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„Pearl millet (*Pennisetum glaucum* [L.] R. Br.) – a suitable drought tolerant crop in times of global warming?“

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Abstract

The assurance of food security and further reduction of hunger are major challenges connected to climate change and increasing world population. Along with increasing mean global temperature, climate change causes increase in frequency, duration and intensity of extreme weather events. Drought is worldwide the most important hazard reducing crop yields. Obviously, crops with high drought tolerance will gain in importance. C_4 plants are generally better adapted to water limitation and have a higher water use efficiency (WUE). Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is known for its high tolerance against drought and salt stress. In this study genotypes 'ICTP 8203' (drought tolerant) and '843-22B' (drought sensitive), provided by the 'International Crops Research Institute for the Semi-Arid Tropics' (ICRISAT), were investigated for their physiology and growth strategies during drought stress. Plants were grown controlled greenhouse conditions, in polyethylene pipes/HT pipes with timely irrigation regime and also in the university garden outside the department. We observed a substantial higher growth height and aboveground biomass for 'ICTP 8203'. Therefore, 'ICTP 8203' was more limited in soil volume and could not make use of its higher drought tolerance. Based on soil respiration measurements and investigations on rhizosphere (Chaturvedi et al. in prep.), it can be hypothesized that 'ICTP 8203' can build up a higher BNI activity (biological nitrification inhibition) by root exudation. To verify that, additional and more detailed research is necessary. Moreover, an experiment with higher soil volume should be considered for better simulation of field conditions.

Contents

1. Acronyms and abbreviations

α	electrons/photons: initial slope of light curve which is related to quantum efficiency of photosynthesis
A	CO_2 assimilation rate [µmol*m^{-2}*s^{-1}]
ABA	abscisic acid
abs.	absolute
ADP	adenosine diphosphate
approx.	approximately
ASW	available soil water
ATP	adenosine triphosphate
BNI	biological nitrification inhibition
BR	basal soil respiration
c_i	intercellular CO_2 concentration [ppm]
cm	centimeters
CO_2	carbon dioxide
DAS	days after sowing
DLI	daily light integral
DUDS	days under drought stress
DW	dry weight
EB	error bars
e.g.	exempli gratia (for example)
ETR_{max}	maximum electron transfer rate in µmol electrons/[m^2*s]:
Fig.	Figure
FTSW	fraction of transpirable soil water
gH_2O	total water vapor conductance [mmol*m^{-2}*s^{-1}]
HI	harvest index
HT	high temperature
H_2O	water

i.e.	id est (that is)
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IRGA	infrared gas analyzer
I_k	minimum saturation irradiance in µmol photons/(m^2*s):
LEF	linear electron flow (Phi2 * light intensity PAR x 0.42)
L	liters
LSD	least significant difference
m	meters
µE	micro Einstein: unit defined as the energy in one mole of photons
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
N	nitrogen
N_2O	nitrous oxide
NUE	nitrogen use efficiency
PAM	pulse amplitude modulation
PAR	photosynthetic active radiation
Phi2	quantum yield of photosystem II = fraction of light energy captured by photosystem II, converting light energy, producing ATP and NADPH
PhiNO	PhiNO is the combination of a number of unregulated processes whose by-products can inhibit photosynthesis or be harmful to the plant.
PhiNPQ	fraction of incoming light (excited electrons) that goes towards non-photochemical quenching, in order to protect from the adverse effects of high light intensity.
PPFD	photosynthetically active photon flux density
ppm	parts per million
prep.	preparation
PS	photosystem
RESP	% Response = SIR-BR (in % of BR)
ROS	reactive oxygen species
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
SIR	substrate induced soil respiration
SIR_{max}	maximal substrate induced respiration (monitored 14 h after start of IRGA measurement
SLW	specific leaf weight

TTSW	total transpirable soil water
WUE	water use efficiency (=fixed CO_2 by Rubisco/transpiration)
%rH	relative air humidity

Key words:

Pearl millet, *Pennisetum glaucum*, drought tolerance, climate change, C_4 photosynthesis, plant productivity, arid and semi-arid regions, ICTP 8203, 843-22B

