month	Mean air temperature (°C)	Minimum air temperature (°C)	Maximum air temperature (°C)	Rainfall (mm)	Mean relative humidity (%)
2011					
April	15.5	5.5	26.3	22.8	58
May	14.9	-1.2	28.9	23.8	66
June	17.3	5.0	31.3	60.8	71
July	16.8	8.7	28.3	70.4	76
Mean or total	16.1	4.5	28.7	177.8	68
2012					
April	8.9	-2.0	27.7	32.6	75
May	14.7	2.4	29.4	49.6	71
June	15.2	4.8	29.5	63.6	81
July	17.6	8.3	33.3	129.4 ²	80
Mean or total	14.1	3.8	30.0	275.2	77

Table 2 Climatic conditions¹ in the cropping seasons of 2011 and 2012

¹Climatic data were recorded with the iMETOS weather station outside the foil tunnel.

²A heavy rain event with 38 mm occurred on 5 July 2012.

IRT-H gave the strongest differentiation between drought-stressed and well-watered conditions. There were clear and significant differences in the levels of CTD measured with the three devices. In 2011, for example, the mean CTD under well-watered conditions amounted to -0.13 , 1.08 and -1.83 °C for IRT-R, IRT-H and IRC, respectively. The seasonal course of the CTD measurements showed strong variability in both years (Fig. 3). The drought-stressed plants had a consistently and significantly ($P < 0.001$) lower CTD than the well-watered plants on all measurement days. The CTD measured with the three IR devices had significantly different temperature bases on almost all measurement days, but the CTD curves largely follow a parallel course. IRT-H gave the highest CTD values of all IR devices, followed by IRT-R. IRC showed the lowest CTD

values, which were never positive even under well-watered conditions.

Effects of genotype on CTD

The CTD of the 16 genotypes showed a greater variation under drought stress ($2.7 - 3.6$ °C) than under well-watered conditions ($0.8 - 1.5$ °C). However, no significant effect of genotype on CTD was observed in 2011, neither under drought-stressed nor well-watered conditions (data not shown). In the year 2012, a significant ($P = 0.025$) variation among the genotypes under drought stress conditions was only found on one measurement day (DOY 142) with the IRT-H. Significant genotype \times water regime interaction for CTD was non-existent in both years.

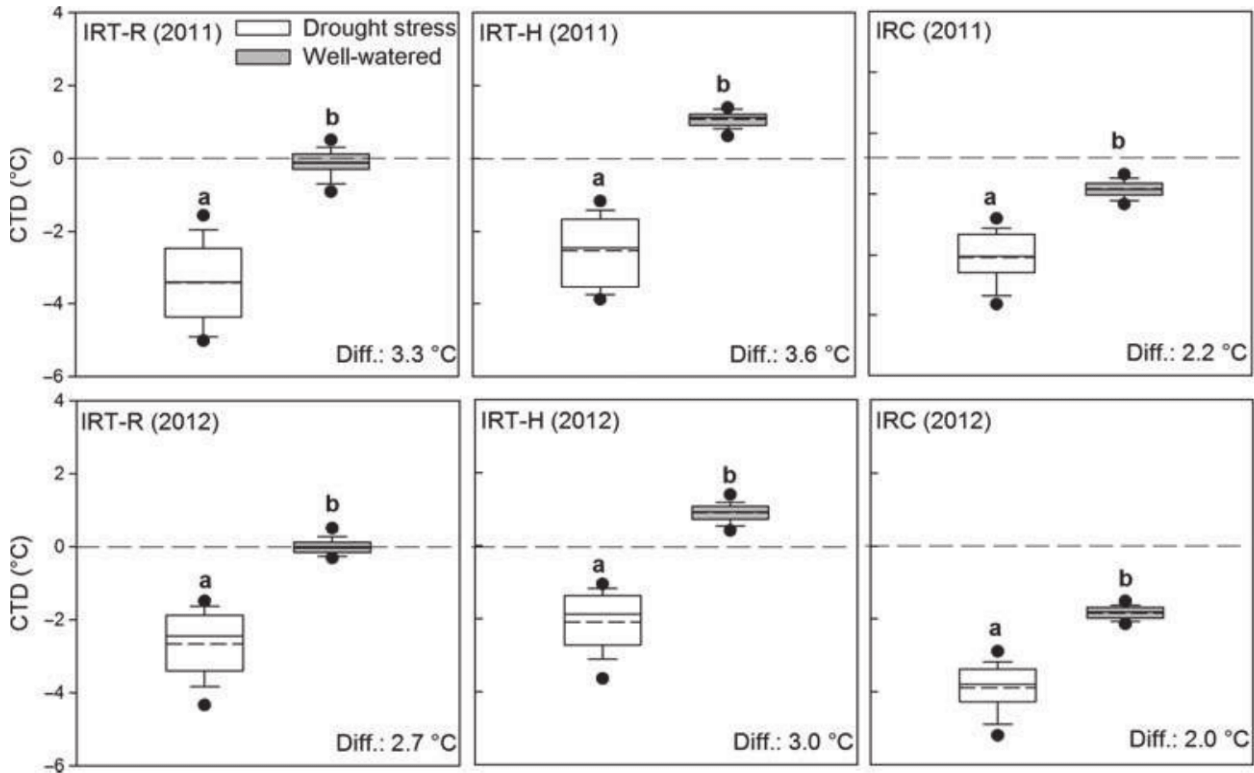


Fig. 2 Boxplots of mean seasonal canopy temperature depression (CTD; mean of all measurement days per year) under stress and well-watered conditions for three infrared devices during the 2011 and 2012 season. The dotted lines within the boxplots indicate the arithmetic means. 'Diff.' refers to the difference between the mean CTD of drought-stressed and well-watered plants. The dotted line within the graph implies $T_{air} = T_{canopy}$. Bars with different letters are significantly different ($P < 0.05$).

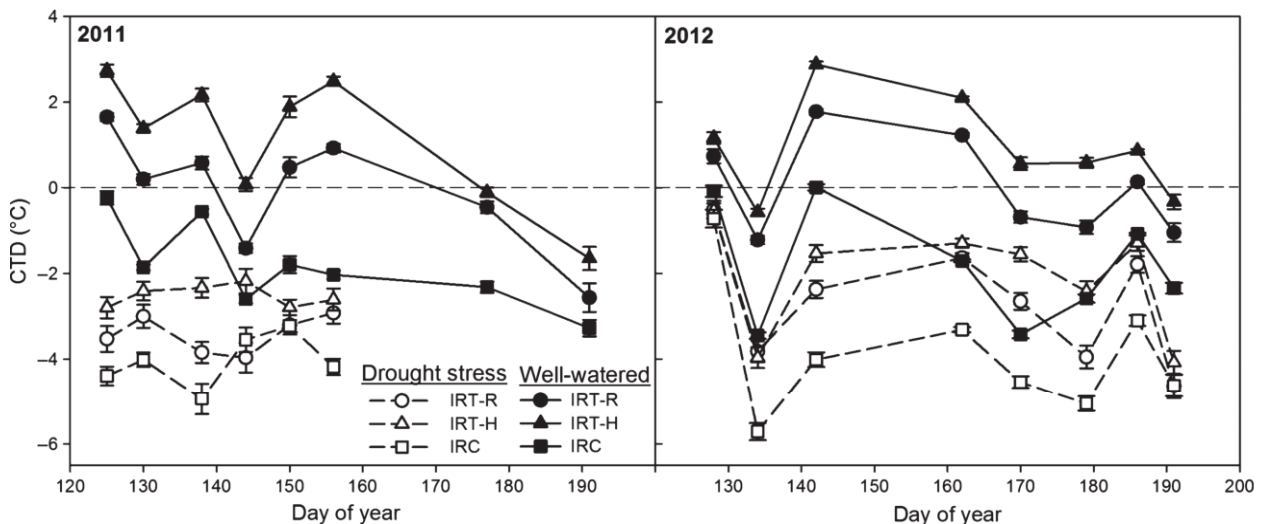


Fig. 3 Seasonal course of the drought-stressed and well-watered mean canopy temperature depression (CTD) for three infrared (IR) devices in the 2011 and 2012 cropping season. On the last two measurement days in 2011, the measurements were not taken for plants under drought-stressed conditions, as the plants were already completely senescent. The dotted line implies $T_{air} = T_{canopy}$. Vertical bars represent ± 1 standard error. Differences between the CTD of drought-stressed and well-watered conditions for the three IR devices were significant at all days ($P < 0.05$).

Relationship between CTD and grain yield

Significant relationships between CTD of single measurement days and grain yield under drought stress conditions were found in 2011 on four measurement days with IRT-R and IRT-H, and on five measurement days with IRC (Table 3). In 2012, CTD was significantly positively correlated with grain yield under drought-stressed conditions on two of the eight measurement days. All three devices also showed a significant negative correlation on one day. Under well-watered conditions, a significant relationship between CTD and grain yield did not occur in 2011, but in 2012 on one measurement day with IRT-R and IRT-H, and on four measurement days with IRC. When CTD data were pooled over both water regimes, the positive relationship between grain yield and CTD was significant on every measurement day in both years (Table 3).

In 2011, an effect of solar irradiance on CTD was clearly evident. Significant relationships between CTD and grain yield were achieved with IRT-R and IRT-H only on days with a solar irradiance $>550 \text{ W m}^{-2}$. The IRC detected a significant correlation also on a day with a solar irradiance of 176 W m^{-2} during the measurement period. In the cropping season 2012, no clear influence of the solar irradiance was notable. Unlike in 2011, a significant relationship between CTD and grain yield was not generally achieved on days with a high solar irradiance. Air temperature, relative humidity, vapour pressure deficit and wind speed showed no clear influence on the relationship between CTD and grain yield in 2011 and 2012.

Discussion

In this study, the CTD of 16 winter rye genotypes was examined with three different IR devices under drought-stressed and well-watered conditions. To achieve a good differentiation, precipitation was completely kept out from the beginning of stem elongation under drought-stressed conditions, whereas the well-watered control was optimally supplied with water. The effects of water regime and genotype on CTD and the relationship between CTD and grain yield are discussed first. Thereafter, the differences among the IR devices are summarised and the suitability of CTD as a secondary trait for plant breeders in a temperate climate is discussed.

Effects of water supply on CTD

The significantly lower CTD of the plants under drought-stressed conditions was caused by drought-induced stomatal closure (Farooq *et al.* 2009). The well-watered plants on the contrary were able to maintain a higher CTD through transpiration cooling (Maes and Steppe 2012). A higher

CTD due to transpiration cooling is reported for wheat by Rashid *et al.* (1999) in the north-western USA and by Hackl *et al.* (2012) in Germany, for instance. The air temperatures were overall higher in 2011, leading to a greater evaporation demand. As a result, a better differentiation of the CTD between the drought stress and well-watered treatments could be achieved. In Denmark, Jensen *et al.* (1990) also found a larger difference between the surface temperatures of drought-stressed and well-watered wheat and barley crops under higher air temperatures compared to lower ones. Similar results were reported for wheat in Israel by Blum *et al.* (1989). The strong variability in the seasonal course of the CTD in both years was caused by the instable and variable weather pattern during the cropping season. Recommended conditions for canopy temperature measurements in the literature include 'days with mean solar irradiance of $>500 \text{ W m}^{-2}$ ' (Balota *et al.* 2007), 'cloudless days' (Rashid *et al.* 1999) and 'full sunshine' (Ayeneh *et al.* 2002). Due to the lack of cloudless days, especially in the year 2012, measurements were also taken under partially cloudy conditions. The decline of the CTD on the last measurement days in both years is rather caused by leaf senescence than by weather conditions.

Effects of genotype on CTD

The fact that no significant genotypic effects on CTD occurred under drought stress on most measurement days might have resulted from the rapidly changing weather conditions during the measurement period. The differences of the CTD among the genotypes, which have to be detected, are small. Therefore, a differentiation of genotypes is especially difficult in a temperate climate, where the weather conditions are unstable (Jones 1999). However, it is assumed that the biggest limitation was the plant material used in this study. The genotypes were selected because of their different grain yield performance under drought in previous breeding trials. However, we were not able to detect these differences in the present study. For example, significant differences in grain yield among the genotypes could only be found under drought stress conditions in 2012. Therefore, significant variation in CTD could not be detected even on days with favourable weather conditions. In arid and semi-arid environments, some authors found significant effects of genotype on CTD under drought stress and some did not. Balota *et al.* (2007) reported significant differences among the CTD of three wheat cultivars under dry land conditions in Texas, USA. Hackl *et al.* (2012) were able to detect significant genotypic differences in CTD between two wheat cultivars grown in containers under drought-stressed conditions in the temperate climate of southern Germany. Rashid *et al.* (1999) on the contrary could not detect any genotypic differences of CTD in wheat

Table 3 Relationship between canopy temperature depression (CTD) and grain yield under drought-stressed and well-watered conditions and pooled over both water regimes. The CTD was measured on several days with contrasting weather conditions¹ with three infrared devices

Day of year	Water regime and measurement device												Weather conditions				
	Drought stress			Well-watered			Pooled (both water regimes)			Measurement period (MEZ)	Air temp. (°C)	Irradiance (W m ⁻²)	RH ² (%)	Wind speed (m s ⁻¹)	VPD (kPa)		
	IRT-R ³	IRT-H	IRC	IRT-R	IRT-H	IRC	IRT-R	IRT-H	IRC								
2011																	
125	0.64***	0.70***	0.76***	0.04	0.13	0.07	0.90***	0.93***	0.90***	11:30–12:30	20.0	790	38	2.2	2.2		
130	0.44*	0.54**	0.62***	0.08	0.16	0.01	0.81***	0.90***	0.85***	11:30–12:30	20.0	565	70	1.4	1.0		
138	0.51**	0.50**	0.10	-0.18	-0.13	-0.10	0.87***	0.88***	0.80***	10:15–11:15	21.9	664	62	1.4	1.5		
144	0.29	0.33	0.38*	-0.30	-0.28	-0.15	0.65***	0.64***	0.40**	11:30–12:30	18.4	669	45	2.7	1.8		
150	0.16	0.22	0.50**	0.04	0.04	0.00	0.80***	0.87***	0.61***	11:00–11:45	22.1	176	58	2.5	1.6		
156	0.67***	0.72***	0.63***	-0.28	0.08	-0.01	0.87***	0.92***	0.81***	11:15–12:15	27.1	831	51	2.0	2.4		
177	-	-	-	0.11	0.15	0.07	-	-	-	11:30–12:00	25.4	886	51	2.9	2.3		
191	-	-	-	-0.06	0.01	-0.36	-	-	-	11:15–11:30	21.6	745	53	2.3	1.7		
2012																	
128	-0.36*	-0.38*	-0.05	0.00	0.01	-0.21	0.56***	0.69***	0.41**	12:45–13:30	18.9	519	50	2.4	1.6		
134	-0.18	-0.04	-0.05	0.14	0.20	0.19	0.65***	0.83***	0.74***	12:30–13:00	16.7	829	40	2.6	1.7		
142	0.46**	0.51**	0.38*	0.05	-0.15	0.10	0.94***	0.95***	0.95***	13:30–14:00	28.8	767	41	3.7	3.2		
162	-0.06	-0.05	-0.10	0.18	0.07	0.47**	0.73***	0.85***	0.83***	12:45–13:30	19.1	320	55	1.8	1.5		
170	-0.22	-0.22	-0.12	-0.09	-0.24	0.45**	0.44***	0.65***	0.27*	12:30–13:00	22.6	510	58	1.5	1.8		
179	-0.18	-0.18	-0.16	0.47**	0.46**	0.54**	0.70***	0.76***	0.79***	13:00–13:30	25.7	651	62	1.0	2.0		
186	0.44*	0.48**	0.43*	0.29	0.22	0.49**	0.65***	0.81***	0.87***	10:45–11:15	22.8	342	82	2.5	0.9		
191	-0.29	-0.35	-0.42*	-0.23	-0.32	-0.29	0.79***	0.81***	0.78***	10:45–11:30	21.3	511	62	2.7	1.5		

¹The weather conditions refer to the measurement period and were measured outside the foil tunnel with the IMETOS weather station.

²Relative humidity.

³IRT-R: Raytech Raynger MX; IRT-H: Heitronics KT17; IRC: FLIR ThermoCam PM675.

