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Analysis of drought stress effect on wheat in interaction with high temperatures

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Abstract:

The production of wheat is greatly threatened by the global increase in heat and drought stress. The individual effects of these two stressors on different growth and yield traits of wheat have been studied, but the response of wheat to a combination of heat and drought stress cannot be directly extrapolated from the response to each stress factor alone. Previous research has shown that there is a lack of information regarding:

1. Response of wheat to a combination of heat and drought stress
2. Interactions between heating method, soil substrate and temperature measurement point, and heat stress under climate change,
3. Effects of heat and drought interactions before anthesis on grain filling rate and duration.

Three experiments were carried out to improve the understanding on the aforementioned knowledge gaps:

1. An experiment was conducted to understand how wheat yield and component yield responded to high temperature, drought stress and combined heat and drought. The reproductive stage of development in wheat proved to be the most sensitive stage, and the effects of combined heat and drought on the physiological and yield traits were considerably stronger than those of the individual stress factors alone. However, the magnitude of the effects varied for specific growth- and yield-related traits. In addition, cultivar responses were similar for the heat but different for the drought and combined heat and drought treatments. Generally, heat stress as imposed in this study was less detrimental than the effect of drought and of heat and drought combined.
2. Three different sites studies were set up to improve the understanding of the reasons for the above findings by testing the response of wheat yield and yield components to differences in heating method, temperature measurement points and soil substrate under sole heat and combined heat and drought stress around anthesis. Grain yield was significantly reduced by heat stress in plants grown on a sandy soil substrate but not in those grown on a soil with a high soil water holding capacity with two heating systems (climate chamber and infrared heaters). Combined heat and drought had a stronger detrimental effect on yield than heat stress alone.

Grain number was significantly reduced in all experiments by heat stress and combined heat and drought stress at anthesis. Single grain weight was increased by heat stress around anthesis, and partly compensated lower grain numbers in pots containing soil with a high soil water holding capacity but not in experiments with a sandy soil substrate.

3. A further experiment was conducted to study the impact of heat and drought stress at anthesis on grain filling rate, grain filling duration, photosynthesis rate, chlorophyll content, yield and yield components of three wheat cultivars. The reduction of grain filling duration was fully compensated by increased grain filling rates, so that single grain weights were not significantly different (2 cultivars) or even higher for the heat treatment (1 cultivar) under heat stress. In addition, heat and drought stress at anthesis reduced the grain number and grain yield. Drought stress accelerated earlier flag leaf senescence and reduced photosynthesis rates during grain filling but not in the plants exposed to heat. This indicates that source limitations may explain the differences in grain filling rates between the treatments. The results demonstrate that the impact of stress at anthesis on the subsequent grain filling period differs between heat and drought stress.

Based on the above findings, several conclusions can be drawn. More consideration should be given to the individual and combined impacts of heat and drought stressors on wheat productivity and in crop models. The crop models must also consider the difference in crop response to heat and drought stress during anthesis in various phonological phases. Finally, it is necessary to place more emphasis on substrate soil properties, temperature measurement point and heat imposing procedure.

Kurzfassung:

Die Produktion von Weizen ist durch die weltweite Zunahme von Hitze- und Trockenstress stark gefährdet. Die einzelnen Auswirkungen dieser Stressfaktoren auf verschiedene Wachstums- und Ertragsmerkmale von Weizen wurden untersucht, allerdings kann die Reaktion von Weizen auf eine Kombination von Hitze- und Trockenstress nicht direkt aus der Reaktion auf jeden einzelnen Stressfaktor extrapoliert werden. Frühere Forschungen haben einen Mangel an Informationen in den folgenden Bereichen aufgezeigt:

1. Reaktion von Weizen auf eine Kombination von Hitze- und Trockenstress,
2. Wechselwirkungen zwischen Erhitzungsmethode, Bodensubstrat und Temperaturmesspunkt und durch den Klimawandel verursachten Hitzestress,
3. Auswirkungen der Wechselwirkungen von Hitze und Trockenheit vor der Anthese auf die Kornfüllungsrate und -dauer.

Es wurden drei Versuche durchgeführt, um die oben genannten Wissenslücken zu schließen:

1. Der erste Versuch dient dem Verständnis drüber, wie der Weizenertrag und die Ertragsanteile auf hohe Temperaturen, Trockenstress und die Kombination von Hitze und Trockenheit reagieren. Insbesondere das Reproduktionsstadium des Weizens erwies sich als das empfindlichste Stadium. Die Auswirkungen der Kombination von Hitze und Trockenheit auf die physiologischen und ertragsrelevanten Merkmale waren erheblich stärker als die der einzelnen Stressfaktoren allein. Das Ausmaß der Auswirkungen variierte jedoch bei bestimmten wachstums- und ertragsbezogenen Merkmalen. Außerdem reagierten die Sorten ähnlich auf die Hitze, aber unterschiedlich auf die Trockenheit und die Kombination von Hitze und Trockenheit. Im Allgemeinen war der Hitzestress, wie er in dieser Studie eingesetzt wurde, weniger schädlich als die Auswirkungen von Trockenheit oder von Hitze und Trockenheit zusammen.
2. Um die Gründe für die oben genannten Ergebnisse besser zu verstehen, wurden Studien an drei unterschiedlichen Standorten durchgeführt. Dort wurde jeweils die Reaktion des Weizenertrags und der Ertragskomponenten auf Unterschiede bei der Heizmethode, dem Temperaturmesspunkt und dem Bodensubstrat unter alleinigem Hitzestress und

kombiniertem Hitze- und Trockenstress um die Anthese herum getestet. Der Kornertrag wurde durch Hitzestress bei Pflanzen, die auf einem sandigen Bodensubstrat angebaut wurden, signifikant verringert, anders als bei Pflanzen, die auf einem Boden mit hoher Wasserhaltekapazität und mit zwei Heizsystemen (Klimakammer und Infrarotstrahler) angebaut wurden. Die Kombination aus Hitze und Trockenheit wirkte sich stärker nachteilig auf den Ertrag aus als Hitzestress allein.

Die Anzahl der Körner wurde in allen Versuchen durch Hitzestress und kombinierten Hitze- und Trockenstress zum Zeitpunkt der Anthese signifikant reduziert. Das Einzelkorngewicht wurde durch Hitzestress um die Anthese herum erhöht und kompensierte teilweise die geringere Kornzahl in Töpfen mit Böden mit hoher Wasserspeicherkapazität, im Gegensatz zu Versuchen mit sandigem Bodensubstrat.

3. In einem weiteren Versuch wurden die Auswirkungen von Hitze- und Trockenstress um die Anthese auf die Kornfüllungsrate, die Kornfüllungsdauer, die Photosyntheserate, den Chlorophyllgehalt, den Ertrag und die Ertragskomponenten von drei Weizensorten untersucht. Die Verkürzung der Kornfüllungsdauer wurde durch eine erhöhte Kornfüllungsrate vollständig kompensiert, so dass sich die Einzelkorngewichte unter Hitzestress nicht signifikant unterschieden (2 Sorten) bzw. bei der Wärmebehandlung sogar höher waren (1 Sorte). Darüber hinaus verringerten Hitze- und Trockenstress in der Anthese die Korn Zahl und den Kornertrag. Trockenstress beschleunigte die frühere Seneszenz der Fahnenblätter und verringerte die Photosyntheseraten während der Kornfüllung, anders als bei den Pflanzen, die Hitze ausgesetzt waren. Dies deutet darauf hin, dass die Unterschiede in den Kornfüllungsraten zwischen den Behandlungen durch eine Einschränkung der Quelle erklärt werden können. Die Ergebnisse zeigen, dass sich die Auswirkungen von Stress in der Anthese auf die nachfolgende Kornfüllungsperiode zwischen Hitze- und Trockenstress unterscheiden.

Auf der Grundlage der obigen Ergebnisse können mehrere Schlussfolgerungen gezogen werden. Die einzelnen und kombinierten Auswirkungen von Hitze- und Trockenstress auf die Produktivität von Weizen sollten auch in Anbaumodellen stärker berücksichtigt werden. Die Anbaumodelle müssen auch die unterschiedliche Reaktion der Pflanzen auf Hitze- und Trockenstress, während der Anthese in verschiedenen phonologischen Phasen berücksichtigen. Schließlich muss mehr Gewicht auf die Eigenschaften des Bodensubstrats, den Temperaturmesspunkt und das Verfahren zum Aufbringen der Wärme gelegt werden.

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Chapter 1

General Introduction

1.1 Wheat production and global food security

The contribution of wheat, as one of the most important source of calories and proteins, to food security and to local and international diets has not been in disguise for many years now. Wheat, with the annual global production about 676 million tons, feeds about one-fifth of humans (FAO, 2011c; Farooq et al., 2014). Compare to two other important cereal crops, i.e., paddy rice (Oryza sativa L.) and of maize (Zea mays L.), wheat has 1.4 times bigger cultivated area with the global acreage about 207 million hectares (FAO, 2011a; Pradhan, 2011). Thus wheat has an indisputable role in securing the adequate access of the world's population to food. However, to continue feeding this rising world population, increasing the wheat production is a must which can better be achieved by the higher yields rather than the acreage cultivated of the crop (Farooq et al., 2014). The anticipated increase in global demand for wheat and other cereals is due to the expected increases in population growth, economic development, and urbanization (Godfray et al., 2010; Eyshi Rezaei, 2016). Agricultural production therefore needs to double to meet the growing demand for food by 2050 (Ray et al., 2013), with at least a 38% increase in wheat yield expected amidst such agricultural production increment (Ray et al., 2013; Eyshi Rezaei, 2016).

Plants are often exposed to abiotic stresses which hinder their growth, development, and productivity. Drought and heat stress are reportedly the major threats to wheat production (Semenov and Shewry, 2010; Pradhan et al., 2012; Zhang et al., 2016). Beside extreme temperatures, drought limits crop production through lower and more irregular precipitation patterns, high evapotranspiration, and large vapour pressure deficit (Vignjevic et al., 2015). According to the prediction by the Intergovernmental Panel on Climate Change (IPCC), more regions are expected to experience frequent episodes of hot days and nights and erratic precipitation with frequent droughts in the future (IPCC 2007). According to documented evidence by climatologists on global maximum and minimum temperature trends, while increments are noted in both minimum and maximum temperatures, greater changes are found in the daily minimum temperatures than in the daily maximum (Pradhan, 2011). The increase in frequency and magnitude of heat and drought events are deemed the most critical yield reduction factors under climate change conditions (Eyshi Rezaei, 2016). As earlier mentioned, climate change and associated extreme weather conditions will pose a great threat to global efforts to meet the food needs of the increasing world's population (Bishop et al., 2016). Drought and heat stress often happen simultaneously at anthesis and grain filling duration of the wheat crop development stage causing severe yield reduction in most of the wheat growing areas of the world (Pradhan et al., 2012). The impacts of drought and high temperature stress on wheat productivity have mostly been studied independently but the simultaneous effects of both stresses on crop performance and yield may be quite different than the individual stress, but there are limited studies on this topic (Pradhan et al., 2012).

Despite being a major food crop in Europe, West and Central Asia, and North Africa over the last 8000 years (Curtis, 2002; Pradhan, 2011), wheat is mostly cultivated under rainfed conditions. In the year 2000 for example, about 70% of the global wheat was produced under rainfed conditions and this made the crop vulnerable to droughts. Droughts led to considerable yield and income losses in major wheat producing regions of the world. About 50% to 70% of the area under wheat production in developed and developing countries is subjected to drought stress (Trethowa and Pfeiffer, 1999; Pradhan, 2011). For example, about 5.16 million hectares of the area under winter wheat is affected by drought stress in China (FAO, 2011b). In 2010, drought damaged at least 10.3 million hectares of cropland in Russia and wheat harvest declined by about 50 million MT (Pradhan, 2011).

Besides the impact of drought, high temperature is one of the main abiotic threats to cereal yield, especially during the reproductive stage of development and it is an increasingly serious threat to agriculture in many regions of the world. Heat stress created by elevated temperatures induces morphological, anatomical, physiological, biochemical, and genetic responses in plants which lead to considerable reductions in crop yield and quality (Zhou et al., 2017). Heat stress is also a primary constraint to wheat (*Triticum aestivum L.*,) production and profitability in many wheat-growing regions of the world (Feng et al., 2014). In developing countries, 7 million ha of wheat growing area is affected by continual high temperatures, whereas in temperate climate, terminal stress often affects about 36 million ha of wheat crop (Reynolds et al., 2001; Pradhan, 2011). Terminal stress refers to high temperature after anthesis, and continual stress is suffered when the mean daily temperature is more than 17.5°C in the coolest month of the season (Fischer, 1991; Pradhan, 2011).

Wheat is very susceptible to high temperatures at the flowering period, and high temperature during this period is deemed a principal yield reducing factor in the European wheat region (Semenov and Shewry, 2010). Based on previous studies for wheat, a threshold temperature of 31 °C was considered for grain number reduction in the period around anthesis but other studies assumed 30 °C or 27 °C as heat stress threshold temperature in wheat (Eyshi Rezaei et al., 2018).High temperatures hinder growth during the heading and grain filling period (Abdalla et al., 2010; Pradhan, 2011). Based on reports from Kansas, USA, high temperature at grain filling period is found to reduce wheat quality and quantity (Paulsen, 1997; Lott et al., 2011; Pradhan, 2011), while in other areas, increasing frequency of high temperatures have been associated with significant reduction in wheat yield (up to 70%) (Fokar et al., 1998; Gibson and Paulsen, 1999; Prasad et al., 2008b). Additionally, drought can have effect on wheat growth during all phenological stages, the reproductive and grain-filling phases are the most sensitive. For example, post-anthesis mild drought decreased the wheat yields by 1–30% while prolonged mild drought at flowering and grain filling decreased the grain yields by 58–92% (Farooqet al., 2014). In other studies, significant yield losses for wheat have been associated with the joint occurrence of drought and heat stress (Mittler, 2006; Lott et al., 2011). The combined effects of drought and high temperature on physiology, growth, water relations, and yield are reported to be significantly higher than the individual effects (Nicolas et al., 1984; Machado and Paulsen, 2001; Shah and Paulsen, 2003; Sharma and Kaur, 2009; Grigorova et al., 2011; Prasad et al., 2011b).

On the other side, a few studies that reported the combined effects of high temperature and drought suggested that a combination of drought and heat stress had either synergistic (combination of stresses being more severe than either stress alone or added), antagonistic (combination of stresses being less severe than either stress alone or added) or hypo-additive (the effect of combined stress is higher than the individual effects but lower than their sum) effects on grain filling, growth and yield traits (Mahrookashani al., 2017). Also, previous research indicated that the impact of heat and drought on crop growth and yield varied between wheat cultivars but variability in terms of high temperature and/or drought stress effects on wheat grain number and size appears to be related to genotypic differences in heat and drought tolerance need to be more studied.

1.2 Description of heat and drought stress

According to Taiz and Zeiger (2006) "stress is usually defined as an external factor that exerts a disadvantageous influence on a plant and is measured in relation to plant survival, crop yield, growth (biomass accumulation), or the primary assimilation processes, which are related to overall growth" (Pradhan, 2011, p 9). Drought has been defined based on classification by the National Drought Mitigation Center at the University of Nebraska, Lincoln, USA into four categories, namely, meteorological drought, agricultural drought, hydrological drought, and socio-economic drought. In this dissertation, we emphasize on agricultural drought, which is assumed to have occurred when the volume of water in the soil is inadequate for normal growth and development of crops (NDMC, 2006; Pradhan, 2011). According to Wahid (2007), "high temperature stress may be defined as the increase in air temperature well above a threshold level for a period of time sufficient to cause irreversible damage to plant organ, growth and/or development" (Pradhan, 2011, p 10).

1.3 Effect of heat and drought stress on wheat

1.3.1 Impact of drought stress on wheat

Carbon fixation is affected by drought stress through stomatal closure, which reduces CO_2 influx into mesophyll cells (Farooq et al., 2014). Drought stress causes a substantial reduction in the rate of net photosynthesis (P_N) due to stomatal closure, which limits the diffusion of CO_2 into the leaf and/or due to non-stomatal factors, such as inhibition of Rubisco or ATP synthesis. Under drought stress, the disturbance of photosynthesis at the molecular level is associated with low electron transport through photosystem II (PSII) and/or with structural injury to photosystem II and the light-harvesting complexes (Guan et al., 2015). Drought stress around anthesis decreases the floret set of grains. This reduction causes a decrease in the water content within the shoot and increases abscisic acid, leading to a reduction in the quantity of grains produced (Foulkes et al., 2007; Rajala et al., 2009; Westgate et al., 1996; Eyshi Rezaei, 2016). Drought stress affects leaf initiation and expansion, as these processes are severely disturbed due to soil water deficit. Consequently, total leaf area reduces drastically (Farooq et al., 2017). Chlorosis, leading to a reduction in photosynthesis, is the primary sign of leaf senescence (Farooq et al., 2014). Drought stress accelerates senescence by enhancing chlorophyll degradation, nitrogen loss, and lipid peroxidation (Yang et al., 2001). Liu et al., (2006) reported significant increase in electrolyte leakage and decline in chlorophylls (Chl) a and b in wheat cultivars subjected to water stress. In addition, drought stress during grain filling accelerates leaf senescence and therefore decreases single grain weight (Plaut et al., 2004; Rajala et al., 2009). In other studies, drought is reported to reduce photosynthesis in wheat and *Aegilops* species (Shah and Paulsen, 2003; Liu et al., 2006). Drought stress decreases photosynthesis by lowering stomatal and mesophyll conductance, or by oxidative damage of the chloroplast (Zhou et al., 2007; Prahdan, 2011).

Exposure of wheat to drought in the early stage of growth leads to a decrease in germination rate and crop establishment (Prahdan, 2011). Drought has harmful effects in any stage of wheat development (Rovo, 2004). During early stage of growth, drought decreases germination rate and reduces crop establishment. During vegetation phase, drought adversely affects stomatal conductance by hindering leaf expansion and detrimenting leaf area (Shah and Paulsen, 2003). This may intervene with the primary events with in the photosynthetic process. In addition, drought stress harmfully affects tillering (Blum et al., 1990). In summary, during vegetation phase drought stress reduces the relative water content, leaf area, and biomass production (Dulai et al., 2006; Liu et al., 2006). During meiosis and anthesis phase, drought, even if it happens in a brief episode, disrupts succeeding microspore genesis, resulting in pollen sterility, which can decrease grain set by 40-50% (Farooq et al., 2014). Also, during early stages of reproductive development, i.e., meiosis in pollen mother cells, drought affects pollen sterility, leading to lower grain numbers (Saini and Aspinall, 1981; Ji et al., 2010). During the reproductive phase of the wheat growth drought stress causes abortion of kernels, by reducing the supply of carbohydrates. In this stage, drought also reduces the number of endosperm cells and amyloplasts in the grain (Saini and Westgate, 1999; Shah and Paulsen, 2003).

During grain filling period drought can cause source limitation (Farooq et al., 2014). In this phase, drought stress reduces nitrogen uptake causing an acceleration in the rate of leaf senescence. This leads to source activity limitation and it assimilates supply to the grains. Thus, it leads to grain yield reduction (Liu et al., 2017). During post anthesis, drought reduces single grain weight which it ends up in grain yield reduction as well (Ahmadi and Baker, 2001; Ji et al., 2010). It is worth to mention that drought in the grain filling period has no effect on the grain number in wheat and a minimum effect on the rate of grain filling. However, it has been reported that terminal drought could shorten the duration of grain filling, thereby reducing the size of individual grains in wheat (Farooq et al., 2014). However, it has however been reported that terminal drought could shorten the duration of grain filling, thereby reducing the size of individual grains in wheat (Farooq et al., 2014).

Responses to drought stress are extremely different according to the genetic background. Previous research also indicated that the impact of heat stress on crop growth and yield varied between wheat cultivars. Wheat cultivars which are tolerant to high temperatures have been specified by the maintenance of photosynthesis, chlorophyll content and an extended grain-filling duration (Al-Khatib and Paulsen 1984, Wardlaw et al. 1989, Tahir and Nakata 2005, Feng et al., 2014). For this reason, it is necessary to investigate that the existing variability currently available in gene pools must be properly characterized and understood at physiological, morphological and genetic levels of wheat genotypes to drought stress.

1.3.2 Impact of high temperature on wheat

Heat stress influences photosynthesis and related processes through disruptions in the structure and function of the chloroplasts and reduction of chlorophyll content in wheat leaves. Viable leaf area also reduces under high temperature conditions. These changes lead to a significant reduction in carbon assimilation capacity in wheat plants (Liu et al., 2017). Also, photosynthesis is the most sensitive physiological process to elevated temperature and any reduction in photosynthesis affects growth and grain yield of wheat. Heat stress reduces photosynthesis through disruptions in the structure and function of the chloroplasts, and reduction in chlorophyll content. The inactivation of chloroplast enzymes, mainly induced by oxidative stress, may also reduce the rate of leaf photosynthesis (Farooq et al., 2011).

Photosystem II of the photosynthetic apparatus is particularly sensitive, and injury to some components is irreversible (Shah and Paulsen, 2003). High temperature causes electrolytic leakage of thylakoid membrane, thus, resulting in significant reduction in photosynthetic rate in wheat genotypes from major areas of the world (Al-Khatib and Paulsen, 1990). High temperature leads to damage to thylakoid membranes, which causes chlorophyll loss and decreases efficiency of photosystem II (PSII) and photosynthesis. At the cellular level, high temperature leads to decreased antioxidant activity and causes increased production of reactive oxygen species (ROS) and oxidative damages such as lipid peroxidation and membrane damage (Narayanan et al., 2015). Parthenocarpy, abortion, and shrinking of kernels happen soon after anthesis. Starch synthesis in the kernel endosperm is ceased by the inhibition of several enzymes, resulting in chalky, opaque kernels (Shah and Paulsen, 2003). Final grain weight is determined by both photosynthesis during grain filling and the water-soluble carbohydrates (WSC) stored previously in the stem.

Wheat is a sensitive crop to unusually high temperatures at almost every developing stage. A higher temperature usually accelerates phenological development causing to have shorter durations of growth phases such as grain filling (Vignjevic et al., 2015) but the growth rate reduces when stress intensity and duration become higher (Shpiler and Blum, 1986; Wollenweber et al., 2003). High temperature reduces time to flowering, grain set, and physiological maturity specially during spring when wheat is growing at high nighttime temperature (Prasad et al., 2008b).

If heat stress happens during the late vegetative development phase (double ridge stage to anthesis), then spikelet formation can be interfered. In addition, it induces ovule and pollen sterility causing a reduction in grain number per spike at meiosis (Saini and Aspinall, 1982; Prasad et al., 2006b, 2008a, b). At anthesis, high temperature decreases the number of grains by adversely affecting ovary development, pollen germination and pollen tube growth (Pradhan et al., 2012). High temperatures during flowering lead to a decrease in the number of grains that are formed, thus reducing yield potential.

Heat stress during reproductive development reduces photosynthesis and promotes premature senescence. The most sensitive stage to heat stress in wheat is 8 to 6 d before anthesis and anthesis stages (Prasad et al., 2014). Consequently, heat stress decreases yield by inducing pollen sterility and seed abortion, and subsequently lowers seed weight, grain yield and dough quality. Wheat varieties with good tolerance to high temperatures have been noticed and are defined by the maintenance of photosynthesis, chlorophyll content and an extended grain-filling duration even at elevated temperatures (Hays et al., 2007; Feng et al., 2014). High temperature also causes low grain to set in several other crop species due to low pollen production and viability. High day- and night-time temperature at anthesis has a negative effect on pollen production and reception, and lead to increased floret sterility in rice (Prasad et al., 2006*b*; Mohammed and Tarpley, 2009; Pradhan et al., 2011). In sorghum and maize, high temperature reduces pollen production and viability, and pollen longevity and shedding, and consequently decreases grain set (Prasad et al., 2006a; Prasad et al., 2011a).

High temperature stress during grain filling accelerates leaf senescence, thus reducing availability of current assimilates to growing grain. It also inhibits starch synthesis and deposition, which eventually lead to a decline in single grain weight (grain size). In addition, high temperature at this stage also reduces grain filling duration, which outweighs the increase in grain filling rate (Prasad et al., 2006a; Prasad et al., 2006b; Prasad et al., 2011b). Reduction in grain weight under high temperature at GS3 (third growing season) has been well documented (Gibson and Paulsen, 1999). The combination of heat and drought during grain filling increases water use efficiency of wheat but leads to a decrease in grain yield. Yield decreases through shortening of the grain filling period due to heat stress, with drought stress however playing a limited role (Wardlaw, 2002).

Several studies have investigated that the reasons of reduction in seed numbers under heat and drought stress could be linked to decreased functionality and structural abnormalities of pollen and/or pistil. On the other hand, the rate of photosynthesis has significant effect on pollen tube growth in wheat, implying that photosynthetic rate during anthesis is critical in maintaining reproductive success (Prasad et al., 2014).

High temperature and drought stress at grain filling period decreased grain yield due to lower the single grain weight. Single grain weight in wheat depends on current assimilates production through photosynthesis and/or remobilization of stored assimilates from vegetative tissues to developing reproductive tissues (grain). Additionally, the reason of reduction in grain yield under heat and drought stress during grain filling stage could be due to accelerated development, and/or leaf senescence associated with decreased photosynthetic rate (Prasad et al., 2014).

As previous research has explained that wheat experiences heat and drought stress to varying degrees at different phenological stages, but the effect of high temperature and drought stress during the pre-anthesis or anthesis stage on grain filling period is necessary to more investigate and find the link between drought stress before anthesis and duration of grain filling.

Also, due to use crop models to analyze climate change effects especially heat and drought stress, the scientist have to calibrate it with experimental data showing a high heat sensitivity or lower sensitivity of wheat to heat stress (Ewert et al., 2002; Eyshi Rezaei et al., 2015). To reduce corresponding in model results, it is therefore fundamental to understand better the potential source of this inconsistency indifferent heat stress studies. The sources of uncertainties in heat stress at anthesis may be explained by varying experimental setup such as the temperature measurement point (ear, leaf, canopy or ambient air), method of heating, soil texture and pot size which have not been investigated systematically before (Eyshi Rezaei et al., 2018). Hence, there is a need for further research to gain an understanding the uncertainties of heat stress experiments setup during the anthesis stage that exacerbate negative effects on grain numbers, single grain weight and grain yield.

1.4 Interactions between heat and drought stress

In multiple-stress environments, crop performance in terms of development, growth and yield depends on the plants' ability to resist, tolerate, or recover from environmental stressors. Heat and drought stress impact source-sink relationships by decreasing the rate of carbon (C) assimilation and respiration, as well as the partitioning and redistribution of carbon and nitrogen (N) within plants. This altered availability of C and N affects starch and protein metabolism in leaves, which consequently affects grain yield and quality (Vignievic et al., 2015). The combination of drought and heat stress alters physiological and molecular processes such as photosynthesis, accumulation of lipids, and transcript expression, but the impacts on reproductive traits and yield processes are not well understood (Prasad et al., 2011b). Heat and drought stress often occur simultaneously, but they can have different effects on physiological, developmental, growth and yield processes. The effects of these two stresses on crops have been studied independently but responses of plants to a combination of heat and drought stress are unique and cannot be directly extrapolated from the response of plants to each of these different stresses applied solely (Rizhsky et al., 2004; Prasad et al., 2011b).

There is a strong relationship between the plant water status and temperature, thus making it very difficult to separate the contributions of heat and drought stress at the cultivated wheat regions (Prasad et al., 2008b). Thus, it is necessary to understanding of combined effects of heat and drought on physiological traits, yield and its attributes is of special significance for wheat breeding program in order to improve productivity and to predict the consequences of climate change on wheat production.

1.5 Objectives and research questions

In response to the need for new detail into the negative effects of heat and drought stressors on wheat, the general objective of this study is to improve the understanding of the response of wheat genotypes to drought stress in interaction with high temperatures. For this purpose, the three key questions of this thesis are:

Question 1 (Q1): *How does drought stress in interaction with high temperature before anthesis affect the physiological, developmental, growth and yield processes?*

Question 2 (Q2): *Do changes in experimental setup influence the wheat response to heat stress and combined heat and drought stress?*

Question 3 (Q3): *Does heat and drought stress before anthesis affect the rate and duration of grain filling among different cultivars?*

1.5.1 Specific objectives

- Chapter II: (a) Investigate the effects of combined heat and drought stress compared with the exposure of crops to either heat or drought stress, and (b) investigate whether the response differed among the cultivars originating from regions with very different climatic conditions (hot arid climate in Iran, humid temperate climate in Germany).
- Chapter III: (a) To quantify the response of wheat to heat stress and combined heat and drought stress around anthesis using experimental data from different sites, and (b) test whether differences in the sensitivity of wheat yields were associated with differences in temperature measurement, heating method and soil substrate used in the experiments.
- Chapter IV: (a) Evaluate the effects of heat and drought stress before anthesis of wheat on post-anthesis processes such as grain filling rate and duration, (b) test the impact on yield and yield components, and (c) test whether effects can be generalized across

cultivars using a German winter wheat cultivar (Batis), a German spring wheat cultivar (Scirocco), and a spring wheat cultivar from Iran (Kohdasht).

Chapter 2

Independent and combined effects of high temperature and drought stress around anthesis on wheat

Based on:

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Abstract

High temperature and drought stress are projected to reduce crop yields and threaten food security. While effects of heat and drought on crop growth and yield have been studied separately, little is known about the combined effect of these stressors. We studied detrimental effects of high temperature, drought stress and combined heat and drought stress around anthesis on yield and its components for three wheat cultivars originating from Germany and Iran. We found that effects of combined heat and drought on the studied physiological and yield traits were considerably stronger than those of the individual stress factors alone, but the magnitude of the effects varied for specific growth and yield related traits. Single grain weight was reduced under drought stress by 13-27 % and under combined heat and drought stress by 43-83 % but not by heat stress alone. Heat stress significantly decreased grain number by 14-28 %, grain yield by 16-25 % and straw yield by 15-25 %. Cultivar responses were similar for heat but different for drought and combined heat and drought treatments. We conclude that heat stress as imposed in this study is less detrimental than the effects of those other studied stresses on growth and yield traits.

2.1. Introduction

Increased mean temperature and climate variability and more frequent drought events are expected to cause considerable negative effects on crop productivity in many regions threatening food security (Porter and Semenov 2005; Lobell et al. 2013). Wheat (*Triticum* spp.) is one of the most important food crops in the world (FAOSTAT 2010), but wheat yields are notably sensitive to climatic and environmental variations (Porter and Semenov 2005, Asseng et al., 2015). Recent studies have reported about effects of heat events for major global wheat producing regions (Asseng et al. 2011, 2015, Teixeira et al. 2013).

The majority of wheat growing areas in the world experience environmental stresses including drought stress and heat stress that have detrimental effects on yield (Semenov and Shewry 2011, Pradhan et al. 2012). Drought stress reduces expansion of leaves and stomatal conductance and may finally affect primary events in the photosynthetic process (Shah and Paulsen 2003). Drought stress during the reproductive phase causes abortion of kernels, likely by reducing the supply of carbohydrates, and reduces the number of endosperm cells and amyloplasts in the grain (Saini and Westgate 1999, Shah and Paulsen 2003). High temperature influences on photosynthesis and related processes in wheat in several ways. Viable leaf area and chlorophyll content reduce considerably. Photosystem II of the photosynthetic apparatus is particularly sensitive, and injury to some components is irreversible (Shah and Paulsen 2003).

Parthenocarpy, abortion, and shrinking of kernels happen soon after anthesis. Starch synthesis in the kernel endosperm is ceased by inhibition of several enzymes, resulting in chalky, opaque kernels (Shah and Paulsen 2003). Heat stress in the late vegetative development phase (double ridge state to anthesis) adversely affects spikelet formation while at meiosis grain number per spike are reduced by inducing ovule and pollen sterility, and anther indehiscence (Saini and Aspinall 1982, Prasad et al. 2006*b*, 2008*a*, 2008*b*). At anthesis, high temperature decreases the number of grains by adversely affecting ovary development, pollen germination and pollen tube growth (Pradhan et al. 2012).