2. Introduction

2.1. Droughts increased by climate change

In times of global warming and an increasing world population, it gets more difficult to maintain food security, especially in developing countries. Further, battle against hunger is still not won. After decades of steady decline, the number of people suffering from hunger remains virtually unchanged since 2015 and still more than 820 million people around the globe are hungry and struggling for everyday food (FAO, IFAD, UNICEF, WFP and WHO 2019). For a projection of increased human world population from 7 to 10 billion, an increase of food production of 50-70% is required to feed the world (Reynolds 2010). Climate change is resulting in increased daily, seasonal and annual mean temperatures. Beyond that, intensity, duration and frequency of extreme high and low temperatures will increase (Wagner 1999; Tebaldi et al. 2006). The global climate risk index 2020, discussed at the 25th United Nations Framework Convention on Climate Change in Madrid (COP 25), shows that already most parts of South and Central Europe are strongly affected by extreme weather events (Eckstein et al. 2020). “Among the weather-related hazards, drought is probably the most complex and severe due to its intrinsic nature, wide-ranging and cascading impacts” (UNDRR 2019, S.173). Drought affects our world on many different levels ranging from natural ecosystems to agriculture up to public water supply (Ghatak et al. 2017). For the aridity of a region precipitation is often the dominant factor, however, the cumulative effect of the imbalance among precipitation and water demand (i.e. potential evapotranspiration) determines local droughts and wet spells (Dai 2011). To gain high yields in agricultural systems with limited precipitation, artificial irrigation is often necessary. This often leads to severe environmental issues. In China the development (availability) of the groundwater played an increasingly important role in the growth of Chinese agriculture (Wang et al. 2007), however, this led to a decline of the groundwater level by more than 1 m per year in Northern China (Wang et al. 2009). In the context of competing water demands for drinking water, agriculture and natural ecosystems, the management of water is expected to play an increasingly critical role in agriculture (OECD 2015).

2.2. How plants cope with drought stress

Drought can be defined as a period of below average precipitation or water supply which limits plant productivity in natural or agricultural ecosystems (Boyer 1982). However, plants can have very different water demands and a certain amount of precipitation can be limiting for one plant

species but for another one it is not (Ghatak et al. 2017). Plants have adapted to different environments with very different amounts of annual precipitation. Despite that plasticity, in many regions water is a limiting factor, because water demand of vascular plants (Tracheophyta) is very high in contrast to other organisms, e.g. terrestrial animals. Approx. 97% of water taken up by the roots is lost by evapotranspiration (Taiz and Zeiger 2010). This is because of the tradeoff between CO_2 uptake and water loss (approx. 1:10000). Drought stress affects virtually in every aspect of plant physiology and metabolism (Zhu 2002). The cellular homeostasis of a plant is maintained by sufficient uptake of water and nutrients by the roots. However, because water and nutrient availability fluctuate, plants react on many different levels to maintain cellular homeostasis.

The capability of a plant to cope with limited water supply can be defined as drought resistance. Drought resistance can be subdivided into three different strategies: (i) drought escape, (ii) drought avoidance and (iii) drought tolerance (Zhang 2007). Drought escape means to complete the plant life cycle before unfavorable season with lower precipitation starts. For the strategy of drought avoidance, the plant tries to enhance water uptake and reduce water loss. This is mainly done by upregulation of abscisic acid (ABA) and resulting stomatal closure to reduce water loss, and enhancement of root growth to increase water uptake (Osmolovskaya et al. 2018). Drought tolerance is facilitated by osmotic adjustment, higher antioxidant activity and desiccation tolerance (Zhang 2007). These strategies can indicate different ecological specializations of a plant species (Carboni et al. 2016). If drought stress lasts too long or severity is too high for the plant adaptation capacity, this results in diminished water potential, turgor loss and reduced cell expansion and cell division (Jaleel et al. 2009). Plant growth is accomplished by cell division, cell expansion and cell differentiation. Especially for cell elongation sufficient water supply plays a very important role, because absorption of water is necessary to maintain a minimal turgor pressure level to sustain growth (Nonami 1998).

During the evolution of vascular plants, a lot of morphological, anatomical and physiological traits emerged to cope with limited water supply. An important physiological adaptation to drought was the development of C_4 and CAM (Crassulacean Acid Metabolism) out of C_3 photosynthesis. C_3 species represent 85 % of all higher plant species, C_4 species approx. 5 % and CAM species make up for the remaining 10 %. It is hypothesized that C_4 photosynthesis evolved as an adaptation to high temperatures in combination with drought stress. In contrast to C_3, C_4 photosynthesis has an additional biochemical CO_2 concentrating mechanism (PEPC, phosphoenole pyruvate carboxylase). Thus CO_2 concentrations are increased by a factor of 10-

100 at the catalytic sites of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) in bundle sheath as compared to ambient air (Furbank and Hatch 1987; Jenkins et al. 1989). This CO_2 enrichment in bundle sheath cells decreases CO_2 concentration in mesophyll cells and generates a positive concentration gradient from ambient air to mesophyll cells. Diffusion of CO_2 out of bundle sheath cells is limited by a suberin layer in the cell walls. This functional anatomy and enzymatic repertoire enable C_4 plants to fix more CO_2 whilst requiring less water (i.e. water use efficiency [WUE] is increased), as compared to C3 plants. However, to reach a higher photosynthetic rate than C_3 plants, sufficiently high leaf temperature and a higher irradiation (PAR) is needed (Yamori et al. 2013).

2.3. Pear millet as an alternative and drought tolerant crop

Drought causes crop yield reduction depending upon severity and duration of stress period and growth stage of the plant, although there are big differences among different crop plants (Farooq et al. 2009). From the top 150 crops regularly consumed by human beings only 10 are C_4 plants (www.fao.org). Certainly, there are some worldwide economically important crops like maize (*Zea mays*), sugarcane (*Saccharum officinalis*), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*) and other millets.