*, **, ***Correlation coefficients are significant at P < 0.05, P < 0.01 and P < 0.001, respectively.

under drought conditions in Idaho, USA. Genotypic effects on CTD under well-watered conditions were not expected as a sufficiently strong drought stress is required to reveal genotypic differences (Blum *et al.* 1989).

Relationship between CTD and grain yield

Although there were hardly any significant genotypic differences for CTD, and grain yield showed a significant genotypic variation only in 2012, correlation between these two traits was calculated to make the results comparable with related studies. This limitation should be taken into account when regarding the correlations. There existed a significant correlation between CTD and grain yield under drought-stressed conditions on nearly all measurement days in 2011. This is in accordance with the results of Fischer *et al.* (1998), Ayeneh *et al.* (2002) and Balota *et al.* (2007). Under well-watered conditions, however, CTD and grain yield did not show a significant relationship in 2011. Canopy temperature is a function of stomatal conductance, and these two traits were significantly related in our study (L. Kottmann, unpublished data, 2013). The first response of virtually all plants to drought is the closing of stomata that is recognised to be the main determinant for decreased photosynthesis under drought (Cornic and Massacci 1996). Consequently, plants with higher stomatal conductance and cooler canopies under drought are able to maintain higher net photosynthesis rates, leading to higher grain yield. The maintenance of stomatal conductance for a longer period of time under water-limiting conditions in the present study might have resulted, for example, from a deeper rooting system through which water from deeper soil layers was accessible (Sharp *et al.* 2004).

Contrary to the results of 2011, only few and weak significant correlations between CTD and grain yield were found under drought-stressed conditions in 2012. This deviating finding may have resulted from the overall lower temperature and higher relative humidity in 2012; the evaporative demand of the atmosphere was less. Some authors also obtained weak or non-significant correlations between CTD and grain yield under less favourable weather conditions such as low vapour pressure deficit (Royo *et al.* 2002) or low solar radiation and high wind speed (Balota *et al.* 2007). On some measurement days in 2012, the CTD correlated significantly with grain yield under well-watered conditions. Also Amani *et al.* (1996) reported significant correlations of CTD with grain yield under well-watered conditions. Their study was conducted under semi-arid climate with higher mean temperatures than those usually occurring in Germany. In the present study, the canopy temperature measurements were taken several days after irrigation. Mild drought stress might thus have occurred during short periods of time in the well-watered treatment

of 2012, resulting in a significant relationship between CTD and grain yield.

Comparison of the IR measurement devices

Infrared thermometers and IR cameras are using the same measuring principle. The main difference is that IR thermometers display temperature values of a certain measurement spot, whereas IR cameras are able to differentiate between temperatures of larger numbers of surfaces (Jones and Leinonen 2003). Thermal images can be further processed, for example, to separate plant temperatures from soil temperatures (Hackl *et al.* 2012). This separation of plant and soil temperatures was not necessary in the present study because the measurements were only taken when the canopy was closed and no soil was visible. Between the two IR thermometers, IRT-H showed the best differentiation between treatments and the best relationships between CTD and grain yield. This is most likely attributable to the larger size of the measurement area due to the greater field of view. But also the IRT-R, which is much lower-priced than the IRT-H, gave comparable and satisfying measurement results. The IRC took its measurements faster than the IRTs as the camera took one image of two plots and stored it for later data analysis. Canopy temperature readings with the IRTs on the contrary had to be noted by hand, which was more time-consuming. The faster measurement with IRC might explain the more frequent significant relationships between CTD and grain yield obtained on single measurement days because the faster the measurements are taken, the less is the influence of changing weather. A major difference among the three devices is the absolute accuracy of readings and the resulting offsets. IRT-H always showed the highest CTD, followed by IRT-R and IRC. This is possibly caused by the different factory calibration. When Hackl *et al.* (2012) compared an IR thermometer with an IR camera, they also found significant differences between the temperature values of the devices on some measurement days. In summary, all devices are in principle suitable for CTD measurements, although they should not be used in parallel within an experiment due to the different temperature offset.

Canopy temperature measurement in a temperate climate

Most of the above-mentioned studies were conducted under semi-arid climate conditions that are recognised to be most suitable for the canopy temperature measurements due to overall higher temperatures, higher solar radiation and lower relative humidity. Balota *et al.* (2007) reported daily mean temperatures of 17–34 °C on measurement days in 2000 and 2001 in Texas. In contrast, the daily mean air temperatures of measurement days in the present study

ranged from 11 to 23 °C. Balota et al. (2007) also reported a solar radiation over 950 W m⁻² at noon on most measurement days, which implies nearly cloudless conditions. In the present study, solar irradiance exceeded 800 W m⁻² only on three out of 16 measurement days. Some of the major limitations for accurate canopy temperature measurements in a temperate climate are the short-term changes of solar radiation (due to cloud shadows), air temperature and wind speed, which all result in fast-changing leaf temperatures (Jones 1999). For that reason, Balota et al. (2007) suggest to take the measurements as quickly as possible. However, Jensen et al. (1990) showed during their trials in Denmark that the canopy temperature responded quickly to changes of solar radiation and wind speed. For drought-stressed crops, they reported fluctuations of canopy temperature by 2 °C within a 1-h measurement period. This explains why the detection of canopy temperature differences among genotypes is very difficult in a humid climate as small differences are to be detected under noisy atmospheric conditions. An alternative approach to avoid the problem of changing weather conditions would be to assess the canopy temperature of all plots at the same time, for example, with an IR camera carried by an unmanned aerial vehicle (Berni et al. 2009).

Conclusion

The results of this study show that the CTD can be used to assess the plant water status of rye also in a temperate climate, when measurements are taken on days with a cloudless sky, high temperature and low wind speed. Furthermore, it is shown that also lower-priced measurement devices are suitable for the CTD measurements. A concluding statement concerning the suitability of the CTD method for phenotyping in a temperate climate cannot be made, because of the limited genetic variability of the used rye material. For this reason, further research is needed with a more diverse and larger number of rye genotypes.

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5. Suitability of carbon isotope discrimination, ash content and single mineral concentration for the selection of drought-tolerant winter rye.

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Suitability of carbon isotope discrimination, ash content and single mineral concentration for the selection of drought-tolerant winter rye

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Abstract

Carbon isotope discrimination (Δ) has been proposed as selection criterion for grain yield under dry conditions, and ash content (m_a) and mineral concentration were suggested as surrogates for Δ . In this study, the relationship between grain yield, Δ , m_a and mineral concentration (Si, Ca, K, Mg) was examined in 2011 and 2012 on 16 winter rye genotypes grown under severe drought, mild drought (2012 only) and well-watered conditions. Analyses were performed on mature flag leaves and grains. Highly significant differences between water regimes occurred for all measured traits. Δ , m_a and mineral concentrations were significantly correlated with grain yield under severe drought in 2011, but not in 2012 except for Δ in flag leaf. Δ was related to m_a and mineral concentrations. Although the correlations were quite inconsistent, the results indicate that the measured traits can potentially be used as selection criterion for drought tolerance in rye. For a final statement about the suitability of these traits in rye breeding, the results should be secured with a larger and more diverse set of genotypes.

Key words: carbon isotope discrimination — ash content — mineral concentration — grain yield — winter rye — drought stress — selection criterion

Rye is mainly cultivated in Central and Eastern Europe as winter cereal, and it is used for baking, livestock feeding, as well as a renewable energy source (Blumtritt 2007). Because rye is recognized to be comparatively drought tolerant (Hlavinka et al. 2009), it is typically grown on light and sandy soils (Miedaner et al. 2012). Since summer drought events are expected to occur more frequently even in Central Europe (Alcamo et al. 2007), rye could be severely affected by drought-induced yield losses. Therefore, improving drought tolerance is of great importance.

Carbon isotope discrimination (Δ) has been proposed as selection criterion for grain yield of cereals under dry conditions, because Δ is directly related to the transpiration efficiency and integrates the transpiration efficiency across the whole growth period of the sample tissue (Farquhar and Richards 1984). The two existing stable carbon isotopes ^{12}C and ^{13}C account for 98.9% and 1.1% of the total carbon. During photosynthetic gas exchange, C_3 -plants discriminate against the heavier ^{13}C -isotope, leading to a depletion of ^{13}C in plant matter (Farquhar et al. 1989). Δ was found to be related with grain yield of C_3 -cereals such as wheat and barley under water limited conditions. However, the sign and the strength of the correlation varied depending on location, weather condition, crop species and the type and age of the examined plant organ. Analyses were

performed on leaves at booting (Monneveux et al. 2005), anthesis (Hafsi et al. 2003, Monneveux et al. 2006, Zhu et al. 2008) and maturity (Fischer et al. 1998, Merah et al. 2002), as well as on mature grains (AlHakimi et al. 1996, Voltas et al. 1998, Araus et al. 2001, Royo et al. 2002). The reported correlation coefficients between Δ and grain yield range from positive (Merah et al. 2001a, 2002, Monneveux et al. 2006, Kumar et al. 2011) to negative (Condon and Hall 1997, Rebetzke et al. 2002) and some authors did not find a significant relationship between the two traits (Hafsi et al. 2003, 2007). Under drought stress conditions, however, Δ was mostly positive related with grain yield (Merah 2001b, Monneveux et al. 2005, Balota et al. 2008, Misra et al. 2010).

Because the sample preparation and analysis of carbon isotope discrimination are relatively expensive and time consuming, ash content has been proposed as a surrogate for Δ (Araus et al. 2001, Merah et al. 2001a). The reason is that under high evaporative demand, more minerals are passively transported through the xylem via the transpiration stream and accumulate in vegetative plant tissues (Masle et al. 1992). Merah et al. (2001a) and Zhu et al. (2008) found a positive relationship between Δ and ash content in leaves. The grain ash content on the contrary was found to be negatively related to grain yield in wheat, because mineral accumulation in grains is primarily regulated by processes other than transpiration (Merah et al. 2001a). Beside the total ash content, single minerals such as silicon, calcium, potassium and magnesium might also represent suitable surrogates for Δ (Febrero et al. 1994, Zhu et al. 2008).

Neither Δ nor ash content and single mineral concentrations were studied so far on rye in the temperate climate of Central Europe. Therefore, the objectives of this study were (i) to apply the method of carbon isotope discrimination to rye under drought stress and well-watered conditions, (ii) to examine ash content and mineral concentration as surrogates for carbon isotope discrimination and (iii) to test the suitability of these methods for rye breeding.

Materials and Methods

Field experiments: The experiments were carried out during 2011 and 2012 on the experimental field (52.30 N, 10.44 E, 80 metre above sea level) of the Julius Kühn-Institute at Braunschweig, Germany. The soil was characterized as a Haplic Luvisol (FAO 1997) with an available water capacity of 120 mm and a water table 10 m below ground. The plot sizes ranged from 5.6 to 7.2 m², seeding density was 230 seeds/m². Nitrogen was applied as calcium ammonium sulphate, split into 60 kg N/ha at the beginning of vegetation and 70 kg N/ha at the beginning of stem elongation. Growth regulators were used to avoid lodging. Fungicides and pesticides were applied when necessary. In all

Experiments were carried out at the Julius Kühn-Institute (JKI), Institute for Crop and Soil Science, Braunschweig, Germany.

experiments, a 4 × 4 alpha lattice with two replications was used. Plants were exposed to severe drought stress (SD), mild drought stress (MD, 2012 only), and well-watered conditions (WW). Severe drought stress was generated by growing the rye plants under a foil tunnel covered with a 200 µm polythene foil, which was mounted at the beginning of stem elongation (April 14, 2011 and April 05, 2012). Since the plants received no rainfall or irrigation from that time until harvest (July 21, 2011 and July 24, 2012), they were entirely dependent on the field-stored soil moisture. Mild drought stress was created by growing the plants under a movable rain-out shelter. The shelter automatically covered the experimental plots during rainfall events from the beginning of stem elongation to harvest. The plant available soil water (PASW), which was estimated by means of the agrometeorological advisory system 'Agrowetter' from the German Weather Service (DWD 2014), was kept between 20 and 40 mm by means of a shelter-based overhead sprinkling facility. The well-watered plots under field conditions were supplementary irrigated by pressure compensated drip tubes to keep the PASW above 60 mm. Air temperature and natural rainfall were measured with an 'iMETOS' weather station (Pessl Instruments, Weiz, Austria), located on the experimental field near the rain-out shelter.

Plant material: Sixteen winter rye (*Secale cereale* L.) genotypes were used, consisting of three parental inbred lines (Lo115-N, Lo90-N, Lo117-N) and 12 F₂:₄ lines selected from two biparental populations F₁ (Lo115-N × Lo90-N) and F₁ (Lo115-N × Lo117-N). Both parental inbred lines and F₂:₄ lines were outcrossed to the same cytoplasmic male sterile tester. These genotypes were used because of their contrasting grain yield performance in previous breeding trials. The parental line Lo115-N, inter alia, showed a better performance than Lo90-N and Lo117-N under low rainfall conditions (Hübner et al. 2013). Furthermore, the hybrid rye cultivar 'Palazzo' was used which has shown a yield performance similar to the other 15 entries in previous trials. All plant materials were provided by KWS Cereals, Bergen, Germany.

Carbon isotope discrimination: Carbon isotope discrimination was analysed each year in flag leaves at maturity and in 2012 also in mature grains. A 5 g sample of flag leaves and grains were dried at 105°C for 24 h. Flag leaf samples were preground with a rotary mill (Brabender GmbH, Duisburg, Germany) and grain samples with a Retsch ZM 200 rotor mill (Retsch GmbH, Haan, Germany). Preground flag leaf and grain samples were then ground to fine powder using a Retsch MM2 disk mill. The carbon isotope composition was determined on a 0.1 mg sample with a Flash EA 1112 elemental analyser (CE Instruments Ltd, Hindley Green, UK) coupled to a Delta plus isotope ratio mass spectrometer (Thermo Fischer Scientific, Waltham, MA, USA) as:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] * 1000,$$

with Vienna-PDB as standard. The discrimination rate (Δ) was calculated according to Farquhar et al. (1989) as:

$$\Delta(\text{‰}) = \left[\frac{(\delta_a - \delta_p)}{(1 + \delta_p)} \right] * 1000,$$

where δ_p is the $\delta^{13}\text{C}$ of the sample, and δ_a is the $\delta^{13}\text{C}$ of the air, which is approximately -8‰ (Farquhar et al. 1989). Carbon isotope discrimination in flag leaves and grains at maturity is referred to as Δ_L and Δ_G , respectively.

Ash content: Ash content was determined on flag leaves and grains at maturity. Samples were dried at 60°C for 24 h. Flag leaf samples were ground with a Brabender rotary mill and grain samples with a Retsch ZM 200 rotor mill. Approximately 2 g of the ground samples were incinerated at 550°C for 16 h in a muffle furnace. The ash content (%) of flag leaves and grains at maturity determined on the basis of dry mass is referred to as m_aL and m_aG , respectively.

Silicon, calcium, potassium and magnesium concentration: Silicon, calcium, potassium and magnesium concentration was determined on

flag leaves at maturity. Samples were dried at 60°C for 24 h, preground with a Brabender rotary mill, and then ground to a fine powder with a Retsch MM2 disk mill. The samples were pressed into tablets, on which the element concentrations were determined with a S8 Tiger wavelength dispersive X-ray fluorescence spectrometer (Bruker Corporation, Billerica, MA, USA). Silicon, calcium, potassium and magnesium concentration (in %) of flag leaves at maturity are referred to as Si_L , Ca_L , K_L and Mg_L respectively.

Stomatal conductance: The measurement of stomatal conductance (g_s) was performed with a SC-1 diffusion porometer (Decagon Devices, Pullman, WA, USA) on seven (2011) and eight (2012) dates between 10 am and 4 pm. Measurements were carried out on the adaxial surface of the flag leaf on three marked plants per plot. Mean values were then calculated for each plot. The annual mean stomatal conductance is referred to as $g_{s,}$.

Grain yield: Grain yield was determined at maturity. In 2011, the whole plots were hand-harvested. In 2012, 0.5 m² portions of the plots were harvested manually; whereas the rest of the plots were combine harvested using a Wintersteiger Nursery Master (Wintersteiger AG, Ried, Austria). The hand-harvested plants were separated into ears and stems. The ears were threshed and winnowed. Grain samples were oven-dried to constant weight at 105°C for 24 h. Grain yield was calculated in t/ha on the basis of 0% water content.