In multiple-stress environments, crop performance in terms of development, growth and yield depends on the plants' ability to resist, tolerate or recover. Heat and drought stress impact source-sink relationships by decreasing the rates of carbon (C) assimilation and respiration, as well as partitioning and redistribution of carbon and nitrogen (N) within the plant. This altered availability of C and N affects starch and protein metabolism in leaves, finally resulting in distinct grain yield and quality (Vignjevic et al. 2015). The combination of drought and high temperature stress was found to alter physiological and molecular processes such as photosynthesis, accumulation of lipids, and transcript expression, but the impacts on reproductive traits and yield processes are not well understood (Prasad et al. 2011). Heat and drought stress often occur simultaneously, but they can have different effects on physiological, developmental, growth and yield processes. The effects of these two stresses on crops have been studied independently but responses of plants to a combination of heat and drought stress are unique and cannot be directly extrapolated from the response of plants to each of these different stresses applied solely (Rizhsky et al. 2004, Prasad et al. 2011).

A few studies that examined the combined effects of high temperature and drought suggested that a combination of drought and heat stress had either synergistic (combination of stresses being more severe than either stress alone or added), antagonistic (combination of stresses being less severe than either stress alone or added) or hypo-additive (the effect of combined stress is higher than the individual effects but lower than their sum) effects on grain filling, growth and yield traits (Nicolas et al. 1984, Wardlaw 2002,Shah and Paulsen 2003,Prasad et al. 2011, Pradhan et al. 2012). Davidson and Birch (1978) for example, found that an increase in day/night temperature from 18/13 °C to 24/19 °C improved post-anthesis water use efficiency (g grain produced per g water used), although grain yield was reduced (Wardlaw 2002). This effect of temperature was evident under both, mild and severe drought conditions. Therefore, the authors concluded that there was no water × temperature interaction in relation to grain yield per plant. Based on experiments in which high temperature (28/20 °C) following anthesis of wheat was imposed during drought, Nicolas et al. (1984) concluded that the effects of drought and heat on grain yield were additive. In contrast, Zhang et al. (2010) found that a combination of drought and heat stress had a significantly greater detrimental effect on the growth and productivity of crops compared with a stress applied alone. During heat stress, plants open their stomata to cool their leaves by transpiration, but if heat stress is combined with drought and plants have to keep their stomata closed to reduce water loss, the leaf temperature remains high. Such combined stresses often have the largest negative effects on grain yield (Zhang et al. 2010).

Previous research also indicated that the impact of heat and drought on crop growth and yield varied between wheat cultivars. Wheat cultivars which are tolerant to high temperatures have been specified by the maintenance of photosynthesis, chlorophyll content and an extended grain-filling duration even at high temperatures (Al-Khatib and Paulsen 1984, Wardlaw et al. 1989, Tahir and Nakata 2005, Feng et al. 2014). However, little is known about the response of different cultivars to heat and drought interaction.

In this study we investigate the independent and combined effects of heat and drought stress imposed around anthesis on spikelet fertility, grain numbers, single grain weight, dry matter production, grain yield and harvest index of three different wheat cultivars originating from Iran and Germany. Objectives were i) to investigate the effects of combined heat and drought stress in comparison to exposure of the crops to individual stresses of heat or drought and ii) to investigate whether the response differed among the cultivars originating from regions with very different climatic conditions (hot arid climate in Iran, humid temperate climate in Germany).

2.2. Materials and methods

2.2.1. Experimental set-up

The experiment was conducted at the University of Bonn, Germany, (50.7265 °N, 7.0873 °E, 57 m a.s.l.) during the growing season 2013/2014. The site is characterized by a temperate climate with a mean annual precipitation sum of 687 mm and an annual mean temperature of 10.7 °C (long-term mean 1981-2010). Plants were grown under ambient conditions until the beginning of anthesis. For the heat treatment the plants were moved into a glasshouse (pot set 1) or into a growth chamber (pot set 2).

The experiment was laid out in a factorial design according to a complete randomized design (CRD), with four replications and consisted of four treatments: control (C), heat (H), drought

(D) and heat and drought combined (H+D). The complete set of pots was duplicated to allow parallel heat treatments in growth chambers and in a neighboring glasshouse to test for the effect of different humidity and radiation levels. One winter wheat cultivar (Batis) and two spring wheat cultivars (Kohdasht and Scirocco) were grown in this study whereas Batis and Scirocco originated from Germany and Kohdasht from Iran. Sowing dates were 29.10.2013 for Batis (winter wheat) and 20.03.2014 for Kohdasht and Scirocco (spring wheat) respectively. The plants were sown by hand in plastic pots of the size 22 cm × 22 cm × 26 cm in two rows, each of them containing 6 plants. The space between rows was 9 cm wide. We selected relatively small pot sizes in order to reduce the length of the period required to establish the drought stress. With bigger pot size we would have to expand the drought initialization period considerably into the vegetative phase with the problem that periods of drought and heat stress would occur in different development phases.

The soil substrate was a mixture of topsoil, silica sand, milled lava and peat dust (Terrasoil®, Cordel &Sohn, Salm, Germany) consisting of 85 % sand, 12 % silt and 3 % clay with a volumetric water content of 18 % at field capacity and of 4 % at the permanent wilting point, respectively.

Sufficient nutrient supply was ensured by adding water soluble fertilizers such as calcium ammonium nitrate (CAN) to the irrigation water three times after germination and additionally before the drought treatment started. The first fertilizer application direct after germination consisted of 3.6 g of Entec (14 % N, 7 % P_2O_5, 17 % K_2O, 2 % MgO, 9 % S, 0.02 % B and 0.01 % Zn) per pot, while the second and third application consisted of 1.86 g CAN (27 % N) per pot.

A soil water content close to field capacity was maintained by watering the pots three times a day with 300 ml of water per pot by using a drip irrigation system (Netafilm, Adelaide, Australia). Echo2 sensors (Decagon Dev., Pullman, WA, USA) were used to determine the volumetric moisture content (VMC) digitally with the frequency domain technique.

2.2.2. Data recording

A meteorological weather station was installed at the experimental site to measure air temperature, relative humidity, and solar radiation inside the glasshouse, in the growth chambers and under ambient conditions in15-minute intervals (Figure 2.1). Starting at two weeks after germination the leaf area (LA), plant height and aboveground dry matter (ADM) were measured and the phenological development stage was determined in two days interval (Tables 2.1, 2.2). ADM and LA were measured destructively by harvesting the plants from five extra pots treated similar to the control variant. The LA was measured using a LI-3100 (Lincoln, LI-COR, NE, USA) while ADM was recorded after the plant material had been oven dried at 65 °C for 48 hours. The phenological development stage was determined based on the BBCH scale (Meier 2001). At maturity, fertile spikelet number, grain number per main stem, single grain weight, ADM, straw yield (straw + chaff) and grain yield were measured after manually harvesting the plants. Spikelet fertility was checked by pressing the floret between the thumb and the index finger. Spikelet fertility (seed-set) percentage was determined as the ratio of spikelet with grain to the total number of spikelets.

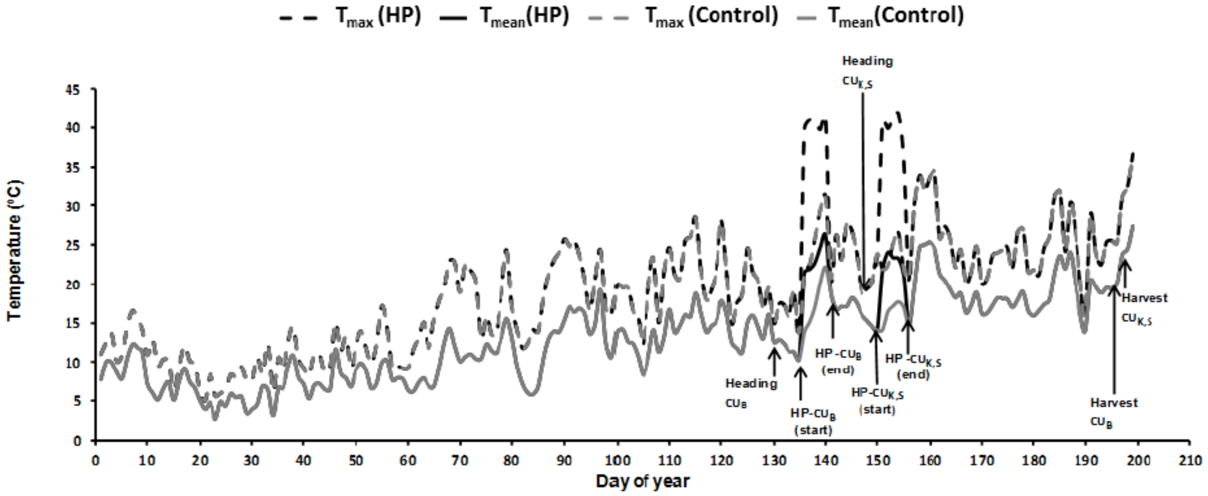

Figure 2.1. Daily mean (T_{mean}) and maximum (T_{max}) temperatures at the experimental site for pot set 2 from 1st of January (day 1) to 10th of July 2014 (day 191). During the heating period (HP) the H and H+D treatments were exposed to higher temperatures (Cultivar Batis: CU_B; Cultivars Kohdasht and Scirocco: $CU_{K,S}$) and T_{max} and T_{mean} refer to climate chamber control.

Table 2.1. Phenological development (BBCH stage), day after planting, thermal time (above a base temperature of 0 °C), leaf area (per plant), aboveground dry matter (per plant) and plant height for the control and the cultivars Batis (CU_B), Kohdasht (CU_K) and Scirocco (CU_S) for different measurements dates across the period between sowing and begin of the heat stress treatment. Data represent means across all pots belonging to the cultivar and for the control (BBCH, plant height) or were destructively measured for sample plants (leaf area, aboveground dry matter).

Date	BBCH			Day after planting			Thermal time (°C day)			Leaf area (cm^2)			Aboveground dry matter (g)			Plant height (cm)		
	CU_B	CU_K	CU_S	CU_B	CU_K	CU_S	CU_B	CU_K	CU_S	CU_B	CU_K	CU_S	CU_B	CU_K	CU_S	CU_B	CU_K	CU_S
07.01.2014	15	-	-	70	-	-	692	-	-	-	-	-	-	-	-	16	-	-
28.01.2014	21	-	-	91	-	-	834	-	-	-	-	-	-	-	-	17	-	-
11.02.2014	22	-	-	105	-	-	906	-	-	-	-	-	-	-	-	17	-	-
11.03.2014	23	-	-	133	-	-	998	-	-	29.7	-	-	0.30	-	-	17	-	-
24.03.2014	25	-	-	146	-	-	1148	-	-	-	-	-	-	-	-	21	-	-
08.04.2014	26	-	-	161	-	-	1366	-	-	181.5	-	-	1.35	-	-	25	-	-
15.04.2014	-	16	14	-	26	25	-	389	389	-	7.6	10.3	-	0.04	0.05	-	16	17
23.04.2014	34	-	-	176	-	-	1650	-	-	330.2	-	-	3.10	-	-	56	-	-
01.05.2014	41	24	24	184	42	42	1835	733	733	325.0	68.5	91.1	3.20	0.35	0.47	71	36	38
17.05.2014	56	41	41	200	58	58	2047	945	945	524.2	215.5	236.7	6.36	1.38	1.69	72	56	58
31.05.2014	-	56	57	-	72	72	-	1240	1240	-	-	-	-	-	-	-	-	-
11.06.2014	75	69	69	225	83	83	2276	1442	1442	427.5	333.7	320.9	8.36	6.18	5.00	98	88	88
30.06.2014	85	75	75	244	102	102	2633	1799	1799	232.0	269.0	299.0	9.07	7.20	6.40	98	88	88

The BBCH-scale is a scale used to identify the phenological development stages of a plant.

Table 2.2. Phenological development (BBCH stage), day after planting (DAP), thermal time (TT) (above a base temperature of 0 °C), and plant height (HT) for different measurement dates in the period between end of flowering (end of the heat treatment) and maturity of the winter wheat *cv.* Batis and the spring wheat *cv.* Kohdasht and Scirocco (Plant height did not change after 11.06.2014). Data represent means across all pots belonging to the cultivar and treatment (C: control, H: heat, D: drought, H+D: combined heat and drought).

Date		Batis				Kohdasht				Scirocco			
		C	H	D	H+D	C	H	D	H+D	C	H	D	H+D
11.06.2014	BBCH	75	77	83	85	69	71	75	77	69	71	75	77
	DAP	225	225	225	225	83	83	83	83	83	83	83	83
	TT	2276	2329	2276	2329	1442	1465	1442	1465	1442	1465	1442	1465
	HT	98	98	87	84	88	88	79	77	88	88	79	77
20.06.2014	BBCH	77	87	92	97	73	77	89	92	73	77	89	92
	DAP	234	234	234	234	92	92	92	92	92	92	92	92
	TT	2444	2497	2444	2497	1610	1637	1610	1637	1610	1637	1610	1637
30.06.2014	BBCH	85	92	-	-	75	83	92	-	75	83	92	-
	DAP	244	244	244	244	102	102	102	102	102	102	102	102
	TT	2633	2686	2633	2686	1809	1826	1799	1826	1799	1816	1767	1803
07.07.2014	BBCH	92	-	-	-	92	97	-	-	92	97	-	-
	DAP	251	251	251	251	109	109	109	109	109	109	109	109
	TT	2927	2980	2927	2980	2093	2146	2093	2146	2093	2146	2093	2146

2.2.3. Stress treatments

Ten days before the expected beginning of flowering, the sprinklers were withdrawn from the D and the H+D treatments to initiate the drying out of the soil in these treatments. When soil moisture declined to 40 % of the total available water capacity (TAWC) of the soil, the pots were watered manually with 200 ml of water to avoid permanent wilting (Figure 2.2). For the first time, irrigation was applied nine days after drought imposing and repeated, if required, on subsequent days to maintain soil moisture content between 20 % and 40 % of the total available water capacity (Figure 2.2). When end of heading was observed, the H and H+D pots of the first pot set were put inside the glasshouse in which the doors were closed to increase the temperature. Every evening, the temperature sum above a threshold of 31°C stress thermal time (STT) was calculated based on the temperature loggings inside the glasshouse. The pots of the second pot set were moved into growth chambers. The temperature curve for the growth chamber containing the H and H+D pots was adjusted every day to ensure that the STT in the heat climate chamber was like the STT observed in the glasshouse on the day before (Figure S2.2) while the temperature curve in the growth chamber containing the C and D pots (normal chamber) was adjusted to mimic the temperature curve observed for the C and D pots under ambient condition (pot set 1) one day before (Figure S2.2). When STT in the glasshouse accumulated to 12000 °C min, the pots of the H and the H+D treatment were placed outside the glasshouse to stop the heat treatment. Irrigation was applied to the corresponding D and H+D treatments immediately to increase the soil water content to field capacity and to stop the drought treatment. One day later, STT of the H and H+D treatment reached 12000 °C min for the pots in the climate chamber belonging to pot set 2 so that heat and drought stress was stopped for the second pot set as well.

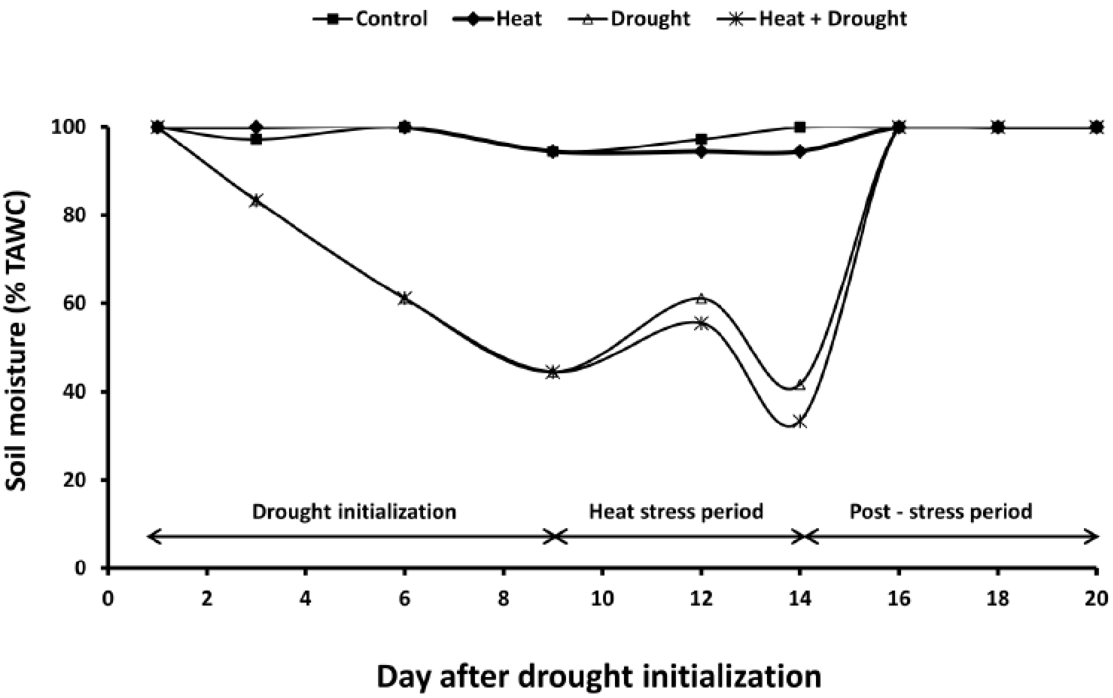

Figure 2.2. Soil moisture content (Total available water capacity (TAWC)) during the drought treatment, the heat treatment period and the post-stress period calculated as the mean of the pots planted with the three cultivars.

2.2.4. Statistical analysis

The significance of differences between treatments was analyzed using analysis of variance (ANOVA) techniques appropriate for factorial design according to a complete randomized design (CRD). The statistical analyses for physiological, growth and yield traits were conducted by using the GLM procedure in the software package SAS Enterprise 9.3 (SAS Institute 2008). Cultivar (CU), heat (H), drought (D) and combined heat and drought (H+D) were used as class variables. Differences among means were tested using the least significant difference (LSD) ($P < 0.05$). Associations between physiological, growth and yield traits were tested across cultivars and treatments with linear regression analysis by using the PROC REG procedure of SAS. The Tukey–Kramer adjustment was used to separate the treatment means across growth and yield traits.

2.3 Results

2.3.1. Effect of heat and drought on growth and yield traits

Drought stress caused an earlier senescence of leaves of the affected plants, while the leaves of the plants in the H treatment did not show any obvious difference from the control treatment for leaf senescence (Figure S2.3). The heat stress treatment and the corresponding increase in thermal time accelerated crop development and resulted in a shortening of the generative phase (flowering to maturity) by 5-7 days (Table 2.2). Like this, drought stress also accelerated the phenological development of the plants to a similar extent (Table 2.2). Pots treated with heat and drought showed an additional of the effects (Figure S2.3, Table 2.2).

The comparisons of straw yield, fertile spikelet number, grain number per main stem, single grain weight, grain yield and harvest index across treatments showed a general pattern with highest values in the control, low to moderate reduction in H, moderate to strong reduction in D and extreme low values in the H+D treatment (Figure 2.3). Differences between the C and D or the C and H+D treatments were highly significant for all growth and yield traits while differences between C and H were not significant for fertile spikelet number, single grain weight and harvest index (Table 2.3).

Compared to the control, straw yield was reduced, depending on the cultivar, by 15-25 % in the H treatment, 21-56 % in the D treatment and 26-60 % in the H+D treatment, respectively (Figure 2.3e). Fertile spikelet number was very similar for C and the H treatment but was reduced by 22-39 % in the D treatment and 80-95 % for the H+D treatment (Figure 2.3c). Grain number per main stem was reduced for all treatments by 14-28 % for the H treatment, 39-61 % for the D treatment and 91-98 % in the H+D treatment while single grain weight was less sensitive to the treatments with almost no reduction for H, a 13-27 % decline for D and a 43-83 % decline for H+D (Figures 2.3a,b). Consequently, grain yield declined by 16-25 %, 48-67 %, and 92-98 % in the H, D, and H+D treatments, respectively (Figure 2.3d). The harvest index showed little difference between C and H but was reduced by 21-30 % for D and 73-95 % for the H+D treatments (Figure 2.3f). Importantly, a combination of heat and drought stress at the levels applied in this experiment has had synergistic effects with regard to fertile spikelet number, grain number per main stem, single grain weight, grain yield and harvest index but antagonistic effects (Batis) or hypo-additive effects (Kohdasht, Scirocco) for straw yield (Figure 2.3).

Table 2.3. Test of the significance of differences in grain number, single grain weight, fertile spikelet number, grain yield, straw yield and harvest index caused by cultivars (CU), heat stress around anthesis (H), drought (D), combined heat and drought (H+D) as well as interactions between cultivars and the stressors performed by an analysis of variance. The crop specific measurements refer to the main stems only, results are shown for pot set 2 (pots moved to growth chambers for the heat treatment).

Source of variation	Grain number	Single grain weight (mg)	Fertile spikelet number	Grain yield (g)	Straw yield (g)	Harvest index (%)
CU	ns	ns	ns	ns	***	ns
H	***	ns	ns	***	***	ns
D	***	***	***	***	***	***
H+D	***	***	***	***	***	***
CU×H	ns	ns	ns	ns	*	ns
CU×D	*	ns	ns	**	*	ns
CU×(H+D)	***	*	***	***	ns	ns

(ns: non-significant. ***, **, * significant at $P \leq 0.001$, 0.01 and 0.05, respectively)

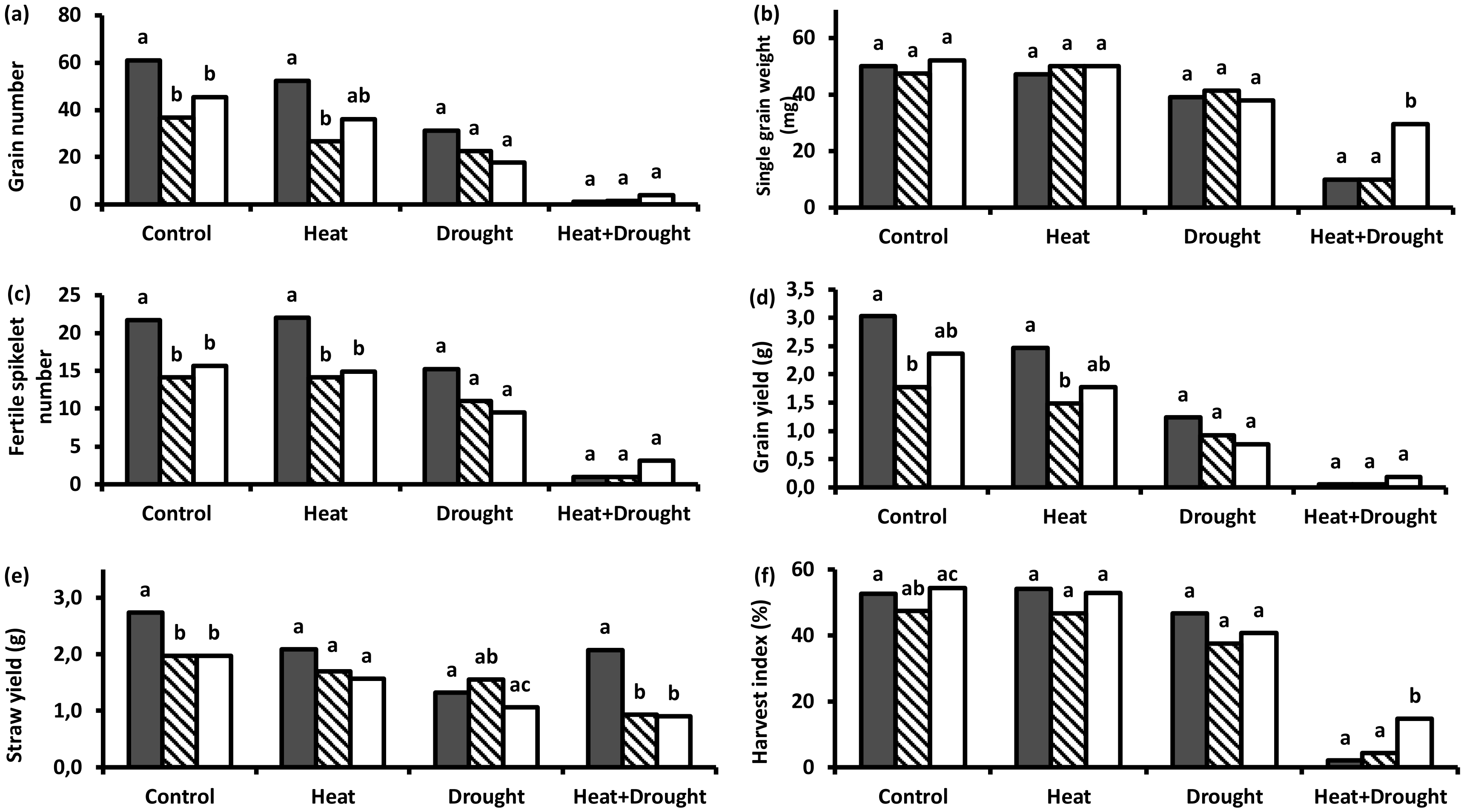

Figure 2.3. Effects of heat, drought, and combined heat and drought stress on grain number (a), single grain weight (b), fertile spikelet number (c), grain yield (d), straw yield (e) and harvest index (f) per main stem. Filled, dark downward diagonal and open columns indicate winter wheat (Batis) and two spring wheat cultivars (Kohdasht and Scirocco) respectively. Data belong to pot set 2 (heat treatment in growth chambers) and LSMEANS estimates with same letters within a cultivar are not significantly different at $P = 0.05$.

2.3.2. Sensitivity of cultivars to heat and drought

In the control pots, the cultivars showed significant differences with respect to all analyzed growth and yield traits, except for single grain weight. The highest values for grain number, fertile spikelet number, grain yield and straw yield were observed for winter wheat *cv*. Batis and lowest values for the Iranian spring wheat *cv*. Kohdasht. The harvest index and single grain weight were highest for the German spring wheat *cv*. Scirocco, but differences for single grain weight among the cultivars were not significant (Figure 2.3). Differences of the cultivars in their sensitivity to heat and drought were mainly found for the combined H+D treatment in which the spring wheat *cv*. Scirocco showed, compared to the other cultivars, less pronounced reductions in fertile spikelet number, grain number, single grain weight, grain yield and harvest index (Figures 2.3, S2.4, Table 2.3). The winter wheat *cv*. Batis showed smaller declines in straw yield than the other two cultivars in the H+D treatment (Figures 2.3, S2.4). In contrast, the response of the three cultivars to heat stress was similar but not for drought and combined heat and drought treatments (Figures 2.3, S2.4).

2.3.3. Relationships between growth and yield traits under heat and drought

Grain yield in the control and in all treatments was closely related to grain number while a significant relationship between grain yield and single grain weight was determined for the combined H+D treatment only (Figures 2.4c, d). Regressions for straw yield and grain yield were significant for the control and D treatments but not for the H and combined H+D treatments (Figure 2.4a). However, regression coefficients were lower for the regressions of straw yield and grain yield as compared to the regression coefficients for the regression of grain number and grain yield. ADM development showed a response to drought and heat already shortly after the heat treatment at anthesis with lowest ADM in the H+D treatment and smallest reduction in the H treatment. Regressions between ADM measured at anthesis, and grain yield were not significant for all treatments (Figure 2.4b). Regressions of straw yield at harvest and grain number were significant for the control and the D treatment while regressions between straw yield and single grain weight were not significant (Figures 2.4e, f). The plot of single grain weight on grain number showed a logarithmic alignment of the data points with lowest values for grain number in the H+D treatment. The logarithmic curve was close to linear for low grain numbers; consequently, the linear regression between grain number and single grain weight was significant for the H+D treatment (Figure 2.5a). Interestingly, there is a small but not significant negative trend of single grain weight for increasing grain numbers in the H treatment indicating a small compensation effect, while no relationship between grain number and single grain weight was found for the control and the D treatment (Figure 2.5a). Trends and significance levels for the relationship between single grain weight and fertile spikelet number and grain yield were similar to those described before for the relationship between single grain weight and grain number (Figures 2.4, 2.5) which is not surprising because fertile spikelet number and grain number, fertile spikelet number and grain yield and grain number and grain yield were highly correlated for the control and all treatments (Figures 2.4, 2.5). In contrast, trends between straw yield and single grain weight were not significant for the control and all treatments (Figure 2.4f). The relationships between yield and growth traits described before were very similar across cultivars when the observations for the different treatments were pooled for the three cultivars (Figures S2.5- S2.7).

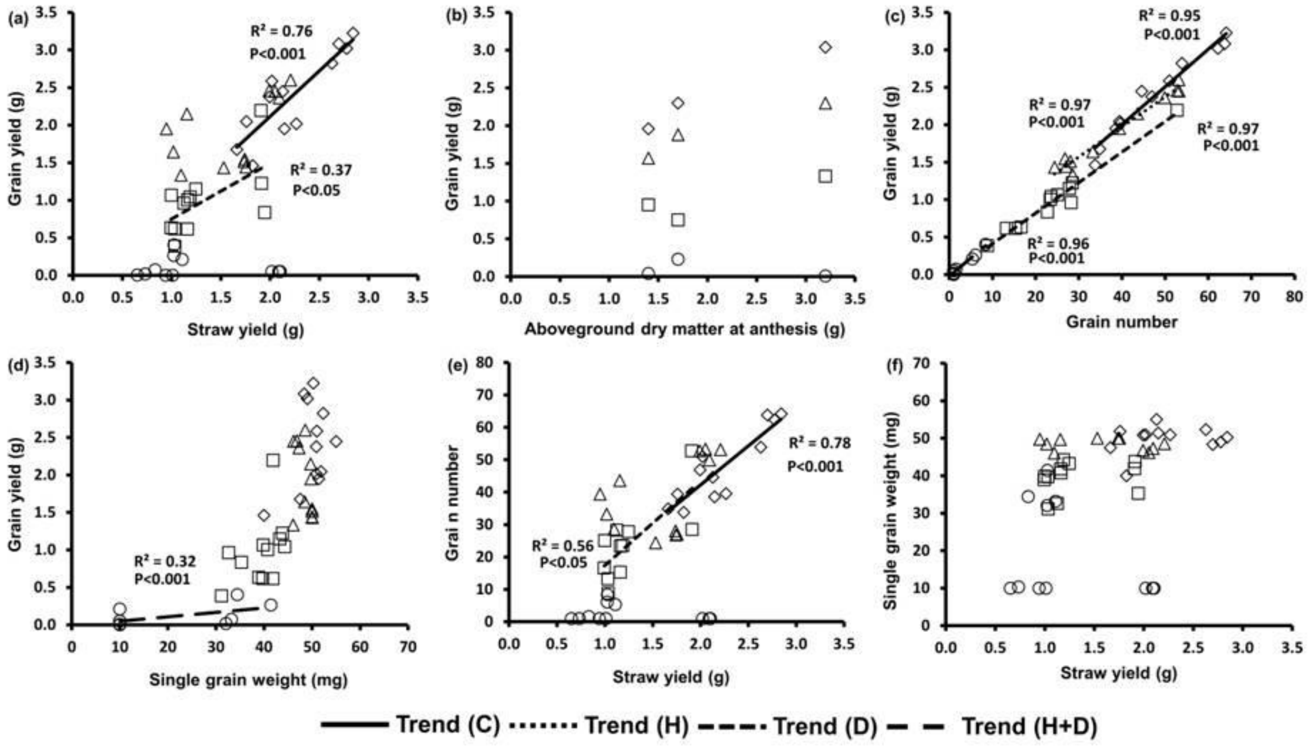

Figure 2.4. Grain yield in response to straw yield (a), aboveground dry matter at anthesis (b), grain number (c) and single grain weight (d) and straw yield plotted against grain number (e) and single grain weight per main stem (f). Diamond, triangle, square and circle indicate control, heat, drought and combined heat and drought treatments, respectively. Data are shown for pot set 2 (heat treatment in growth chambers), linear trends are shown for relationships when are significant p<0.05.