Pearl millet *(Pennisetum glaucum* [L.] R. Br.), belonging to the group of millets (cereals of the subfamily Panicoideae), originated from the wild progenitor of pearl millet *Pennisetum glaucum* ssp. *monodii* (Brunken 1977a; Harlan 1992). Most probably first domestication has taken place in Africa (Harlan et al. 1976; Harlan 1992), although different geographical origins along the Sahelian zone are proposed ranging from Mauritania to Sudan. Today pearl millet is mainly cultivated in India (9.3 million hectares), West/Central Africa (14.2 million hectares) and South/East Africa (more than 2 million hectares) (exploreit.icrisat.org). Among the major cereals pearl millet is highly drought and salt tolerant (Brunken et al. 1977b; Allouis et al. 2001; Massino et al. 2015; Toderich et al. 2018). *P. glaucum* has a short life cycle with a short grain-filling period with small seeds sizes compared to other cereals, however, it can grow in areas of low precipitation (300-500mm per year) and low soil fertility (Vadez et al. 2012). It is a highly cross-pollinated diploid (2n = 14) with a large genome size of over 2,000 Mb and estimated 38,000 genes (Varshney et al. 2017).

The response to drought is strongly depending on species, however, genotypes within a species often also vary substantially (Hall et al. 1982; Campos et al. 2004). Ongoing climate

change, competition for land and water resources, together with increased world population and food demand will provide novel challenges for cultivated plant species. To cope with all these issues, we are probably in need of a new ‘agricultural revolution’ (Chapman et al. 2012). Therefore, smart decision for breeding crop genotypes are necessary to reduce demand of fossil fuels and protect water and nutrient cycles. One possibility to reduce ecological impact of food production is to establish more drought tolerant crops such as pearl millet. These crops are underutilized but have high nutritional properties with high yield in abiotic stressed land. It needs to be focused more in the molecular breeding areas which in turn can overcome the problem of malnutrition in undeveloped or underdeveloped nations. Millets can also be an alternative to high iron and zinc content crops used in various medicinal properties. In order to assess, which crop fits best to certain environmental conditions, it is necessary to understand plant responses to drought and breeding plants for increased drought tolerance.

In this study, I investigated the potential impact of drought stress on the two pearl millet genotypes ICTP 8203 and 843-22B, measuring several physiological parameters of biomass, productivity and soil respiration to understand their response mechanisms. I have chosen just two genotypes in order to be able to do an in-depth comparison employing many promising physiological monitoring methods with enough replicates instead of many genotypes scratching at the surface with many loose ends.

3. Material and methods

3.1. Plant material

The two *Pennisetum glaucum* genotypes ICTP 8203 (drought-tolerant) and 843-22B (drought-sensitive) were chosen for evaluation of their respective drought tolerance. These frequently cultivated genotypes were provided by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (www.icrisat.org). These genotypes are very widely used for consumption and research purpose in India and Africa.

3.2. Preliminary experiment

A first preliminary experiment was set up with just eight plants (four for ICTP 8203 and four for 843-22B) in order to optimize physiological measurement procedures and to establish best conditions for the main experiment. The analytical devices are explained in chapter 3.5. At the start of the measurements, plants were a few weeks old, growing in normal plant pots of approximately 5 L of soil volume. Subsequently, they were monitored over a period of 35 days once per week. Plants were watered always sufficiently, to avoid drought stress. One plant of each genotype was taken to the lab, growing under artificial light and especially low humidity (35% rH) for 24h/7d measuring primary and secondary photosynthesis processes at severe drought stress by means of *MINI-PAM II* and the *GFS 3000* both from WALZ ™ (www.walz.com).

3.3. Main experiment

The main experiment was set up on March 25th, 2019. Four untreated seeds were sown in each pot in approx. 1 cm soil depth. The used soil was a mixture of 3 parts 'Kranzinger Balkon- und Blumenerde' (www.kranzinger-erde.at), i.e. a common flower soil, 2 parts quartz sand and 1 part perlite. The 'ficote total N/P/K 17/9/11+2MgO' fertilizer (www.icl-sf.com) was added at the start of the experiment with a concentration of 0.01 %. After germination only the strongest seedling was kept in the pot. The plants were growing under controlled glasshouse conditions (see Figure 1B). Nevertheless, as the weather outside partly influenced the climatic conditions we recorded all important microclimatic parameters (air temperature, relative air humidity, light intensity, soil humidity and soil temperature). The plants were grown in pipes made of rigid polyethylene. One pot (Figure 1A) consisted of three elements of these pipes glued together

with a hot glue gun. Each pot had a total height of 45 cm and an inner diameter of 10.3 cm and therefore a total volume of approx. 3.75 L. Also, there were two access openings in each pot to insert sensors for measuring soil humidity and soil temperature fitting exactly in diameter to accommodate the DeltaT ™ ML3 theta probe soil rH sensors, in order to minimize water loss by evaporation. Figure 1B shows all pots of the experiment in the glasshouse at the starting day of the experiment and Figure 1C shows the growing plants 67 days after sowing (DAS).