Statistics: Analyses of variance were carried out with the GLIMMIX procedure of SAS 9.3 (SAS Institute, Cary, NC, USA). Individual years were analysed separately. In these analyses, genotype and water regime were considered as fixed effects, replication as random effect. Correlations were calculated with the CORR procedure of SAS. All graphs were created with SIGMAPLOT 12 (Systat Software Inc., Chicago, IL, USA).

Results

Growth conditions, phenology and grain yield

The year 2011 was overall warmer and drier than 2012, with a higher average temperature especially in spring, and less rainfall (Fig. 1). The PASW under severe drought stress conditions from April to end of July amounted to 128 and 135 mm in 2011 and 2012, respectively. Under mild drought stress in 2012, plants received a total of 228 mm water, including 85 mm from irrigation. Under well-watered conditions, PASW amounted to 376 mm in 2011 and 463 mm in 2012, including 196 and 165 mm of additional irrigation in 2011 and 2012, respectively. The water scarcity under severe drought led to a 4 days earlier flowering in the year 2011, and a 12 and 11 days earlier maturity in the years 2011 and 2012, respectively. Under mild drought, plants reached maturity 7 days earlier than plants under well-watered conditions. Differences in phenology between the genotypes were not observed. The mean grain yield under well-watered conditions was 9.5 t/ha in 2011 and 11.9 t/ha in 2012. Severe drought reduced the grain yield to 4.2 t/ha in 2011 and to 4.6 t/ha in 2012. Grain yield under mild drought stress was 10.2 t/ha in 2012. The grain yield differences among the genotypes were generally small.

Effects of water regime on measured traits

Highly significant (0.001 level) differences between the water regimes occurred for all measured traits in both years (Table 1). The carbon isotope discrimination in flag leaves at maturity (Δ_L) and in mature grains (Δ_G) was highest under well-watered condi-

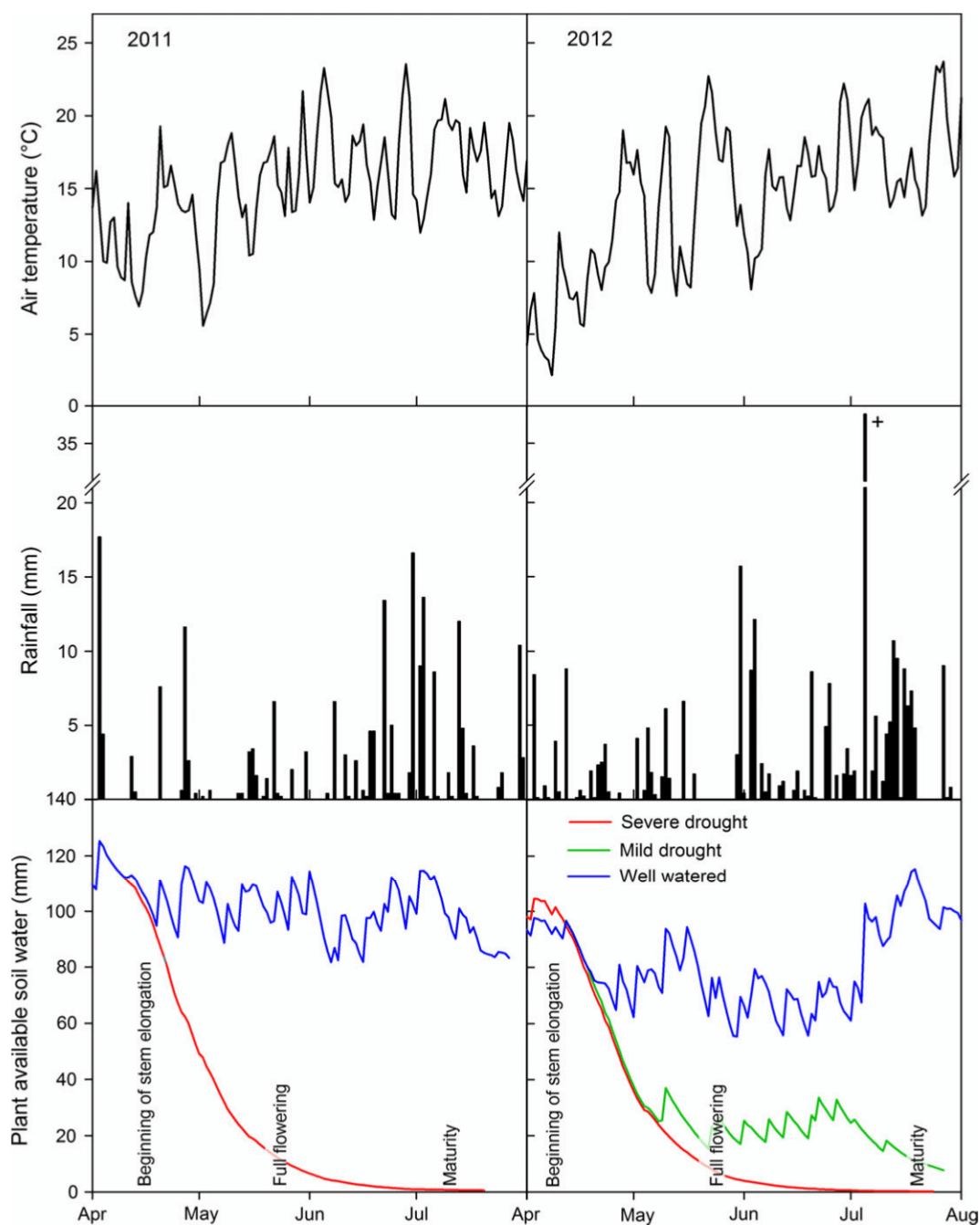


Fig. 1: Weather conditions in the cropping seasons 2011 and 2012: mean daily 2 m air temperature, rainfall and plant available soil water (PASW) in the different water regimes. The date of beginning of stem elongation, full flowering and maturity refer to the plants under severe drought. \dagger : heavy rain event with 38 mm within 2 h

tions, and declined with increasing drought stress. Δ_L was generally higher than Δ_G . The ash content of flag leaves at maturity (m_aL) followed the same pattern as the carbon isotope discrimination, except for severe drought stress conditions in 2012, where m_aL was higher than under well-watered conditions. The ash content in mature grains (m_aG) was lowest under well-watered conditions, and highest under severe drought (2011) and mild drought (2012). Regarding the single minerals, the silicon concentration increased, and the potassium concentration decreased with increasing water supply. Calcium and magnesium showed the highest concentration under mild drought stress conditions, followed by severe drought stress and well-watered

conditions. The stomatal conductance clearly increased with higher water availability.

Relationship between grain yield and measured traits

In 2011, all traits except m_aL and K_L were significantly related to grain yield under severe drought stress condition (Figs 2 and 3). The relationship was positive for Δ_L , Si_L , Ca_L , Mg_L and g_s , and it was negative for m_aG . In 2012, only Δ_L was significantly positive correlated to grain yield. Under mild drought conditions, no significant correlation between the measured traits and grain yield could be observed. Under well-watered conditions, only

Table 1: Mean values for grain yield (GY), carbon isotope discrimination in flag leaves (Δ_L) and grains (Δ_G) at maturity, ash content in flag leaves (m_aL) and grains (m_aG) at maturity, silicon (Si_L), calcium (Ca_L), potassium (K_L) and magnesium (Mg_L) concentration in flag leaves at maturity and stomatal conductance (g_s , mean of all measurements in one season)

	GY (t/ha)	Δ_L (‰)	Δ_G (‰)	m_aL (%)	m_aG (%)	Si_L (%)	Ca_L (%)	K_L (%)	Mg_L (%)	g_s (mmol/m ² /s)
2011										
Severe drought	4.2 ^a	20.4 ^a	—	8.4 ^a	1.90 ^a	0.52 ^a	1.02 ^a	2.00 ^a	0.12 ^a	212 ^a
Well watered	9.5 ^b	22.7 ^b	—	9.0 ^b	1.73 ^b	2.32 ^b	0.78 ^b	0.61 ^b	0.10 ^b	789 ^b
G	ns	*	—	**	ns	*	ns	ns	ns	ns
W	***	***	—	***	***	***	***	***	***	***
G × W	ns	ns	—	ns	ns	ns	ns	ns	ns	ns
2012										
Severe drought	4.6 ^a	22.0 ^a	17.1 ^a	10.3 ^a	1.94 ^a	0.48 ^a	1.49 ^a	2.02 ^a	0.14 ^a	158 ^a
Mild drought	10.2 ^b	22.6 ^b	19.2 ^b	9.7 ^b	2.08 ^b	1.10 ^b	1.92 ^b	0.92 ^b	0.28 ^b	230 ^b
Well watered	11.8 ^c	23.5 ^c	21.3 ^c	9.8 ^b	1.86 ^c	2.57 ^c	0.77 ^c	0.63 ^c	0.10 ^c	618 ^c
G	*	ns	**	**	**	**	**	**	ns	**
W	***	***	***	***	***	***	***	***	***	***
G × W	ns	ns	**	**	*	*	*	**	ns	*

Mean values followed by different letters for a given trait within a year are significantly different at the 0.05 level.

G, genotypic effects; W, effects of water regime; G × W, interaction; ns, not significant.

*, **, ***Indicate significance at 0.05, 0.01 and 0.001 levels, respectively.

m_aG was significantly correlated with grain yield in 2012. Pooled over all water regimes and years, the strongest positive relationships with grain yield could be found in Δ_L , Δ_G and Si_L , the strongest negative relationships were found in K_L and m_aG (data not shown).

Relationship between carbon isotope discrimination and its potential surrogates

Δ_L was negatively correlated to m_aG under severe drought stress in 2011, and Δ_G was negatively correlated to m_aG under severe and mild drought conditions (Table 2). Under severe stress conditions, Δ_G was positively related to m_aL , and under well-watered conditions, the correlation between Δ_G and m_aL was negative. With regard to the individual minerals, Δ_L was only significantly related to Si_L under severe drought in 2011. Δ_G , however, was significantly positive related to Ca_L and Mg_L under severe drought, and significantly negative related to Si_L and Mg_L under well-watered conditions. Δ_L was positively correlated with g_s in the year 2011, with the strongest correlation existing under well-watered conditions. In the year 2012, neither Δ_L nor Δ_G were significantly related to g_s .

Discussion

Effects of water regime on measured traits

When water is scarce, one of the first reactions of plants is the closing of stomata, in order to reduce transpiration water losses (Condon et al. 1990). This could be shown by the considerable decrease in g_s under drought stress, which affected all measured traits. The carbon isotope discrimination in flag leaves and grains at maturity decreased significantly with increasing drought stress. Discrimination against ¹³CO₂ takes place during the diffusion of CO₂ from the ambient air through the stomata to the sites of carboxylation, as well as during CO₂ fixation by Rubisco (Farquhar et al. 1989). When soil water is limited, the supply of CO₂ is reduced by stomatal closure, which causes a decrease in the intercellular to atmospheric CO₂ concentration ratio (C_i/C_a). Because the carbon isotope discrimination is directly related to the C_i/C_a ratio (Farquhar et al. 1989), Δ decreases with increasing drought stress (Condon et al. 1990, Araus et al. 2003, Mon-

neux et al. 2005). The carbon isotope discrimination in flag leaves was generally higher than in grains, as also observed by Merah (2001b) and Monneveux et al. (2006). This is caused by the high starch content of the grains compared to the high lipid content of leaves, because starch has a lower carbon isotope fractionation than lipids (Condon et al. 2006).

The ash content of the flag leaves decreased with increasing drought stress in 2011. When the evaporation rate is high under well-watered conditions, more minerals are passively transported through the xylem by the transpiration stream and accumulate in the vegetative plant organs (Masle et al. 1992). Under drought stress, on the contrary, the evaporation and consequently the passive mineral uptake are limited, causing a decrease in leaf ash content (Araus et al. 2001, Merah et al. 2001a, Misra et al. 2010). In 2012, however, the ash content in flag leaves at maturity was higher under severe drought stress than under well-watered conditions, which was also observed by Zhu et al. (2008). This is in contrast to the 2011 results, and might be caused by a better nutrient availability or a better nutrient uptake under severe drought in 2012. However, the nutrient availability should have been the same for all water regimes, as the experimental plots were fertilized uniformly. The ash content in mature grains was generally higher under drought stress than under well-watered conditions. In grains, the mineral accumulation primarily depends on the photosynthetic rate during grain filling and the remobilization of minerals from lower plant parts after the onset of senescence (Wardlaw 1990). Drought stress affects remobilization to a lesser extent than it affects photosynthesis (Loss and Siddique 1994), and the retranslocation of minerals from vegetative organs into the grain is much higher under drought stress than under well-watered conditions.

Regarding the individual minerals in flag leaves at maturity, the silicon concentration was highest under well-watered conditions and declined with increasing drought stress. Although silicon can be actively accumulated by crop plants (Liang et al. 2007), it accumulates mainly passively via the transpiration stream under field conditions (Walker and Lance 1991). The accumulation of silicon is thus primarily linked to the transpiration rate. Potassium on the contrary had the highest concentrations under severe drought, and the lowest concentrations under well-watered conditions, as the uptake of potassium is highly selective (Mars-

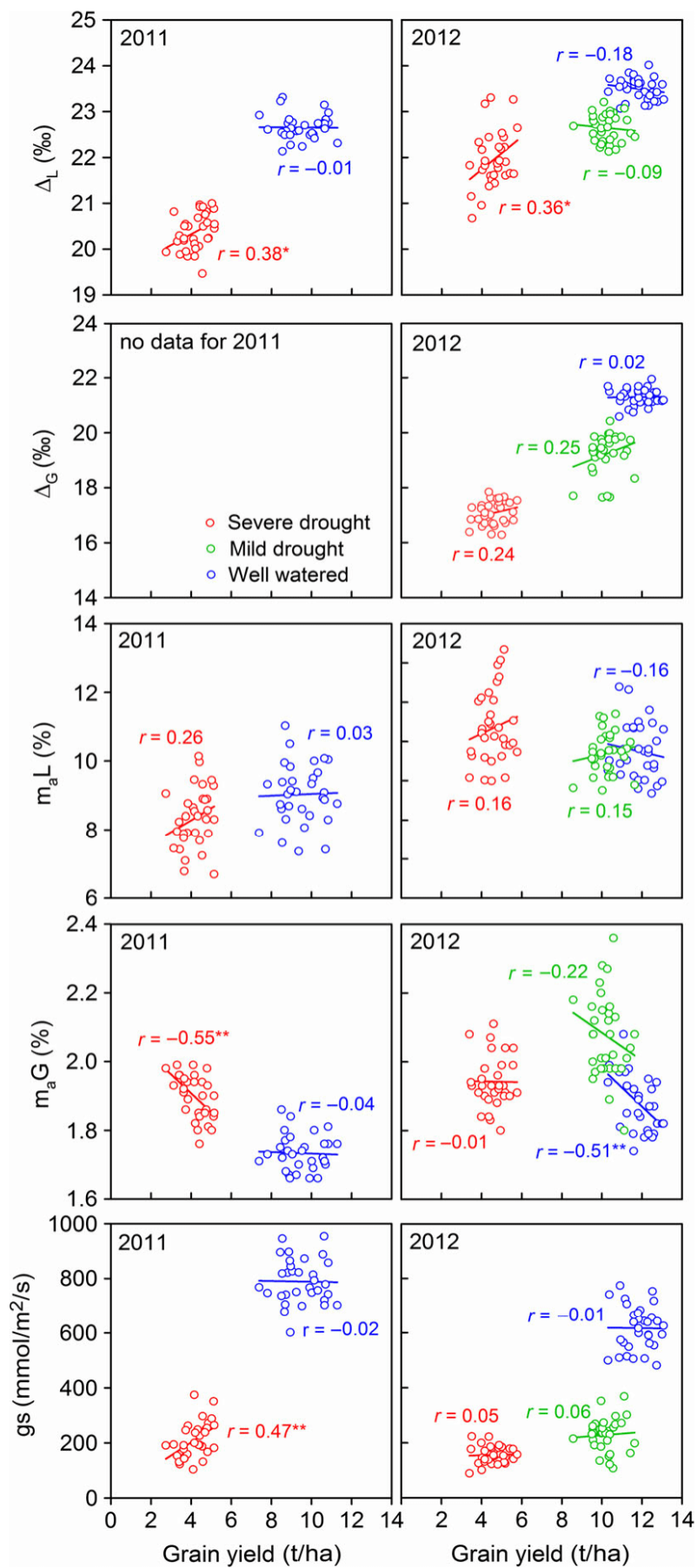


Fig. 2: Correlations of grain yield with carbon isotope discrimination in flag leaves (Δ_L) and grains (Δ_G) at maturity, ash content of flag leaves (m_{aL}) and grains (m_{aG}) at maturity, as well as stomatal conductance (g_s , mean over all measurements in one season). *, **, *** indicate significance at 0.05, 0.01 and 0.001 levels, respectively