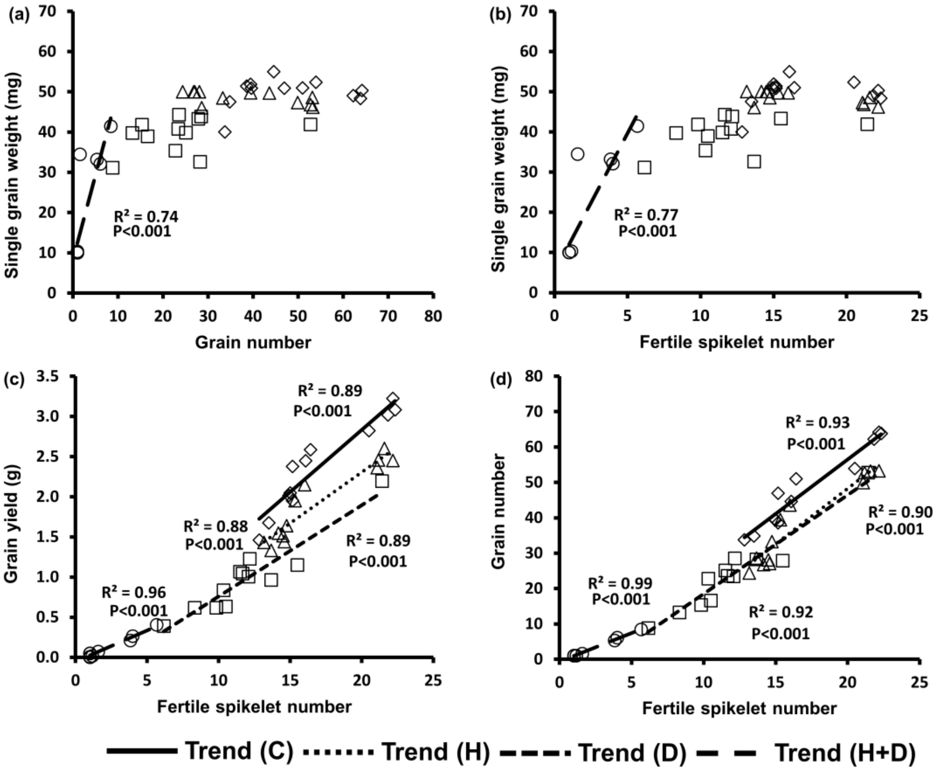

Figure 2.5. Single grain weight in response to grain number (a) and fertile spikelet number (b) and fertile spikelet number affecting grain yield (c) and grain number (d) per main stem. Diamond, triangle, square and circle indicate control, heat, drought and combined heat and drought treatments, respectively. Data are shown for pot set 2 (heat treatment in growth chambers), linear trends are shown for relationships when are significant p<0.05.

2.3.4. Comparison of results obtained from glasshouse and growth chambers

Differences across cultivars in the control as well as the response of cultivars to heat, drought and combined heat and drought stress were very similar when pot set 1 with heat treatment in the glasshouse was compared to pot set 2 with the heat treatment in growth chambers (Figures 2.3, S2.4; Tables 2.3, S2.1). The test of the significance of differences in the mean of grain numbers, single grain weight, fertile spikelet number, grain yield, straw yield, and harvest index across cultivars and of treatments with the control yielded exactly the same significance levels for grain number and fertile spikelet number for pot set 1 (Table S2.1) or pot set 2 (Table 2.3). In addition, the significance level of differences in all growth and yield traits caused by heat, drought and combined heat and drought were the same when comparing the results for pot set 1 and pot set 2 (Tables 2.3, S2.1). Relationships between yield and growth traits pooled for cultivars or treatments were also very similar when results for pot set 1 are compared to those for pot set 2 (Figures 2.4, 2.5, S2.5-S2.9). This indicates that differences in the diurnal temperature curve, relative humidity, or radiation during the period of the heat stress treatment between the pots located in the glasshouse and those in the growth chambers have had only very little impact on the findings of the study.

2.4. Discussion

The results of this study show significant effects of heat, drought and combined heat and drought applied at around anthesis on most growth and yield related traits particularly for grain number and grain yield across the tested cultivars. Effects of combined heat and drought stress on the studied traits were stronger than the effects of individual stress factors but the magnitude of effects varied for the specific yield traits. All growth and yield related traits measured in this study showed a synergistic response to combined heat and drought stress except for straw yield which showed a hypo-additive response. This result is in agreement with the findings by Shah and Paulsen (2003) who reported that interactions between heat and drought were pronounced, and consequences of drought on all physiological (photosynthetic rate, stomatal conductance), growth (plant biomass) and yield traits (yield and single grain weight) were more severe at high temperatures than at low temperatures (Prasad et al. 2011).

The number of fertile spikelet was decreased by high temperature stress and/or drought. This agrees with previous research which showed that either heat or drought stress impacted reproductive processes, mainly pollen fertility or ovule fertility, negatively (Saini and Aspinall 1981, 1982, Praba et al. 2009, Prasad et al. 2011). However, in our study, the effect of heat stress on fertile spikelet number was only very minor.

The combination of drought and high temperature decreased grain number per main stem of all cultivars with greater magnitude than under drought or high temperature stress alone and the decrease by drought stress was at least two times higher than the one caused by high temperature. Westgate (1994) also reported that in corn, both heat stress and drought stress directly influence grain formation (Prasad et al. 2011). However, reductions in grain numbers were the result of the effects on different reproductive processes. High temperature stress decreased pollen viability, whereas drought stress inhibits pistillated flower development and ovule function. Drought, with or without heat, can increase spike and floret concentrations of abscisic acid (ABA), which can be related closely to poor grain set (Westgate et al. 1996, Weldearegay et al. 2012, Semenov et al. 2014). Additionally, Barnabás et al. (2008) reported that heat and drought can reduce photosynthesis, and the subsequent dilution of sucrose in the ear can be associated with floret abortion. Furthermore, temperatures above 30 °C during meiosis can interfere with division and lead to abnormal pollen development (Semenov et al. 2014).

Single grain weight was mainly reduced by drought and combined heat and drought while the effect of high temperature applied solely was not significant. It has been shown before that drought and high temperature stress decrease single grain weight by decreasing grain filling duration and grain filling rate (Wardlaw and Willenbrink 2000, Ehdaie et al. 2008, Prasad et al. 2008*a*, Pradhan et al. 2012). On the other hand, there are reports indicating an increase in grain filling rate under high temperature stress. However, this increase was not enough to compensate for the loss due to decreased grain filling duration (Prasad et al. 2006a, 2006b, 2008a, Pradhan et al. 2012).In contrast, Borrás et al. (2004) reported that assimilate availability from actual photosynthesis during grain filling as well as reserve remobilization was larger than the demand from the growing seed; like our study differences in single grain weight were therefore not significant and seed growth and seed yield were sink-limited during seed filling. In the present study, heat stress decreased time to physiological maturity by ~5 to 7 days while drought and combined stress decreased it by ~10 to 20 days on average across all cultivars respectively. This is one of the reasons for greater decrease in single grain weight under drought and combined stress of drought and high temperature compared with high temperature stress alone. One reason for the strong effect of drought stress in the present study could be that the soil water capacity of the sandy soil was low so that the soil water content during the drought treatment was often close to the permanent wilting point.

Combined effects of drought and high temperature stress decreased grain yield per plant of all cultivars with a greater magnitude than under drought or high temperature stress alone and this reduction by combined drought and high temperature stress was synergistic as well. This result can be expected because grain yield represents a combination of the effects of heat and drought on grain number per main stem and on single grain weight as discussed before.

High temperature and drought also reduced the straw yield but compared to the other traits, reductions by drought or combined heat and drought were relatively small. One reason is certainly that straw yield is mainly determined in the vegetative development phase of the crop which was almost finished when the drought treatment started.

The harvest index was less sensitive to temperature and/or drought stress when compared with grain yield or straw yield. This indicates that ADM partitioning also plays an important role in yield formation under stress conditions (Prasad et al. 2011).

Previous studies showed cultivar-specific responses to drought and high temperatures in wheat (Al-Khatib and Paulsen 1990, Lopez et al. 2003, Reynolds et al. 2007, Ristic et al. 2008, Prasad et al. 2011). In the present study, the responses to heat stress were very similar across the three studied cultivars while we found significant differences in the responses of the cultivars to drought and combined heat + drought. It is possible that other cultivars may respond differently to heat and combinations of heat with other stresses. The lack of differences in the heat stress responses among the considered cultivars originating from very different climatic regions may deserve more attention in future studies. Slafer et al. (2014) found a strong negative relationship between grain number and single grain weight among cultivars with varying patterns of resource allocation in comparison with environment variability which is not consistent with results in this study. However, our results about the relationship between grain yield and grain number is consistent with Slafer et al. (2014) expecting the relationship to be stronger for environmental than for genetic drivers. Our findings show that differences in incoming radiation or humidity during the stress period as well as interaction of these differences with treatments do not have any effect on the studied growth and yield traits.

This experiment was carried out under controlled ambient environmental conditions to ensure that the stress factors analyzed in the study had exactly the magnitude that was foreseen in the experimental plan and to exclude or at least minimize other factors constraining plant growth. This is different from field experiments where a confounding impact of other factors is much more difficult to exclude. Weather conditions in controlled environment studies do not change from year to year unless they are explicitly investigated. In addition, the stress treatments applied in climate chambers were repeated in a glasshouse to test for effects of the modified incoming radiation and relative humidity in the chambers. We found very similar results suggesting that the relationships obtained for the stress factors tested in the experiment are robust. We are therefore convinced that the data obtained in one season are sufficient.

The three cultivars were selected for this study due to their different geographical origin. We expected that the Iranian cultivar might be better adapted and more tolerant to heat and drought stress than the German cultivars due to the hot arid climate in Iran. However, our results did not confirm our hypothesis. The wheat cultivars grown in Iran and other regions with similar climate are spring wheat cultivars that don't require vernalization. In contrast, the cultivars in Germany are mainly winter wheat cultivars. To make sure that potential differences in the response to heat and drought between the Iranian and the German wheat were not caused by the difference between spring and winter wheat, we added the third cultivar Scirocco to the experiment which is a German spring wheat.

This study showed that drought, high temperature and combination of drought and high temperature stress at anthesis were detrimental to growth and yield traits under controlled conditions. However, the findings should not simply be generalized for field conditions. Under field conditions often a very specific canopy microclimate is established, for conditions with low wind speed. Under those conditions, canopy temperatures, for example, can then deviate considerably from temperatures measured in the surrounding of the field and result in additional interactions between heat and drought stress (Siebert et al. 2014, Webber et al. 2017) which cannot be studied in pot experiments. Variability in weather and soil conditions coupled with biotic and abiotic stress factors makes it however extremely difficult to set up and monitor experiments to study drought and heat stress under field conditions, in particular, when possible, differences among varieties should also be studied.

In summary our results clearly show that both high temperature and drought stress decreased ADM, harvest index, spikelet fertility, grain number and grain yield. Importantly, the combined effects of heat and drought were more severe than the additive effects of heat and drought alone, thus interactions between the two stress factors were synergistic for most traits. This research shows that interactive effects between heat and drought differed across various growth and yield related physiological processes. Drought stress and the combination of heat and drought, but not heat stress alone, affected single grain weight across all cultivars. In contrast, high temperature stress caused significant decreases in grain number, grain yield and biomass under both irrigated and drought conditions. More emphasis should therefore be given in future research to comparisons with cultivars originating from breeding programs aiming to develop cultivars that can tolerate both stresses (Barnabas et al. 2008, Prasad et al. 2011).

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Chapter 3

Quantifying the response of wheat yields to heat stress: the role of the experimental setup

Based on:

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Chapter 3- The response of wheat yields to heat stress: the role of the experimental setup

Abstract

Previous studies suggested a wide range of sensitivities of wheat yields to heat stress around anthesis. The aim of this study was to improve the understanding of the reasons of the disagreement by testing the response of wheat yield and yield components to differences in the method of heating, the temperature measurement points and soil substrate under sole heat and combined heat and drought stress around anthesis. Growth chamber experiments performed at different sites showed that increasing of the ambient air temperature at anthesis corresponding to a temperature sum of 12000 °C min above 31 °C resulted in a significant yield reduction of -24 % for plants grown on sandy soil substrate but not for those grown on a soil with high soil water holding capacity. The grain yield of wheat also declined by -16 % for sandy soil substrate but at a much lower level of heat stress when the temperature of the ears was increased by infrared heaters (a temperature sum of 1900 °C min above 31 °C). The yield reduction increased significantly under combined heat and drought compared to sole heat stress. Grain number significantly declined in all experiments with heat stress and combined heat and drought stress at anthesis. Single grain weight increased with heat stress around anthesis and partly compensated for lower grain numbers of pots containing a soil with high soil water holding capacity but not in experiments with sandy soil substrate. We demonstrate, based on data from previous heat stress studies, that statistical relationships between crop heat stress and yield loss become stronger when separating the data according to the soil used in the experiments. Our results suggest that the differences in the yield response to heat may be caused by additional drought stress which is difficult to avoid in heat stress experiments using sandy soil substrate. We conclude that differences in the experimental setup of heat stress experiments substantially influence the crop response to heat stress and need to be considered when using the data to calibrate crop models applied for climate change impact assessments.

3.1. Introduction

Climate is one of the most important yield determining factors explaining30-50 % of global yield variability (Frieler et al., 2017; Ray et al., 2015; Zampieri et al., 2017). The response of crop growth and yield to increasing temperatures under climate change conditions (Delworth and Knutson, 2000; Karl et al., 2015) received therefore increasing attention (Lobell et al., 2011b). For example it was estimated, that a 1°C increase in global temperature could reduce the global wheat yield by 4.1% to 6.4% depending on the method used for yield projection (Liu et al., 2016b).

Increase in mean temperature results mainly in a shortening of the length of the growing season by acceleration of the development rate (Asseng et al., 2015). Climate change does not only increase the mean temperature during the growth season but also intensifies the frequency of extreme heat events (Teixeira et al., 2013). There is growing experimental evidence that short episodes of very high temperature around anthesis of cereal crops can significantly reduce the grain yield (Eyshi Rezaei et al., 2015; Talukder et al., 2014). The major negative effect of heat stress around anthesis (HS_A) on crop yield is that grain number per ear is reduced (Ferris et al., 1998) because of pollen abortion and sterile grains (Calderini et al., 1999b; Farooq et al., 2011; Wheeler et al., 1996). In most of the studies for wheat, a threshold temperature of 31 °C was assumed for grain number reduction in the period around anthesis (Eyshi Rezaei et al., 2015) but other studies indicated 30 °C(Liu et al., 2016a) or 27 °C (Tashiro and Wardlaw, 1989) as heat stress threshold temperature in wheat. The period around anthesis is often defined as a time interval from mid of heading to end of anthesis (Ferris et al., 1998; Wheeler et al., 1996), corresponding to a period of nearly 15 days before to 5 days after anthesis (Ferris et al., 1998; Fischer et al., 1985; Ortiz-Monasterio et al., 1994; Wheeler et al., 1996). Experimental studies to evaluate the effect of heat stress on wheat yield and yield components have mainly been conducted in growth chambers (Hays et al., 2007; Narayanan et al., 2015; Prasad et al., 2011) as pot experiments, while temperature gradient tunnels (Ferris et al., 1998; Wheeler et al., 1996) and temperature free-air controlled enhancement (T-FACE) approaches (Kimball, 2005; Kimball et al., 2008) were less frequently used.

The results of previous HS_A studies performed under controlled conditions (Figure 3.1and Supplementary Table 3.1) are extremely diverse in terms of the response of wheat yield to different levels of heat (Ferris et al., 1998; Hays et al., 2007; Liu et al., 2016a; Narayanan et al., 2016; Tashiro and Wardlaw, 1989; Wollenweber et al., 2003; Zhang et al., 2013). For instance, grain yield loss of wheat amounted to 20 % at a stress thermal time (STT) of 12000 °C min at ambient air temperature (T_{air}) above 31 °C (Wollenweber et al., 2003) and to 95 % at an almost similar stress thermal time of 14400°C minat ambient air temperature above 31 °C(Zhang et al., 2010).

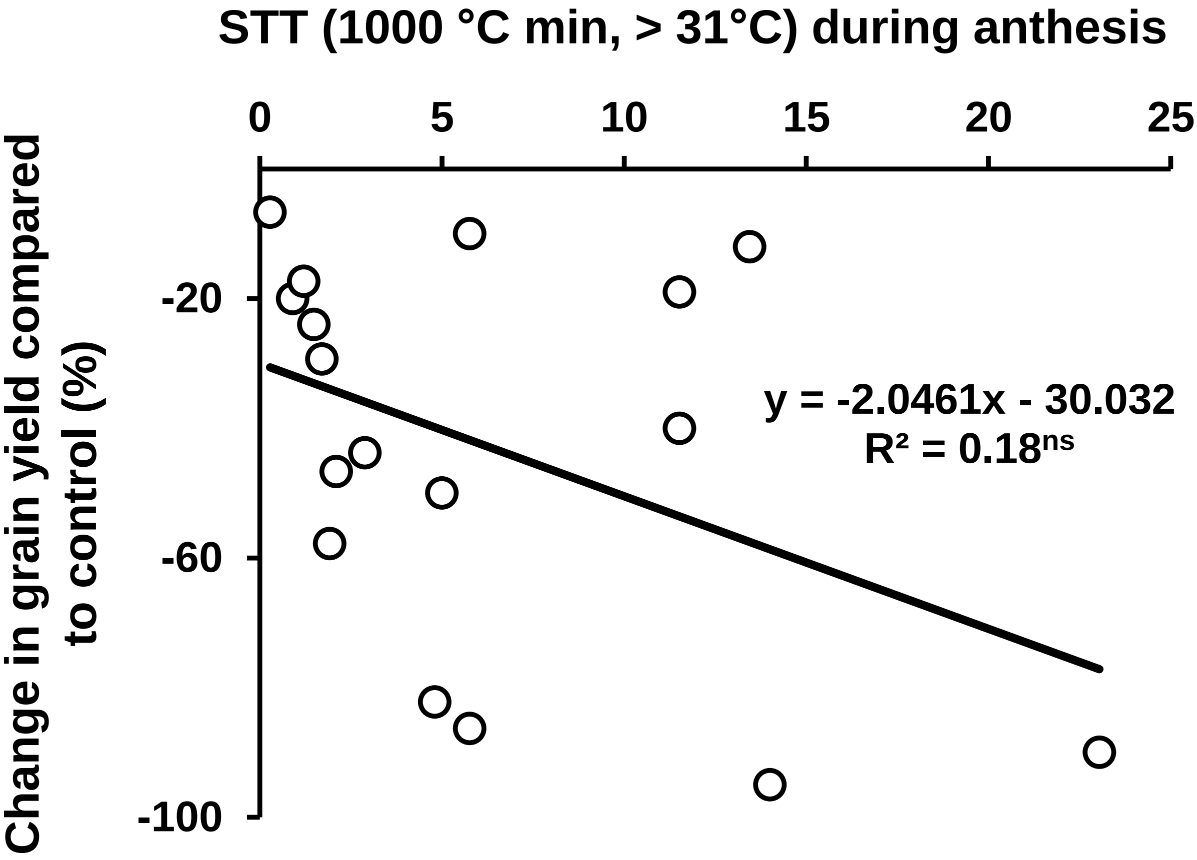

Figure 3.1. The relationship between relative yield reduction and stress thermal time (STT) for 8 published studies about heat stress effects around anthesis on crop yield which provided detailed information of soil, experimental setup and applied treatments(Ferris et al., 1998; Hays et al., 2007; Liu et al., 2016a; Narayanan et al., 2016; Tashiro and Wardlaw, 1989; Wollenweber et al., 2003; Zhang et al., 2010, 2013).

Crop models used in climate change impact assessments (Ewert et al., 2015) have been calibrated with experimental data showing a high heat sensitivity(Semenov and Shewry, 2011; Trnka et al., 2014) or lower sensitivity of wheat to heat stress (Liu et al., 2016a). To reduce corresponding uncertainties in model results, it is therefore fundamental to understand better the potential source of this inconsistency in different heat stress studies.

The sources of uncertainties in HS_A may be explained by varying experimental setup such as the temperature measurement point (ear, leaf, canopy or ambient air), method of heating (ambient air heating in growth chambers or direct heating of ears by infrared heaters), soil texture and pot size which have not been investigated systematically before. Under well-watered conditions, evapotranspiration will result in a cooling of soil, vegetation surface and air inside the canopy while canopies may heat up under drought because of the reduced transpiration. Consequently, there might be considerable differences in organ temperature, canopy temperature, air temperature above the canopy and heat stress intensities calculated based on these temperatures. For example, canopy temperature was up to 7 °C higher than air temperature measured 2 m above the canopy in drought stressed rye plots, while canopy temperature was up to 6 °C lower than ambient air temperature for irrigated plots (Siebert et al., 2014). The findings of HS_A studies can also be influenced by undesirable occurrences of drought due to exacerbated impact of combined heat and drought in comparison to the sole heat effect on wheat yield (Grigorova et al., 2011; Mahrookashani et al., 2017; Wang et al., 2010). Objectives of the present study are (i) to critically compare and summarize the results of a series of independent studies with similar objectives which used different experimental setups to quantify the response of wheat yield to heat stress and combined heat and drought stress around anthesis, and (ii) to investigate whether the differences found in the sensitivity of wheat yield to the stress treatments could be associated with differences in the experimental design, in particular with respect to the temperature measurement point, heating method and the soil substrate used in the experiments.

3.2. Materials and methods

3.2.1. Experimental design and treatments setup

3.2.1.1. Overview

A series of 6 pot experiments was carried out from 2013 to 2015 under controlled conditions at the University of Bonn (50.72 N, 7.08 E), University of Halle (51.25 N, 11.45 E) and Thünen Institute of Biodiversity Braunschweig (52.18 N, 10.26 E) to study the impact of heat stress and combined heat and drought stress on wheat yield. The experiments originally were not designed to systematically evaluate the effects of different experimental setups on crop response to heat stress around anthesis. However, experimental data were collected systematically, differences between the experiments regarding the temperature measurement point, the heating method and the soil substrate were documented and the effect on yield response to heat and drought stress was tested. The ambient air temperature close to the wheat canopy (T_{air}) in the growth chamber was measured in the experiments performed at Bonn and Braunschweig, while ear surface temperature (T_{ear}) was measured in the Halle experiments. Based on these temperature measurements, heat stress was calculated as the temperature sum (°C min) above the threshold temperature of 31 °C. At the Braunschweig experiments, heating was not only applied during anthesis as in the other experiments but also at heading and after anthesis. The experiments in Halle were explicitly designed to test the effect of the heating method and temperature measurement point on the HS_A (Tables3.1 and 3.2). A soil substrate with relatively low water holding capacity was used in the experiments at Bonn and Halle, but not in Braunschweig where the water storage capacity of the substrate was high. The plant developmental stage was determined using the BBCH scale (Lancashire et al., 1991). The experiments were performed using the winter wheat cultivar Batis or the spring wheat cultivar Ethos, which are genetically quite similar but differ in vernalization demand.

Table 3.1. Pot size, water supply and climatic conditions in the growth chambers during heating treatments in Bonn, Halle and Braunschweig.

Experimental conditions	Bonn	Halle	Braunschweig
Pot height, bottom, and top edge (cm)	26; 22; 22	16; 13; 16	20; 9; 9
Soil volume (lit)	9.0	2.4	1.2
Plants per pot	12	6	5
Soil texture	sandy	sandy loam	Mixture of peat and clay
UL (vol%)	20	38	70
LL (vol%)	5	7	15
aSWC (vol%)	18	27	55
bSWC (vol%)	5	14	30
Appointed time of watering (during the heating period)	Once daily	Once daily	Twice daily
PAR at plant height (µmol m^{-2} s^{-1})	500	300	500
Temperature range at heating (°C)	32-40 (air)	32-36 (ear)	36-40 (air)
Duration of heating (h per day; days)	6; 5	2; 5	15; 2-3
Relative air humidity (%)	45-65	50-60	55-65
Cultivar	Batis	Batis	Ethos

UL: Maximum soil water holding capacity in the pot, LL: Soil water content at the permanent wilting point, aSWC: Adjusted soil water content after watering, bSWC: Minimum of soil water content just before watering

Table 3.2. Summary of experimental setup and arrangement of sole heat and combined heat and drought stress treatments in Bonn, Halle and Braunschweig experiments.

Location	Experiment	Treatment	Start of heating (BBCH)	Heat dose (STT, °C min)	Heating method	Set-point drought intensity (SAW)	Temperature measurement point
Bonn	E1 (2014)	Control	-	-	-	-	Air
		Heat	60	12000	Growth chamber	-	
		Heat + Drought	60	12000		40	
	E2 (2015)	Control	-	-	-	-	
		Heat	60	12000	Growth chamber	-	
		Heat + Drought	60	12000		40	
Halle	E1 (2013)	Control	-	-	-	-	Ear
		Heat	60	1400	Infrared heater	-	
		Heat + Drought	60	800		30	
	E2 (2015)	Control	-	-	-	-	
		Heat1	60	500	Infrared heater	-	
		Heat2	60	1900		-	
		Heat + Drought	60	2600		30	
Braunschweig	E1 (2014)	Control	-	-	-	-	Air
		Heat1	50	13000	Growth chamber	-	
		Heat2	60	8000		-	
		Heat3	68	10000		-	
	E2 (2014)	Control	-	-	-	-	
		Heat1	50	12000	Growth chamber	-	
		Heat2	60	12000		-	
		Heat3	68	16000		-	

SAW: Soil available water

3.2.1.2. Bonn experiments

Two pot experiments were conducted in season 2013/2014 (October-June) and 2014/2015 (November-June) in a greenhouse and growth chambers. The treatments were heat stress and combined heat and drought stress for both years in 4 replications. Plants of winter wheat (cv. Batis) were grown in pots (height 26 cm, bottom edge length 22 cm, top edge length 22 cm, 12 plants/pot) filled with a soil substrate of low water holding capacity containing 85% sand and 3% clay (Table 3.1). The plants were cultivated at ambient conditions until beginning of the anthesis and then moved to a growth chamber for the heat stress treatment. Watering was carried out by an automatic drip irrigation system. All macro and micronutrients were supplied by using the irrigation system. The heat stress was imposed by heating the air in the growth chamber starting at anthesis (BBCH 60) and continuing for 5 days. T_{air} was gradually increased and kept between 32 °C and 40 °C for 6 hours on those 5 days resulting in a stress thermal time of 12000 °C min at T_{air} above 31 °C. The temperature measurement point was located inside of the chamber close to the plants. The drought stress for the combined heat and drought treatment was initiated 10 days before heading and lasted until the end of the heat stress period 5 days after anthesis. Soil moisture was kept close to 40% of total plant available soil water capacity (Tables3.1 and 3.2). The relative air humidity in the growth chamber was between 45% and 65%.

3.2.1.3. Halle (Saale) experiments

The climate chamber experiments were carried out in period January-June in 2013 and 2015 and comprised three levels of heat stress and two levels of combined heat and drought stress. Plants of winter wheat (cv. Batis) were grown in pots (height 16 cm, bottom edge length 13 cm, top edge length 16 cm, 6 plants/pot) on sandy loam soil (Table 3.1). After vernalisation, the photon flux density was set to 300 mol m^{-2} s^{-1} at plant height. After beginning of shooting, day (16 h)/night T_{air} was set to 16°C/12°C and after beginning of heading to 22°C/18°C. The pots were watered in daily intervals and macro and micronutrients were supplied by irrigation water. The heating was performed on 5 days (2 h/day) during anthesis by two infrared radiators (2 kW each). The ambient air temperature was elevated to 28 °C during the heating hours. The infrared radiators were arranged on two sides above the treated plants at an angle of 45 degree and at a distance of 40 cm to 60 cm to get different treatment groups of the temperature of the ears of the main tillers (32 °C to 36 °C). Related temperature sums based on T_{ear} ranged from 500 to 2600 °C min and corresponded to those reported by Ferris et al. (1998) based on T_{air}-T_{ear} was measured by an infrared thermometer (Testo 845, Testo GmbH & Co., 79849 Lenzkirch, Germany) on three ears (main tillers) of three pots representing 9 replications per treatment. For simultaneous drought, the watering in the morning was adjusted during five days before and then during 5 days at anthesis to obtain an upper value of soil water content of 30% of soil water capacity (SWC) during the heat treatment days. Thereafter, soil moisture was increased to 50 % of SWC (Tables 3.1 and 3.2). Since water was supplied to reach the above-mentioned target values only once per day in the morning, the soil water content usually dropped from these maximum values to lower ones during the day, where the minimum was obtained next morning before watering. The relative air humidity was between 50% and 60% under different heating treatments.

3.2.1.4. Braunschweig experiments

Two pot experiments were conducted under controlled conditions from December 2013 until May 2014. The treatments were roughly similar levels of heat stress at three different phenological stages including heading (BBCH 50), anthesis (BBCH 60) and after anthesis (BBCH 68) with 4 replications. Plants of spring wheat (cv. Ethos) were grown in pots (height 20 cm, bottom edge length 9 cm, top edge length 9 cm, 5 plants/pot) on a soil substrate containing a mixture of peat and clay with high water holding capacity (70 vol%) (Table 3.1). All macro and micronutrients were supplied to the soil before the start of the experiments. The plants were grown up in a greenhouse and then moved at heading, anthesis and after anthesis to a growth chamber for heat stress treatments. All the side tillers were cut during the early growth period. During the heating treatments pots were watered at the start and end of the light period to a constant weight. The heat stress treatments were imposed by increasing T_{air} and adjusting air humidity with a humidifier in the growth chambers. T_{air} and relative humidity inside the growth chamber were measured with a ventilated thermistor located near the plants at the height of the flag leaves, and data were continuously recorded with a logger (Manderscheid et al., 2016). The heat treatments (15 h per day) were as follows at BBCH 50, E1: 36°C × 3 days, E2: 36°C × 2 days; at BBCH 65, E1: 36°C × 2 days, E2: 38°C × 2 days; at BBCH 68, E1: 38°C × 2 days, E2: 40°C × 2 days (Table 3.2). The relative air humidity inside of the growth chamber was between 55% and 65% under different heating treatments.

3.2.2. Plant measurements

Grain yield, grain number and single grain weight were obtained from main stems when plants reached maturity. The harvested grains were dried for 48 h at 100 °C to measure the dry weight. Images of plant surface temperature during the heating treatment in the growth chamber were taken using a thermographic camera (Fluke Ti32, Fluke Cooperation, USA) at Braunschweig climate chambers.

3.2.3. Data analysis

The design of all experiments was a completely randomized. A one-way ANOVA was performed to test the significance of the applied heat and heat + drought combinations on wheat yield and yield components. The Fisher's protected least significant difference (LSD) test was employed to identify the mean differences between the treatments. The letters *a*, *b* and *c* represented significant difference in mean values. The relationships between study variables were tested using linear regression. The "agricolae" package embedded in R language (R Development Core Team 2012) was used to perform the statistical tests.

3.3. Results

3.3.1. Sole effects of heat stress on yield and yield components

Grain yield: Results of the experiments in Bonn and Halle showed a significant decline in grain yield of wheat (-24% to -16%) by imposing STT of12000°C min (T_{air}>31°C) and 1900°C min (T_{ear}>31°C) at anthesis stage, respectively (Figure 2a). A low heat stress (STT of 500 °C min (Tair > 31°C) did not result in significant differences of wheat yield in Halle experiments compared with the control. Application of similar heat intensity (STT of 8000 to 16000°C min (T_{air}>31°C)) at different phenological stages from heading to end of anthesis did not significantly influence the grain yield of wheat in Braunschweig experiments (Figure 3.2a).