Figure 1: Pots for main experiment. A: pot for one plant composed of 3 HT pipes; B: pots in the glasshouse on the day of sowing (March 25th, 2019); C: plants 67 days after sowing (DAS);

Figure 2 shows all major events during the experiment. We performed two early response physiological measurements: the first after 21 days and the second 38 DAS. At day 30 the plant pots were transferred to a slightly lower position in the greenhouse to maintain light intensity for middle and uppermost leaves as plants were already quite tall. Until DAS 56 all plants were irrigated sufficiently (approx. 30 %vol water content). Every 4 weeks 0.01 % of 'wuxal' fertilizer (www.wuxal.com) was given to avoid N/K deficiency. From that time point on we induced drought stress: treatment plants were only given 200 ml water per day instead of 400 ml (control groups). We measured/harvested leaves once per week from DAS 63 (7 days after drought stress induction) until DAS 92 (36 days after drought stress induction). At DAS 94 we stopped irrigation of all plants, at DAS 101 soil was almost completely dry (below 5% abs. soil humidity). Experiment was terminated at DAS 134. We measured aboveground biomass of the already completely dry shoots and took soil samples that were stored at 4 °C.

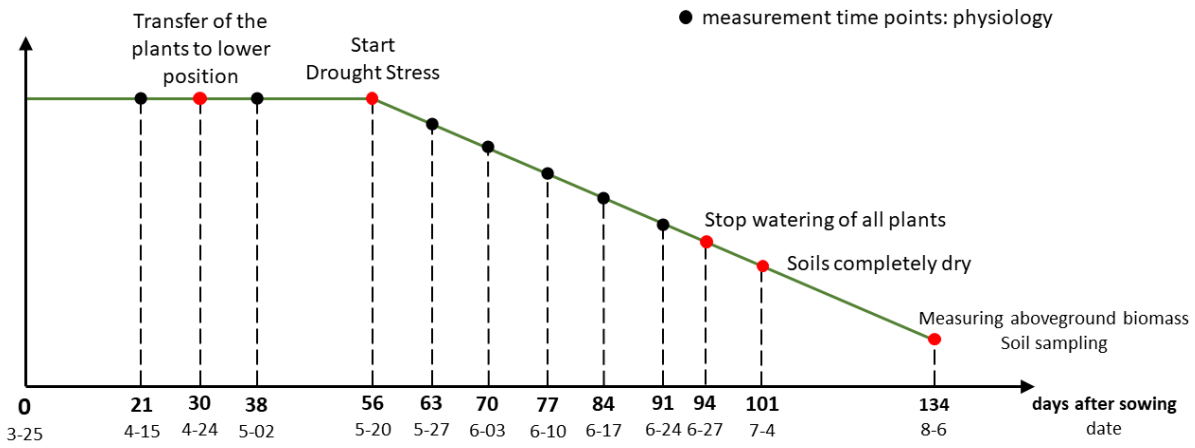

Figure 2: Time scale of the main experiment: Shows important events during the whole experiment; black dots are delineated for physiological measurement time points.

3.4. Outdoor garden experiment

The outdoor garden experiment was initiated on May 29th, 2019 by sowing the seeds in our outdoor garden. A few square meters per genotype was used. The soil in the outdoor garden is a typical brown earth with a loamy sand soil texture, a field capacity of approx. 30 % and a pH

around 7. There were two aims for this experiment: seed proliferation and for comparison among field growing in Austria and glasshouse growing in pots. Harvest was done on Oct. 30th and 31st, 2019 by cutting the whole aboveground biomass, weighing it (FW), counting of the panicles per plant and measuring the shoot height.

3.5. Devices for physiological measurements

Climatic conditions

To monitor and control growth conditions for the plants we measured air temperature, relative air humidity, light intensity soil humidity and soil temperature. Air temperature and relative air humidity and air CO_2 were recorded using the *testo Saveris™ 160 IAQ* data logger and cloud (www.testo.com). Light intensity was measured using a full-spectrum (400-700 nm) smart-quantum sensor *Apogee™ SQ-520* (www.apogeeinstruments.com). Soil humidity was the most important factor, to monitor level of water stress of the plants and keep the soil humidity constant by adapting the irrigation regime. Here we used *DeltaT ™ s ML3 Theta Probe Soil humidity and Temperature Sensors* together with two *GP2 Data Logger Controllers (www.delta-t.co.uk).*

Stomatal conductance

Stomatal conductance defines the rate of passage of carbon dioxide (CO_2) entering, or water vapor leaving through the stomata of a leaf. For measuring the stomatal conductance of our plants, we used the *DeltaT ™ s AP4 porometer (www.delta-t.co.uk)*. It's a transient diffusion porometer (Ghatak et al. 2016). This means that the measured conductance is based on a comparison with the calculated conductance of a standardized perforated calibration plate with a water saturated filter paper.