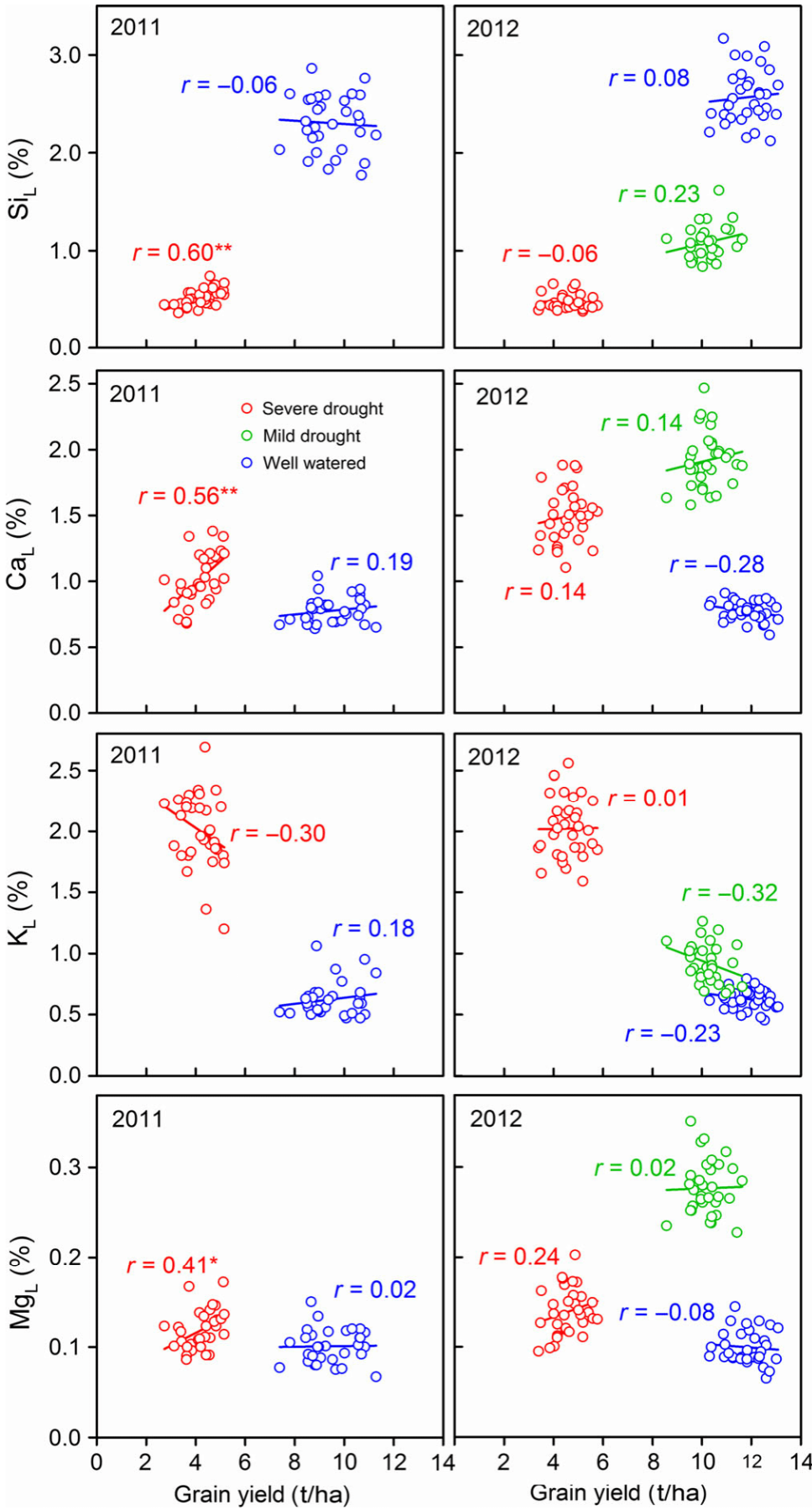


Fig. 3: Correlations of grain yield with silicon (Si_L), calcium (Ca_L), potassium (K_L) and magnesium (Mg_L) concentration in flag leaves at maturity *, **, *** indicate significance at 0.05, 0.01 and 0.001 levels, respectively

Table 2: Correlations between carbon isotope discrimination in flag leaves (Δ_L) and grains (Δ_G) at maturity with its potential surrogates under severe drought stress (SD), mild drought stress (MD) and well-watered conditions (WW): ash content in flag leaves (m_{aL}) and in grains (m_{aG}) at maturity, silicon (Si_L), calcium (Ca_L), potassium (K_L) and magnesium (Mg_L) concentration in flag leaves at maturity and stomatal conductance (g_s , mean of all measurements in one season)

	Δ_L			Δ_G		
	SD	MD	WW	SD	MD	WW
2011						
m_{aL}	0.24	–	–0.03	–	–	–
m_{aG}	–0.49**	–	–0.11	–	–	–
Si_L	0.42*	–	0.06	–	–	–
Ca_L	0.31	–	–0.02	–	–	–
K_L	–0.35	–	0.08	–	–	–
Mg_L	0.29	–	0.00	–	–	–
g_s	0.35*	–	0.66***	–	–	–
2012						
m_{aL}	–0.01	0.16	0.14	0.51**	0.09	–0.59**
m_{aG}	0.15	–0.04	0.01	–0.41*	–0.43*	0.15
Si_L	–0.26	–0.06	0.12	0.32	–0.09	–0.46**
Ca_L	0.04	0.28	0.11	0.74***	0.16	–0.08
K_L	–0.03	–0.09	0.29	–0.21	–0.12	0.17
Mg_L	0.07	0.30	–0.17	0.66***	–0.17	–0.48**
g_s	–0.12	0.05	–0.09	0.25	–0.08	0.11

*, **, *** indicate significance at 0.05, 0.01 and 0.001 levels, respectively.

chner 1995). Potassium is essential for many physiological processes, for example photosynthesis, maintenance of turgescence and activation of enzymes. Under drought stress, the demand for potassium increases, especially for the maintenance of photosynthetic CO_2 fixation and protection from oxidative damage (Cakmak 2005). Calcium and magnesium showed, like potassium, the lowest concentrations under well-watered conditions. As also observed by Zhu et al. (2008), the highest Ca_L and Mg_L concentrations were found under mild drought stress conditions. Both, magnesium and calcium play an important role in acclimation to stress (Palta 1990, Waraich et al. 2011). The higher concentration of these two macronutrients under water limited conditions implies a partly active uptake (Marschner 1995, Yang and Jie 2005). The observation that the Ca_L and Mg_L levels already peaked at mild drought stress might be attributable to the fact that plants under severe drought stress were no longer able to actively take up calcium and magnesium from the soil.

Relationship between grain yield and measured traits

Grain yield was significantly positive correlated with Δ_L under severe drought in both years, but not with Δ_G . The closing of stomata under drought stress results in a decline of the photosynthesis rate. This caused a reduced biomass and grain yield, which could be shown by the significant relationship between grain yield and g_s . High- Δ genotypes are able to keep their stomata more open and thus transpire more water (Araus et al. 2003), leading to a higher photosynthesis rate. The better water status of high- Δ genotypes is for example caused by a deeper and better developed rooting system, which allows these genotypes to attain water from deeper soil layers. It can also be caused by other stress adaptive (e.g. osmotic adjustment) or morphological and phenological traits (Araus et al. 2002). Positive correlations between grain yield and Δ under water limited

conditions have been frequently reported for wheat, for example in flag leaves at anthesis (Merah et al. 2001a, Monneveux et al. 2005, Misra et al. 2010) and in mature grains (Merah et al. 2002, Balota et al. 2008, Kumar et al. 2011). The flag leaves at maturity have been rarely examined in studies concerning carbon isotope discrimination; most studies used the flag leaf at anthesis. Sampling the flag leaves at maturity provides information over a longer growing period. A possible limitation of sampling the flag leaf at maturity is the retranslocation of photoassimilates into the grain (Voltas et al. 1998). The lack of correlation between grain yield and Δ of flag leaves and grains at maturity under well-watered conditions in the present study agrees with findings of Monneveux et al. (2004, 2005). The C_i/C_a ratio, to which Δ is directly related is influenced by changes in either stomatal conductance or photosynthetic capacity. Under well-watered conditions, the stomatal conductance is high and results in a high C_i/C_a ratio. Simultaneously, the photosynthetic capacity increases under favourable conditions, resulting in a decrease of the C_i/C_a ratio. These two effects offset each other and result in a reduced or non-existing correlation between grain yield and Δ (Monneveux et al. 2005). The mild drought stress was probably not strong enough to reveal a significant relationship between grain yield and Δ . Also Hafsi et al. (2003) did not observe a significant correlation between grain yield and Δ under semi-arid conditions with PASW levels similar to the present study.

The negative correlation between m_{aG} and grain yield under severe drought agrees with previous findings by Voltas et al. (1998), and Misra et al. (2010). Genotypes with higher grain ash content were more affected by drought during grain filling, and therefore filled their grains rather through retranslocation of photoassimilates from preanthesis reserves. Low grain ash content therefore indicates a better photosynthesis rate during grain filling, and consequently a better adaption to drought. Grain yield and m_{aL} were not significantly correlated in any of the practiced water regimes. Grain yield and m_{aL} were found to be positively related under irrigated conditions (Monneveux et al. 2004), and under drought stress with stored soil moisture only (Monneveux et al. 2004). Misra et al. (2010) on the contrary did not observe a relationship between grain yield and m_{aL} in any water regime practiced. The lack of a significant correlation suggests that the flag leaf ash content at maturity is a poor indicator for transpiration rate and grain yield, most likely due to the depletion of minerals in leaves, which are transported via the phloem into the grain.

Among the minerals, Si_L , Ca_L and Mg_L were significantly positive correlated with grain yield in 2011. The r -values were even higher than those for the total ash content. This indicates that silicon, calcium and magnesium are not only passively accumulated via the transpiration stream, but that there exists also an active mineral uptake (Yang and Jie 2005, Liang et al. 2007). For that reason, high silicon, calcium and magnesium content is not only associated with higher transpiration rates, but also with a higher ability to alleviate drought stress effects. The missing correlation between K_L and grain yield in the present study is in contrast to the results of Febrero et al. (1994) and Merah (2001b), who reported a positive association between grain yield and potassium concentration. However, Febrero et al. (1994) used mature wheat grains for potassium analyses, and Merah (2001b) wheat awns. The retranslocation processes described earlier could explain the missing relationship between the potassium concentration and grain yield in the present study.

Relationship between carbon isotope discrimination and its potential surrogates

The negative correlation between Δ_L/Δ_G and m_aG under drought stress agrees with results of Merah et al. (2001a) and Zhu et al. (2008), and indicates that genotypes which are able to maintain a higher stomatal conductance during grain filling under drought conditions (higher Δ_L/Δ_G), need less minerals from preanthesis organs for grain filling, resulting in lower grain ash content (Merah et al. 2001a). In the present study, Δ_G and m_aL were positively correlated under severe drought stress, which is in accordance with Araus et al. (2001) and Monneveux et al. (2005). Also, Δ_G was positively correlated with the calcium and magnesium content of flag leaves under severe drought stress. The correlation coefficient was even higher for the individual minerals than for the total ash content. This is most likely caused by the partly active uptake of calcium and magnesium as already mentioned above. The correlation of Δ_G with m_aL , Si_L and Mg_L , however, became negative under well-watered conditions in 2012. This could indicate that under optimal soil moisture generally more minerals are transported into the grain, either via the transpiration stream or through phloem transport, which decreases the mineral content in the leaves. In accordance with Fischer et al. (1998), Δ_L and g_s were significantly positive correlated under severe stress ($r = 0.35^*$) and well-watered ($r = 0.66^{***}$) conditions in the 2011 experimental year. Stomatal conductance directly affects carbon isotope discrimination through C_i/C_a ratio. However, one would expect a stronger relationship between Δ and stomatal conductance under drought stress, as the C_i/C_a ratio (and therefore Δ) is more affected by stomatal conductance than by photosynthetic capacity under water limited conditions (Morgan et al. 1993). The weak correlation under drought stress in 2011 and the lack of significant correlation in 2012 are most likely caused by the long duration of the g_s -measurements. The measurements, which took several hours at highly variable weather conditions especially in the year 2012, might explain the missing significant correlation in that year.

Are carbon isotope discrimination and its potential surrogates valuable traits for improving drought tolerance in rye?

The results of this study show that carbon isotope discrimination on mature flag leaves can potentially be used as secondary trait in rye breeding. Also, ash content in mature grains and the concentrations of potassium, silicon, calcium and magnesium offer a promising approach as surrogates for Δ . However, the observed correlations were quite variable between the two experimental years. This variability might have been caused by the contrasting weather conditions, the low genotypic variability, or both. The weather conditions differed notably between the years. Although the rain was completely kept out by rain-out shelters in the severe drought stress treatment, natural rainfall from April to end of July outside the shelter was higher in 2012, leading to a higher relative humidity than in 2011. Also the mean air temperature in April, May, June and July was by 2°C lower in 2012 than in 2011. This caused a lower evaporative demand and thus a somehow lower drought stress level in 2012, which is also indicated by a higher grain yield under severe drought stress in 2012. Most of the studies cited above were conducted under arid (Fischer et al. 1998, Monneveux et al. 2006), semiarid (Rebetzke et al. 2002, Hafsi et al. 2003, Balota et al. 2008) and Mediterra-

nean (Febrero et al. 1994, Voltas et al. 1998, Merah 2001b, Monneveux et al. 2006) climate conditions. These climates are characterized not only by low rainfall during the summer months, but also by high temperatures and low relative humidity. One might assume that the climate conditions of the present study (especially in 2012) were unfavourable for Δ -, ash- and mineral analyses under drought stress, as no trait except for Δ_{Lm} was significantly related to grain yield in 2012. Another reason which might account for the inconsistent correlation coefficients is the genetic material. Although the 16 genotypes were chosen because of their diverse grain yields in previous breeding trials, large genotypic grain yield differences did not occur in the present study. In order to make a more precise statement about the suitability of these methods, the results should be secured with a larger and more diverse set of genotypes.

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6. How do timing, duration, and intensity of drought stress affect the agronomic performance of winter rye?

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Abstract

Winter rye (*Secale cereale* L.) will be especially affected by drought induced yield losses in Central and Eastern Europe in the future, because it is predominantly cultivated on low-fertile soils with a poor water-holding capacity. In order to examine the performance of winter rye under different drought conditions, field experiments were carried out during the years 2011, 2012, and 2013 near Braunschweig, Germany. Two sets of genotypes were tested under severe, mild, pre-anthesis, and post-anthesis drought stress in rain-out shelters as well as under rainfed and well-watered conditions. The grain, straw, and total aboveground biomass yields, grain yield components, leaf area index (LAI), and phenological characteristics were examined, as well as phenotypic correlations between grain yield and further characteristics. Drought induced grain yield reduction ranged from 14 to 57%. The straw yield was lesser affected by drought than the grain yield. Under drought conditions, fully ripe was reached up to twelve days earlier than under non water-limited conditions. Pre-anthesis drought mainly reduced spikes m^{-2} and kernels spike⁻¹ while drought during grain filling reduced the 1000-kernel weight (TKW) only. The grain yield was positively associated with straw yield, spikes m^{-2} and kernels spike⁻¹ under water limited conditions while the TWK was only positively associated with grain yield under drought during grain filling. Consequently, high pre-anthesis biomass as well as high numbers of spikes m^{-2} and kernels spike⁻¹ are especially important for

obtaining high grain yields under water-limited conditions. Focusing on these traits is therefore recommendable for developing drought tolerant rye genotypes.