Grain number: Imposing of heat at anthesis stage significantly reduced the grain number in all experiments (Figure 3.2b). However, the magnitude of reduction in grain number was different across the experiments. Application of heat stress during the anthesis stage reduced the grain number by -11% to -22% across the experimental sites, however, heating at heading stage reduced the grain number by -38% at Braunschweig (Figure 3.2b).

Single grain weight: The single grain weight showed a small non-significant decline or no change under different heat stress intensities at anthesis and after anthesis stages in all experiments (Figure 3.2c). However, application of heat stress at heading stage in Braunschweig increased the single grain weight by +35% (Figure 3.2c).

3.3.2. Combined effects of heat and drought stress on yield and yield components

Grain yield: Combined heat and drought at anthesis stage caused a remarkable yield decline (-85%) compared to sole heat treatment in Bonn experiments (Figure 3.2a). The magnitude of yield decline under combined heat and drought treatments was slightly smaller (-50%) but still significant in Halle experiments (Figure 3.2c). Even a small amount of heat stress at anthesis (STT of 800 °C min (T_{ear}>31°C)) caused a significant yield decline in combination with drought in Halle experiments (Figure 3.2a).

Grain number: The grain number was strongly reduced under combined heat and drought stress at anthesis by -87% to -80% in Bonn and Halle experiments, respectively (Figure 3.2b). The variability of grain number across replications was also increased under combined heat and drought stress compared to control and sole heat treatments (Figure 3.2b).

Single grain weight: The single grain weight of wheat showed a diverse response to combined heat and drought stress in Bonn and Halle experiments (Figure 3.2c). It increased by +30% under combined heat (STT of 2600 °C min (T_{ear}>31°C)) and drought stress in Halle experiments (Figure 3.2c). In contrast, the combined heat (STT of 12000 °C min (T_{air}>31°C)) and drought stress reduced the single grain weight by -80% in Bonn experiments (Figure 3.2c).

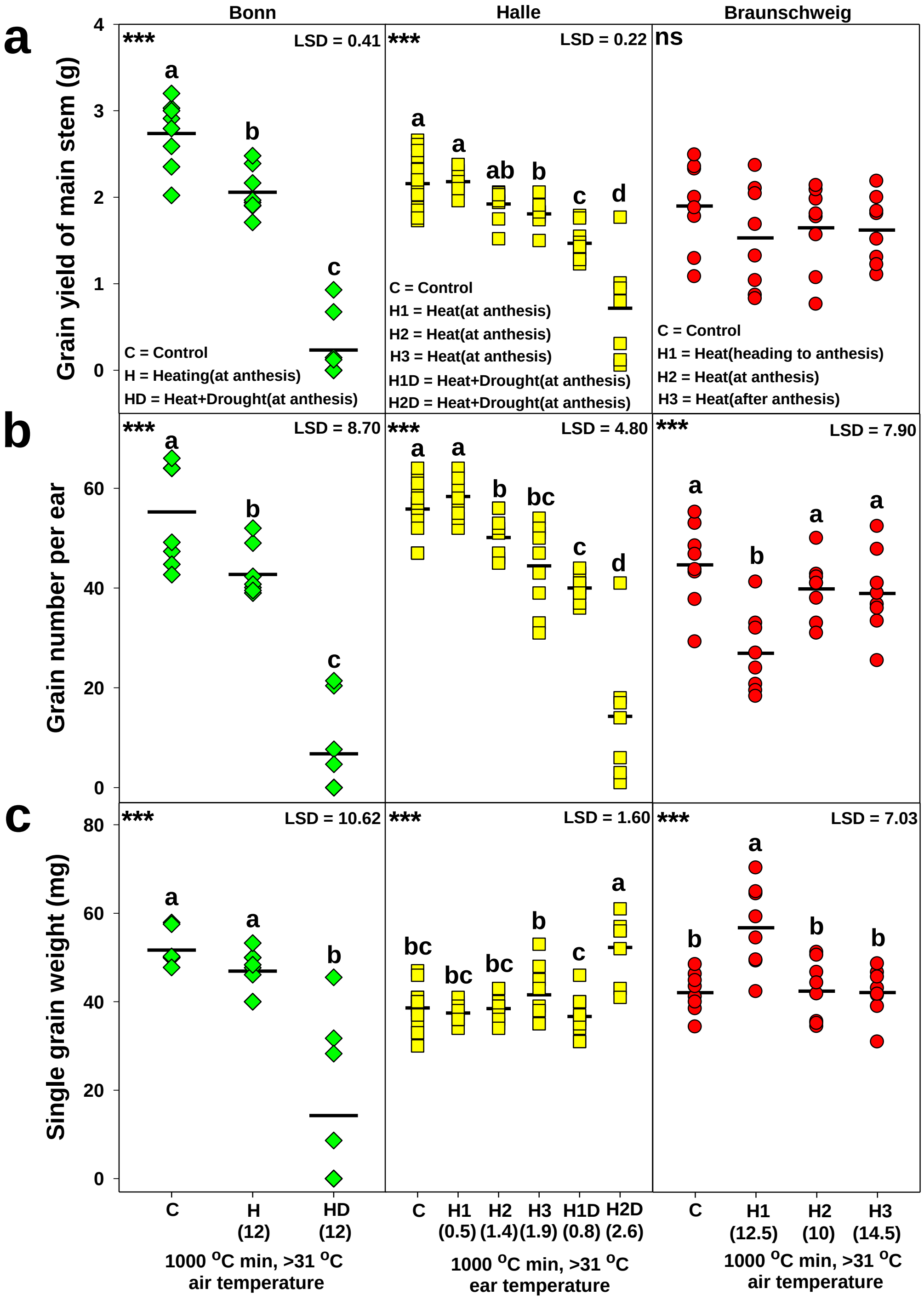

Figure 3.2. The observed grain yield (a), grain number (b) and single grain weight (c) of wheat under control, different levels of heating and combined heat and drought stress in Bonn, Halle and Braunschweig experiments. Each point represents one replication; the black line indicates the mean value. ns = non-significant trend and *** = significance at 0.1% probability level, respectively. Differences between treatments were obtained using Fisher's Least Significant Difference (LSD) test. Different letters indicate statistically significant difference ($P < 0.05$) between treatments. C = control, H = sole heat, and HD = combined heat and drought.

3.3.3. The relationships between yield and yield components under sole heat and combined heat and drought stress

As expected, there was a positive relationship between grain yield and grain number in all experiments. This relationship was strong and significant for the Bonn ($R^2 = 0.84$ to 0.93) and Halle ($R^2 = 0.72$ to 0.93) experiments under sole heat and combined heat and drought at anthesis (Figures 3.3a, b), and less strong ($R^2 = 0.51$) but still significant under heat stress in Braunschweig experiments (Figure 3.3c). On the other hand, there was no significant relationship between grain yield and single grain weight ($R^2 = 0.004$ to 0.19) under sole heat stress in all experiments (Figure 3.4). Combined heat and drought stress resulted in a strong significant positive relationship between grain yield and single grain weight for Bonn ($R^2 = 0.82$) and Halle ($R^2 = 0.25$) experiments (Figures 3.4a, b).

Negative non-significant and significant relationships between grain number and single grain weight were found under heat stress at anthesis stage in Bonn ($R^2 = 0.09$) and Halle ($R^2 = 0.59$) experiments, respectively (Figures 3.5a, b). However, heat stress at anthesis stage caused a non-significant positive relationship ($R^2 = 0.11$) between grain number and single grain weight in Braunschweig experiments (Figure 3.5c). Relationships between grain number and single grain weight under heat stress before and after anthesis (Figure 3.5c) were all non-significant. The combined heat and drought stress at anthesis resulted in a positive relationship between grain number and single grain weight at both Bonn ($R^2 = 0.78$) and Halle ($R^2 = 0.15$) experiments (Figures 3.5a, b).

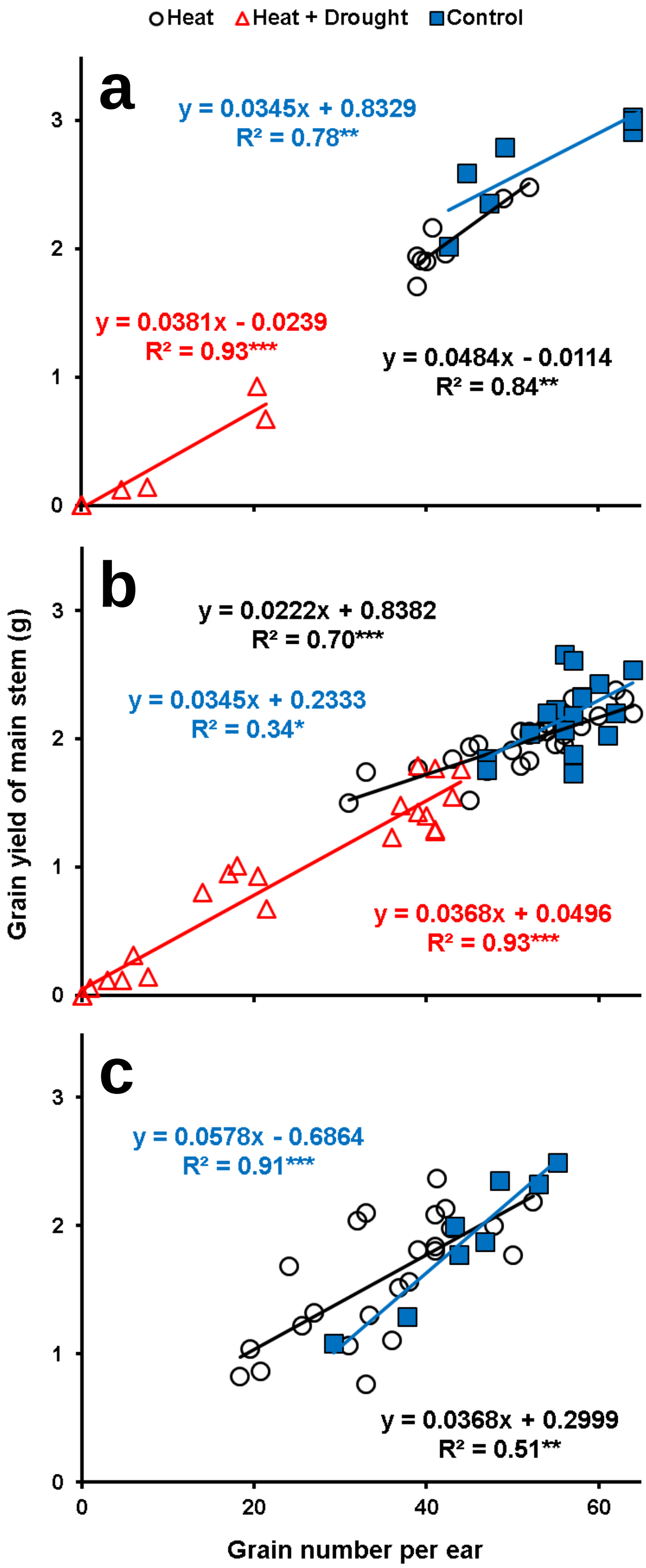

Figure 3.3. The relationship between grain yield and grain number under control, sole heat and combined heat and drought stress in Bonn (a), Halle (b) and Braunschweig (c) experiments. ** and *** = significance at 1 and 0.1% probability levels, respectively.

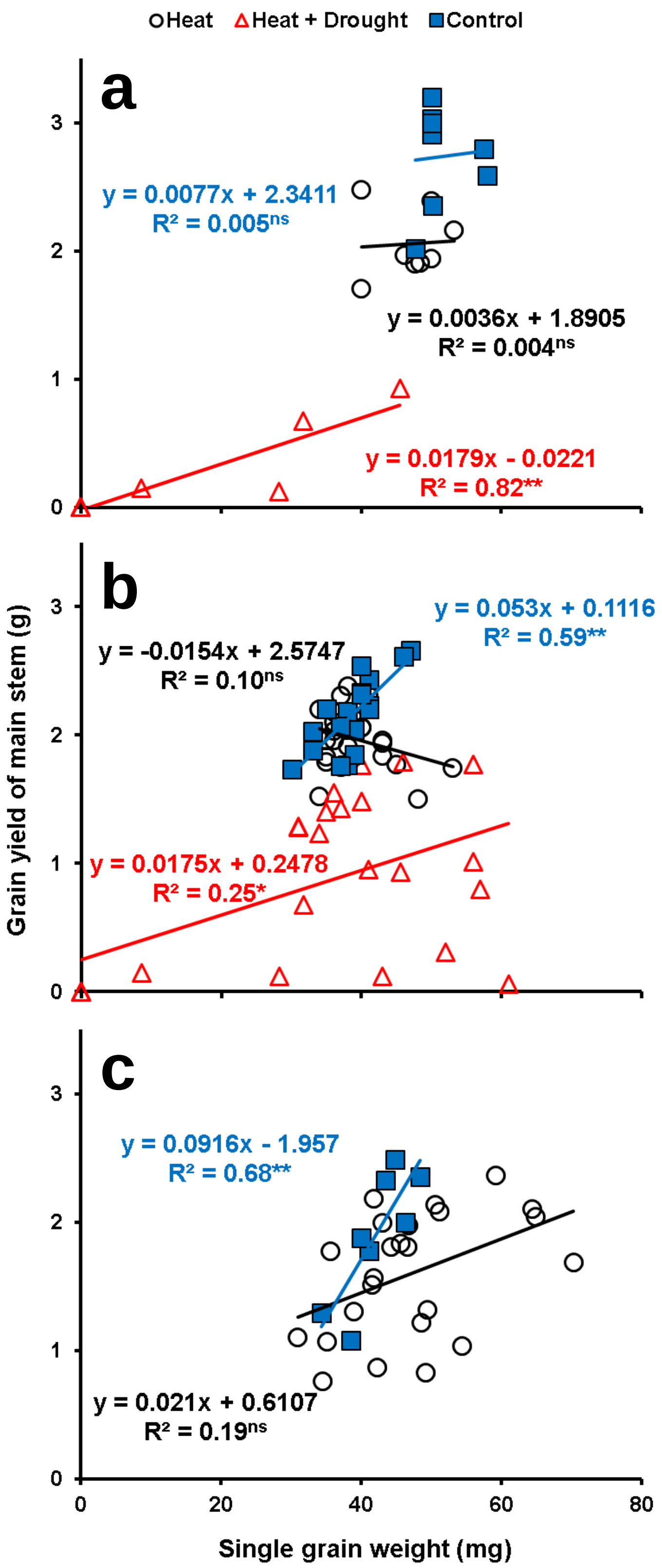

Figure 3.4. The relationship between grain yield and single grain weight under control, sole heat and combined heat and drought stress in Bonn (a), Halle (b) and Braunschweig (c) experiments. ns = non-significant trend, * and ** = significance at 5 and 1% probability levels, respectively.

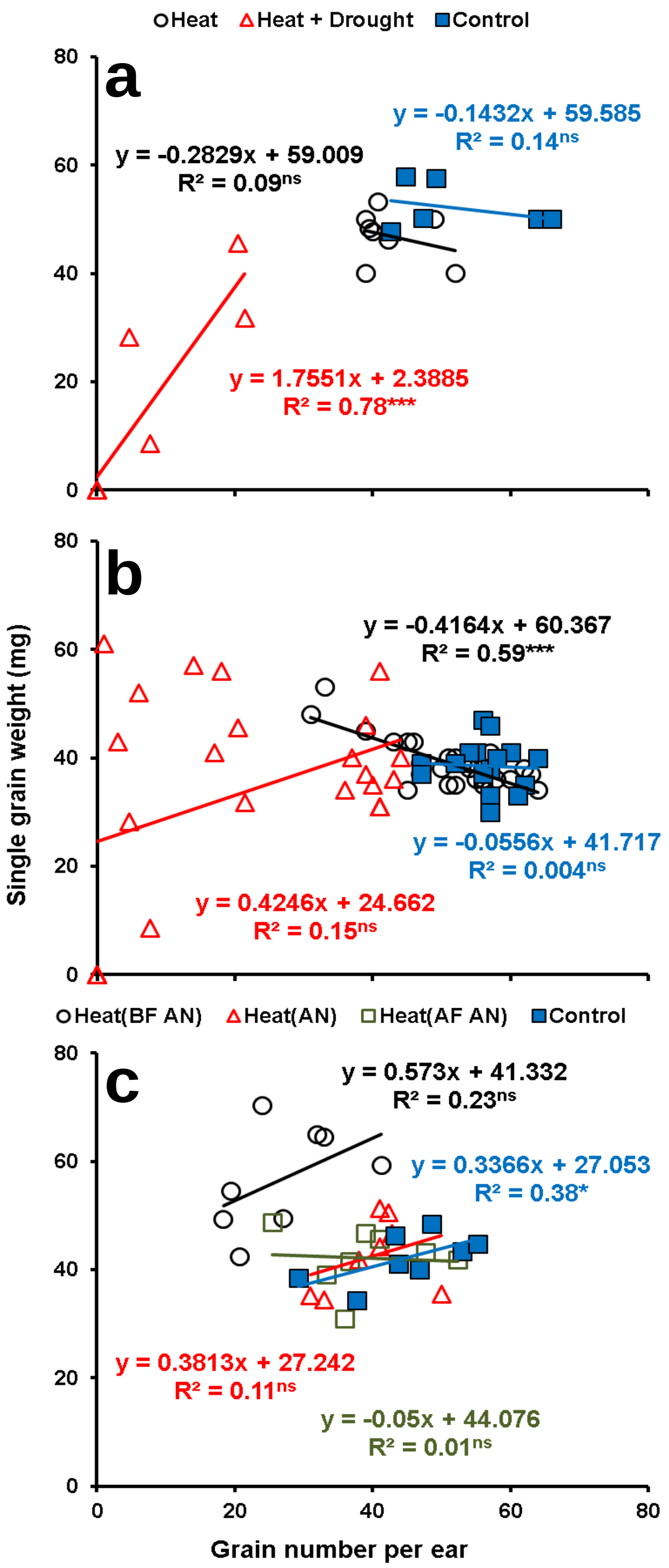

Figure 3.5. The relationship between grain number and single grain weight under control, sole heat and combined heat and drought stress in Bonn (a), Halle (b) and Braunschweig (c) experiments. ns = non-significant trend and *** = significance at 0.1% probability level, respectively. BF AN = before anthesis, AN = anthesis and AF AN = after anthesis.

3.4. Discussion

3.4.1. Heat stress experiments and temperature measurement point and method

Our results show that it is essential to consider differences in the temperature measurement point and, in the method, used to measure temperature when interpreting the response of crops to heat stress. The relative grain yield reduction was almost similar for stress thermal times of 12000 °C min or 1900 °C min when measuring the ambient air of the growth chamber and the tissue temperature of the ear, respectively (Figure 3.2a). This could be related to substantial differences between tissue, canopy and ambient air temperature at different conditions (Siebert et al., 2014) impacted by soil water status and crop transpiration rate (Jackson et al., 1981; Van Oort et al., 2014).

Our temperature measurements under heat stress in Braunschweig experiments revealed differences of 4-5 °C between ambient air temperature and leaf surface temperature and 3 °C between ambient air temperature and ear surface temperature (Figure 3.6). The temperature of air, ear and leaf surface were 37.3 °C, 34.5 °C and 33.5 °C, respectively (Figure 3.6).However, considering a 3 °C difference between air and ear surface temperature according to the data from the Braunschweig experiments in the calculation of STT for the Halle and Bonn experiments could only partly remove the differences in the sensitivities of wheat yields to heat. This implies that other factors than only the temperature measurement point (see next section) might be responsible for the different findings in the three experiments. We also want to highlight that the air temperature also depended on the distance of the measurement point to the plant tissue with higher air temperature at larger distance (Figure 3.6b). In any case, stress thermal time and the corresponding sensitivity of wheat yields to it depends highly on the temperature measurement point and the method used to measure temperature (infrared sensor versus thermometer). Unfortunately, we did not measure air, canopy and tissue temperatures simultaneously in all experiments. However, it may be assumed that the tissue temperature could have been higher under combined heat and drought stress, such that this could be one of the reasons for the larger decline in grain yield under that treatment. It was shown before that stomatal closure under combined heat and drought stress resulted in significantly higher leaf temperature and decline in photosynthesis rate compared to effects of individual heat and drought (Mittler, 2006).

Under field conditions and for regional assessments another uncertainty is introduced by the fact that temperature measurements in the canopy or tissue temperature data are often not available. Instead, usually air temperature measurements from weather stations are used (Lobell et al., 2011a; Lobell and Asseng, 2017) although air temperature at the weather station is a poor indicator of canopy temperature(Siebert et al., 2017, 2014; Webber et al., 2017). However, the difference between air and tissue temperature observed in a growth chamber may not be appropriate to represent the relationships between air and canopy temperatures in the field because of the effects of air turbulence (wind speed), diverse soil water conditions, radiation, and energy transfer between air and canopy. For instance, the canopy temperature of groundnut and rice were about 4 °C cooler than air temperature under well-watered conditions(Jagadish et al., 2007; Prasad et al., 1999). The occurrence of combined heat and drought stress can reverse the relationship between air and canopy temperature. The leaf temperature was 4 °C higher in a combined heat and drought treatment in comparison to sole heat stress under well-watered conditions (Rizhsky et al., 2002).

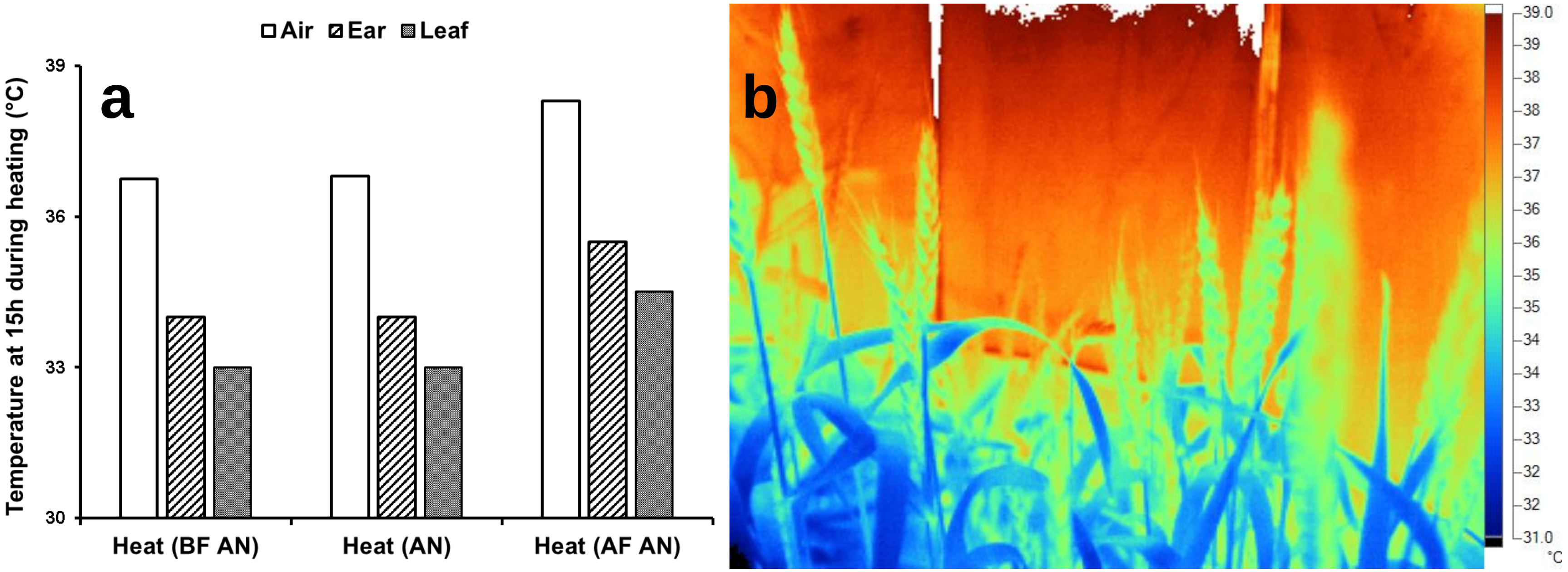

Figure 3.6. The average temperature (a) of air, leaf and ear of wheat plants during the heating treatment and the example of thermal picture (b) during the heating period in Braunschweig experiments. BF AN = before anthesis, AN = anthesis and AF AN = after anthesis.

3.4.2. Impact of the heating method on heat stress sensitivity

Our results indicate that the heating method could be another source of uncertainty in HS_A experiments. Heating of the ears directly by infrared heaters as in the Halle experiments could have led to the lesser amount of heat (STT of 1900 °C min (T_{ear}>31°C)) required to reduce the grain number and in consequence yield (Figure 3.2), even in case of 3-5°C temperature difference between tissue and air temperature. In contrast, whole plants will need much more heating in growth chamber experiments to eliminate the transpiration cooling effect under high temperature (Crawford et al., 2012). Another side-effect of heating of the whole plants by elevating the air temperature in growth chambers may result from the fact that also the roots will be heated up from different sides, which is not the case under field conditions. The root growth and functioning is more sensitive to heat stress than the shoot (Heckathorn et al., 2013) and can be strongly influenced by heat stress (Huang et al., 2012). However, own investigations in Braunschweig with only pot heating up to about 36°C did not affect grain yield of Batis or Ethos (data not shown). Moreover, the quite small effect on grain yield of heating after anthesis when roots are still functioning implies that roots might not be impaired by these high temperatures.

Using infrared heaters was claimed to be the best method to reproduce natural heating effects because of minimizing the canopy disturbance under well-watered conditions (Kimball, 2011). One possible reason for higher heat response in Halle as compared to Bonn and Braunschweig may be related to the difference in leaf vapour pressure deficit (LVPD). The air temperature was not increased in Halle experiments; thus, vapour partial pressure was much lower in Halle than in Bonn and Braunschweig. It seems possible that LVPD in Halle was extremely high causing a decrease of stomatal conductance and thus a decrease of photosynthesis. However, further studies and accurate measurements of LVPD are required to confirm this hypothesis. On the other hand, it may be argued that this approach may not be a suitable mimic of temperature effects related to climate change because of the strong modification of the vapour pressure gradient from leaf to atmosphere under heat and under combined heat and drought conditions and the general change in relationships between air and canopy temperature (Amthor et al., 2010; Aronson and McNulty, 2009).

3.4.3. Impact of the soil substrate on heat stress sensitivity

We found a different yield response at similar intensity of heat stress (STT of 12000 °C min (T_{air}>31°C)) and the method of heating (growth chamber) when comparing the results between the Bonn and Braunschweig experiments. There was a significant yield reduction in Bonn experiments but not in the Braunschweig experiments (Figure 3.2a). In addition, the significant reduction in grain number under heat stress around anthesis in the Braunschweig experiments was compensated by increase in single grain weight (Figures 3.2 b and c). This compensatory effect was not observed in the Bonn experiments (Figures 3.2 b and c). The ultimate grain weight of wheat is determined by the rate and duration of the grain filling period (Liu et al., 2014). There is a negative relationship between grain number and individual grain weight of wheat at non-stress conditions (Dreccer et al., 2009). However, the relationship between grain number and single grain weight after imposing heat stress around anthesis varied across the studies. The reduced number of grains due to heat stress at anthesis did not consequently increase the single grain weight (Wheeler et al., 1996), likely due to heat stress damage of photosynthetic apparatus (Narayanan et al., 2016; Wollenweber et al., 2003). However, other studies showed that the single grain weight significantly increased if grain number was reduced under heat stress conditions (Stone and Nicolas, 1995b). In the present experiments at Braunschweig flag leaf senescence as measured by chlorophyll content was not accelerated under the heat treatments indicating that photosynthesis was unaffected.

Our results suggest that the discrepancy in those two experiments may be caused by differences of the soil substrates. The HS_A experiments in Braunschweig, were performed using a soil with very high soil water capacity (55 vol%), while the soil water capacity was reasonably low (18 vol%) in Bonn experiments. The tillers which are the potential source of transpiration to reduce the available water in a pot for each plant were cut off in Braunschweig experiments but not in the Bonn and Halle experiments. The daily maximum available water per plant during the heat experiment just after watering was ~ 170 ml in Braunschweig experiments and ~75 ml in Bonn experiments. Differences in the setup of the experiments with respect to pot size, plant density and water holding capacity of the soil will affect the competition for water and nutrients within a pot and thus also the crop response to heat and combined heat and drought stress. Therefore, more systematic analyses under controlled conditions are required to confirm the results of the current study.

Soil water status and transpiration rate play a key role in control of the temperature of crops under heat stress (Reynolds et al., 1994). The light soil substrates such as sandy soil which we used the in Bonn and Halle experiments could have resulted into some amount of unintended drought stress as shown for the Halle experiments (Figure S3.1) and thus could have limited the transpiration (Jones and Tardieu, 1998; Passioura et al., 2006). Possibly, a mild drought might have been induced by the high temperature and high plant water requirements during the heating period at Bonn. An additional increase of the ear and leaf temperature could then have resulted in an additional reduction of the grain yield due to the direct effects of drought stress on grain number and yield (Rajala et al., 2009). On the other hand, this explanation will not apply for the Halle experiments, because the evapotranspiration for the heat stress treatments was not remarkably increased in comparison to the control and drought treatments without heating (Figure S3.2). Therefore, in the Halle experiment the accelerating effect of drought on the response to heat stress might be attributed to a direct impairment of fertility (pollen growth) by drought during anthesis. Finally, the high proportion of peat of the soil used in Braunschweig experiments probably avoided the effect of unintended drought that might have been led to yield reduction by an extra heat effect as assumed above for the Bonn study as well by a direct drought effect on fertility as supposed for the Halle analysis.

Based on these considerations, we divided the studies that we used for meta-analysis in the introduction (Figure 3.1) plus the results of our series of experiments into studies which were performed by the soil substrate with either high or low water holding capacities. There was a significant relationship between the relative reduction in grain yield and $STT_{>31°C}$ after dividing the studies to low ($R^2 = 0.36$) and high ($R^2 = 0.69$) water holding capacity of soil substrates of pot experiments (Figure 3.7) while the relationship was not significant when analyzing the data in combination (Figure 3.1). Differences in sensitivity of specific wheat cultivars are well documented and some of the variability presented Figure 3.7 could be caused by cultivar differences. In the present study cultivars of wheat with a similar genetic background have been used, which do not strongly differ in their response of yield and yield components to STT as indicated by the results obtained in Bonn and Braunschweig in the sole heat treatments. Moreover, experimental data from two Chinese wheat cultivars recently published in Liu et al., (2016) indicated that 50% grain yield loss due to heat around anthesis occurred at a stress thermal time of about 40000 – 50000 °C min (Tair > 30°C), which corresponds to the results for our cultivar if the data are linearly extrapolated.