Photosynthesis primary processes: MultispeQ

The MultispeQ™ device (https://photosynq.org) can measure many photosynthesis and microclimate values. It combines functionality of a fluorometer, a chlorophyll meter and a spectrophotometer. It measures relative chlorophyll content and fluorescence based parameters:, Phi2 (fraction of light energy captured in Photosystem II), PhiNPQ (non-photochemical quenching), PhiNO (light energy lost by unregulated processes), LEF (linear electron flow) and absorbance-based parameters like v_{H^+}, g_{H^+}, ECS_t which are describing the

accumulation of protons in the thylakoid and their flow through ATP synthase which converts ADP to ATP known as proton motive force. Also, abiotic factors including barometric pressure and altitude, light intensity (PAR) ambient temperature, relative humidity, leaf direction, leaf angle and leaf temperature are recorded. Also, a leaf temperature differential can be calculated by the device which corresponds to stomatal conductance (Fauset et al. 2018; Zhang et al. 2019).

The photosynthetic linear electron flow (LEF) is the electron flow from H_2O to NADP. LEF is calculated from Phi2 and light intensity of photosynthetic active radiation (PAR) internally (LEF = Phi2 * PAR * 0.4). Therefore, if PAR is constant LEF and Phi2 are correlating with each other. F_v/F_m is the ratio between variable fluorescence and maximum fluorescence and defines the photosynthetic efficiency of quantum yield of PS II. For healthy, unstressed plant leaf tissue F_v/F_m ratio is around 0.8-0.7 (Masojídek et al. 1991; Baker 2008; Sharma et al. 2015; Toderich et al. 2018).

Photosynthesis primary processes: WALZ™ GFS-3000

The *GFS-3000 (www.walz.com)* basically measures the CO_2 uptake and the H_2O release of a leaf. Leaf or part of a leaf is enclosed in a rubber gasket sealed climatically controlled microchamber (leaf clamp). All relevant environmental parameters for photosynthesis (CO_2, H_2O, temperature, light, ventilation and flow) can be controlled automatically within this chamber. Programming of experimental protocols for automatic light or CO_2 curves is easily possible (Surova et al. 2016).

Photosynthesis primary processes: WALZ™ MINI-PAM II

Mini-PAM II (pulse amplitude modulation) (www.walz.com) has been developed for saturation pulse analysis of photosystem II. We used it for measuring light curves (Fig. 3). The Light curve program exposes the sample to a stepwise increasing intensity of actinic illumination. The light curve shows the electron transfer rate (ETR) dependent on Photosynthetically Active Photon Flux Density (PPFD). The Software (*WinControl 3.26*) extrapolates a light curve based on the measuring points and then calculates α (electrons/photons: initial slope of light curve which is related to quantum efficiency of photosynthesis), ETR_{max} (µmol electrons/[m^2*s]: maximum electron transfer rate) and I_k (µmol photons/(m^2*s): minimum saturation irradiance). ETR at ambient light conditions corresponds to LEF: (Phi2 * ambient light intensity PAR x 0.42).

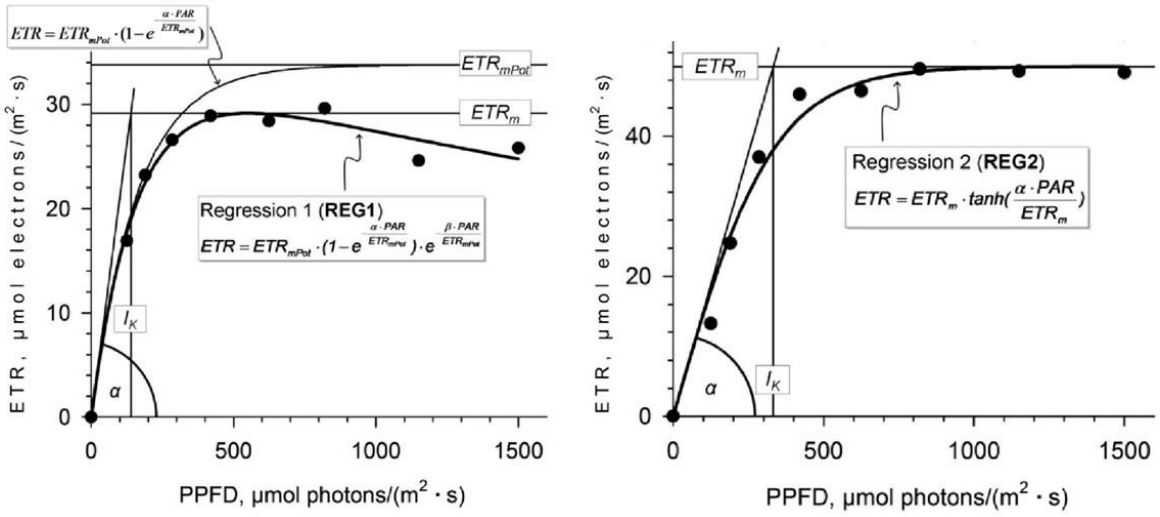

Figure 3: Model functions of rapid light curve of MINI-PAM II (www.walz.com)

Soil respiration: Infrared Gas Analyzer (IRGA)