Keywords: drought stress, winter rye, drought tolerance, grain yield, yield components, breeding, leaf area index, phenology

Introduction

Rye (*Secale cereale* L.) is an important food, feed, and whole-plant energy crop in Central and Eastern Europe. In these regions, rye is primarily cultivated as winter cereal on 4.8 million hectares (FAO, 2014). Rye has been recognized to be relatively drought tolerant compared to other crops (Schittenhelm et al. 2014, Hlavinka et al. 2009). Therefore, it is predominantly grown on infertile and sandy soils, which are characterised by a low water holding capacity. Although Central and Eastern Europe have a humid climate, climatologists predict more future summer drought events even for these regions as well as an overall temperature rise (IPCC 2014). Therefore, rye could be especially affected by drought events.

Drought is the most yield-limiting abiotic stress and affects cereal crops on all levels during all phenological stages. The extent of grain yield loss is depending on the intensity and timing of water shortage as the different stages of development vary in their sensitivity to drought stress (Cattivelli et al. 2008). Grain yield can be dissected into the yield components spikes m^{-2} , kernels spike $^{-1}$, and 1000-kernel weight (TKW). These yield components are not equally susceptible to water deficits because they are determined at different stages of plant development (Slafer and Savin 2004). For example, drought stress during the vegetative phase will affect grain yield mainly by reduced crop density and kernel number (Dolferus et al. 2011) while drought during grain filling results in reduced kernel weight caused by a reduced grain filling duration (Gooding et al. 2003). Generally, grain number is considered to be the

main determinant for final grain yield whereas grain weight is less important (Slafer et al. 2014, Chmielewski and Köhn 2000, Dencic et al. 2000, Lopezcastaneda and Richards 1994, Giunta et al. 1993).

As there is hardly any information about the effect of various types of drought on winter rye, the main objectives of this study were to (1) identify the effect of timing, duration, and intensity of water deficit on phenological, morphological and agronomical characteristics, and (2) to examine the relationship between grain yield and other crop characteristics.

Materials and methods

Field experiments

The trials were conducted during the 2010/11, 2011/12, and 2012/13 growing seasons on the experimental field of the Julius Kühn-Institute near Braunschweig, Germany (52.30 N, 10.44 E, 80 meters above mean sea level). The soil was characterized as Haplic Luvisol (FAO, 1997) with an available water capacity of 120 mm (0-90 cm), and a groundwater level 10 m below ground. Sowing dates were September 30 in 2010, September 26 to October 5 in 2011, and September 26 to 28 in 2012. Seeding density was 230 seeds m⁻² and plot sizes ranged from 5.6 to 7.2 m². A total of 130 kg nitrogen ha⁻¹ was applied as calcium ammonium nitrate, split into 60 kg N ha⁻¹ at the beginning of vegetation, and 70 kg N ha⁻¹ at the beginning of stem elongation. Growth regulators were used to avoid lodging. Fungicides and pesticides were applied when needed. The trials were divided into two experiments. In Experiment I (2011 and 2012), the winter rye was grown under three levels of water supply: severe drought stress, mild drought stress (2012 only) and well-watered conditions. In Experiment II (2013), four water regimes were practiced: early drought stress with water deficit during the vegetative phase (stem elongation to anthesis), late drought stress with water deficit during

the generative phase (grain filling to fully ripe), rainfed-, and well-watered conditions. All experiments were set up as a 4x4 alpha lattice with two replications. Further details about the water regimes are given in Table 1. Severe drought in 2011 and 2012, and early drought in 2013 were created by growing the winter rye crops under 50 m long, 10 m wide, and 4 m high foil tunnels (CASADO, Douville, France). These stationary rain-out shelters were covered by a 200 µm polythene foil, which was mounted at the beginning of stem elongation in each year. The front and the sides of the foil tunnels were open in order to attain good ventilation. Mild (2012) and late drought stress (2013) were attained by means of mobile rain-out shelters (Götsch & Fälschle, Alerheim, Germany) which were 24 m long, 12 m wide, and 5 m high. The mobile shelters automatically covered the experimental plots during rainfall events. Under well-watered conditions and in the stationary rain-out shelter (early drought, irrigation during grain filling only), plants were additionally watered by drip irrigation. The plants grown in the mobile rain-out shelters were irrigated by a shelter-based overhead sprinkling facility.

Plant material

In both experiments a different set of each 16 winter rye (*Secale cereale* L.) genotypes were examined. In Experiment I (2011-2012) 15 genotypes were composed of three parental inbred lines (Lo115-N, Lo90-N, and Lo117-N) as well as 12 F_{2:4} lines selected from the two biparental F₁ populations Lo115-N x Lo90-N and Lo115-N x Lo117-N. These 15 genotypes were out-crossed to the same cytoplasmic male sterile tester. In Experiment II (2013), 15 advanced breeding populations were studied. The hybrid rye cultivar ‘Palazzo’ was used as a standard in both experiments. All plant materials were provided by the KWS LOCHOW GmbH, Bergen, Germany.

Climate conditions

Air temperature and precipitation were recorded at 2 m height with a iMETOS weather station (Pessl Instruments, Weiz, Austria) located on the experimental field. The agrometeorological advisory system 'Agrowetter' from the German Weather Service (DWD, 2014) was used for irrigation scheduling.

Phenological data

Plant development was recorded using the BBCH scale for cereals (Hack et al. 1992). The beginning of stem elongation (BBCH 30), beginning of anthesis (BBCH 61), and fully ripe (BBCH 89) were expressed as day of year (DOY). Additionally the DOY when all leaves were senescent recorded was.

Agronomic data

Harvest took place at fully ripe (BBCH 89) in each year. Whole plants of the entire plots were hand-harvested in 2001. In 2012 and 2013 only the whole plants of a 0.5 m² portion of the plots were hand-harvested while the rests of the plots were harvested with a Nursery Master plot combine (Wintersteiger, Ried, Austria). The hand-harvested plants were separated into ears and straw; the ears were threshed and winnowed and the chaff added to the straw fraction. Grain and straw samples were oven-dried to constant weight at 105 °C for 24 h. Grain yield, straw yield, and total aboveground biomass yield (hereafter referred to as biomass yield) were calculated on the basis of 0% water content in t ha⁻¹. The yield components spikes m⁻², kernels spike⁻¹, and 1000-kernel weight (TKW) were determined from the hand-harvested plant samples.

Leaf area index

Starting at the beginning of stem elongation, the green leaf area index (hereafter referred to as LAI) was measured weekly with a SunScan canopy analysis system (Delta-T Devices, Cambridge, UK). Eight measurements were taken per plot in 50 cm intervals while maintaining a distance of 50 cm to the front and back edges of the plot; the eight values were averaged. When senescence started, the SunScan was held over the senescent leaf layer to record the photosynthetic active leaves only. The mean of all LAI measurements in one season is referred to as LAI_{mean}. Additionally, the LAI that intercepts 95% of the incoming photosynthetic active radiation (PAR) was calculated according to Brougham (1958).

Soil water content

The course of the soil moisture was recorded in 2012 and 2013 using the portable soil moisture probe Diviner 2000 (Sentek Technologies, Stepney, Australia). Plastic tubes with a diameter of 5 cm were installed to a depth of up to 150 cm in 24 and 32 plots in 2012 and 2013, respectively. Soil moisture readings at 10 cm intervals from 5 to 125 cm were taken twice a week from beginning of vegetation to harvest. The soil water content was also determined gravimetrically on several occasions, in order to obtain a site-specific calibration ($R^2 = 0.64$).

Statistics

Analyses of variance were carried out with the GLIMMIX procedure of SAS 9.3 (SAS Institute, Cary, NC, USA). Experiment I and II as well as individual years were analyzed separately because of different water regimes and different genotypes between the years. Genotype and water regime were considered as fixed effects; replication as random effect. Correlations were calculated with the CORR procedure of SAS. Graphs were created with SigmaPlot 12 (Systat Software Inc., Chicago, IL, USA).

Results

Climatic conditions and water supply

The weather conditions in the three experimental years were quite different (Figure 1). In 2011, the spring was warm and dry while the summer was mild. Yearly total rainfall amounted to 484 mm with an average temperature of 10.6 °C. The year 2012 started with low temperatures, followed by a cool spring and high amounts of rainfall especially during the summer month. Total rainfall (547 mm) and average air temperature (9.7°C) were lower than in 2011. The beginning of 2013 was also accompanied by cold temperatures, causing a late start of the vegetation. The May was especially wet with over 200 mm rainfall. The subsequent summer, however, was hot and dry. The total rainfall amounted to 684 mm with an average air temperature of 9.7 °C. By the use of rain-out shelters and additional irrigation a clear differentiation between the water regimes could be achieved with total plant available water ranging from 101 to 508 mm between beginning of April and end of June. While the soil moisture remained high under well-watered conditions, the soil moisture under severe drought steadily decreased from the beginning of stem elongation until harvest (Figure 2). The soil moisture under mild drought decreased to a lesser extent than under severe drought. In 2013, the soil moisture clearly increased under rainfed, late drought, and well-watered conditions in spring. Under early drought, however, the soil moisture decreased at the beginning of stem elongation until irrigation started after anthesis. Simultaneously, the soil moisture decreased under late drought as the irrigation was terminated at this time.

Response of phenology to drought

The plant development was significantly accelerated under drought (Table 2). In Experiment I, both severe and mild drought resulted in earlier anthesis, earlier full senescence, and earlier fully ripe. The acceleration effects were stronger under severe than mild drought. In

Experiment II, the plants under early drought started flowering before the well-watered plants, but fully ripe was reached at the same time. The plants exposed to late drought exhibited a significantly reduced grain filling period whereas plants under rainfed conditions showed a similar phenology as the well-watered plants.

Responses of agronomic and morphologic characteristics to drought

Across the three experimental years, a close relationship between the mean grain yield and the total plant available water was observed (Figure 3). In Experiment I, the grain yields were overall higher in 2012 than in 2011. Severe drought significantly affected grain, straw, and biomass yield in both years, but grain yield was more affected than straw and biomass yield (Table 3). Furthermore, drought negatively affected all yield components, but mild drought was much less detrimental than severe drought. In Experiment II, grain yield was significantly reduced in all drought stress regimes with the highest reduction found under early drought. Under early drought, straw and biomass yield, spikes m^{-2} , and kernels spike $^{-1}$ were also significantly decreased while the TKW was significantly increased. Late drought did not affect spikes m^{-2} and kernels spike $^{-1}$, but significantly reduced the TKW. Although the plants grown under rainfed conditions showed no significant reduction in any yield component compared to the well-watered plants, the grain yield was significantly reduced.

Responses of leaf area index to drought

Drought significantly reduced the LAI in varying degrees depending on the intensity of the water deficit (Figure 4). The strongest reduction in Experiment I was found under severe drought where the maximum LAI amounted to 4.5. The highest value of the LAI under mild drought was higher than that of the well-watered plants on the first measurement dates, but decreased faster later in the season. In Experiment II, the lowest LAI was found under early drought. The 95% PAR interception was reached at LAI = 4.9 in both experiments.

Relationship between grain yield and other agronomic and morphologic characteristics

Under severe drought in Experiment I, grain yield was significantly and positively associated with straw yield and all yield components with the strongest correlation found for spikes m^{-2} (Table 4). No significant correlations among these characters were observed under mild drought. When the plants were well-watered, significant relationships of grain yield with straw yield, spikes m^{-2} , and kernels spike^{-1} occurred in 2011 but not in 2012. In Experiment II, grain yields of the plants grown under early drought were positively related to straw yield and spikes m^{-2} . Under the influence of drought during grain filling, grain yield correlated significantly positive with TKW. The LAI_{mean} was related to grain yield under severe drought in 2011 and 2012, as well as in all water regimes in 2013.

Discussion

The plants in the different water regimes were treated uniformly until the beginning of stem elongation. At that time, the differentiation among the water regimes started either by withholding rainfall or by providing additional irrigation, which resulted in a wide range of water regimes. While the effect of different drought intensities was studied in Experiment I, the influence of drought during clearly defined development stages was examined in Experiment II.

Rain-out shelter effects

The use of rain-out shelters allowed the establishment of clearly defined water regimes. As a side effect, the microclimate inside the shelters was somewhat different from the outside conditions. This was especially true for the stationary rain-out shelter which permanently covered the experimental plots. Under these conditions the photosynthetic active radiation was decreased by about 10% while the air temperature was slightly increased. Comparable effects were reported by Brisson and Caslas (2005) who used similar rain-out shelters in their

experiments. The slightly changed light and temperature conditions also affected crop growth, albeit to a much lesser extent than the contrasting levels of water supply provided in the different water regimes.

Changes in phenology under drought stress.

The shortening of the growth period by up to 12 days under severe drought agrees with findings of Brisson and Casals (2005) who reported a 15 day earlier maturity in drought stressed spring wheat (*Triticum aestivum* L.) grown in Southern France. Li et al. (2011) reported an up to 14 day earlier maturity in spring wheat when the crops received amounts of water comparable to the severe drought in the present study. The degree of phenological acceleration was, however, dependent on the intensity of the water deficit. Continuous mild drought, for example, resulted in a seven day earlier fully ripe while plants under late drought reached fully ripe only four days earlier. The strongest effects on phenology was observed at the senescence of the plants with an 18 days earlier fully senescence under severe drought. According to Hafsi et al. 2007, the leaf senescence is especially sensitive to water stress. Because an accelerated plant development represents a trade-off between plant survival and yield, a shorter growth period is linked to grain yield losses (Gooding et al. 2003). Li et al. (2011) found a significantly positive relationship between grain yield and days to physiological maturity in spring wheat. Grain yield was found to be positively related to late flowering dates in winter wheat (Foulkes et al. 2007). Both results indicate that a shorter growth period goes along with reduced grain yield. In the present study, an 11 to 13 days shorter growth period under severe drought caused grain yield losses of up to 57%. Mild drought, which shortened the growth period by seven days, resulted in a grain yield reduction of only 14%.

Sensitivity of agronomic and morphologic characteristics to drought stress

The positive correlation between water regime means for grain yield and total available water (Figure 3) illustrates the importance of water availability for plant growth and grain yield. Plants respond to drought at molecular, cellular, and physiological levels, for example by stomatal closing, reduced photosynthesis rates, limited carbohydrate synthesis and diminished cell division and expansion (Barnabas et al. 2008). All these effects result in reduced growth rates and grain yield losses while the degree of response is depending on timing, duration, and intensity of the water deficit (Araus et al. 2002). The strongest decrease in grain yield of -57% under severe drought when the winter rye crop received no water from stem elongation onwards is comparable to results by Brisson and Casals (2005), who reported a 57% grain yield reduction in spring wheat grown in a rain-out shelter under drought conditions from emergence to harvest. Schittenhelm et al. (2014) observed a 60% grain yield decrease in winter rye grown on field-stored soil moisture only. In their study as well as in the present investigation, drought intensity in the most severe stress treatment increased continuously during the growth period. This pattern of drought stress caused a stronger reduction of grain than of straw yield, because during the vegetative development phase the plants could still benefit from the stored soil moisture originating from winter rainfall.

When regarding the yield components it could be shown that drought stress during pre-anthesis development stages seriously affected spikes m^{-2} and kernels spike^{-1} . Drought stress solely during grain filling affected the TKW only. While spikes m^{-2} and kernels spike^{-1} are already set before anthesis, the TKW is solely determined after anthesis (Slafer and Savin, 2004). Because crops are especially susceptible to drought during the period from three weeks before anthesis to a few days after anthesis (Fischer 1985), a reduced kernel number (through spikes m^{-2} and kernels spike^{-1}) is recognized to be the main determinant for yield reduction under drought stress (Slafer et al. 2014, Dolferus et al. 2011) caused by a general decrease in

fertility (Giunta et al. 1993). The strong reductions of spikes m^{-2} and kernels spike^{-1} under drought in the present study together with the subsequent reduction in grain yield confirms the strong influence of these yield components on grain yield, which is in line with similar studies (Ivanova and Tsenov 2011, Chmielewski and Köhn 2000, Dencic et al. 2000, Giunta et al 1993). When the rye crops were solely dependent on field-stored soil moisture under conditions of severe drought, the TKW was also significantly reduced. However, the TKW was reduced to a lesser extent than spikes m^{-2} and kernels spike^{-1} although the drought was more severe during grain filling than in the period before. This lesser reduction was most likely caused by the fact that a smaller number of kernels had to compete for assimilates as the kernel weight is negatively related to kernel number (Slafer et al. 2014, Acreche and Slafer 2006). Furthermore, the lower reduction in kernel weight might also have resulted from the fact that the plants filled their grains to a larger extent from the pre-anthesis reserve pools. The contribution of assimilate remobilization from pre-anthesis reserves is especially important under drought. When current assimilates are limited, for example, through reduced photosynthesis rates under drought, pre-anthesis reserves from stems, leaf sheaths, and leaves can account for up to 100% of the assimilates for grain filling (Foulkes et al. 2007, Yang and Zhang 2006). Ehdaie et al. (2008) reported an up to 65% contribution of stem reserves to final grain weight under drought. Yang et al. (2001) observed that 75 – 92% of ^{14}C -labeled carbon stored in the straw of winter wheat was reallocated to grains under drought stress, which represented an increase of 50 – 80% compared to well-watered conditions.