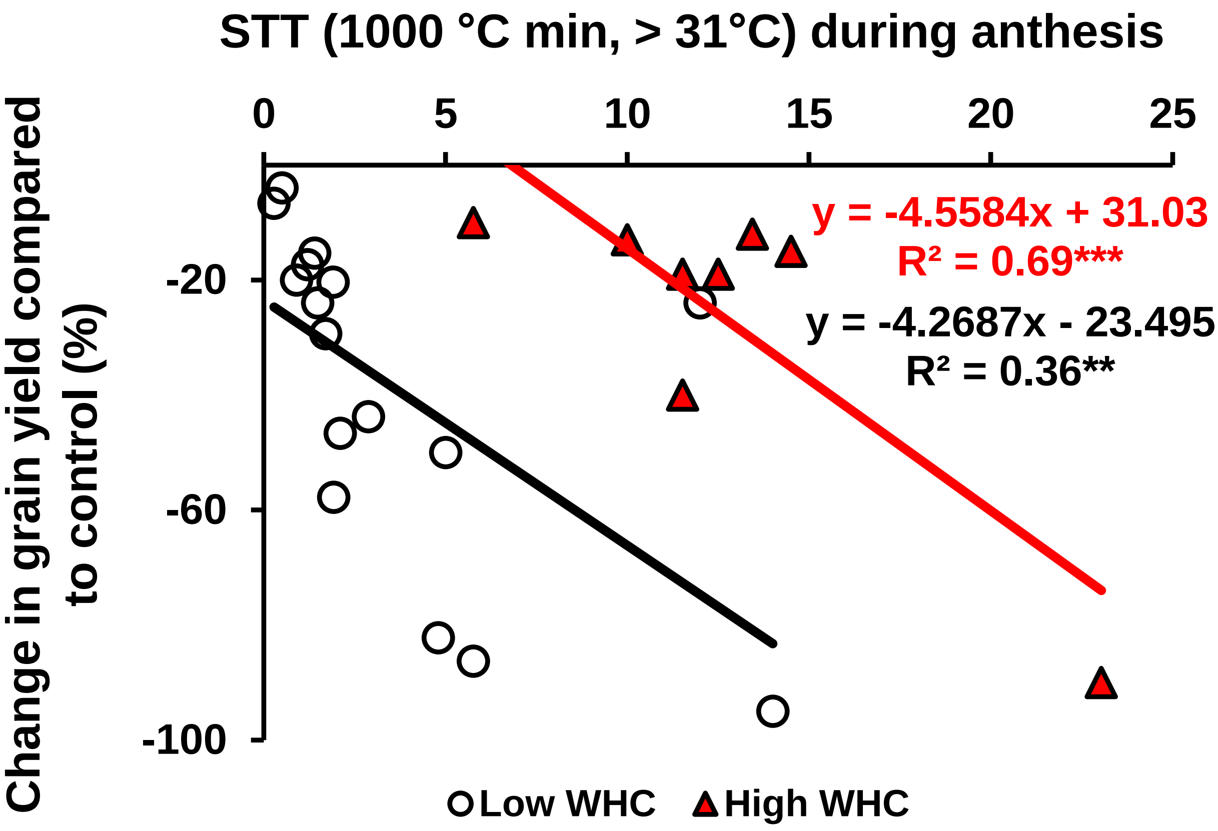

Figure 3.7. The relationship between relative yield reduction and stress thermal time (STT) for 8 published studies about heat stress effects around anthesis on crop yield which provided detailed information of soil, experimental setup and applied treatments(Ferris et al., 1998; Hays et al., 2007; Liu et al., 2016a; Narayanan et al., 2016; Tashiro and Wardlaw, 1989; Wollenweber et al., 2003; Zhang et al., 2010, 2013) and results of Bonn, Braunschweig and Halle experiments. The studies were divided based on the soil substrate of pots to substrate with high and low water holding capacity (WHC). ** and *** = significance at 1 and 0.1% probability levels, respectively.

Based on our results the simultaneous occurrence of drought stress under high temperature (as combined heat and drought treatment) may amplify the heat effect by reducing the transpiration and photosynthesis due to stomatal closure (Nankishore and Farrell, 2016). Such amplifying effect of combined heat and drought was also observed for other crops including groundnut, maize and cotton (Cairns et al., 2013; Dabbert and Gore, 2014; Hamidou et al., 2013). Additionally, a direct impairment of pollen growth that may occur under drought (Barnabas et al., 2007) may overlay and strengthen the effect of heat stress. This could be the main reasons of the higher yield reduction in combined heat and drought stress in Bonn and Halle experiments. We found an increase in single grain weight under combined heat and drought treatment by reducing of grain number in the Halle H2D experiment, but this compensatory effect was not observed in Bonn experiments. The different response could be related to longer duration of drought stress in Bonn experiments (10 days before anthesis) in comparison to Halle experiments (4 days before anthesis).

We suggest that because of the predominant mechanism of interaction of heat and rising CO_2 concentration a precise analysis of the sole heat effect on grain yield is necessary. If grain yield will be affected already at small levels of heat stress, then the small increase of tissue temperature due to the reduction of transpiration under elevated CO_2 would intensify the effect of high air temperature and there would be a positive interaction of heat and CO_2 as observed in studies with rice and sorghum (Jagadish et al., 2014). If grain yield is only impacted at high levels of heat stress, detrimental effects of heat only seem rather unlikely for the moderate climate zone of Europe even under future climate change. Such high levels of STT of the plant tissue can be expected only under restriction of transpiration by low soil moisture due to lack of rainfall. However, plants grown under elevated CO_2 use less water and thus soil drying is delayed. Consequently, it is conceivable that rising CO_2 concentration can also mitigate the effect of heat especially when the high tissue temperature results from restricted transpiration cooling due to soil drought.

3.5. Conclusion

The magnitude of heat effect on grain yield of winter wheat can be substantially affected by the experimental setup including the temperature measurement point, the method of heating, and the soil substrate used. Combined heat and drought reinforced the negative effect of high temperature on crop yield and yield components and in this way reduced the grain yield significantly even under very moderate intensity of heat. Therefore, it is fundamental to understand the source of uncertainties in such experiments in order to make proper used of the data and results obtained. Crop modellers should carefully select experimental data to be used for model calibration and testing depending on the process representation in the specific model. Data obtained from experiments differing in heating method, temperature measurement and soil substrate should not be merged but be analysed separately. The findings of our experiments imply that the observations reported in some previous studies on HS_A did not only result from heat stress impact but also from a combination of heat and drought. Climate change impact assessments performed using crop models indicated that heat will be a major threat to wheat yield under climate change. However, we suggest that many of those models have been calibrated based on studies that included a combination of heat and unintended drought. Thus, threat to wheat yield by future heat periods might have been overestimated at least for some regions such as central Europe. A more typical scenario in such regions will be peak heat phases occurring during drought periods rather than heat stress under conditions with sufficient water availability. Our results show that this combination, in particular for soils with low water holding capacity, may lead to much more dramatic yield losses than drought or heat on its own.

Acknowledgements

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Chapter 4

Impact of heat and drought stress at anthesis on post-anthesis growth of wheat

Abstract

Short episodes of heat and drought stress at the anthesis stage of cereals can have a substantial impact on crop growth and yield, mainly by reducing grain number. However, little is known about the effects of heat and drought stress at anthesis on the subsequent grain filling period of wheat. This study was carried out to evaluate the impact of heat and drought stress at anthesis on grain filling rate and duration, photosynthesis rate, chlorophyll contents, yield and yield components of three wheat cultivars. We found that heat and drought stress at anthesis reduced the grain number by 54% to 17% and shortened the length of the grain filling period of the different wheat cultivars by 28% to 13%. The plants exposed to heat compensated for lower grain filling duration by increased grain filling rates so that single grain weights were not significantly different (2 cultivars) or even higher for the heat treatment (1 cultivar). In contrast, grain filling rates did not increase in plants exposed to drought stress at anthesis. Earlier flag leaf senescence and reduced photosynthesis rates during grain filling were observed for the drought-stress treatment but not for the plants exposed to heat. This indicates that source limitations may explain the differences in grain filling rates between the treatments. Heat at anthesis significantly reduced the grain yield of the German winter wheat cultivar Batis by 25% and of the Iranian spring cultivar Kohdasht by 23%. In contrast, heat at anthesis did not significantly reduce grain yield of the German spring wheat cultivar Scirocco (by 9%) because reduced grain number and reduced grain filling duration were partly compensated by the increased grain filling rate. The findings show that the impact of stress at anthesis on the subsequent grain filling period differs between heat and drought. Further research is needed to understand the physiological mechanisms causing different responses of specific cultivars before the processes can be implemented and parameterized in crop models.

4.1. Introduction

The frequency and magnitude of extreme events such as heat and drought may increase under climate change conditions (Jentsch et al., 2007; Meehl and Tebaldi, 2004) and negatively affect crop growth and yield (Challinor et al., 2014; Rosenzweig et al., 2014). It was estimated, for instance, that the global wheat production will decline by 6% with each one-degree increase in temperature (Asseng et al., 2015). To develop comprehensive adaptation strategies to climate change it is therefore fundamental to better understand crop response to heat and drought (Lobell, 2014; Olesen et al., 2011; Reidsma et al., 2010).

Photosynthesis is one of the growth processes that is most sensitive to heat and drought (Fischer et al., 1998). Heat stress reduces the regeneration of chlorophyll, manipulates the activities of enzymes active in photosynthesis, and increases the photorespiration (Law and Crafts-Brandner, 1999; Sairam et al., 2000; Xu et al., 1995). Drought causes stomata closure and thereby affects the photosynthesis rate (García-Mata et al., 2001).

Previous research shows that the effects of heat and drought on crop growth and yield differ depending on the development stage in which the crop is exposed to stress (Calderini et al., 1999a, Rajala et al., 2009). The occurrence of heat stress around anthesis can reduce the grain number by increased pollen abortion and increased number of sterile grains (Calderini et al., 1999a; Farooq et al., 2011) with associated grain yield reductions in wheat of up to 40% (Ferris et al., 1998). Number of grains and tillers can also be reduced by occurrence of drought in the period around anthesis (Foulkes et al., 2007) due to decline in floret set and increase in the abscisic acid level (Dolferus et al., 2011).

Heat stress events during the grain filling period can reduce the single grain weight by accelerating leaf senescence and by shortening the grain filling period (Dias and Lidon, 2009; Zhao et al., 2007). Temperatures above 34 °C showed a significant impact on leaf senescence and a remarkable contribution to yield loss in wheat in India (Lobell et al., 2012). Increasing the mean temperature from 20 °C to 25 °C shortened the grain filling period by 12 days (Yin et al., 2009). On the other hand, the grain filling rate may accelerate as temperature rises, but this compensation also depends on the activity and strength of sinks (Sofield et al., 1977). However, Al-Khatib and Paulsen(1984) observed that the increased grain filling rate did not fully compensate the reduction in the length of the grain filling period under severe heat stress. Terminal drought can also significantly accelerate crop development and shorten time to maturity (Rosales-Serna et al., 2004). The grain filling rate under drought can be restricted due to reduced photosynthesis, accelerated leaf senescence, and sink limitations (Bogard et al., 2011; Farooq et al., 2014; Khanna-Chopra, 2012). However, a mild level of drought can increase the grain filling rate of wheat (Yang et al., 2003).

Previous studies suggest remarkable cultivar differences in the response to heat and drought (Ayeneh et al., 2002; Stone and Nicolas, 1995a; Viswanathan and Renu, 2001).These differences can be explained by various mechanisms including stability of membrane, protein synthesis, higher leaf chlorophyll content, nitrogen remobilization to flag leaf and production of heat shock proteins in thermos-tolerant cultivars of wheat (Krishnan et al., 1989; Reynolds et al., 1994; Ristic et al., 2007; Stone and Nicolas, 1995a; Tahir and Nakata, 2005). A stronger osmotic adjustment is one of the main characteristics of drought-tolerant wheat cultivars (Mattioni et al., 1997; Nayyar and Walia, 2003). Larger reserves of assimilates in the stem can also contribute to higher tolerance of wheat cultivars to drought stress (Foulkes et al., 2007).

Most of the previous studies focused on the effect of heat and drought at pre-anthesis stage on grain number and impact of heat and drought during the post-anthesis stage on grain filling rate and duration and on single grain weight. Little is known on the effects of heat and drought stress at pre-anthesis or anthesis stage on subsequent growth processes including grain-filling period and rate, and the findings in literature are contradictory. In a study on wheat, barley and triticale no effect of pre-anthesis heat stress on grain filling duration was found (Ugarte et al., 2007), while a significant decline in grain filling period and yield of wheat was detected as the result of imposing heat stress around the anthesis stage (Lizana and Calderini, 2013). Drought caused an acceleration of crop development and decline of 12% in thermal time between emergence and maturity for winter wheat with the largest response in the phase between anthesis and maturity. However, there was little impact of pre-anthesis drought on the length of the subsequent phase between anthesis and maturity (McMaster and Wilhelm, 2003). Therefore, the drought escape mechanism (ability of plants to complete their life cycle before the onset of drought) is mainly discussed and considered relevant for terminal drought (Farooq et al., 2011). In crop models, heat and drought stress before or around anthesis usually influence the grain number but not the length of the following growing stages such as the grain filling period (Ewert et al., 2002; Eyshi Rezaei et al., 2015). In contrast, wheat plants exposed to heat or drought between heading and anthesis responded with a shortening of the development phase between anthesis and maturity by 5-7 days in an experiment with three wheat cultivars in Germany (Mahrookashani et al., 2017).

In the current study, we extended previous research (Mahrookashani et al., 2017) by repeating the experiment for a second year to (i) systematically evaluate the effects of heat and drought stress before anthesis of wheat on post-anthesis processes such as grain filling rate and duration, (ii) test the impact on yield and yield components, and (iii) to test whether effects can be generalized across cultivars by using a German winter wheat cultivar (Batis), a German spring wheat cultivar (Scirocco) and a spring wheat cultivar from Iran (Kohdasht).

4.2. Materials and methods

4.2.1. Plant husbandry and growth

The experiment was carried out at the University of Bonn, Germany (50.73 °N, 7.10 °E), during two growing seasons (2013/2014 and 2014/2015). The experimental site is characterized by a temperate climate with a mean annual precipitation of 687 mm and an annual mean temperature of 10.7 °C (1981-2010). The treatments were control, drought and heat stress between heading and anthesis of wheat. The sowing of the German winter wheat cultivar Batis was on 29 October

2013 and 5 November 2014, while the sowing of the Iranian spring wheat Kohdasht and of the German spring wheat cultivar Scirocco was on 20 March 2014 and 5 March 2015. Twelve (12) plants were sown in plastic pots (22 cm × 22 cm × 26 cm) in two rows with 9-cm spacing between rows. The pots were filled with a mixture of topsoil, silica sand, milled lava and peat dust (Terrasoil®, Cordel & Sohn, Salm, Germany) consisting of 85% sand, 12% silt and 3% clay with a volumetric water content of 18 % at field capacity and of 4% at the permanent wilting point. Crop nutrients were added to the irrigation water and sufficient amounts supplied at three-time steps. The first fertilizer application direct after germination consisted of 3.6 g Entec (14 % N, 7% P_2O_5, 17% K_2O, 2% MgO, 9% S, 0.02% B and 0.01% Zn) per pot, while the second and third application consisted of 1.86 g CAN (27% N) per pot. The pot watering system was operated by an automatic drip irrigation system. Plants were grown under ambient conditions until the beginning of anthesis (Figure 4.1).

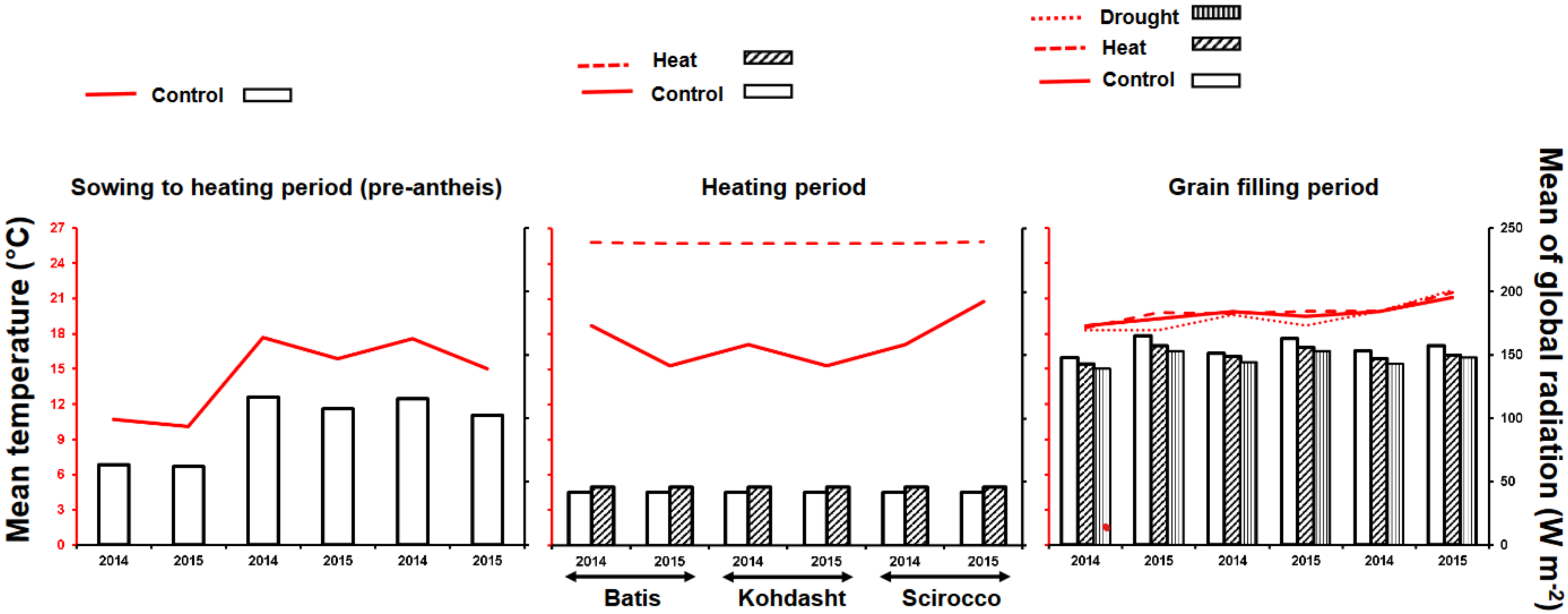

Figure 4.1. Mean temperature and mean global radiation during the pre-anthesis, heating and grain filling period for the cultivars Batis (German winter wheat), Scirocco (German spring wheat) and Kohdasht (Iranian spring wheat) for the growing seasons (2013/2014 and 2014/2015).

4.2.2. Treatment setup

The drought stress treatment was initiated ten days before the expected anthesis date in both years. In the first year (2014), the soil moisture content was reduced to 20% of the plant-available water capacity and then 200 ml water was added to avoid permanent wilting. However, in the second year (2015), a similar drought intensity could not be established until begin of anthesis because of lower temperatures and less evapotranspiration. Therefore, plants experienced different magnitudes of drought stress in the two years of the experiments (Figure 2).

The pots of control (normal chamber, C), drought (D) and heat (H) treatments were moved into a normal and a heating growth chamber at the beginning of anthesis. The air temperature in the heating chamber was gradually increased to 38 °C for about 8 hours so that a total stress thermal time (STT) of 12000 °C min above a critical threshold of 31 °C (Ferris et al., 1998) accumulated in the period of five days. The temperature of the normal chamber was adjusted by a daily procedure to mimic the ambient temperature for the control and drought treatments (Figure S1.4). To avoid drought in the control and heat treatment, pots in the normal chamber (C) were irrigated with 300 ml per day while the pots in the heat chamber (H) received 400 ml water per day. After approaching a STT of 12000 °C min in the heat chamber, the pots of all treatments were moved to ambient conditions and full irrigation was ensured until maturity (Figures 4.1 and 4.2).

4.2.3. Data collection

A portable meteorological station was installed at the experimental site and growth chambers to record temperature, relative humidity, and solar radiation at 15-minute intervals in both growing seasons. The phenological development was observed after emergence using the BBCH scale (Lancashire et al., 1991) to detect the phenological stages including heading (55), anthesis (65), and maturity (92). The grain filling duration was the phase between end of flowering (69) and maturity (92). The grain filling rate was calculated by dividing the single grain weight by the duration of grain filling (growing degree days), assuming that grain weight was 0 at anthesis (Dias and Lidon., 2009). After maturity, plants were harvested, dried at 65 °C for 72 hours, and aboveground biomass, grain number, single grain weight and grain yield of main stems were measured. Light response curves of flag leaf photosynthesis were measured under 0, 15, 30, 60, 120, 250, 500, 1000 and 1500 µmol $m^{-2}s^{-1}$ of photosynthetic photon flux (PPF) after 20 minutes dark adaptation using a portable LI-COR 6400 photosynthesis system (LI-COR, Lincoln, NE, USA) for control, heat and drought treatments in the second year

(2015). The CO_2 concentration of ambient conditions was 400 µmol mol^{-1}. A portable chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, Illinois, USA) was used to measure chlorophyll concentration. Measurements were performed at three different points along the flag leaf in the second year (2015).

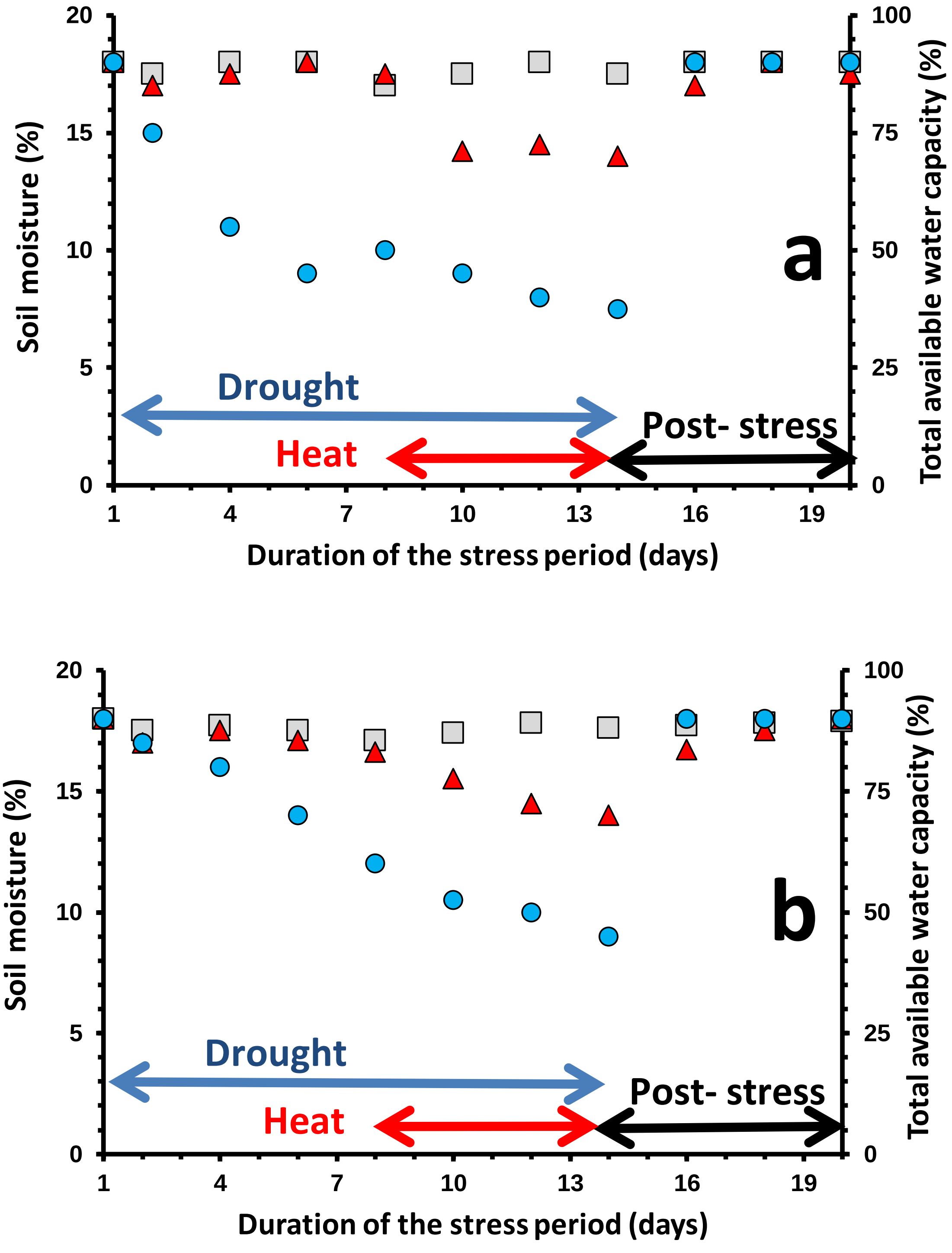

Figure 4.2. Volumetric soil moisture content (%) and available soil water (% of total available water capacity) during the heat and drought stress treatment in years 2014 (a) and 2015 (b).

4.2.4. Experimental design and statistical analysis

The experimental design comprised a factorial design with two levels of heat stress (0 and 12000 STT°C min) as a first factor and three cultivars (Batis, Kohdasht and Scirocco) as a second factor based on a completely randomized (CRD) design with 4 replications. We excluded the drought stress treatment from the statistical analysis because the intensity of drought stress varied considerably between the two years of the study. However, we analysed the SPAD and photosynthesis measurements under drought stress in the second year (2015). The significance test was performed for each measured variable by a mixed-effect model fit by restricted maximum likelihood (REML) implemented in the*nlme* package of R (R Development Core Team 2012) v3.1-131. Heat stress, cultivars and interactions between heat and cultivars were treated as fix effects and years treated as a random effect in the model. The Fisher's protected least significant difference (LSD) test, implemented in the *agricolae* package of R v1.2-4, was employed to identify the mean differences between heat stress and cultivar treatments. If the interaction was significant, the interaction between the two factors heats stress and cultivars (the response of a factor within each level of the interacting factor) was tested using the differences of least squares means implemented in the *lsmeans* package of R v2.25.

4.3. Results

4.3.1. Effects of heat and drought at anthesis on grain filling duration and rate

Heat and drought at anthesis resulted in a remarkable shortening of the grain filling period in both years of the study with a reduction of the thermal time needed for grain filling of 23% by drought and of 19% by heat compared to the control (Figure 4.3a). The thermal time required for grain filling of the Iranian cultivar Kohdasht showed the highest sensitivity to heat and drought stress (-28% to -22%, Figure 4.3a), while the decline in grain filling duration was smallest for the German spring wheat cultivar Scirocco (-20% to -13%, Figure 4.3a). With an increase of 25%, the grain filling rate of the plants exposed to heat around anthesis was much higher than that of the control (Figure 4.3b), while there was little difference between that of the plants in the drought treatment as compared to the control (3% increase, Figure 4.3b). Consequently, higher grain filling rates in the heat treatment overcompensated the shorter grain filling duration as compared to the control, so that single grain weight at harvest was 5% higher for the heat treatment than for the control (Figure 4.3b). In contrast, single grain weight in the drought treatment was mainly determined by the shortening of the grain filling duration and therefore lower than the single grain weight of the control.

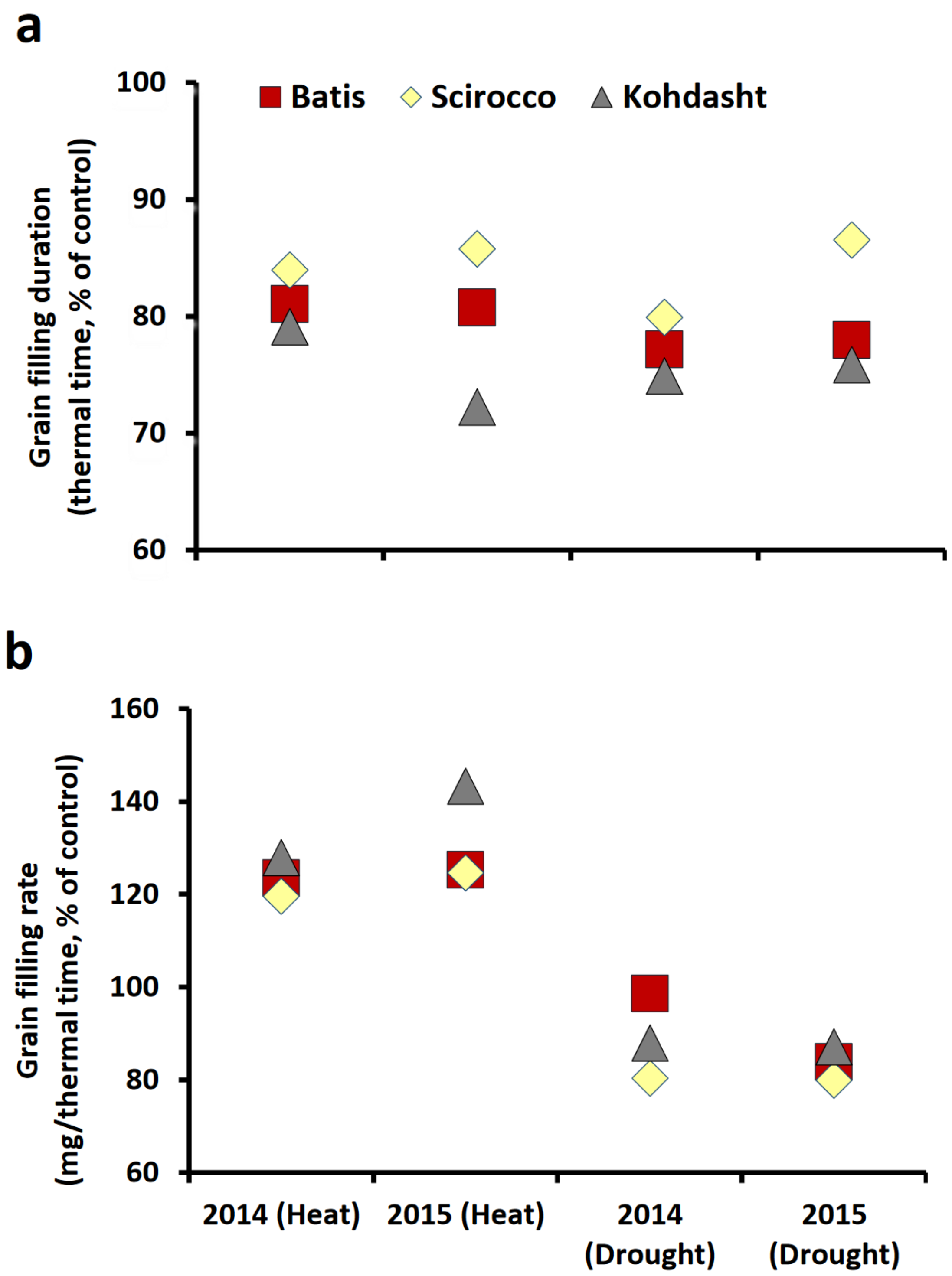

Figure 4.3. Relative change in the length of the grain filling period (a) and in grain filling rate (b) of the cultivars Batis (German winter wheat), Scirocco (German spring wheat) and Kohdasht (Iranian spring wheat) for the heat and drought treatments compared to the control in 2014 and 2015.

4.3.2. Impact of heat stress and cultivar on yield and yield components

The aboveground biomass, grain yield, and grain number per ear were significantly influenced by the heat and cultivar treatments, while the interaction between heat and cultivar effects was significant for grain yield and single grain weight (Table 4.1). Heat at anthesis reduced aboveground biomass and grain yield by about 20% and grain number per ear by 25% (Figure 4.4a). In the control, the Batis cultivar showed a significantly higher grain yield (2.4 g plant) and grain number per ear (49) compared to the Kohdasht (1.6 g plant and 40 grain per ear) and Scirocco (1.8 g plant and 32 grains per ear) cultivars (Figure 4.4b).

Heat stress around anthesis significantly reduced the grain yield of the Batis cultivar by 25% and of the Kohdasht cultivars by 22% but yield reductions for the cultivar Scirocco were smaller and not significant (9%; Table 4.2). Grain number per ear was reduced significantly by heat stress for all cultivars (Table 4.2). The single grain weight was not significantly influenced by heat stress for in the Batis and Kohdasht cultivars. In contrast, single grain weight was significantly higher (19%) for the cultivar Scirocco in the heat treatment (Table 4.2) and partly compensated therefore for the effect of the reduction in grain number on grain yield.

4.3.3. Chlorophyll concentration and photosynthesis under heat and drought stress

Drought stress at anthesis resulted in smaller absolute values of SPAD readings (as an estimation of chlorophyll content) during the grain filling period compared to the readings for the heat and control treatments (Figure 4.5). The SPAD readings were relatively similar until mid-grain filling period for the control but declined considerably for the drought treatment and cultivars Batis and Kohdasht. In contrast, there was little effect of heat and drought around anthesis on SPAD readings until mid-grain filling period for the cultivar Scirocco (Figure 4.5). The area below the SPAD readings curve is relatively similar for the control and the heat treatment but considerably smaller for the drought treatment, which indicates potential source limitations during grain filling for the plants exposed to drought at anthesis (Figure 4.5).

Table 4.1. Significance levels (linear mixed-effects model fit by REML) of study treatments on above ground biomass, yield, yield components and grain filling rate across two years (2014, 2015).