To measure soil respiration, I used the Infrared Gas Analyzer *EGA60p* from *ADC BioScientific Ltd ™ (www.adc.co.uk)*. I measured basal respiration (BR) and substrate induced respiration (SIR) by adding 0.2% glucose, referred to fresh soil weight. I measured soil respiration at two time points: previous to the main experiment (plant unaffected soil) and at the end of the experiment (134 DAS, where soils were completely dry). The soil samples were stored in closed plastic bags at 4°C. Previous to measurements, soil samples were re-moisturized to approx. 20% volumetric water content and left over 2 days for stabilization at 4°C. Weighed portion of soil sample was 30 or 50 g, respectively. Respiration rate is given in mg CO_2 kg soil FW^{-1} h^{-1}. Further I calculated the response or respiratory quotient in % of BR RESP (% Response = SIR-BR % of BR) percentage of SIR referring to BR (basal soil respiration), i.e., it defines how strong the soil respiration can be maximized by adding glucose as a substrate for microorganisms.

3.6. Biometry

Biometrical data were measured non-destructively by folding meter stick, caliper rule and balance. Based on shoot height, stem thickness, length of the longest leaf, breadth of the broadest leaf, thickness of the thickest leaf and number of leaves a total aboveground biomass was estimated by calculation of leaves and stem as geometrical shapes and the usage of the density of water for plant tissue and corrected using leaf water content analysis of leaves

harvested earlier. This was later corrected by actual gravimetric water content measurement at harvest. The calculation for the estimated biomass at day 42 is shown in Equation 1. The correction factor (Equation 2) was calculated by using the vegetative DW biomass at the end of the experiment of the drought treated groups, since there was just very marginal growth in vegetative biomass under drought stress.

$$biomass\ [g] = \left(0.75 * shoot\ height\ [cm] * \left(\frac{stem\ thickness\ [cm]}{2} \right)^2 * \pi + \frac{longest\ leaf\ [cm]}{2} * \frac{broadest\ leaf\ [cm]}{2} * \frac{thickest\ leaf\ [cm]}{2} * leaf\ number \right) * 1 \left[\frac{g}{cm^3} \right] * correction\ factor$$

Equation 1: biometrical estimation of leaf biomass

$$correction\ factor = \frac{\frac{vegetative\ DW\ biomass\ at\ end\ of\ experiment\ [g]}{water\ content\ [\%]} * 100\%}{estimated\ biomass\ without\ correction\ factor\ [g]}$$

Equation 2: correction factor for the estimation of the leaf biomass

3.7. Statistics

For statistical analysis I used the statistics software *Statgraphics XVIII*, mainly employing one-way and multifactorial ANOVA with LSD intervals. I used *Veusz 3.0* for visualization of the recorded environmental parameters. Biological replicate no. 21 (control group of 843-22B [drought sensitive cultivar]) was defined as an outlier because of premature desiccation and death following an accident and thus excluded from the data matrix.

4. Results

4.1. Monitoring of the environmental conditions

Soil humidity, air relative humidity, soil temperature, air temperature and light intensity are the main factors influencing drought stress in plants. We tried to keep these parameters as constant as possible. To that end, it was necessary to monitor all these environmental parameters. Measured climatic conditions are shown in Fig. 4 and 5. Fig. 4A-B shows the means of volumetric soil humidity in %. Soils were irrigated to a median value of 25% (treatments), corresponding to 50- 60% of the field capacity of the utilized soil. Water consumption of the drought tolerant cultivar was approx. 20% higher, as were the CO_2 Assimilation and WUE (Fig. 14) and the biomass harvested at the end of the experiment (Fig 9). Fig. 4C shows the parameters air temperature, air relative humidity, air pressure and atmospheric CO_2 concentration. Air temperature in the greenhouse were kept constant at approx. 30°C during the day and 25°C during nighttime. Air relative humidity was maintained at 58%rH (+/- 15%rH). Air pressure was quite constant at approx. 1000 mbar. In the greenhouse, atmospheric CO_2 concentration was slightly higher than global mean at around 480 ppm in average. Light intensity reached a maximum at around 800 µE (Fig. 5A) at noon. The daily light integral (DLI, Fig 5B) amounted to around 10 moles/day/m^2 with an increase towards the end of the experiment due to outside seasonal irradiation increase.