With the comparison of pre- and postanthesis drought it could be observed that drought during early development stages resulted in higher grain yield losses than drought later in the season: The grain yield reduction was significantly higher under pre-anthesis drought (-34%) compared to post-anthesis drought (-20%). This difference occurred despite the fact that both water regimes had similar water availability. This compares to results of Estrada-Campuzano

et al. (2012), who simulated “monsoonal” (pre-anthesis) and “Mediterranean” (post-anthesis) drought stress with wheat and triticale (*Triticosecale* Wittmack) under rain-out shelters. In their study, the grain yield was 33% lower under pre-anthesis than under post-anthesis drought. In the present study, pre-anthesis drought significantly reduced spikes m⁻² and kernels spike⁻¹. The irrigation after anthesis in this treatment resulted in a significantly increased TKW which could, however, not fully compensate for grain yield losses by the previous reductions of the other two yield components. According to Slafer et al. (2014), kernel weight can only function as fine regulation for grain yield. Therefore, only the formation of a large kernel number per unit area can lead to high grain yields when water is scarce before anthesis. Contrary to the pre-anthesis drought, post-anthesis drought only affected TKW through reduced photosynthesis rates during grain filling, accelerated senescence, and a shorter grain filling period (Barnabas et al. 2007). The relatively low reduction in TKW despite the decrease in current assimilation indicates that the kernels were primarily filled through retranslocation processes as described earlier.

Changes of leaf area index through drought stress

In all water regimes the highest LAI was found at anthesis when the vegetative growth was terminated. The faster LAI decrease under drought stress compared to optimal moisture conditions was caused by the drought-induced acceleration of senescence (Hafsi et al. 2007). The extent of LAI reduction under water deficit in the present study lies in between values indicated in the literature. Brisson and Casals (2005) reported a maximum of LAI = 2 for spring wheat under drought, which was a 75% reduction compared to irrigated conditions. Schittenhelm et al. (2014) on the other hand, found a significant reduction of the maximum LAI for winter rye under severe drought in only one of two experimental years. Because the LAI is linked to light interception, photosynthetic capacity, and aboveground biomass (Breda 2003), a reduced LAI is in turn consequently linked to reduced crop productivity. Brougham

(1958) defined a “critical LAI” as the LAI, where plants are able to intercept 95% of the incoming radiation. Under severe as well as under early drought the critical LAI could not be attained and might thus explain a large part of the grain yield losses observed for these water regimes.

Relationship between grain yield and further agronomic and morphologic characteristics.

The strong positive correlation between grain and straw yield underlines the importance of total aboveground biomass accumulation as a basis of high grain yield under pre-anthesis drought (Estrada-Campuzano et al. 2012, Dodig et al. 2008, van Ginkel et al. 1998). The same holds true for the positive correlation between grain yield and LAI_{mean} under severe drought because LAI_{mean} is an indicator for aboveground biomass (Breda 2003). Schittenhelm et al. (2014) reported a significant relationship between total aboveground biomass and LAI_{mean} , which was also found in the present study (data not shown).

The close association of grain yield with spikes m^{-2} and kernels $spike^{-1}$ under drought stress during vegetative growth in the severe and early drought treatments is in agreement with the results of Dencic et al. (2000), Gonzalez et al. (2007), and Dodig et al. (2008). The number of spikes m^{-2} had a stronger effect on grain yield than kernels per spike, which is in line with results provided by Slafer et al. (2014). Grain yield and TKW were only positively correlated under conditions of drought stress during grain filling in the late drought and rainfed treatments. This indicates that genotypes with a high TKW were able to maintain a high grain filling rate under water deficit. This might have been facilitated by either high current assimilation through high photosynthesis rates or by high retranslocation rates through maintaining a longer period of transport and deposition of assimilates during grain filling (Voltas et al. 1998).

In conclusion it could be shown that variation in grain yield of rye can be better explained by changes in grain number per area land than by changes in grain weight. This could also be illustrated with the pooled correlations across all water regimes and years when both spikes m^{-2} and kernels spike $^{-1}$ showed a highly significant correlation with grain yield. The TKW, on the other hand, was not associated with grain yield when pooled across all years and water regimes. This is in accordance with other studies examining the effect of yield components on final grain yield of cereals under a wide range of environmental conditions (Slafer et al. 2014, Estrada-Campuzano et al. 2012, Chmielewski and Köhn 2000, Dencic et al. 2000, Lopezcastaneda and Richards 1994). Peltonen-Sainio et al. (2007) even reported a stronger influence of grain number per area land on final grain yield in winter rye than in winter wheat.

Conclusion

Breeding for drought tolerance in winter rye is especially important because rye is mainly cultivated on sandy and infertile soils in Central and Eastern Europe and will therefore be strongly affected by the changing climate. This study examined the agronomic performance of winter rye under a wide range of water regimes for the first time. The results emphasize the importance of high numbers of spikes m^{-2} and kernels spike $^{-1}$ as basis for high grain yields under water limited conditions. Furthermore, a strong importance of pre-anthesis reserves for grain filling under drought is suggested. Maybe the reallocation processes explain a large part of the fact that rye is recognized to be the most drought tolerant cereal crop. In order to evaluate the contribution of pre-anthesis reserves for grain filling under water limited conditions for rye in detail, further research on this topic is necessary.

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Table 1. Details about the water regimes.

Year	Water regime	Water supply during		Total available water ⁺ (mm)	Rainfall (mm)	Irrigation (mm)
		Vegetative phase	Generative phase			
2011	Severe drought	None	None	131	23	0
	Well-watered	Optimal [‡]	Optimal	464	158	196
2012	Severe drought	None	None	101	18	0
	Mild drought	Moderate [#]	Moderate	208	35	85
	Well-watered I	Optimal	Optimal	508	279	165
	Well-watered II	Optimal	Optimal	508	279	165
2013	Early drought	None	Optimal	241	49	113
	Late drought	Optimal	None	274	225	55
	Rainfed	Rainfed	Rainfed	284	274	0
	Well-watered	Optimal	Optimal	413	274	175

⁺ Rainfall + Irrigation + $\Delta_{\text{Soil water}}$ – Seepage (early April to late June). $\Delta_{\text{Soil water}}$ ranged from 80 to 115 mm, seepage only occurred in 2013, ranging from 80 to 120 mm.

[‡] Optimal: >60% usable field capacity during the entire growth period.

[#] Moderate: 20 to 40% usable field capacity during the entire growth period.

Table 2. Water regime means for phenological characteristics.

Year	Water regime	Beginning of stem elongation		Beginning of anthesis		All leaves senescent		Fully ripe	
		DOY	Δ (d) ⁺	DOY	Δ (d)	DOY	Δ (d)	DOY	Δ (d)
2011	Severe drought	103 ^a	0	137 ^b	5***	171 ^b	18***	188 ^b	12***
	Well-watered	103 ^a		142 ^a		189 ^a		200 ^a	
2012	Severe drought	95 ^b	0	141 ^c	2***	181 ^c	13***	201 ^c	11***
	Well-watered II	95 ^b		143 ^a		194 ^a		212 ^a	
	Mild drought	88 ^a	0	141 ^c	1***	189 ^b	5***	205 ^b	7***
	Well-watered I	88 ^a		142 ^b		194 ^a		212 ^a	
2013	Early drought	113 ^a	0	156 ^c	1**	202 ^b	3***	216 ^a	0
	Late drought	113 ^a	0	157 ^{bc}	0	198 ^d	7***	212 ^b	4***
	Rainfed	113 ^a	0	157 ^{ab}	0	200 ^c	5***	216 ^a	0
	Well-watered	113 ^a		157 ^a		205 ^a		216 ^a	

Characteristic means within a year followed by different letters are significantly different at $P < 0.05$.

⁺ DOY (well-watered) - DOY (drought stress).

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Table 3. Water regime mean values for agronomical and morphological characteristics.

Year	Water regime	Grain yield		Straw yield		Biomass yield		Spikes m ⁻²		Kernels spike ⁻¹		TKW	
		(t ha ⁻¹)	Δ% ⁺	(t ha ⁻¹)	Δ%	(t ha ⁻¹)	Δ%	(#)	Δ%	(#)	Δ%	(g)	Δ%
2011	Severe drought	4.2 ^b	-56%	5.6 ^b	-36%	9.8 ^b	-46%	426 ^b	-22%	31.8 ^b	-29%	31.2 ^b	-22%
	Well-watered	9.5 ^a		8.8 ^a		18.3 ^a		545 ^a		45.1 ^a		39.9 ^a	
2012	Severe drought	4.6 ^c	-57%	5.8 ^c	-36%	10.3 ^c	-47%	412 ^c	-39%	28.0 ^c	-41%	30.9 ^b	-4%
	Well-watered II	10.6 ^b		9.1 ^b		19.7 ^b		670 ^b		47.5 ^a		32.3 ^a	
	Mild drought	10.2 ^b	-14%	- [‡]	-	-	-	763 ^a	-3%	41.3 ^b	-11%	31.6 ^{ab}	-3%
	Well-watered I	11.8 ^a		11.1 ^a		22.9 ^a		788 ^a		46.5 ^a		32.6 ^a	
2013	Early drought	8.0 ^d	-34%	5.8 ^c	-32%	13.8 ^d	-33%	569 ^b	-27%	38.3 ^b	-16%	36.9 ^a	+18%
	Late drought	9.8 ^c	-20%	9.9 ^a	+16%	19.7 ^b	-5%	790 ^a	+1%	45.1 ^a	-1%	27.7 ^c	-12%
	Rainfed	10.3 ^b	-16%	8.3 ^b	-2%	18.6 ^c	-10%	772 ^a	-1%	44.4 ^a	-2%	30.2 ^b	-4%
	Well-watered	12.2 ^a		8.5 ^b		20.7 ^a		783 ^a		45.4 ^a		31.3 ^b	

Characteristics in one year followed by different letters are significantly different at P<0.05.

⁺Percentage change relative to well-watered in the respective year. In 2012, severe and mild drought was compared with well-watered II and well-watered I, respectively.

[‡]No data for straw and biomass yield.

Table 4. Correlation coefficients for the relationship of grain yield and other agronomical and morphological characteristics.

Year	Water regime	Straw yield	Spikes m ⁻²	Kernels spike ⁻²	TKW	LAI _{mean}
2011	Severe drought	0.81***	0.51**	0.47**	0.43*	0.43*
	Well-watered	0.80***	0.68***	0.39*	-0.16	- ⁺
2012	Severe drought	0.63**	0.66***	0.39*	-0.28	0.40*
	Well-watered II	0.22	0.04	0.22	0.15	0.21
	Mild drought	-	0.24	0.00	0.31	0.35
	Well-watered I	0.28	0.01	0.42	0.07	0.27
2013	Early drought	0.64**	0.52**	0.23	0.14	0.58**
	Late drought	0.26	0.21	-0.02	0.52**	0.48**
	Rainfed	0.13	0.01	0.18	0.49**	0.53**
	Well-watered	0.07	-0.08	0.09	0.34	0.38*
Pooled [‡]		0.79***	0.77***	0.76***	0.04	0.85***

P < 0.05, **P < 0.01 and ***P < 0.001.

⁺ no LAI_{mean} values available.

[‡] across all years and water regimes.

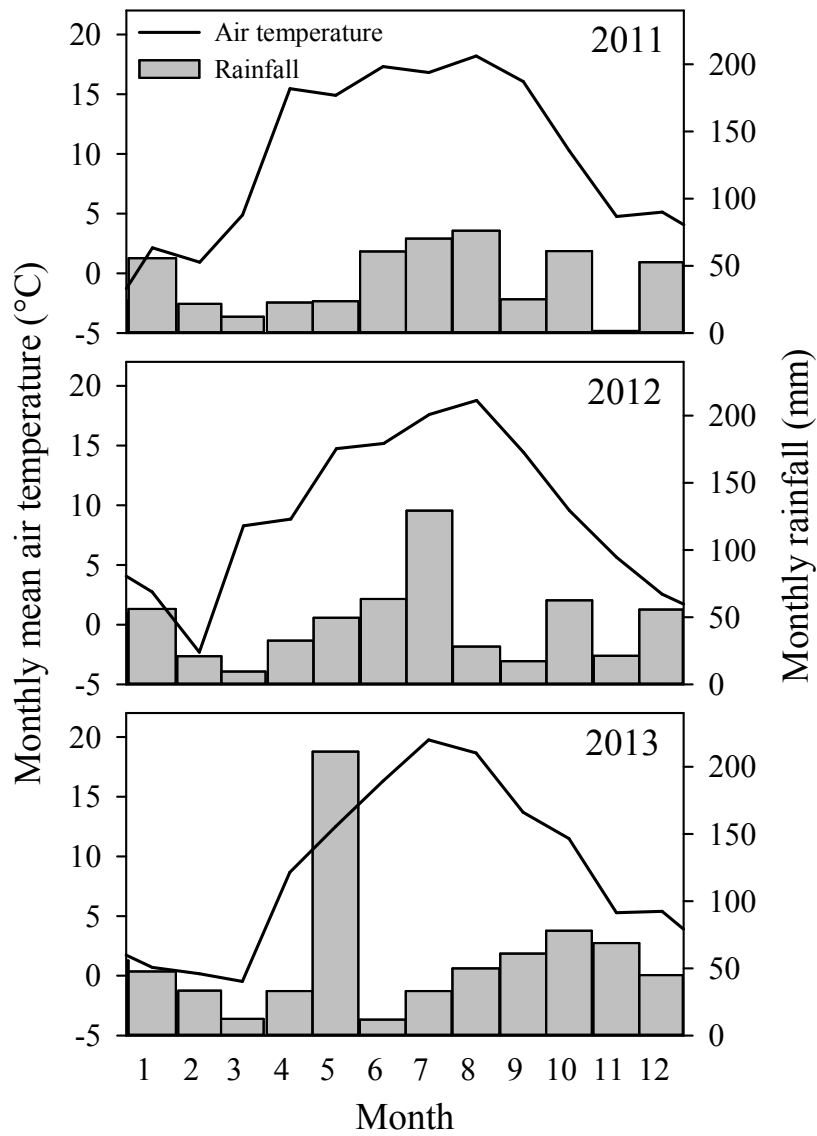


Figure 1. Air temperature and rainfall in the three experimental years.