Source	d.f	Above ground biomass	Grain yield	Grain number	Single grain weight	Grain filling rate
Heat	1	***	***	***	ns	***
Cultivar	2	***	***	***	ns	***
Heat × Cultivar	2	ns	**	ns	***	***

Table 4.2. Comparison of mean of interactions between heat stress × cultivars on grain yield, single grain weight and grain filling rate across two years (2014, 2015).

Cultivar	Heating	Single grain weight (mg)	Grain yield (g plant)	Grain filling rate (mg GDD^{-1})
Batis	Control	52^{ns}	2.74^{a}	0.09^{ns}
	Heat	47^{ns}	2.06^{b}	0.10^{ns}
Kohdasht	Control	49^{ns}	1.84^{a}	0.10^{b}
	Heat	48^{ns}	1.44^{b}	0.14^{a}
Scirocco	Control	43^{b}	1.94^{ns}	0.09^{b}
	Heat	51^{a}	1.77^{ns}	0.12^{a}

Different letters indicate statistically significant difference (LS means, $P < 0.05$) between interactions.

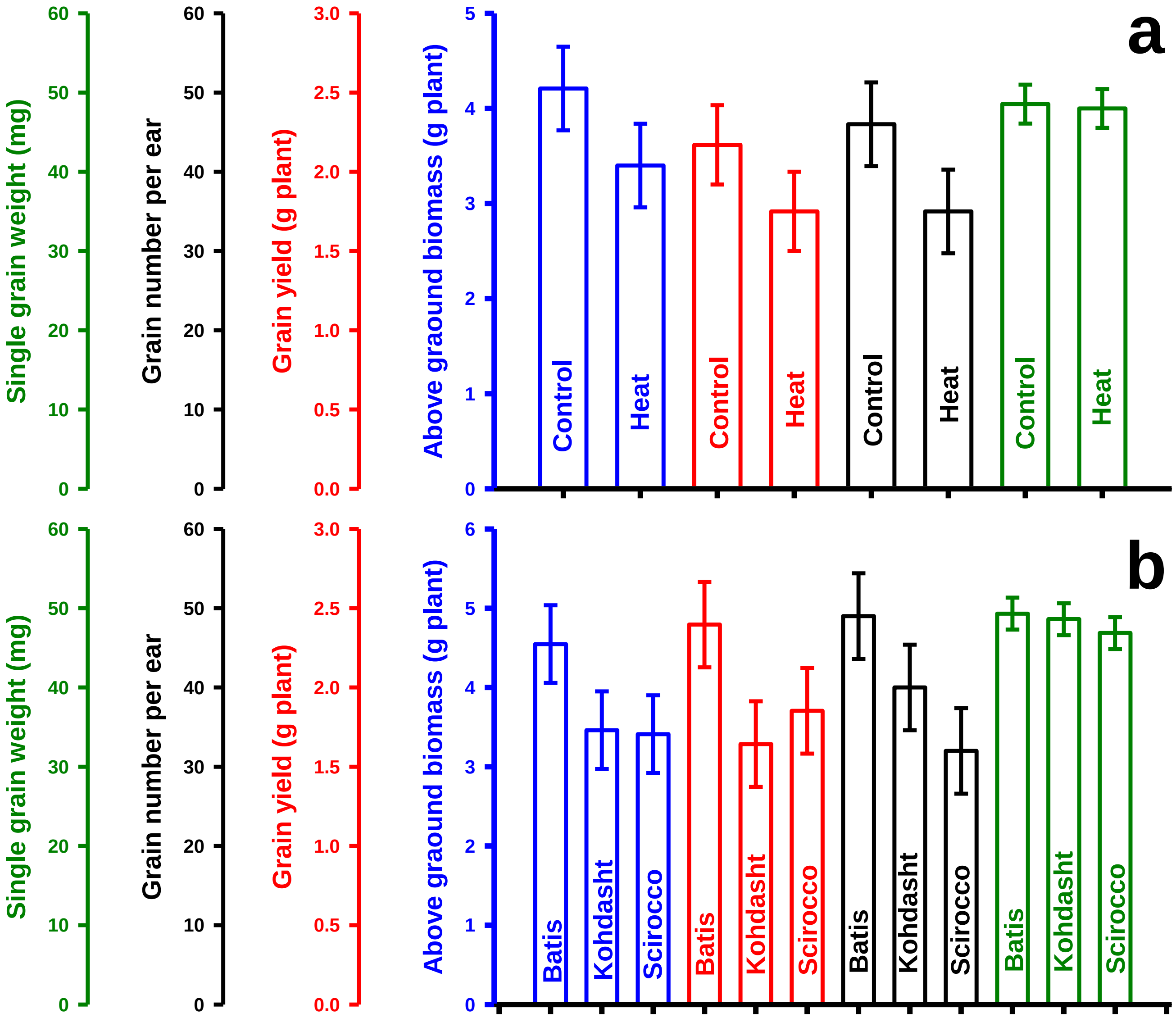

Figure 4.4. Main effects of heat (control and 12000 °C min STT) (a) and cultivar (Batis, Kohdasht and Scirocco) (b) on above ground biomass, grain yield, grain number and single grain weight. The error bars indicate the Fisher protected least significant difference (LSD) for comparing means at P< 0.05 level of significance.

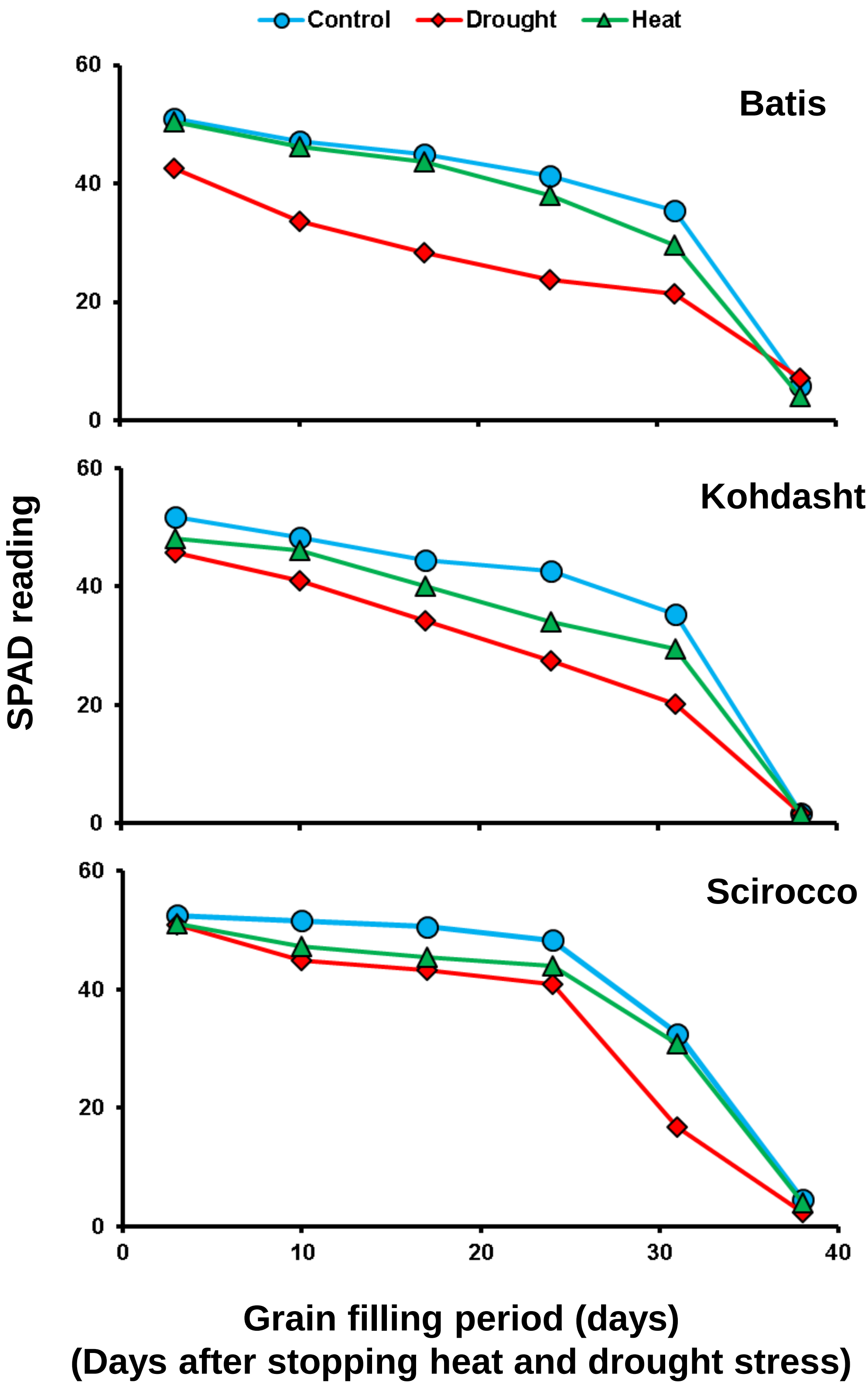

Figure 4.5. SPAD readings during the grain filling period (after stopping heat and drought stress) for the control and the heat and drought treatments for the cultivars Batis (German winter wheat), Scirocco (German spring wheat) and Kohdasht (Iranian spring wheat) in 2015.

We found notable differences in photosynthesis light curves between cultivars and treatments, in particular for mid grain filling (Figure 4.6). At mid anthesis, the photosynthesis rate at the light saturation point was remarkably higher for the heat stress treatment and the cultivar Batis (Figure 4.6). However, the light saturation point was obtained at 500 µmol m^{-2} s^{-1} for all treatments (Figure 6). The photosynthesis rate and light saturation point of Batis detected mid grain filling were considerably lower (250 µmol $m^{-2}s^{-1}$) under drought stress compared to the heat treatment and the control (Figure 4.6).

The photosynthesis light response curves for the spring wheat cultivars Kohdasht and Scirocco were very similar at the mid-anthesis stage (Figure 4.6). In contrast, the net photosynthesis rate of the spring wheat cultivars was higher for the drought and heat treatments under low PPF compared to the control at mid grain filling (Figure 4.6). In addition, the light saturation points were reached at lower light intensities (500 µmol $m^{-2}s^{-1}$) at the mid grain filling stage for the heat and drought treatments of the spring wheat cultivars (Figure 4.6).

4.4. Discussion

4.4.1. Grain yield response to heat stress across the winter and spring wheat cultivars

The results of this study reveal negative effects of heat and drought stress at anthesis on grain yield and yield components, which is in agreement with previous studies. The heat stress (> 31°C) around anthesis significantly reduced the grain number in a range between 30% and 60% (Ferris et al., 1998; Narayanan et al., 2015; Wollenweber et al., 2003; Zhang et al., 2013). We found a significant difference in the response of winter and spring wheat cultivars, especially of Scirocco, to heat stress (Table 4.2). Scirocco is a spring wheat cultivar from Germany and only showed a 9% decline in grain yield under heat stress compared to the control. However, the winter wheat cultivar Batis from Germany showed a remarkably larger decline in grain yield (25%) under a similar level of heat intensity. Higher cell membrane stability and chlorophyll content of the flag leaf (Reynolds et al., 1994; Viswanathan and Renu, 2001), higher nitrogen re-translocation to the flag leaf (Tahir and Nakata, 2005), less damage to thylakoids and lower chlorophyll loss are closely associated with thermo-tolerant cultivars under heat stress (Ristic et al., 2007; Stone and Nicolas, 1995a).

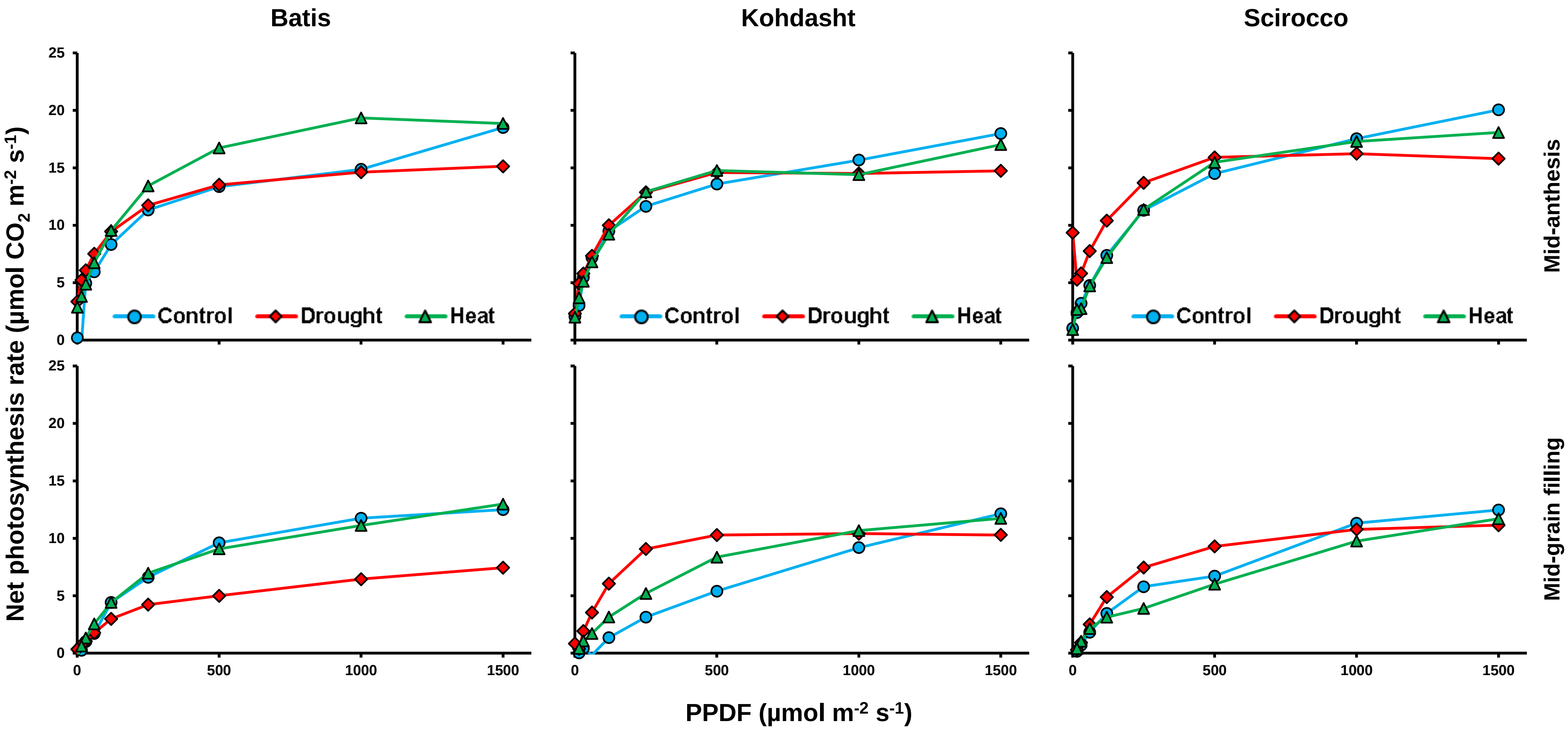

Figure 4.6. Photosynthetic light response curves (the relationship between net photosynthesis rate and photosynthetic photon flux density (PPDF)) of the cultivars Batis (German winter wheat), Scirocco (German spring wheat) and Kohdasht (Iranian spring wheat) for the control and the heat and drought treatments at mid-anthesis and mid-grain filling in 2015.

4.4.2. Effects of heat and drought stress at anthesis on relationships between yield-determinant variables

Application of heat and drought stress at anthesis reduced the grain filling duration by 28% to 13% (5-10 days) depending on the cultivar (Figure 4.3). The length of the grain filling period showed the smallest change in the Scirocco cultivar under heat and drought treatments. This could be also due to the higher single grain weight in the heat stress treatment (Table 4.2) and can be categorized as an adaptation mechanism to heat stress around anthesis. The reduced grain filling duration showed a significant positive relationship with the reduction in grain yield ($R^2 = 0.68^{**}$) under heat stress at anthesis. However, the negative relationship detected between reduction of grain filling duration and grain yield ($R^2 = 0.34^{ns}$) was not significant under drought stress at anthesis (Figure 4.7). The reduction in grain yield was highly dependent on the reduction in grain number at anthesis under both heat ($R^2 = 0.78^{***}$) and drought ($R^2 = 0.24^{*}$) (Figure 4.7). The changed grain filling rate compared to control was not significantly correlated to the change in grain filling duration and grain number (Figure 4.7).There was a significant decline in the length of the grain filling period through heat stress around anthesis especially for a heat-sensitive cultivar (Lizana and Calderini, 2013). The drought stress around anthesis could manipulate the length of the grain filling period (Edmeades et al., 1989) particularly for drought-sensitive wheat cultivars (Moragues et al., 2006).

Based on our results, there was a variability in relationships between grain yield and yield components (grain number and single grain weight) with similar magnitudes of heat stress across the winter and spring cultivars. The Batis (winter wheat) cultivar showed higher grain number and single grain weight compared to the spring wheat cultivars (Kohdasht and Scirocco). However, the reduction rate of grain number and single grain weight was also higher for Batis under heat stress. The reduced grain number due to heat stress at anthesis was compensated for by higher single grain weight (18%) in the spring wheat cultivar Scirocco (Table 4.2). The final grain weight of wheat is controlled by length and rate of grain filling period (Liu et al., 2014). There is a negative association among single grain weight and grain number of wheat under conditions without stress (Dreccer et al., 2009).

Nevertheless, there is an inconsistency in relations between single grain weight and grain number under heat and drought stress for different wheat cultivars. The reduced grain numbers could not increase the grain weight of a winter wheat cultivar under different levels of heat stress (Wheeler et al., 1996). Such a crop response could be related to permanent damage to the photosynthetic apparatus through heat stress (Narayanan et al., 2016; Wollenweber et al., 2003). On the other hand, the reduction in grain number was offset by an increase in single grain weight of a heat- and drought-tolerant spring wheat cultivar (Rajala et al., 2009; Stone and Nicolas, 1995a).

The heat and drought stress at anthesis significantly changed the grain filling and photosynthesis rate under the normal range of radiation intensity (15 µmol $m^{-2}s^{-1}$ to 500 µmol $m^{-2}s^{-1}$) during the grain filling period mainly for the spring wheat cultivars (Table 4.2 and Figure 4.6). The grain filling rate was increased by 11% for Batis under heat stress. However, the increase in the heat stress treatment did not compensate for the reduction in grain number. The grain filling rate of Scirocco and Kohdasht increased by 25% and 40% under heat stress, respectively. The photosynthesis rate in the grain filling period also indicates a larger increment for spring wheat cultivars under drought and heat stress. The grain filling rate could be significantly associated to grain yield under heat stress for heat-tolerant cultivars (Dias and Lidon, 2009; Tewolde et al., 2006). The transcendence in the photosynthesis rate of spring wheat cultivars in the drought and heat stress treatments disappeared under higher radiation intensity (500 µmol $m^{-2}s^{-1}$ to 1500 µmol $m^{-2}s^{-1}$). The effect of heat and drought at anthesis on grain filling and photosynthesis rate at the post-anthesis stage of the spring wheat cultivars could be associated to high-temperature acclimation of those cultivars to high temperature at anthesis. The pre-acclimation to heat stress significantly increases the photosynthesis rate in the post-anthesis period by increasing antioxidant activities (Wang et al., 2011).

4.4.3. Improving modeling of heat and drought stress effects on wheat growth

Crop models are the most widely used tools for evaluating the effects of climate change on crop production (Asseng et al., 2013). However, the growth processes and/or factors influenced by heat and drought stress at anthesis are limited to reduction in radiation use efficiency, grain filling duration, harvest index, net photosynthesis and change in biomass partitioning and stomatal conductance (Asseng et al., 2011; Brisson et al., 2009; Ewert et al., 2002; Holzworth et al., 2014; Jones et al., 2003; Lizaso et al., 2007; Stockle et al., 2014). However, none of the crop models consider the effects of heat and drought stress at anthesis on the duration of the grain filling period (Barlow et al., 2015; Eyshi Rezaei et al., 2015; Jin et al., 2016). The shortening of the crop phenology due to drought stress is only considered in a very limited number of crop models such as CropSyst (Stockle et al., 2014).

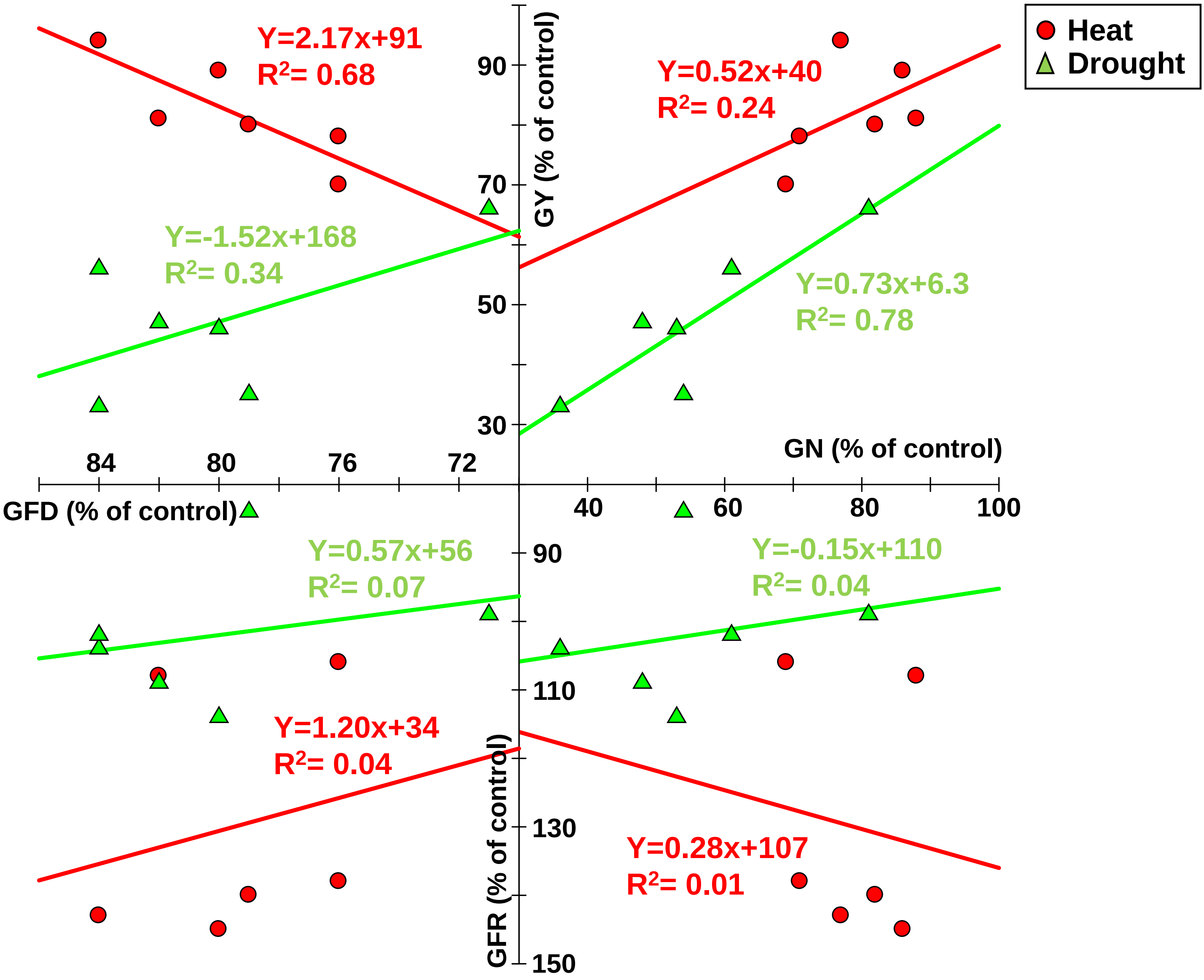

Figure 4.7. The relationships between relative change in grain yield (GY), grain number (GN), grain filling rate (GFR) and grain filling duration (GFD) compared to the control across the two years of study and study treatments.

The direct impact of heat stress on grain number is also only implemented in a few crop models such as MONICA and GLAM by using simple empirical equations (Challinor et al., 2005; Nendel et al., 2011). Nevertheless, there is no compensatory routine in the crop models to reflect the production of larger grains under heat stress at anthesis due to the reduction in grain number that we found in this study. Based on our results, we propose cultivar-specific empirical routines to consider both effects in crop models. We could not apply a dynamic approach to simulate the effect of heat and drought stress at anthesis on the length of the growing period because the involved mechanism for such response is still not clear, and further studies are required.

Chapter 5

General discussion

5.1. Main findings

This chapter we summarize the results of the studies presented in chapter 2 to 4. As mentioned before the purpose of this dissertation is to address the effect of drought in interaction with high temperatures on wheat before and during anthesis. The findings of this study can help scientists and farmers to obtain higher productivity rates in the presence of heat and drought stressors. The first section of the current chapter reviewed the findings related to our investigation triggered by the research questions from chapter 1. We present the results of our assessments about the effects of drought and/or heat stressors on wheat before anthesis in section 2 and in the subsequent section, on wheat during anthesis. Exploiting experimental data equipped us with a method by which we quantified the response of wheat to drought, heat, and their combination around anthesis. In section 5.4 we reviewed our discovery which explains how the heat and drought stressors during anthesis can affect the rate and duration of grain filling. This chapter discusses the main results of each study presented in chapters 2 to 4. The aim of this study is to better understand of the drought stress effect around anthesis on wheat in interaction with high temperatures. The findings can help scientists and farmers to better quantify and reduce the detrimental effects of heat and drought stressors on wheat productivity. Finally, sections 5.5 and 5.6 include final conclusions and suggestions for future research.

Heat and drought stress were categorized as a yield reduction factor in the introduction of this thesis. Crops respond differently to heat and drought stress during different growth and developmental stages, but they are most susceptible during the key reproductive stages, i.e., gametogenesis and flowering (Prasad et al., 2017). As mentioned in chapter 2 heat and drought stress can reduce grain number and grain weight so heat and drought stresses can be classified as reduction factors. In response to Q1, the importance of detrimental effect of combined heat and drought stress on wheat was highlighted. Furthermore, the detrimental effects of heat and/or drought stressors are related to crop sensitivity, and duration and intensity of stress exposure (Chapter 2). In answering Q2 it was found that differences in the experimental setup of heat stress experiments substantially influence the crop response to heat stress (Chapter 3). In response to Q3, the results of the related study showed that the impact of stress at anthesis on the subsequent grain filling period differs between heat and drought (Chapter 4).

5.2. High temperature and drought stress around anthesis among different crops

Heat, drought, and their combination combined heat and drought around anthesis significantly affected growth and yield-related traits particularly grain number and grain yield across the tested wheat cultivars (Chapter 2).Wheat, one of the most important crops worldwide, adapts to a wide range of ecological conditions. However, heat and drought stress have reduction effects on yield, especially when associated heat and drought simultaneously (Balla et al., 2019). Simultaneously, occurrence of drought stress and high temperatures is frequently reported under field conditions. Severe drought stress causes increased tissue temperature which has negative effects on the floral organs (Prasad et al., 2017). Under tropical conditions, increased floral organ temperature during flowering stage which in early noon has detrimental effects (Jagadish et al., 2011; Prasad et al., 2017). Therefore, it is essential to intensify research on the effects of combined heat and drought stress in wheat.

The flowering stage of wheat is the most sensitive developing stage to heat and drought stress (Balla et al., 2019) because both meiosis and pollen growth are negatively affected. Interestingly, among different field crops including wheat, sorghum, peanut and rice with different growth habits and flowering patterns, there is a narrow window of extreme sensitivity ranging between 5 and 9 days before anthesis (coinciding with gametogenesis) and more so during anthesis (Figure 5.1) (Prasad et al., 2017). Complex interactions between the timing of phenological stages and the sensitivity of different growth phases to the environment affect the final yield (Balla et al., 2019). The detrimental effects of heat and drought depend on the magnitude, timing and the duration of the stress. Higher temperatures accelerate the onset of anthesis, with the consequence that there are fewer spikelets per spike. Additionally, high temperature during anthesis reduces pollen fertility or sterile grains due to the negative effect of heat (>30˚C) on pollen viability, leading to poor fertilisation, abnormal ovary development, slower pollen growth and thus a reduction in seed setting (Balla et al., 2019). Therefore, heat and drought stress around anthesis considerably decreases grain number and grain weight in wheat, and consequently has a negative effect on grain yield (Semenov et al., 2014; Eyshi Rezaei, 2016). Based on the results of the current thesis, heat and/or drought stress around anthesis should be considered as the most abiotic stress, which represent a greater danger to wheat production.

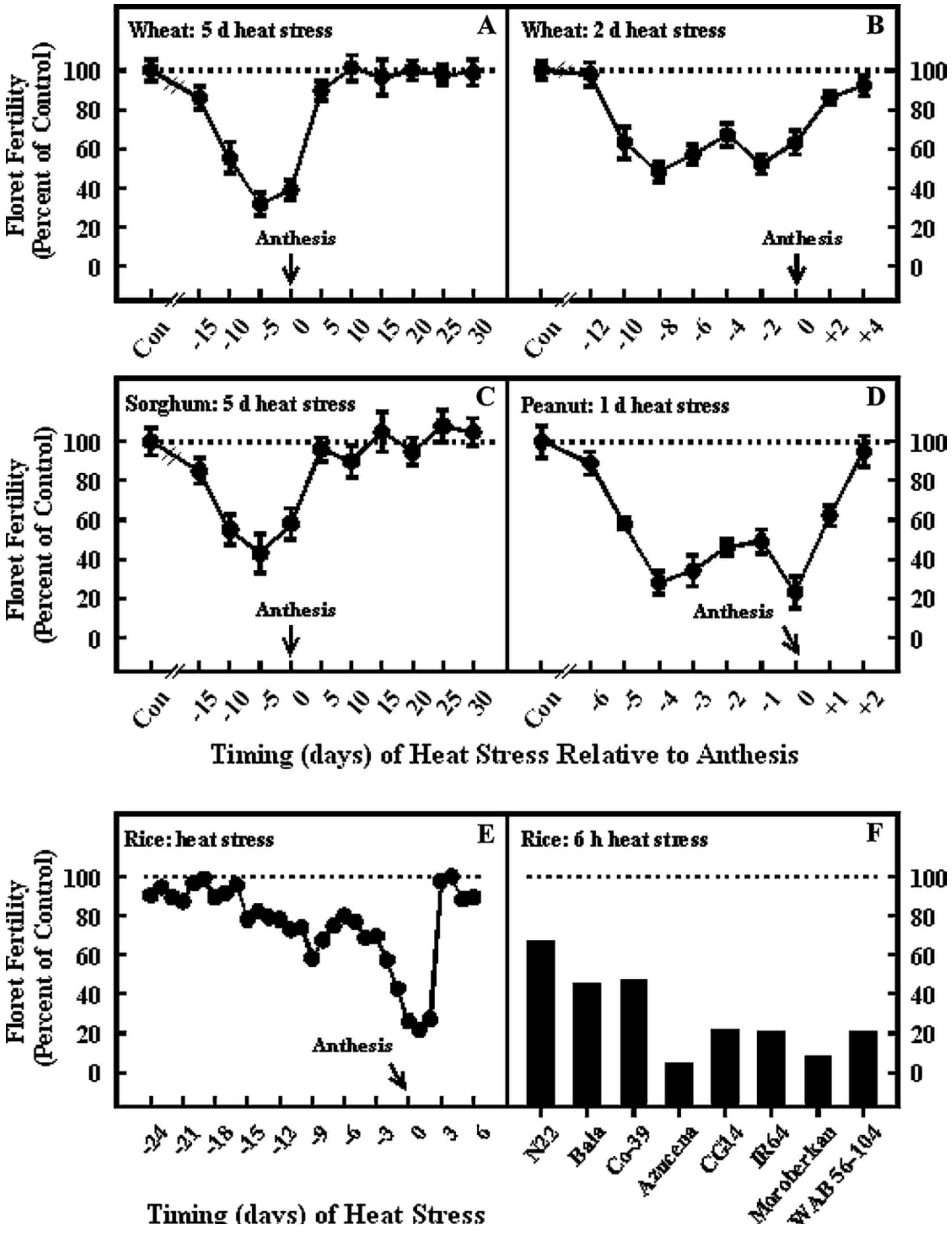

Figure5.1. Comparative assessment of the magnitude of sensitivity on a developmental timescale in wheat, sorghum, peanut, and rice to heat stress coinciding with reproductive stages (gametogenesis and flowering). Daily optimum (control) and heat stress treatments for wheat were 20˚C (day/night: 25˚/15˚C) and 31˚C (36˚/26˚C) for 5 d (A) and 2 d. The corresponding values for sorghum were 25˚C (30˚/20˚C) and 31˚C (36˚/26˚C), respectively, for 5 d; for peanut were 25˚C (28˚/22˚C) and 31˚C (40˚/22˚C) for 1 d; for rice were 30˚C and 35˚C; and for genotypic responses for rice were 30˚C (30˚/24˚C) and 38˚C (38˚C/24˚C) for 6 h during anthesis" (Prasad et al., 2017, p116).