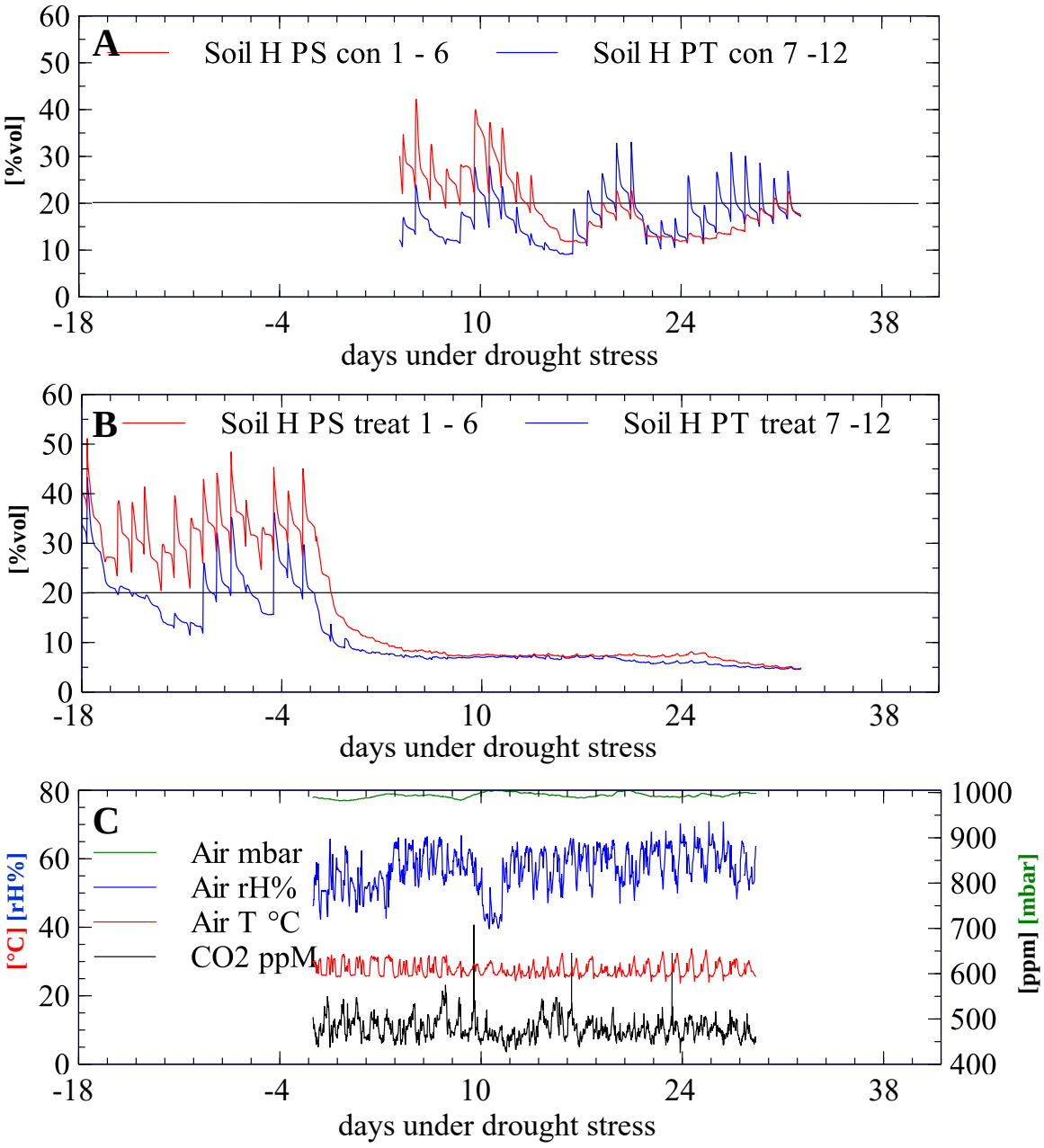

Figure 4: Measurement of environmental parameters; A: medians of soil humidity (Soil H) in [%vol] of drought sensitive genotype 843-22B (PS) in red and drought tolerant genotype ICTP8203 (PT) in blue for the control groups; B: same as A, but for treatment groups; C: air relative humidity in [rH%] in blue and air temperature (T) in [°C] in red belong to the left scale, air pressure in [mbar] in green and CO_2 concentration in [ppm] in black belong to the right scale; (soil humidity of controls was not measured automatically before stress treatment started);