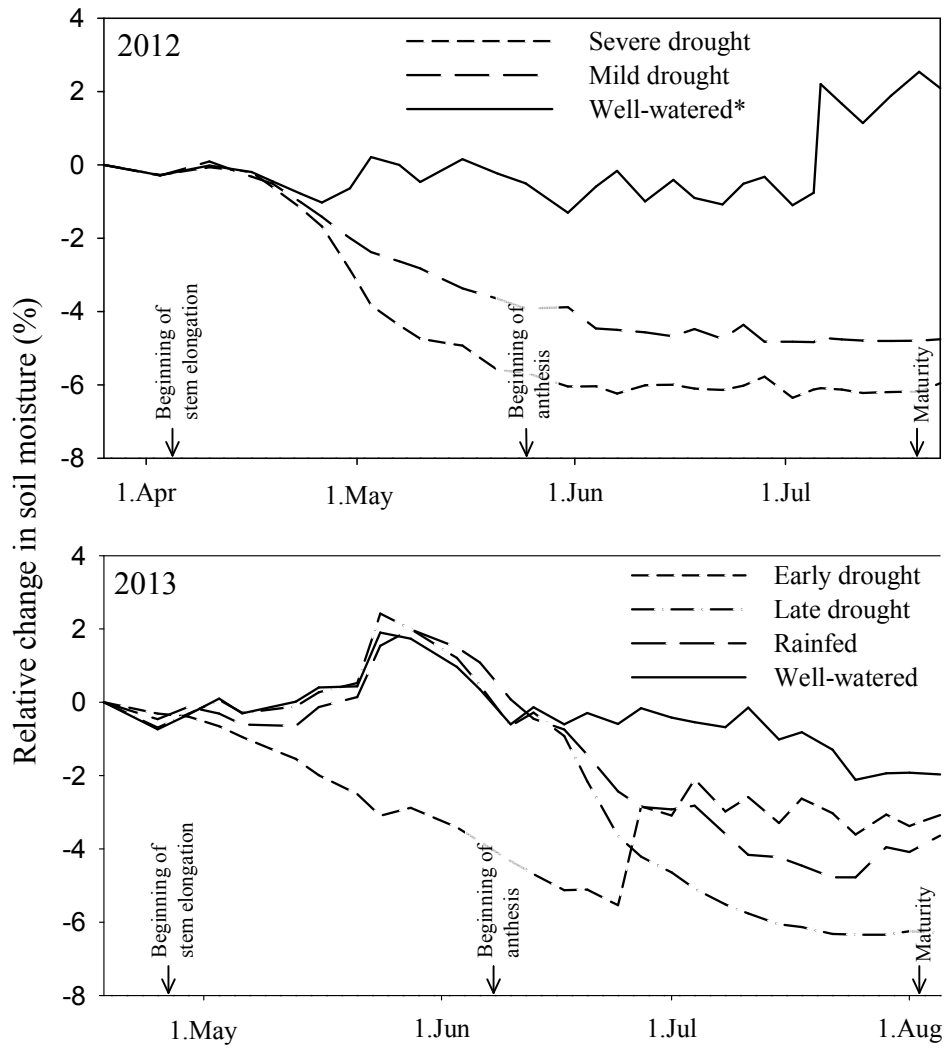


Figure 2. Relative change of soil moisture (%) in 0 – 120 cm soil depth during the season for the different water regimes in 2012 (above) and 2013 (below). * Only the water regime “well-watered I” is shown because of close similarity with “well-watered II”. Arrows indicate some main phenological stages.

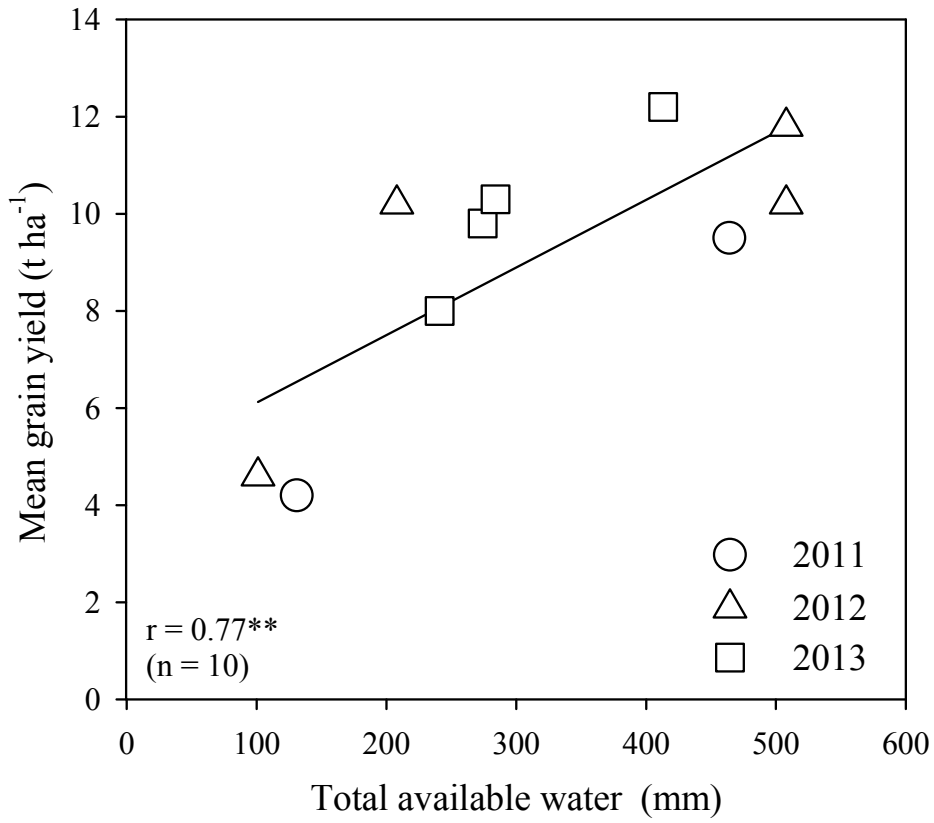


Figure 3. Relationship between the mean grain yield and the total available water.
****P<0.01.**

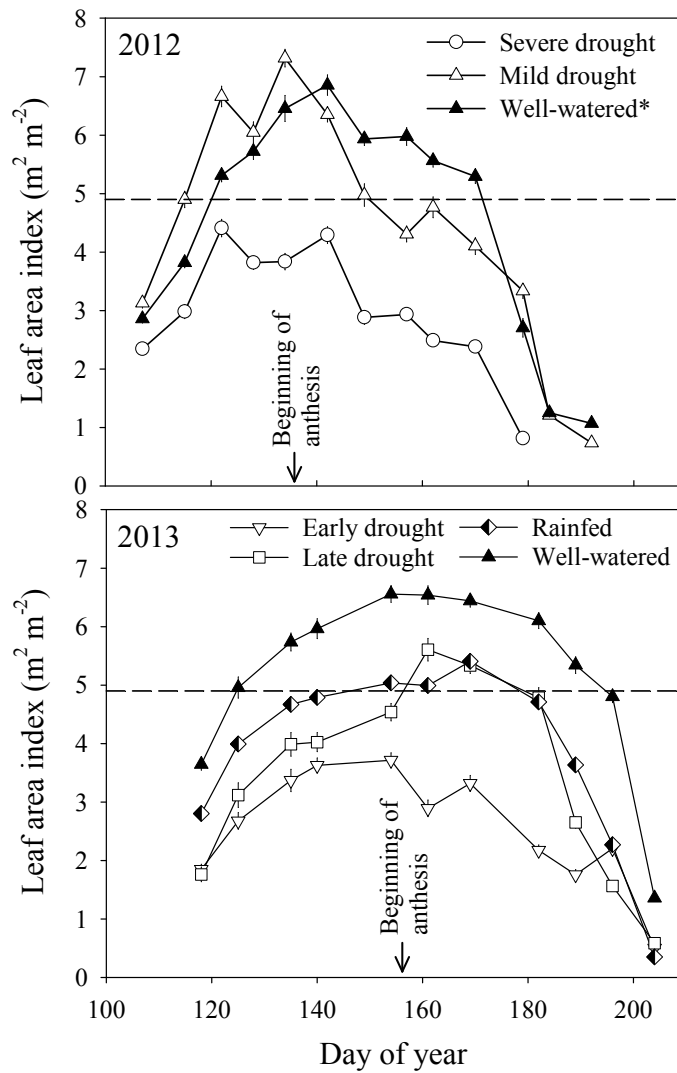


Figure 4. Water regime means of the leaf area index course in 2012 (top) and 2013 (bottom). Error bars indicate ± 1 standard error (only shown when they exceed the size of the symbol). Arrows indicate beginning of anthesis. *The water regimes “Well-watered I” and “Well-watered II” showed a quite similar curve course for the LAI, therefore only “Well-watered I” is shown. The dashed horizontal line indicates 95% PAR-Interception.

7. General discussion

While the suitability of canopy temperature depression and carbon isotope discrimination as secondary traits for the selection of drought tolerant winter rye was examined in the first part of this work (sections 4 and 5), the second part (section 6) deals with the effects of different drought events on agronomy, phenology, and morphology of winter rye. The primary findings are therefore presented and discussed in the scientific papers. This general discussion now aims to draw an overall picture of the results obtained in this study. It deals with a brief overview of the impact of different droughts on the measured crop characteristics, as well as a comparison between canopy temperature depression and carbon isotope discrimination. The genotypes studied in the present work exhibited a quite low genetic variability, especially in 2011 and 2012. For that reason, genotypes are not discussed individually.

7.1 Effects of drought stress on measured crop characteristics

The rye crops were optimally supplied with water under well-watered conditions. Because also nutrient supply was optimal, and pests, diseases, weeds, and other stresses were effectively controlled, the crops were able to express their full yield potential under well-watered conditions. By the use of stationary and mobile rain-out shelters, a wide range of clearly defined water regimes could be established. The resulting drought stress levels affected all measured physiological, phenological, morphological, and agronomical characteristics to varying degrees, resulting in mean grain yield reductions from 14 to 57%. As a side effect of the rain-out shelters, the microclimate inside the shelters was slightly different compared to the outside conditions, especially in the stationary rain-out shelter. Here, the photosynthetic active radiation was decreased by about 10% while the air temperature was slightly increased. Crop growth was also affected by the slightly changed

irradiation and temperature conditions, albeit to a much lesser extent than by the different drought stress levels.

7.1.1 Effects of drought stress on physiological characteristics

A reduced stomatal conductance (g_s) through stomatal closing is one of the first reactions of plants to reduced water availability in order to reduce transpiration water loss (Condon et al. 1990). A strong sensitivity of g_s to water deficit could be clearly shown by the strong decrease in g_s of up to 74% under severe drought. While stomatal regulations provide optimal levels of internal CO_2 concentration to feed the demand for CO_2 fixation under optimal water supply, stomatal closing under water deficit prevents excessive water loss in order to maintain a functional water status of the plants (Cornic and Massacci, 1996). A reduced g_s under drought stress was also indicated by means of a lower canopy temperature depression ($CTD = T_{air} - T_{canopy}$) under drought, because the CTD is an indicator of g_s (Fischer et al. 1998). CTD and g_s were significantly positively correlated under drought in the present study (data not shown), which compares to the results provided by Balota et al. (2007) and Fischer et al. (1998). Under optimal water supply, the CTD mostly had a positive sign in the present study, which implies that the canopy was cooler than the ambient air. The lower canopy temperature (= higher CTD) under adequate water supply was caused by the energy-demanding water evaporation, which reduces the plant surface temperature (Maes and Steppe, 2012). Under drought stress conditions, the CTD was 1.9 - 2.7 °C lower than under well-watered conditions because evaporation and, consequently, transpiration cooling was reduced. A similar decrease in CTD under drought stress was reported for wheat by Balota et al. (2007) and Rashid et al. (1999). Variation in g_s could also be described by changes in carbon isotope discrimination (Δ). Both characteristics were significantly related in the present study, which was also reported by Fischer et al. (1998). Unlike the CTD, Δ provides information about the transpiration efficiency (and therefore indirectly g_s) integrated over the whole growth period

of the sampled plant organ (Farquhar and Richards, 1984). Under drought, stomatal closing reduces the supply of CO₂ for carboxylation, which results in a decrease of the intercellular to atmospheric CO₂ concentration ratio (C_i/C_a). Because the discrimination rate is directly related to the C_i/C_a ratio, Δ is negatively affected by water deficit (Farquhar et al. 1989). Δ was strongly affected by drought stress, leading to significant decreases: Δ of flag leaves at maturity (Δ_L) was reduced by 6% under severe drought, whereas Δ of mature kernels (Δ_G) was reduced by 20% under severe drought. This reduction compares to the results provided elsewhere. Monneveux et al. (2005), for example, reported a 15% decrease of Δ_G under residual soil moisture. Δ analyzed on flag leaves at anthesis was 8% lower under residual soil moisture compared to full irrigation conditions in an experiment by Misra et al (2010). Δ_L was generally higher than Δ_G . This can be explained by the high starch content in grains compared to the high lipid content in leaves because the carbon isotope fractionation is lower in starch than in lipids (Condon et al. 2006). Beside CTD and Δ , changes in g_s also affected ash content and mineral concentrations in leaves and grains. The higher the transpiration rate, the higher is the amount of minerals passively transported through the xylem via the transpiration stream and accumulated in vegetative plant organs (Masle et al. 1992). As a result, drought stress reduced the evaporation rate and consequently the passive mineral uptake. The ash content in flag leaves at maturity (m_aL) was therefore significantly reduced under drought, which was also observed by Misra et al. (2010) and Araus et al. (2001). The ash content in mature grains (m_aG), on the other hand, increased significantly under water limited conditions. The mineral accumulation in grains depends on two main factors: the photosynthetic rate during grain filling and the remobilization of minerals from vegetative plant parts such as stems, leaves, and leaf sheaths (Wardlaw 1990). The photosynthetic rate is more affected by drought stress than by remobilization of minerals. As a result, the retranslocation of minerals from vegetative plant parts into the grain is much higher under water limited conditions than under

optimal water supply (Loss and Siddique 1994). The concentrations of single minerals showed different reactions to water deficit. The silicon concentration in flag leaves (Si_L) was highest under well-watered conditions and decreased significantly up to 81% under water limited conditions. This indicates a mainly passive uptake of silicon via the transpiration stream, which is in accordance with Walker and Lance (1991). The potassium concentration in flag leaves (K_L), on the other hand, was more than doubled under drought compared to well-watered conditions. This indicates a highly selective uptake of potassium (Marschner, 1995). The reason for the higher potassium concentration under water limited conditions is most likely the higher demand for potassium under drought, for example for the maintenance of photosynthetic CO_2 fixation and the protection from oxidative damage (Cakmak, 2005). Regarding the concentration of calcium (Ca_L) and magnesium (Mg_L) in flag leaves, there was no clear trend as for Si_L and K_L . Both calcium and magnesium are recognized to be important for the acclimation to stress (Waraich et al. 2011, Palta 1990). The highest concentrations of these minerals were found under mild drought stress while the lowest concentrations were found under well-watered condition, which implies a partly active uptake of calcium and magnesium (Yang and Jie, 2005, Marschner 1995). However, because calcium and magnesium are assumed to be important for the acclimation to stress, the highest concentrations of these minerals are expected under severe drought. The observation that the Ca_L and Mg_L levels already peaked at mild drought stress might be attributable to the fact that plants under severe drought stress were no longer able to actively take up calcium and magnesium from the soil.

7.1.2 Effects of drought stress on phenological characteristics

The shortened phenological development under drought confirms the strong sensitivity of phenology to water deficit. Anthesis begun up to 5 days earlier and fully ripe was reached up to 12 days earlier under drought compared to well-watered conditions, which is in line with

findings by Brisson and Casals (2005) under similar conditions. The duration of the growth period was related to the severity of the drought. The lesser the amount of plant available water, the shorter was the growth period. Li et al. (2011) found a significant positive correlation between grain yield and days to physiological maturity. This indicates that a reduced duration of the growth period results in yield reductions, which is in line with the results provided in the present study. The 7 and 12 days earlier fully ripe under mild and severe drought were, for example, accompanied by grain yield reductions of 14 and 57%, respectively. The observed phenological acceleration under drought in the present study should, however, not be confused with the drought adaption mechanism escape. Drought escape is rather the matching of phenological development with periods of soil moisture availability in environments with terminal drought stress, than a phenological acceleration per se (Araus et al. 2002).

7.1.3 Effects of drought stress on morphological and agronomical characteristics

Water deficit severely affected total aboveground biomass and straw yield, resulting in up to 47% less aboveground biomass and up to 36% less straw yield. When stomatal closing reduces the intake of CO₂ into the cells and decreases the photosynthesis rate, carbohydrate synthesis is diminished and cell division and expansion are decelerated, which leads to reduced plant growth (Barnabas et al. 2008). The green leaf area index (LAI), which is directly related to aboveground biomass (Breda 2003), was in a range of 8 under well-watered conditions and was more than halved under drought. Because the LAI is linked to light interception and photosynthetic capacity, a reduced LAI is consequently linked to reduced crop productivity (Breda 2003). Beside the reduction of the maximum LAI value, the LAI also decreased significantly earlier under drought compared to well-watered conditions, caused by the drought-induced acceleration of senescence (Hafsi et al. 2007).