5.3. Appropriate different experimental heat stress methods on wheat productivity

Heat stress in each development stage, i.e., vegetative, reproductive, and the grain filling, shows distinct differences in the harvest index (HI) and hence on grain yield. The heat stress effects depend on the magnitude, timing, and the duration of the stress. Heat stress around anthesis on wheat significantly reduces grain number per ear because of pollen abortion and sterile grains (Eyshi Rezaei et al., 2018). On the other hand, Porter and Gawith (1999) reported that in the period around anthesis, the maximum temperature that wheat can tolerate without a reduction in grain number is 31˚C. This period is often defined as lasting from approximately 20 days before anthesis to 10 days after anthesis (Balla et al., 2019). Wollenweber et al., (2003) showed that grain yield was reduced to 20% at a stress thermal time (STT) of 12000 °C min at ambient air temperature (Tair) above 31 °C. Also, the same result was reported by Zhang et al., (2010) that to 95% at stress thermal time of 14400 °C min at ambient air temperature above 31 °C (Eyshi Rezaei et al., 2018). Thus, it is necessary to know the effects of heat stress around flowering at a different stress thermal time (STT) at ambient air temperature (Tair) above 31 °C.

Surprisingly, the reproductive organs of cereals such as wheat and rice placed above or at the canopy and directly exposed to the heat load from the sun have low thresholds before the HI starts to drop significantly. Other crops like peanut and soybean have much higher thresholds compared to rice in response to high temperatures in the flowering period. This could be due to the sensitive processes during flowering when protected from direct heat from the sun by the foliage canopy. Also, the canopy transpiration cooling can reduce floral tissue temperature leading to normal fertilization despite extreme high air temperatures (Prasad et al., 2017). On the other hand, canopy temperature has a considerable effect on grain yield in heat stress conditions (Craufurd et al., 2013; Siebert et al., 2014). The difference between air and canopy temperature due to soil water status, time of the day, and transpiration rate could reach up to 7 °C (Ferrise et al., 2011; Siebert et al., 2014; Eyshi Rezaei, 2016). In addition, canopy temperature depends on soil water status and stomatal conductance and must therefore be considered in heat and drought conditions in crop models (Eyshi Rezaei, 2016).

Additionally, Reynolds et al., (1994) demonstrated that soil water status and transpiration rate have a key role in control of the temperature of crops under heat stress (Eyshi Rezaei et al., 2018). Therefore, the author of current thesis has used sandy soil with low soil water content in the Bonn experiment to distinguish the soil substrate effect in heat stress condition. Also, it was identified that some amount of unintended drought stress as shown in this experiment. But not for those wheat plants grown on a soil with high soil water holding capacity in other experiments with the same amount of a temperature sum above 31°C (stress thermal time) (Chapter 3). Thus, it could have limited the transpiration (Jones and Tardieu, 1998; Passioura et al., 2006; Eyshi Rezaei et al., 2018). In addition, we found that, by inducing Heat stress at heading period, there was a mild drought stress led to more plant water requirements in this period at Bonn. Consequently, increase of the ear and leaf temperature should have resulted in an additional reduction of the grain yield due to the direct effects of drought stress on grain number and yield (Eyshi Rezaei et al., 2018).

Most of the heat studies on cereals were carried out in growth chambers (pot experiments), which can hardly reproduce field conditions (Eyshi Rezaei, 2016). The main problem of these studies is due to restricted development of the root system in pot conditions in comparison with field conditions. The temperature gradient tunnel experiment was implemented to overcome root restriction issues. However, incident radiation from the tunnel’s polyethylene cover may decrease crop yield (Kittas et al., 1999). Kimball (2011) reported that the best procedure to mimic field conditions under heat stress is the T-FACE system. Nevertheless, this procedure needs to have both an extensive knowledge and a high energy supply (Eyshi Rezaei, 2016). The results of the current thesis show that it is essential to consider differences in the temperature measurement point and, in the method, used to measure temperature when interpreting the response of crops to heat stress (Chapter 3). Additionally, as was discussed in the Chapter 3 of current thesis that using infrared heaters was claimed to be the best method to reproduce natural heating effects because of minimizing the canopy disturbance under well-watered conditions (Kimball, 2011; Eyshi Rezaei et al., 2018). Surprisingly, in the Bonn experiment, the effect of combined heat and drought stress was investigated by the author of this thesis to compare the impact of the heating method on the heat stress and combined heat and drought sensitivity between growth chamber and infrared heaters procedures (Chapter 3). Therefore, as mentioned before, the experimental setup including method of heating, soil substrate and temperature measurement point have considerable impacts on the magnitude of heat effect on grain yield of winter wheat.

5.4. Influence of heat and drought stress at anthesis on post growing season

In this dissertation we show that during anthesis, heat and drought stress affects the rate and length of the grain filing period. This happens because under heat and drought stress the source-sink relation can be changed. Nevertheless, when average temperature increases it accelerates the development rate which ends up in shorter growing season (Asseng et al., 2015). Heat stress can shorten the grain filling period by 3-12 days, while other authors demonstrated that high temperatures reduced the grain-filling period by 45–60% (Balla et al., 2019).The grain yield of wheat is predominantly accounted for by the concurrent photosynthesis during grain filling. The quantity of the concurrent photosynthesis depends on the performance of photosynthesis in the leaves (Zhang et al., 2016). Drought stress before anthesis was found to accelerate leaf senescence. It has a detrimental effect on assimilation in the grain filling period and reduces the grain filling rate and consequently also decreases single grain weight due to the shorter grain filling period. Heat stress during anthesis and grain filling accelerates the degradation of the leaf chlorophyll content. Also, it led to an increase in the rate of leaf senescence, resulting in a decrease in both leaf photosynthetic activity and in final biomass (Balla et al., 2019).The wheat grain weight is determined by the rate and duration of the grain filling period (Liu et al., 2014). However, in contrast, grain filling rate enhances by heat stress before anthesis and has a compensation effect on single grain weight. This means that this increased grain filling rate compensates the decreased grain filling duration. Thus, heat stress has no significant effect on grain weight (Zhang et al., 2016). Additionally, Dreccer et al., (2009) reported a negative relationship between grain number and individual grain weight of wheat at non-stress conditions (Eyshi Rezaei et al., 2018). Heat stress at anthesis reduced grain number but did not consequently increase the single grain weight, likely due to heat stress damage of photosynthetic apparatus. However, Stone and Nicolas, (1995) reported that the single grain weight significantly increased if grain number was reduced under heat stress conditions (Eyshi Rezaei et al., 2018). However, it is concluded in chapter 4 that the findings show that the impact of stress at anthesis on the subsequent grain filling period differs between heat and drought.

5.5. Conclusions

After having systematically addressed the effects of heat and/or drought stress around anthesis the following general conclusions can be drawn:

1. There exists a need for further experiments to enhance an understanding of the effects of combined heat and drought stress on reproductive processes of wheat that exacerbate

negative effects on yield and component yield. The results presented here that came from control environment conditions. Du to quantifying interactions of high temperature and drought under field conditions, we affirm that the results under field condition may be different. Hence, further research using by new facilities like automated rain-out shelters, heat tents is ensured and may demonstrate useful.

2. To better understanding impacts of the experimental setup including method of heating, soil substrate, and temperature measurement point on grain yield under heat stress is necessary to more investigate.
3. Grain filling rate and period is affected by heat and drought stress around anthesis, which could be related to change in source-sink relations under heat and drought stress. Also, there exists the link between effects of heat and drought stress on before and after anthesis, thus we have to more take care on these developing stages.

5.6. Outlook

The results of this study provide better understanding of the effects of heat and drought on wheat production across different experimental setups. This study helps scientist and farmers to design effective adaptation strategies to cope with climate change. Therefore, breeders and agronomists must try to generate crops with early morning flowering to avoid negative combined abiotic stress effects on sterility.

Furthermore, agricultural productivity is threatened not only by the short duration day maximum temperature affecting reproductive sterility even by high night temperatures in long temperature stress period. Therefore, it is necessary to carry out heat stress studies to better understand the effects of high night temperatures on spikelet sterility leading to yield losses. In addition, the further research is necessary to understand the physiological mechanisms involved in post-anthesis response of crops to the occurrence of extreme heat and drought periods at the anthesis stage. It is also fundamental to implement the new routines in crop models to account for the effect of heat stress and drought stress around anthesis on the duration of the grain filling period in climate change impact assessment.

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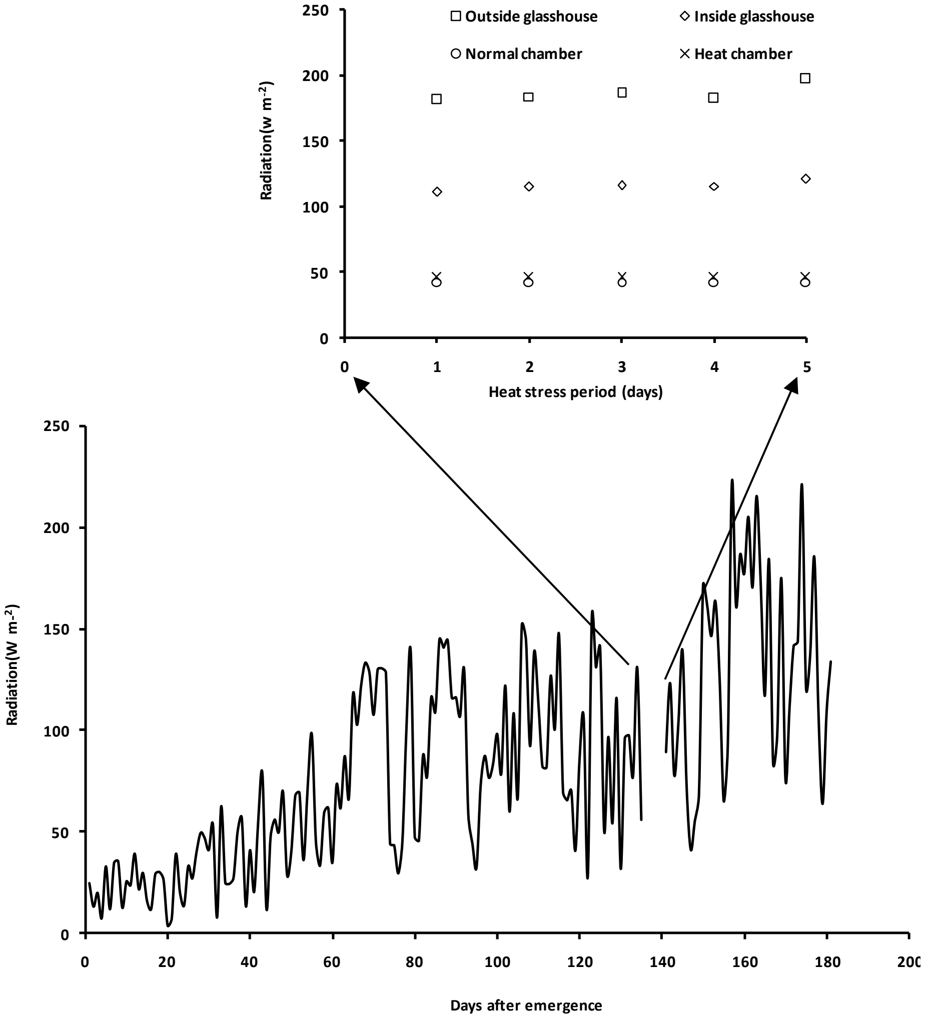
Supplementary material for chapter 2

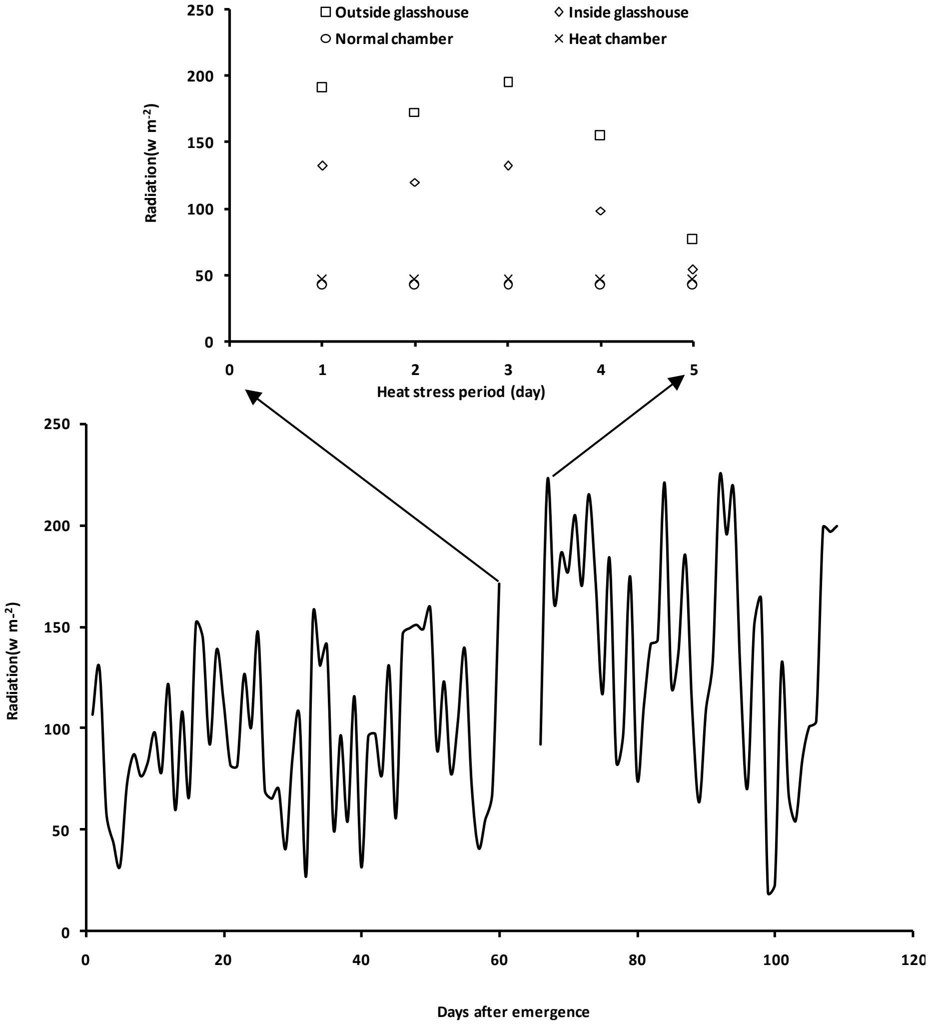
Table S2.1. Test of the significance of differences in grain number, single grain weight, fertile spikelet number, grain yield, straw yield and harvest index caused by cultivars (CU), heat stress around anthesis (H), drought (D), combined heat and drought (H+D) as well as interactions between cultivars and the stressors performed by an analysis of variance. The crop specific measurements refer to the main stems only, results are shown for pot set 1 (pots moved to the glasshouse for the heat treatment).

Source of variation	Grain number	Single grain weight (mg)	Fertile spikelet number	Grain yield (g)	Straw yield (g)	Harvest index (%)
CU	ns	*	ns	ns	***	ns
H	***	ns	ns	***	***	ns
D	***	***	***	***	***	***
H+D	***	***	***	***	***	***
CU×H	ns	ns	ns	ns	ns	ns
CU×D	*	ns	ns	ns	ns	ns
CU×(H+D)	***	***	***	***	*	*

(ns: non-significant. ***, **, * significant at P ≤ 0.001, 0.01 and 0.05, respectively)


□ Outside glasshouse
◇ Inside glasshouse
○ Normal chamber
× Heat chamber
Radiation(w m^{-2})
Heat stress period (days)
0
1
2
3
4
5
0
50
100
150
200
250
Radiation(W m^{-2})
Days after emergence
0
20
40
60
80
100
120
140
160
180
200

CU$_B$


□ Outside glasshouse
◇ Inside glasshouse
○ Normal chamber
× Heat chamber
Radiation(w m^{-2})
Heat stress period (day)
0
1
2
3
4
5
0
50
100
150
200
250
Radiation(w m^{-2})
Days after emergence
0
20
40
60
80
100
120

CU$_{K,S}$

Figure S2.1. Radiation in the growing season with particular reference to the heating period (inside and outside glasshouse, normal and heat chamber) for the cultivars Batis (CU_B), Kohdasht (CU_K) and Scirocco (CU_S).

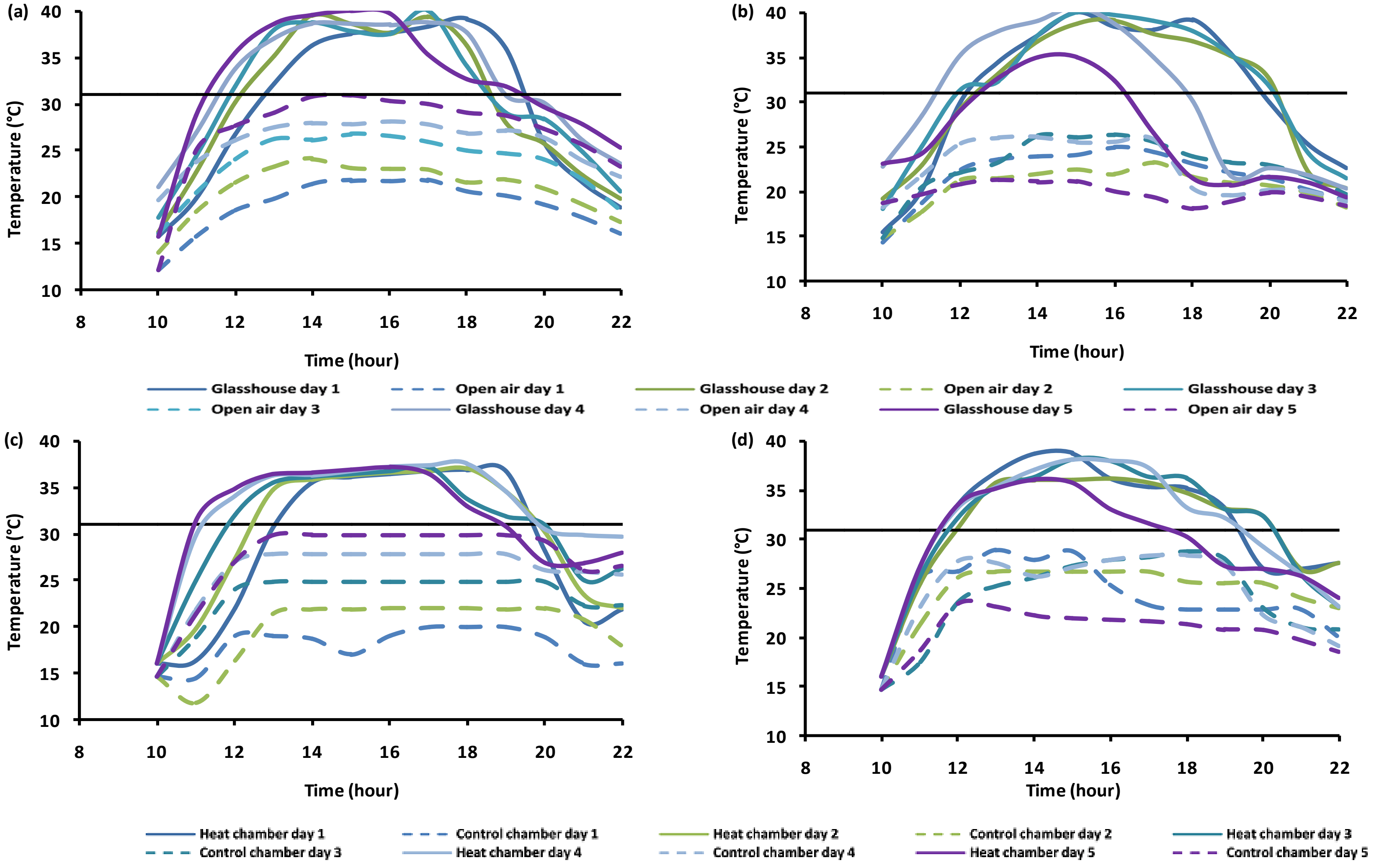
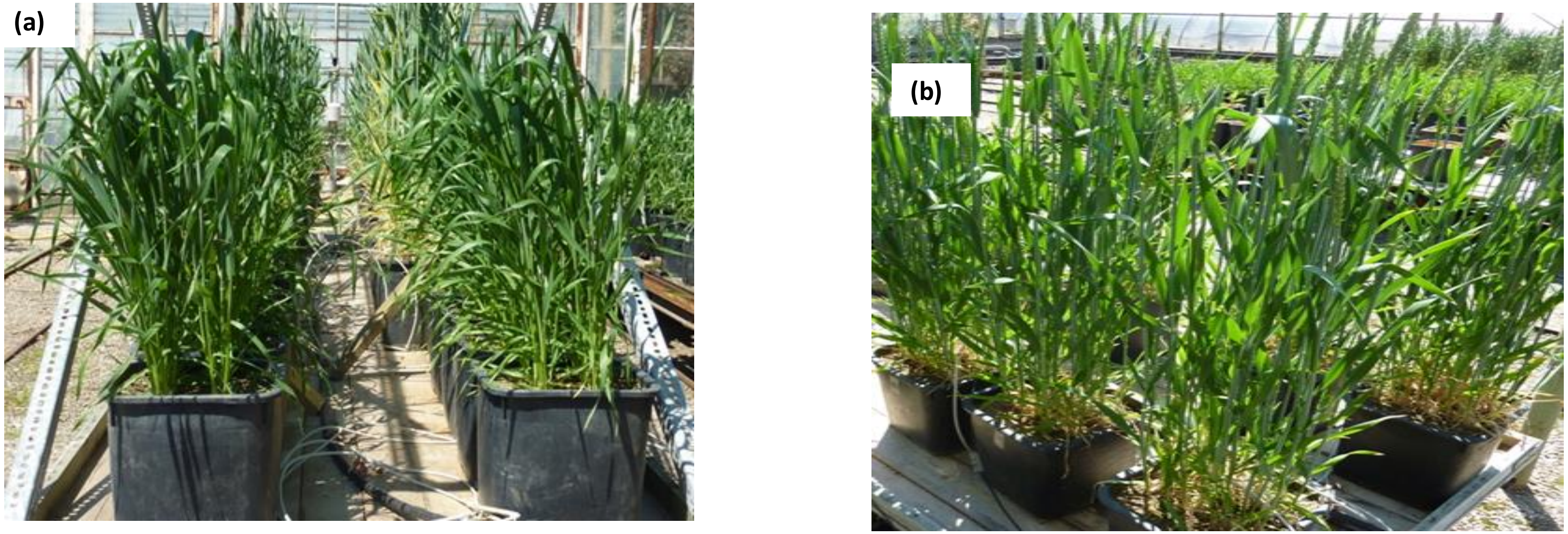
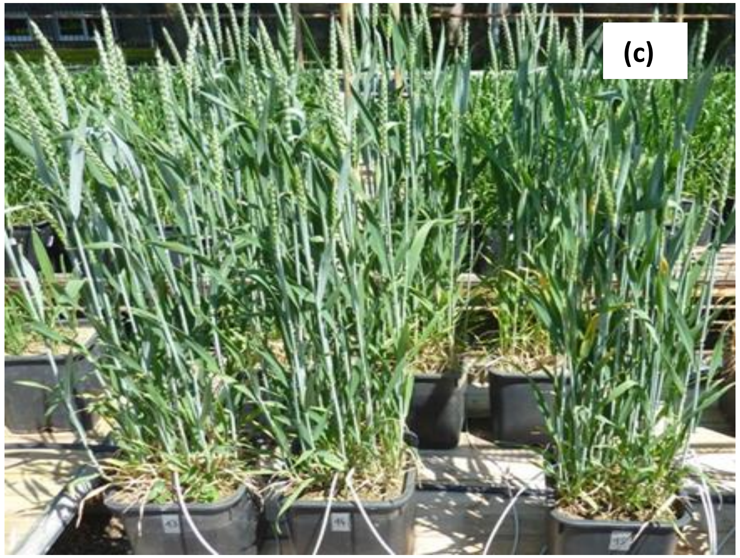


Figure S2.2. Hourly temperature during the heat stress period inside the glasshouse and in the open air for pot set 1 (a, b) and in the growth chambers for pot set 2 (c, d) for *cv*. Batis (a, c) and *cv*. Kohdasht and *cv*. Scirocco (b, d). The black line indicates the threshold temperature for heat stress (31 °C) used in the calculations of stress thermal time.




(c)


(d)


(e)


(f)


(g)

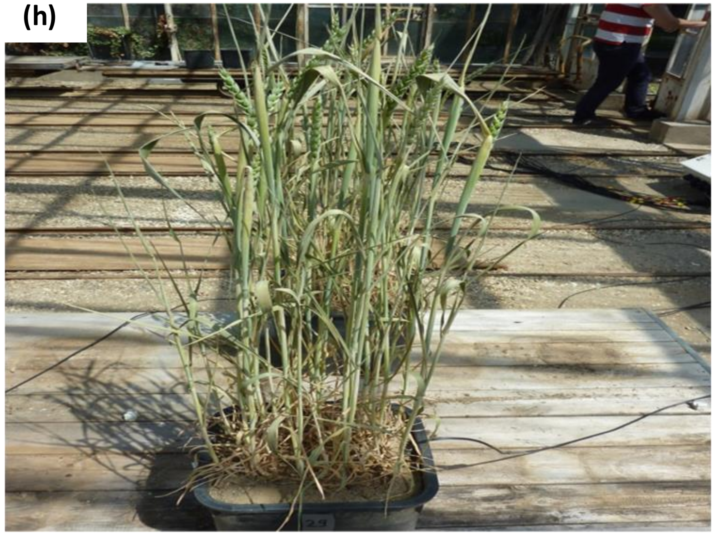
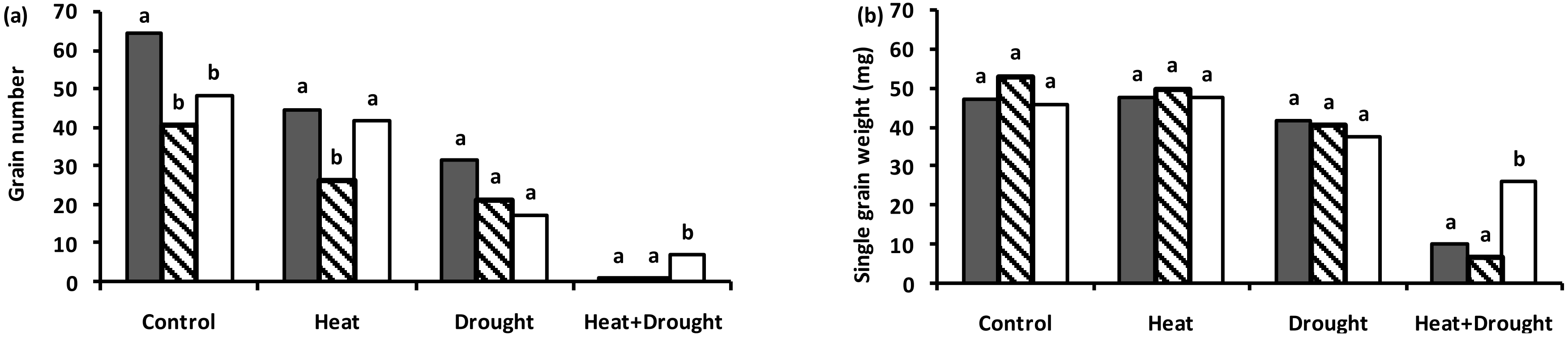

(h)

Figure S2.3. Photographs showing the pots belonging to the control (a, b), to the heat stress treatment (c, d), to the drought stress treatment (e, f), and to the combined heat + drought treatment (g, h) before the application of the stress (a, c, e, g) and immediately after the heat stress period (b, d, f, h).



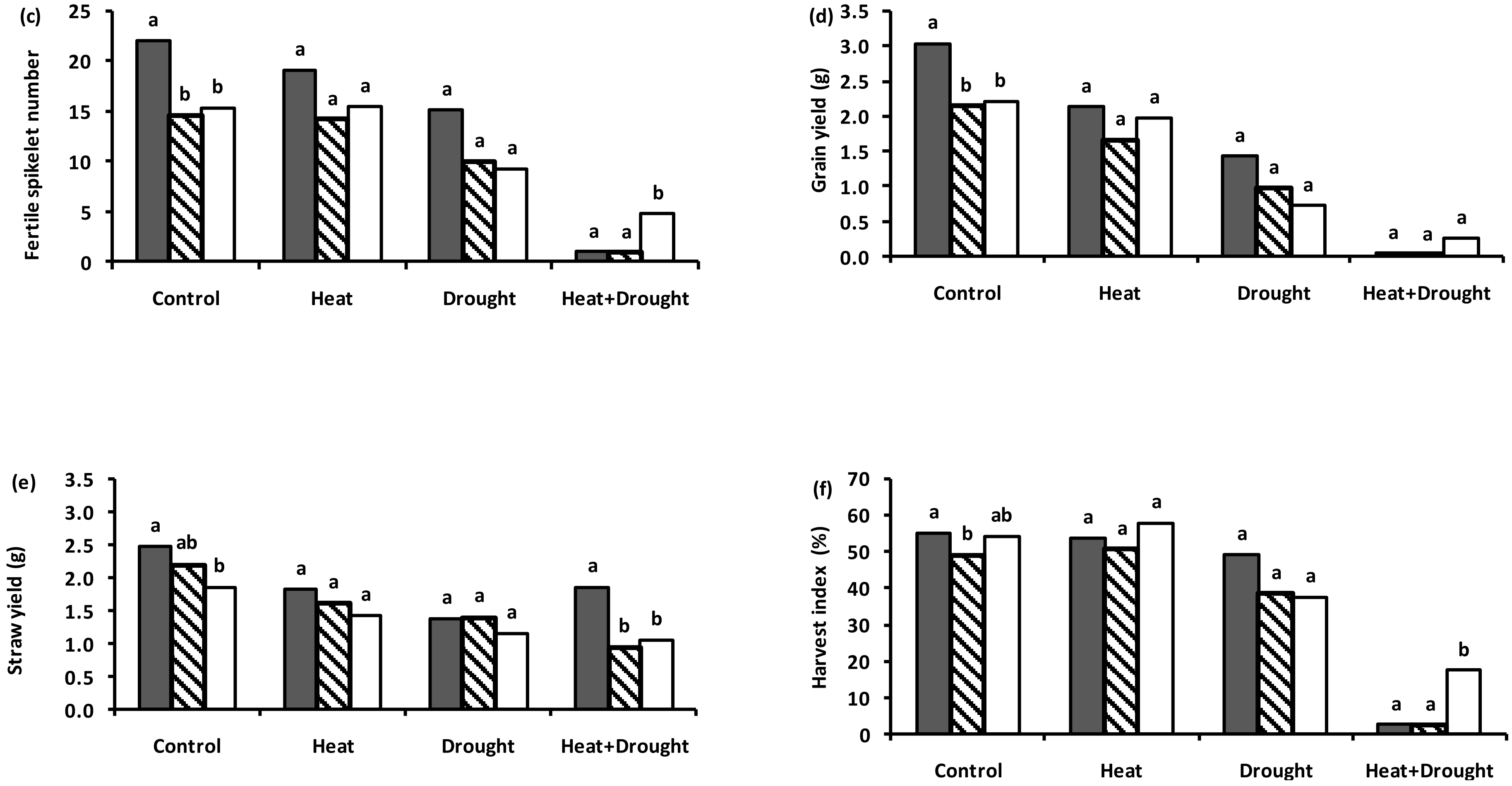


Figure S2.4. Effects of heat, drought, and combined heat and drought stress on grain number (a), single grain weight (b), fertile spikelet number (c), grain yield (d), straw yield (e) and harvest index (f) per main stem. Filled, dark downward diagonal and open columns indicate winter wheat (Batis) and two spring wheat cultivars (Kohdasht and Scirocco), respectively. Data belong to pot set 1 (heat treatment in the glasshouse) and LSMEANS estimates with same letters within a cultivar are not significantly different at *P* = 0.05.

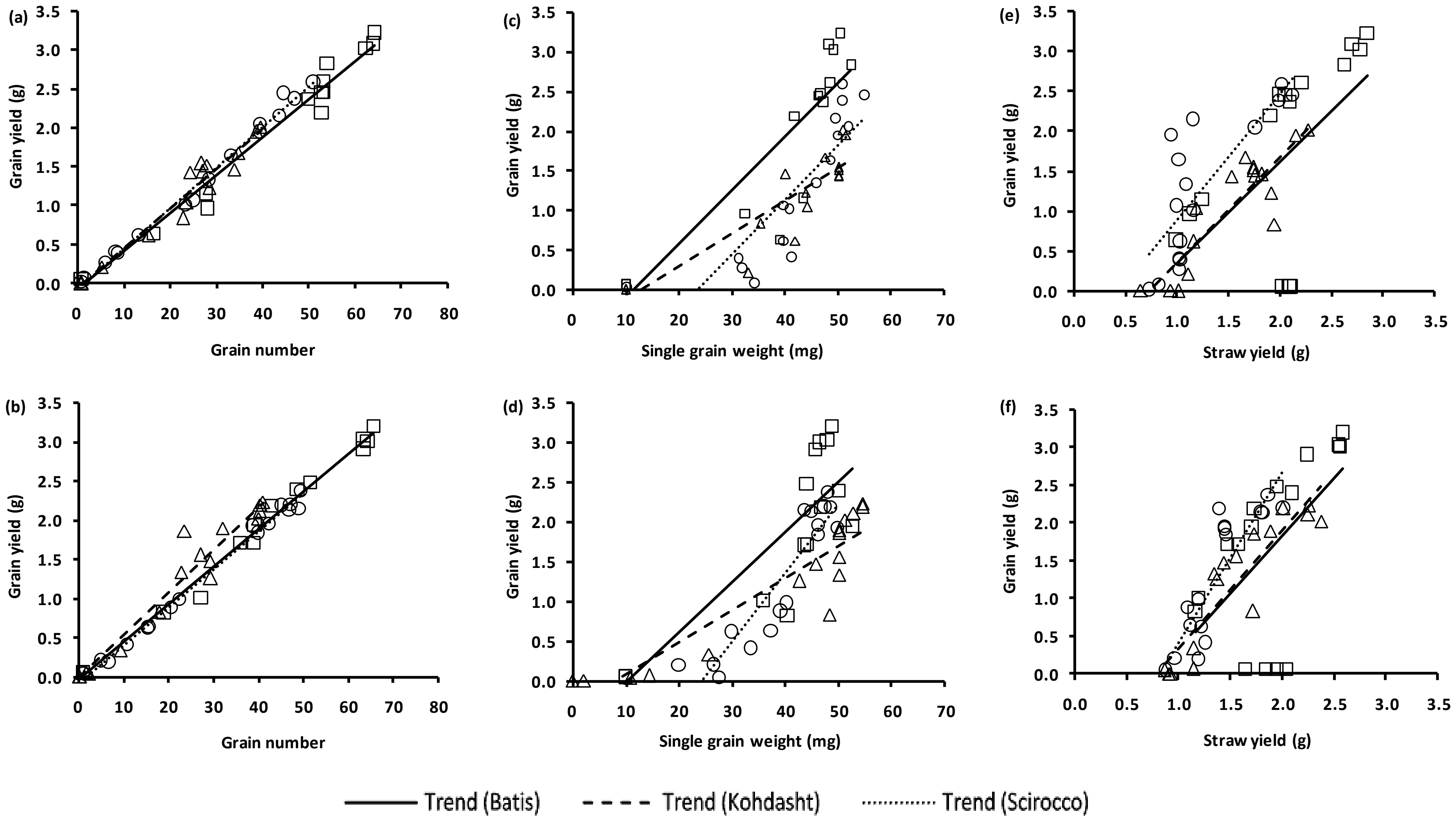
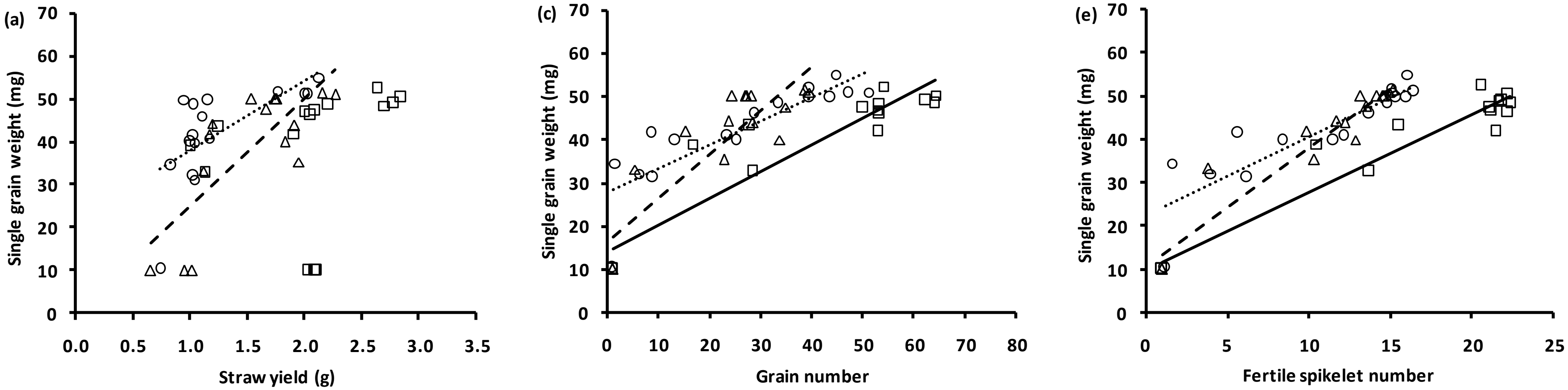

(a)
Grain yield (g)
Grain number
(b)
Grain yield (g)
Grain number
(c)
Grain yield (g)
Single grain weight (mg)
(d)
Grain yield (g)
Single grain weight (mg)
(e)
Grain yield (g)
Straw yield (g)
(f)
Grain yield (g)
Straw yield (g)
Trend (Batis)
Trend (Kohdasht)
Trend (Scirocco)

Figure S2.5. Grain yield plotted against grain number, single grain weight and straw yield between cultivars (square, triangle and circle indicate Batis, Kohdasht and Scirocco cultivars, respectively). Observations for control and the treatments heat, drought and combined heat + drought were pooled, data are shown for pot set 2 with the heat treatment in growth chambers (a, c, e) and pot set 1 with the heat treatment in the glasshouse (b, d, f); linear trends are shown for relationships when are significant p<0.05.



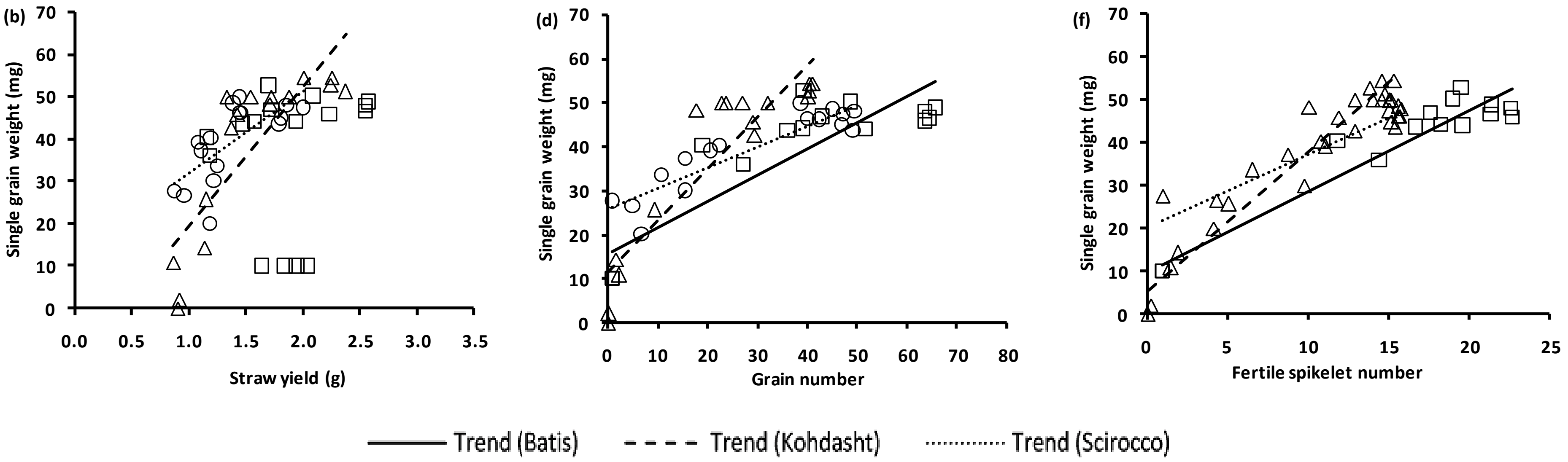


Figure S2.6. Single grain weight plotted against straw yield, grain number and fertile spikelet number between cultivars (square, triangle and circle indicate Batis, Kohdasht and Scirocco cultivars, respectively). Observations for control and the treatments heat, drought and combined heat + drought were pooled, data are shown for pot set 2 with the heat treatment in growth chambers (a, c, e) and pot set 1 with the heat treatment in the glasshouse (b, d, f); linear trends are shown for relationships when are significant $p<0.05$.

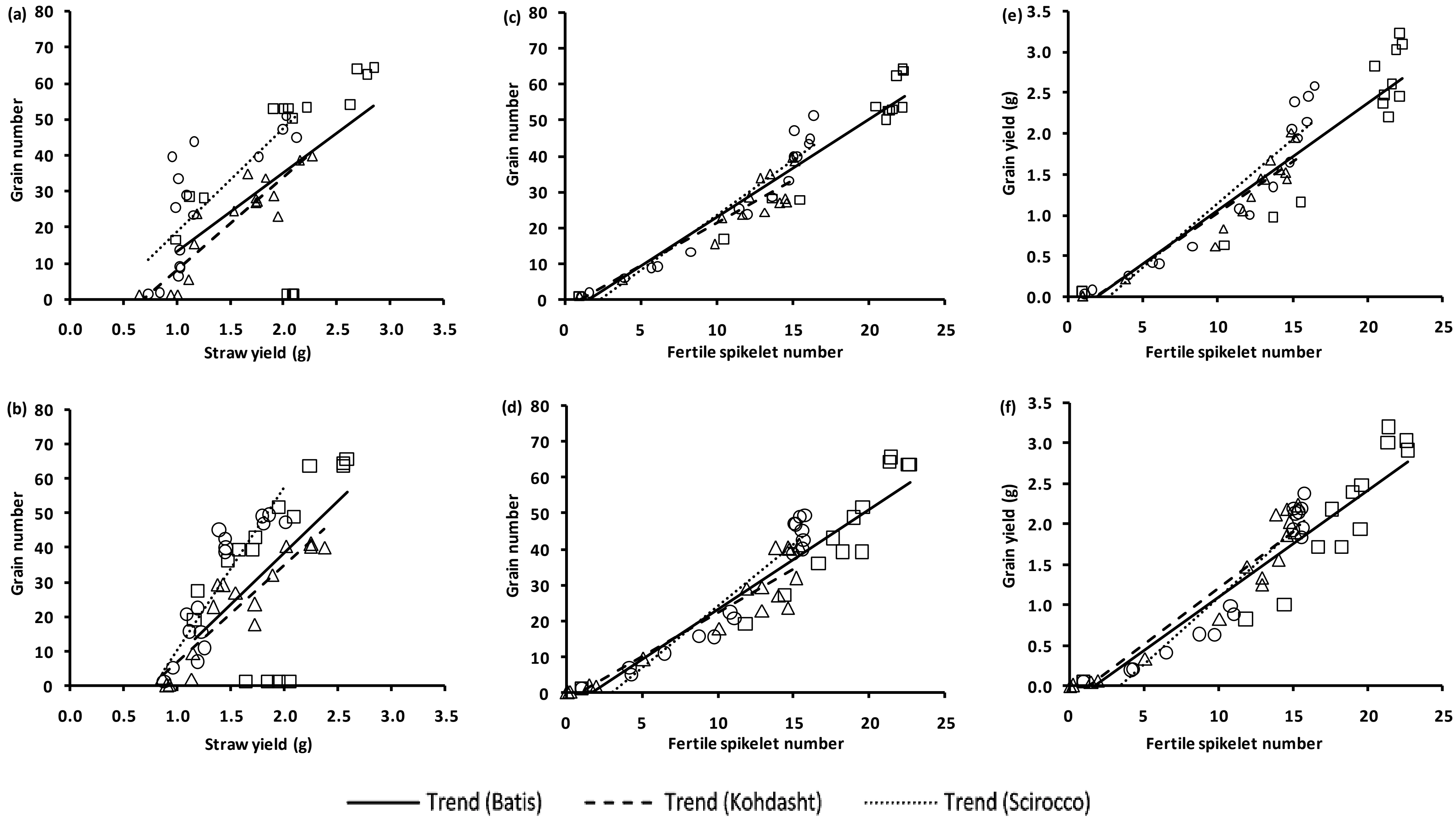

(a)
Grain number
Straw yield (g)
(c)
Grain number
Fertile spikelet number
(e)
Grain yield (g)
Fertile spikelet number
(b)
Grain number
Straw yield (g)
(d)
Grain number
Fertile spikelet number
(f)
Grain yield (g)
Fertile spikelet number
0 10 20 30 40 50 60 70 80
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
0 5 10 15 20 25
Trend (Batis)
Trend (Kohdasht)
Trend (Scirocco)

Figure S2.7. Grain number plotted against straw yield and fertile spikelet number, and grain yield plotted against fertile spikelet number between cultivars (square, triangle and circle indicate Batis, Kohdasht and Scirocco cultivars, respectively). Observations for control and the treatments heat, drought and combined heat + drought were pooled, data are shown for pot set 2 with the heat treatment in growth chambers (a, c, e) and pot set 1 with the heat treatment in the glasshouse (b, d, f); linear trends are shown for relationships when are significant $p<0.05$.

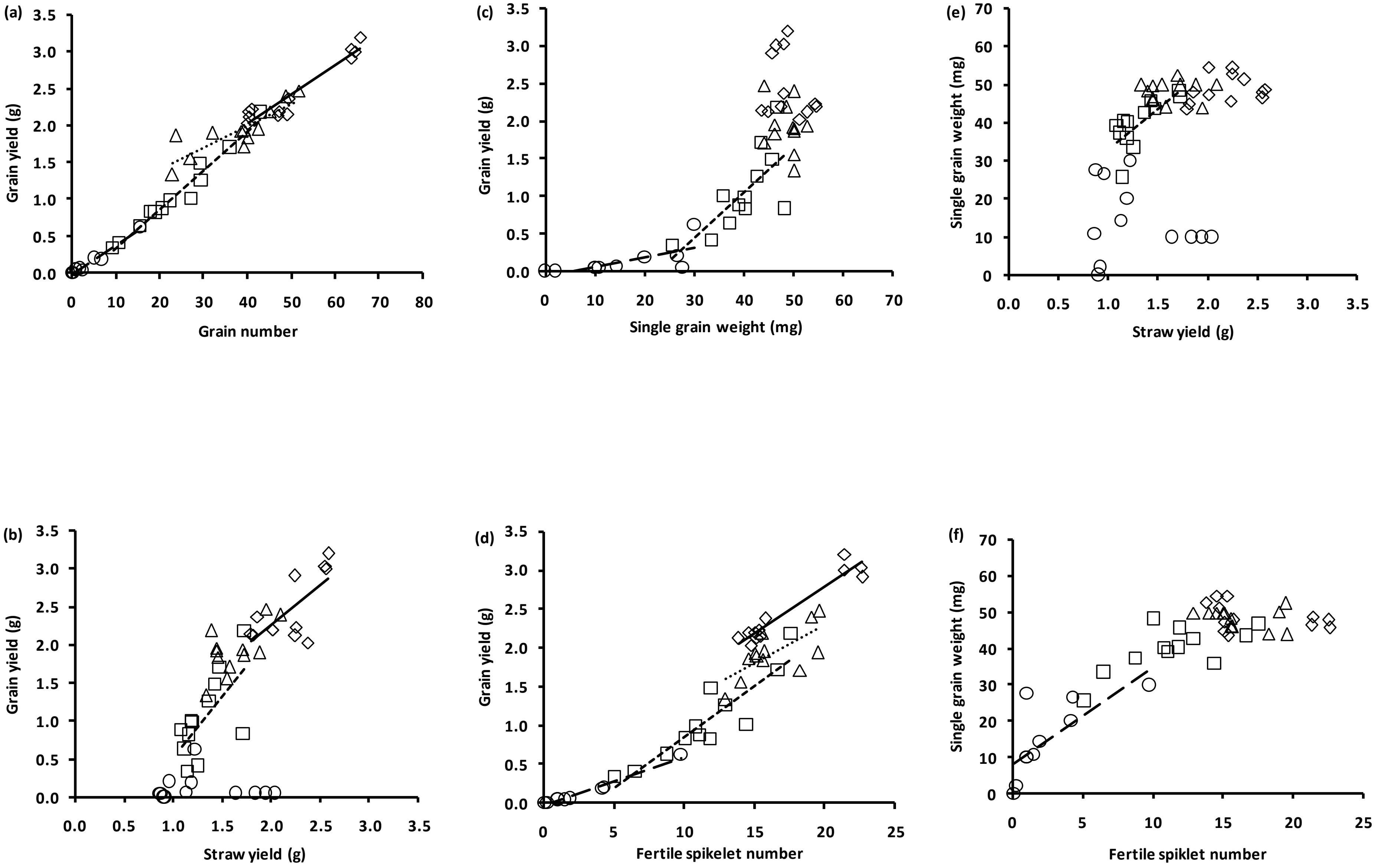

(a)
Grain yield (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
Grain number
(c)
Grain yield (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
Single grain weight (mg)
(e)
Single grain weight (mg)
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Straw yield (g)
(b)
Grain yield (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Straw yield (g)
(d)
Grain yield (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Fertile spikelet number
(f)
Single grain weight (mg)
70
60
50
40
30
20
10
0
0
5
10
15
20
25
Fertile spiklet number

Figure S2.8. Grain yield plotted against grain number (a), straw yield (b), single grain weight (c) and fertile spikelet number (d) and single grain weight plotted against straw yield (e) and fertile spikelet number (f) per main stem. Diamond, triangle, square and circle indicate control, heat, drought and combined heat and drought treatments, respectively. Data are shown for pot set 1 (heat treatment in the glasshouse), linear trends are shown for relationships when are significant p<0.05.

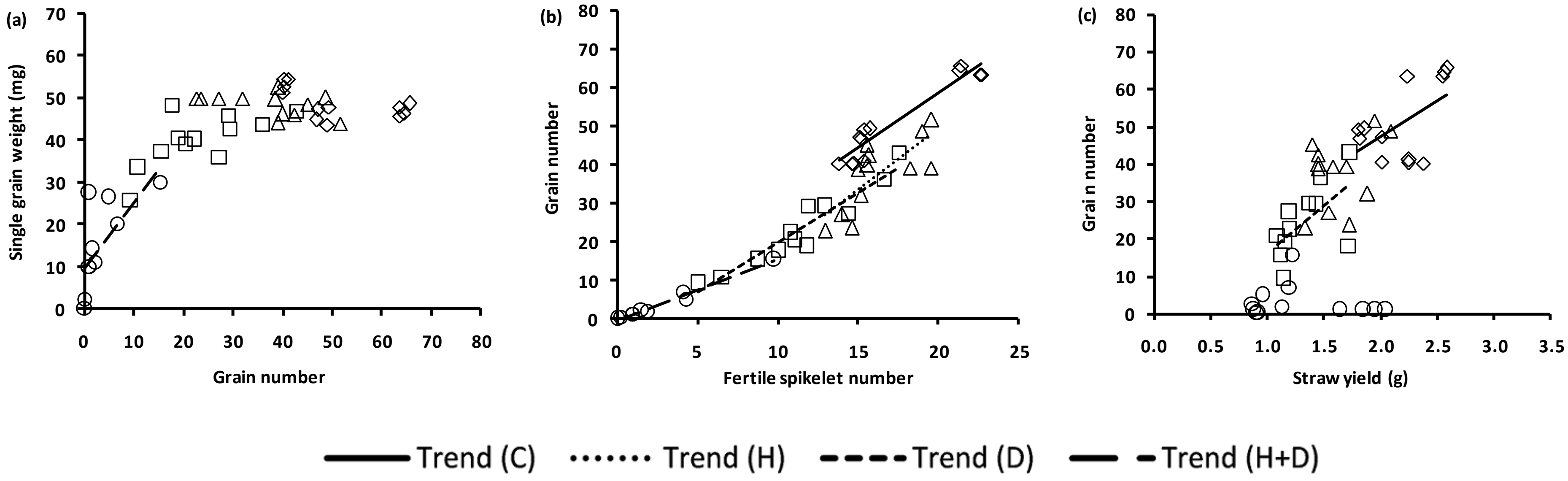


Figure S2.9. Single grain weight plotted against grain number (a) and grain number plotted against fertile spikelet number (b) and straw yield (c) per main stem. Data are shown for pot set 1 (heat treatment in the glasshouse), linear trends are shown for relationships when are significant $p<0.05$.

Supplementary material for chapter 3

Supplementary table 3.1. The heating temperature and duration obtained from 8 published studies about heat stress effects around anthesis on crop yield which provided detailed information of soil, experimental setup, and applied treatments.

Heating temperature (°C)	**Duration of heating**	**Reference**
35	12 days, 16 hours per day	Narayanan et al., 2016
31-39	3-9 days, 14 hours per day	Liu et al., 2016a
32	10 days, 16 hours per day	Zhang et al., 2013
35	4 days, 16 hours per day	Wollenweber et al., 2003
30-33	15 days, 8 hours per day	Tashiro and Wardlaw, 1989
38	2 days, 14 hours per day	Hays et al., 2007
32	20 days, 12 hours per day	Zhang et al., 2010
31-40	12 days	Ferris et al., 1998

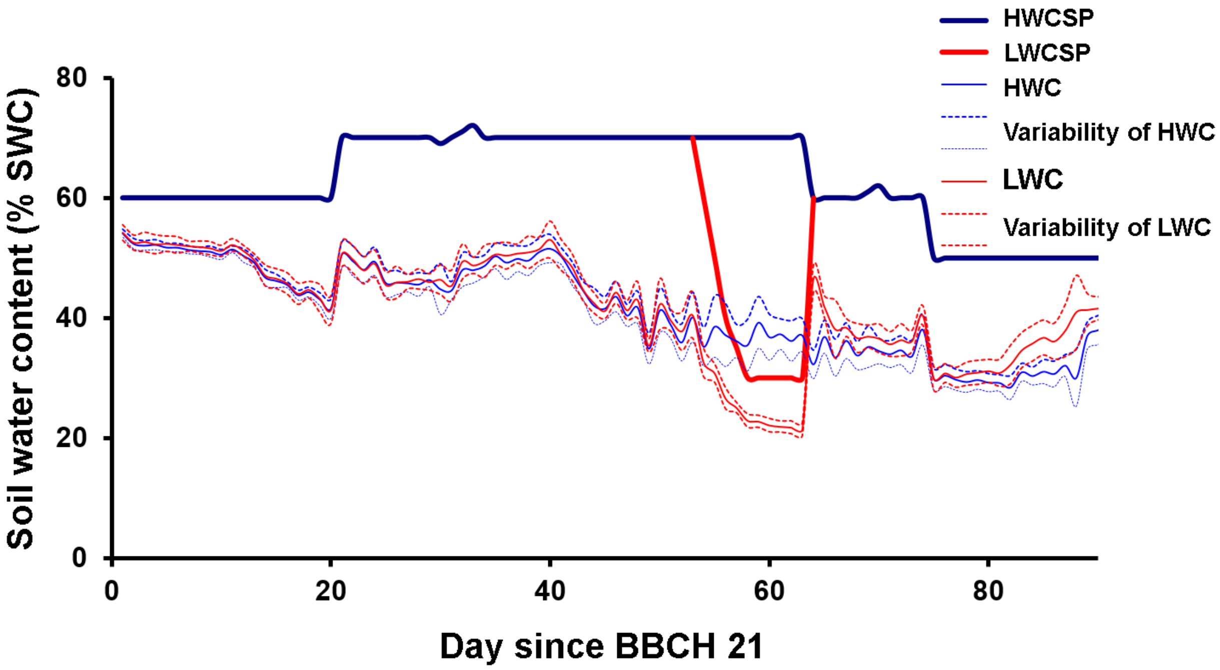


Figure S3.1. Course of soil water content (WC) in the Halle experiment 2015 (in % of soil water capacity SWC). HWC and LWC: Minimum soil water content measured before watering in the morning for treatments with high WC (control or heat at high water supply) or low WC (drought or combined heat and drought). The WC values for the heat stress treatments and the combined heat + drought treatments fit within the range of the corresponding HWC and LWC treatments without heat stress. HWCSP and LWCSP: WC set point values (SP) of the HWC and LWC treatments, i.e.,bnmaximum values obtained after watering in the morning, respectively. Variability: standard deviation.

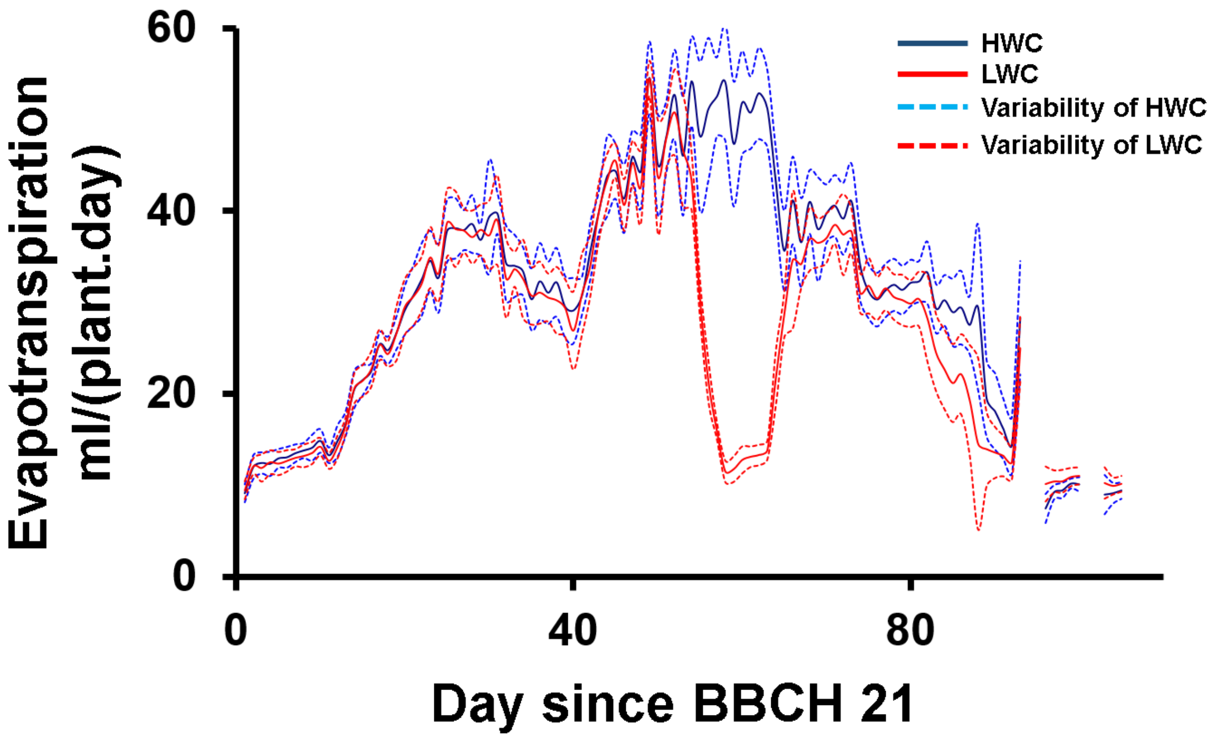


Figure S3.2. Course of evapotranspiration rate in Halle experiment 2015 for the treatments with high (HWC) and low (LWC) soil water content and the variability (standard deviation).

Supplementary material for chapter 4

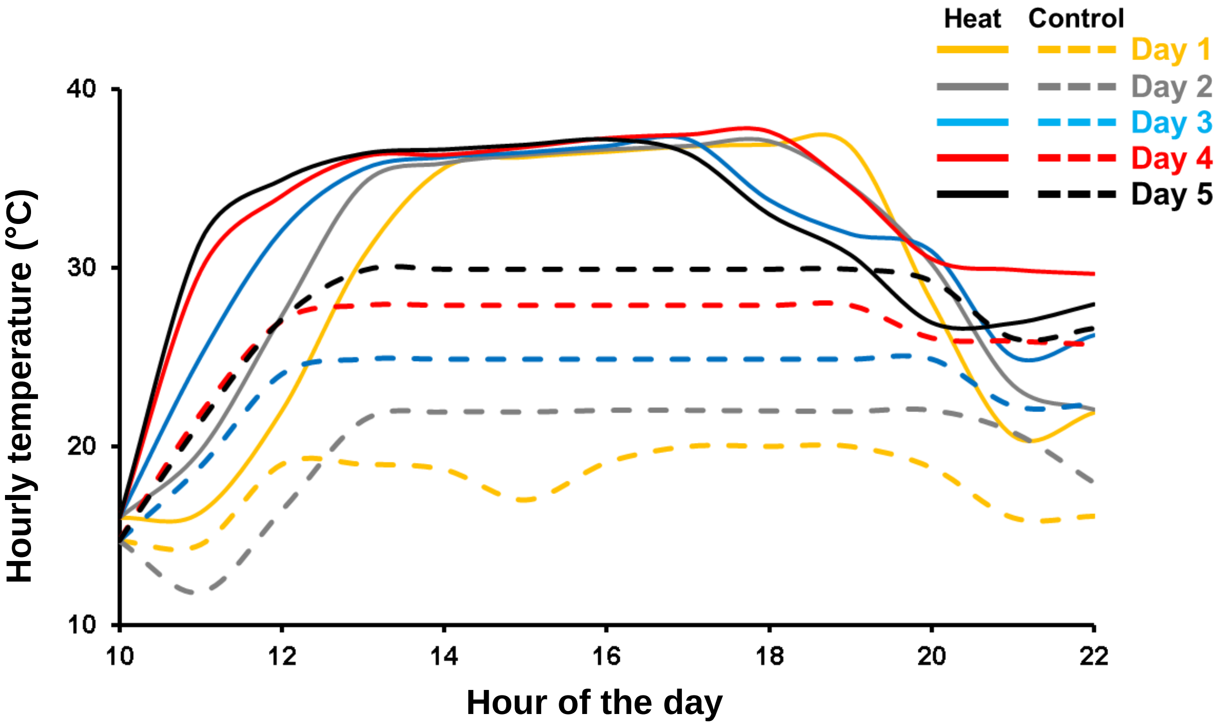


Figure S4.1. The variability of hourly temperature during the heating period at the growth chambers in both years of study.