All previous mentioned physiological, phenological, and morphological drought effects resulted in significant grain yield losses under drought in the end because grain yield is the result of many individual processes reacting to water deficit during crop growth. The strongest reduction in grain yield of 57% was observed when the winter rye crops did not receive any natural precipitation from stem elongation onwards, and were not irrigated during that time. These grain yield reductions are comparable to the results of Schittenhelm et al. (2014), who reported a 60% grain yield decrease in winter rye under similar drought conditions. Brisson and Casals (2005) found a 57% grain yield reduction in spring wheat grown in rain-out shelters with water exclusion from emergence to harvest. As also observed by Schittenhelm et al. (2014), grain yield was more affected by water deficit than straw yield because the water deficit was induced at the beginning of stem elongation and increased steadily with the age of the plants. Plants could therefore still benefit from field stored soil moisture during vegetative growth, caused by adequate winter precipitation. When the grain yield was further dissected into the grain yield components spikes m^{-2} , kernels spike $^{-1}$, and 1000-kernel weight (TKW), the variation in grain yield under drought could be explained by changes in its components: Spikes m^{-2} and kernels spike $^{-1}$ had a considerably higher influence on final grain yield than TKW under water deficit, which was, for example, indicated by the significant positive relationship between grain yield and both spikes m^{-2} and kernels spike $^{-1}$ under drought. The TKW, on the other hand, had a lesser effect on final grain yield under drought. A high TKW, for example, could not compensate for grain yield losses caused by a low kernel number under pre-anthesis drought even when the irrigation was reinstated during grain filling. While the number of spikes m^{-2} and kernels spike $^{-1}$ is already determined at anthesis, the TKW is solely determined after anthesis (Slafer and Savin, 2004). Cereal crops are most susceptible to water deficit from 3 weeks before anthesis to a few days after anthesis (Fischer 1985). This explains why a reduced number of spikes m^{-2} and kernels spike $^{-1}$ are

recognized to be the main determinants for yield reductions under water deficit (Chmielewski and Köhn 2000, Dencic et al. 2000). The observed low impact of TKW on final grain yield is, for example, in accordance with Slafer et al. (2014) who stated that the kernel weight can only function as a fine regulation for grain yield, while solely the number of kernels per area land can be responsible for large changes in final grain yield. This explains why drought during early development stages affected grain yield considerably more than drought solely after anthesis. In the present work, grain yields were reduced under pre-anthesis drought by 34% while post-anthesis drought reduced the grain yield by only 20%, despite similar amounts of plant available water (during the whole growth period). Similar results were obtained, for example, by Estrada-Campuzano et al. (2012) with 33% less grain yield under “monsoonal” (pre-anthesis) drought compared to “mediterranean” (post-anthesis) drought.

7.2 Comparative performance of canopy temperature depression and carbon isotope discrimination for the selection of drought tolerant winter rye.

The suitability of CTD and Δ as secondary traits were examined under German climate conditions in Experiment I in 2011 and 2012. Hereinafter, both methods are compared to each other with regards to their practicability, costs, phenotypic correlation with grain yield as well as their usability in plant breeding.

7.2.1 Practicability and costs

Although both CTD and Δ are indicators of stomatal conductance (Fischer et al. 1998), their implementation is completely different. The CTD represents a snapshot of the plant water status and transpiration rate, which can be assessed non-destructively with IR thermometry and IR photometry. The CTD could, therefore, be performed quickly: the measurement of all 128 plots in the present study took between 30 and 60 minutes, and the CTD measurements could be repeated several times during the vegetation period. Δ on the contrary represents an

integrative measure of the transpiration rate during the whole growth period of the sampled plant tissue (Farquhar and Richards 1984). Since Δ is a destructive measurement, the analyses are much more time and labor intensive. The analyses included the collection of leaf and grain samples, grinding of the samples and the actual analysis using an elemental analyzer coupled to an isotope ratio mass spectrometer (IRMS). Furthermore, the Δ analyses are also quite expensive. Beside the requirement for an elemental analyzer and an IRMS, the material for every single measurement sample costs approximately 5€ (Giesemann, personal communication 2014). The CTD measurements, on the contrary, can be carried out much cheaper. Three different IR instruments were used for the CTD measurements in the present work: two IR thermometers and an IR camera with prices ranging from 1.500 to 30.000 €. It could be shown that all three instruments were in principal suitable for the CTD measurements, despite their greatly different costs. Cossani et al. (2012) even suggested that 200 \$ IR thermometers are well suited for canopy temperature measurements.

7.2.2 Phenotypic correlation with grain yield

The most crucial consideration for the usefulness of a secondary trait as selection criterion is the correlation with grain yield (Fischer et al. 1998). When regarding the correlations of the present study, one limitation must be considered. The genotypes showed a quite low genetic variability, which was inter alia caused by the fact that 15 of the 16 genotypes were out-crossed to the same cytoplasmic male sterile tester. Thus, the genetic variability was already halved. As a result, differences among the genotypes were quite small. Genotypic differences in CTD, for example, could not be observed on most measurement days. Despite this fact, correlations were calculated in order to make the results comparable to related studies. The CTD was significantly positively correlated to grain yield under drought. The maximum value of the correlation coefficient (r_{\max}) was 0.76 under drought, which compares to the results obtained for wheat by Balota et al. (2007) and Rashid et al. (1999). Δ measured on flag leaves

at maturity (Δ_L) was also significantly positively correlated to grain yield under drought. The correlation ($r_{\max} = 0.38$) was, however, weaker than that of the CTD. When Δ was analyzed in mature grains (Δ_G), no correlation with grain yield could be found at all. This is in contrast to the results of Kumar et al. (2011) and Merah et al. (2002), who reported significant positive correlations between Δ_G and grain yield.

The strong correlation between CTD and grain yield implies a good suitability of CTD as selection criterion at first sight. However, the CTD was quite sensitive to environmental conditions. Significant correlations between grain yield and CTD were almost exclusively existent on days with good weather conditions, i.e. high solar irradiation, high air temperature, and low wind speed. These suitable weather conditions occurred more frequently in 2011 than in 2012. Significant positive correlations between CTD and grain yield existed on 5 out of 6 measurement dates in 2011 but only on 2 out of 8 dates in 2012, because the year 2011 was generally warmer and drier and therefore more suitable for assessing the canopy temperature. Optimal conditions for CTD measurements are described in literature as “warm, dry, and clear conditions” (Fischer et al. 1998), “cloudless days” (Rashid et al. 1999), “full sunshine” (Ayeneh et al. 2002), and “days with mean solar irradiance of $>500 \text{ W m}^{-2}$ ” (Balota et al. 2007). All of these mentioned studies were however carried out under arid and semiarid conditions, and such conditions are quite rare in the temperate climate of Germany. The biggest limitation of CTD measurements in a temperate climate is therefore the weather condition, which can also change quite fast (Jones 1999). The effect of weather fluctuation on canopy temperature was further examined in two one-hour-measurements on days with contrasting weather conditions (data not shown). Under the condition of a cloudless sky, the solar irradiation was constant and the canopy temperature largely followed the air temperature. When the sky was partly clouded, the decrease in solar irradiation caused by a cloud passage led to a significant decrease in canopy temperature while the air temperature

was hardly affected. This makes it impossible to compare two genotypes, of which one genotype is measured during a cloud passage, and the other during clear sky. Jensen et al (1990) also reported that the canopy temperature quickly responded to changes in solar irradiation and wind speed. They found a 2 °C fluctuation of the canopy temperature during a one-hour measurement. However, when the measurements are carried out on days with optimal weather conditions, the performance of CTD is satisfying even in a temperate climate. Optimal condition for performing CTD measurements turned out to be a cloudless sky, a solar radiation $>700 \text{ W m}^{-2}$, an air temperature $>20^{\circ}\text{C}$, and wind speeds $<3 \text{ m s}^{-1}$ during the measurements.

The quite weak correlations between grain yield and Δ_L under drought stress, as well as the missing correlations between grain yield and Δ_G suggest a poor suitability of carbon isotope discrimination as a selection criterion for rye in a temperate climate. There are some possible explanations for this assumption. The environmental conditions might have diminished the relationship between Δ and grain yield probably because of quite low air temperatures and high relative humidity during the growing season. Most of the cited studies were carried out under arid, semiarid, and Mediterranean climates (Monneveux et al. 2006, Royo et al. 2002, Merah 2001), which are characterized by higher temperatures and lower relative humidity. Furthermore, the water deficit under severe drought might have been too strong. Hafsi et al. (2007) also could not detect any relationship between Δ and grain yield in wheat grown under similar water availability. These authors suggested that the usability of Δ may be restricted to moderate drought conditions. The reason might be a strong contribution of the reallocation of pre-anthesis reserves for grain filling under severe drought (Foulkes et al. 2007). Assimilates originating from early pre-anthesis growth (when the water supply was still adequate) with high carbon isotope discrimination rates, which were reallocated into the grains under severe drought during grain filling, might have distorted the final Δ -values. This does, however, not

explain why no significant correlations were found under mild drought either. A further explanation for the weak or missing relationship between Δ and grain yield might be the low genetic variability of the material used in this study. A more diverse set of genotypes might have resulted in stronger correlations. Many of the studies on associations between Δ and grain yield used more diverse sets of genotypes. Monneveux et al. (2005) used 20 wheat cultivars of the CIMMYT (International Maize and Wheat Improvement Center), which were chosen based on their different grain yield performance. They reported an r_{\max} of 0.89 under post-anthesis water stress. Royo et al. (2002) analyzed Δ of 25 wheat genotypes with different origin to represent a wide range of genetic variability. In their study, r_{\max} was 0.53. Whether and to what extent these factors (unsuitable weather conditions, too severe drought, limited genetic variability) contributed to the poor performance of Δ could not be fully clarified.

7.2.3 Can CTD and Δ be used in plant breeding?

The CTD seems to be a promising secondary trait for crop improvement even under temperate climate conditions. The CTD was strongly related to grain yield and could be assessed quickly, cheaply, and non-destructively. The CTD could therefore be used at a large scale, for example to screen a breeding nursery with thousands of entries at various stages of crop development. The weather conditions must, however, be considered as a limiting factor in order to get meaningful results. Therefore, measurements should only be performed on days with a cloudless sky, a solar irradiation $>700 \text{ W m}^{-2}$, an air temperature of at least $20 \text{ }^{\circ}\text{C}$, and wind speeds $<3 \text{ m s}^{-1}$. The use of Δ as selection criterion cannot be recommended, because of the weak respectively missing relationship with grain yield. Generally, Δ would have not been suitable to screen large sets of entries because it is an expensive, time consuming, and labor intensive method. It could have been used, however, to screen a smaller set of entries more detailed. Δ has, for example, already been successfully integrated in breeding programs for improving productivity under water limited conditions (Richards et al. 2010). In conclusion,

for a final recommendation of the use of CTD and Δ as selection criterion, the results should be secured with a more diverse set of genotypes. It might then be possible to evaluate whether the missing genotypic differences in CTD and the missing relationship between Δ and grain yield were caused by the method itself or by the low genetic variability.

8. Conclusion

In the light of the ongoing climate change, breeding for drought tolerant cultivars is important for all crops even in temperate climates in order to adapt them to the expected reduced water availability in the near future. The need for drought tolerant cultivars holds especially true for winter rye, which is predominantly cultivated on non-optimal sites in Central and Eastern Europe. The fact that plant breeding is primarily based on the direct selection for grain yield at present, which is quite unfavorable for the selection of drought tolerant genotypes, emphasizes the need for further selection criteria. The suitability of canopy temperature depression and carbon isotope discrimination was therefore assessed to examine their use as selection criterion in rye breeding under temperate climate conditions. Although the results were limited to some extent by the low variability of the genetic material and should therefore be validated with a more diverse set of genotypes, this thesis demonstrates that the CTD can be used as selection criterion in rye breeding also in the temperate climate of Germany. The CTD measurements should, however, only be carried out on days with a cloudless sky, high air temperatures, and low wind speeds in order to get meaningful results. In addition, the comparison of three infrared instruments in greatly different price categories showed that also lower priced infrared instruments were suitable to assess the canopy temperature. The carbon isotope discrimination, on the other hand, could not be recommended as selection criterion at present. However, the poor performance of carbon isotope discrimination might have rather been caused by the low genetic variance of the genetic material than by the method itself. A further examination with a more diverse set of genotypes would therefore be preferable.

The additional examination of the agronomic performance of winter rye under drought provided insights into the reaction of rye to different timings, durations, and intensities of drought. High number of kernels per area land was especially important for high grain yields under reduced water availability in rye. Furthermore, the results suggested a major importance

of pre-anthesis reserves for grain filling. The reallocation of assimilates for grain filling may explain a large part of the relatively good drought tolerance of rye. This assumption would be interesting to examine any further.

For rye breeding, the CTD seemed to be a promising tool. If the CTD can be successfully integrated in the rye breeding process, this method will help to accelerate the breeding progress in order to keep up with the negative effects of the ongoing climate change. To further reduce the effects of fluctuating weather conditions, the measurement of all plots in parallel would be preferable. This could be done, for example, by means of unmanned aerial vehicles (UAV) as described by Munns et al. (2010) and Berni et al. (2009). Furthermore, the suitability of the CTD under German climate conditions may even increase in the future because of the predicted increase in air temperature and the expected lesser amounts of rainfall during the summer months.

9. References

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10. Curriculum vitae

Personal Information

Name	Lorenz Kottmann
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Education

2003	Schubart-Gymnasium Ulm <i>Abitur (A-Level)</i>
2003 – 2004	University Stuttgart <i>Environmental Engineering (two semesters)</i>
2004 – 2009	University Stuttgart-Hohenheim <i>Diploma in Agricultural Biology</i> Thesis title: Effect of elevated CO ₂ and temperature on photosynthesis, growth, and biomass of spring wheat (<i>Triticum aestivum</i> L.)

Work

2010 - 2011	Zeeb & Partner, Ulm <i>Landscape planner (freelancer)</i>
2011 - Present	Julius-Kühn Institute, Braunschweig Institute of Crop and Soil Science <i>Research associate</i> Project title: Field-based innovative measurement techniques for drought tolerance improvement of winter rye in biogas crop rotations. (“Feldbasierte innovative Messtechniken für die Verbesserung der Trockentoleranz von Winterroggen in Biogasfruchtfolgen.“)

11. Publication list

Scientific papers

Kottmann, L., Schittenhelm, S., Wittich, K.P., and Wilde, P., 2013. Suitability of canopy temperature depression in a temperate climate with drought-stressed winter rye, determined with three infrared measurement devices. *Journal of Agronomy and Crop Science* 199, 385-394.

Kottmann, L., Giesemann, A., and Schittenhelm, S., 2014. Suitability of carbon isotope discrimination, ash content, and single mineral concentration for the selection of drought-tolerant winter rye. *Plant Breeding* 133, 579-587.

Kottmann, L., Schittenhelm, S., and Wilde, P., 2014. How do timing, duration, and intensity of drought stress affect the performance of winter rye? Submitted to *European Journal of Agronomy*.

Conference contributions

Kottmann, L., 2014. Trockenstressversuche in Rain-out Shelters. Sommertagung Abteilung Getreide der Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP) am 24. Juni 2014 in Böhnshausen.

Kottmann, L., 2014. Bestandestemperatur als Indikator für den Wasserstatus von Kulturpflanzen. 45. DLG-Technikertagung am 29. Januar 2014 in Soest.

Kottmann, L., Wittich, K.P., Giesemann, A., and Schittenhelm, S., 2013. Bestandestemperatur und Kohlenstoffisotopendiskriminierung als pflanzenbasierte Methoden für die Beurteilung von Trockenstress bei Winterroggen. 56. Jahrestagung der Gesellschaft für Pflanzenbauwissenschaften e.V. (GPW) am 05. September 2013 in Freising-Weihenstephan.

Kottmann, L., Schittenhelm, S., Wittich, K.P., and Giesemann, A., 2013. Bestandestemperatur und Kohlenstoffisotopendiskriminierung als pflanzenbasierte Methoden zur Erfassung von Trockenstress. Sommertagung Abteilung Getreide der Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP) am 25. Juni 2013 in Gießen.

Kottmann, L., Schittenhelm, S., Wittich, K.P., and Giesemann, A., 2012. Feldbasierte innovative Messtechniken für die Verbesserung der Trockentoleranz von Roggen in Biogasfruchtfolgen: Ertragsphysiologische Messungen im Rain-out Shelter. Sommertagung Abteilung Getreide der Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP) am 13. Juni 2012 in Braunschweig.

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