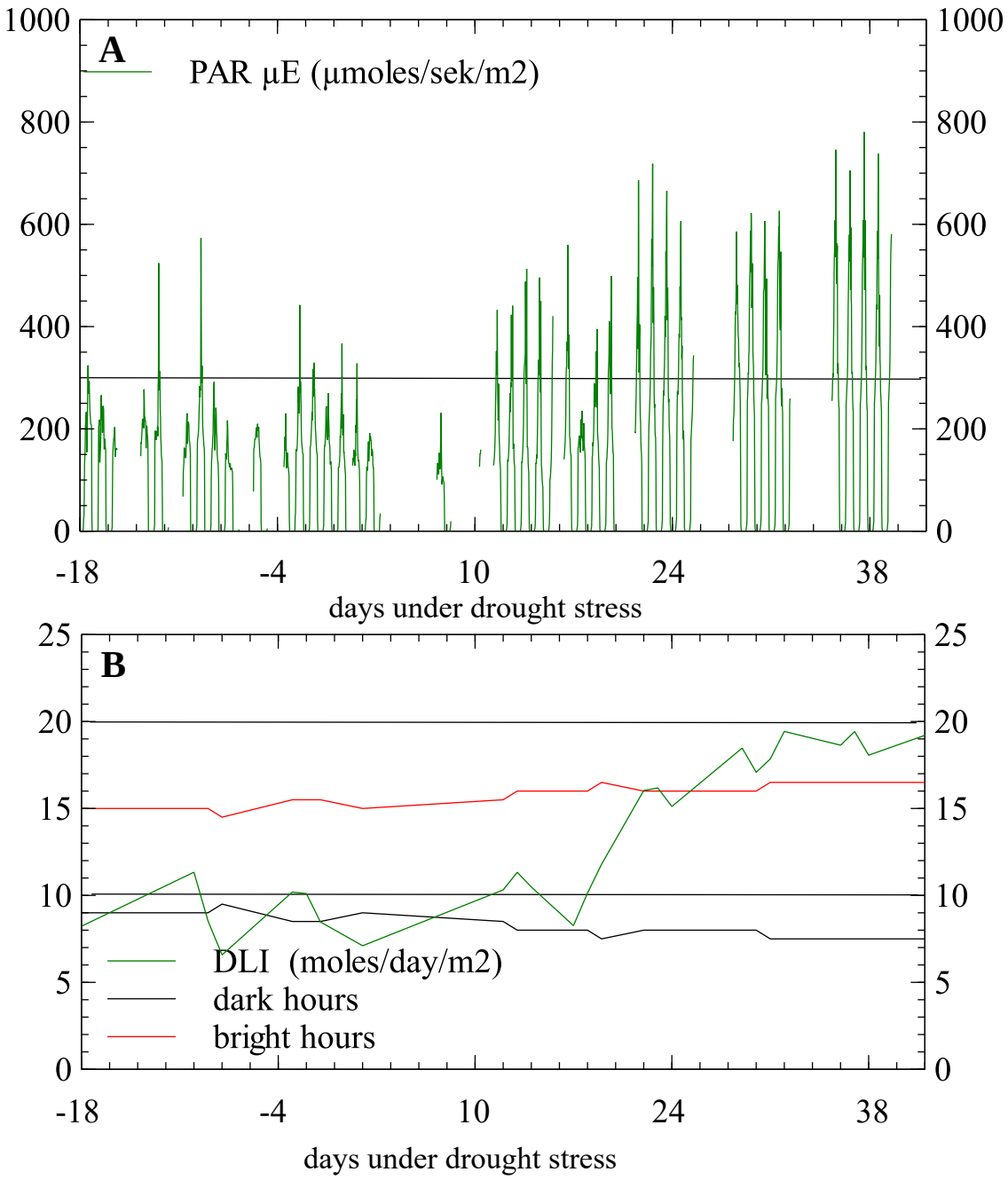

Figure 5: PAR measurement (photosynthetic active radiation); A: temporally exact record of PAR in [µE=µmoles/sec/m^2] with peaks at daytime; B: daily light integral (DLI) in [moles/day/m^2] in green, dark hours per day in black and bright hours per day in red;

4.2. Growth traits

There are some general differences in size among the genotypes ICTP8203 (drought tolerant) and 843-22B (drought sensitive) displayed by biometry. Figure 6 shows traits from main experiment in the glasshouse: shoot height (A), stem thickness at 20 cm above soil surface (B), number of leaves per plant (C), length of the longest leaf (D), thickness of the thickest leaf (E) and breadth of the broadest leaf (F) measured 42 DAS (i.e. 14 days before drought inducement, under well-watered conditions). Our results show that there are significant differences ($p<0.05$) in all traits except of thickness of the thickest leaf. In our parallel garden experiment (see Fig. 21 [appendix]), where we measured plant height, fresh weight (FW) aboveground biomass and number of panicles per plant, differences were even more substantial. Figure 7 shows the estimated FW aboveground biomass (A), estimated FW total leaf biomass (B), estimated FW stem biomass (C) and estimated total leaf area per plant (D). For Fig. 7A and B a correction factor was used (see chapter 3.6). There are tendencies that the values in Fig. 7 are higher for drought tolerant genotype, however, there are no substantial differences and no statistical significances. I measured shoot height and stem thickness a second time 65 DAS i.e. 9 days under drought stress (DUDS). Especially shoot height (Fig. 8A) further increased and differences between genotypes got bigger. Stem thickness increase (Fig. 8B) was only minor. Fig. 8C shows estimated specific leaf weight (SLW) based on the biometrical data collected 42 DAS, whereas Fig. 8D shows true SLW measured by leaf harvesting. Data from Fig. 8C and 8D are not directly comparable, however, magnitude of values is quite similar. There are no big differences in SLW among genotype and also not among control and treatment groups. However, after 22 DUDS SLW of all plants decreased. Figure 9 shows results at the end of the experiment. Regarding the number of panicles per plant (A), the gap between control and treatment is higher for tolerant genotype. Total panicle weight per plant (B) and total DW aboveground biomass (D) are following the same pattern. The weight per panicle (C) seems not to be influenced by increased drought stress. Our data even indicate a slight increase. Also 1000 seed weight (F) did not change due to drought stress. Our results indicate that harvest index (E) is in general lower for tolerant genotype. Interestingly, drought stress did not reduce the harvest index for the sensitive genotype in our experiment, whereas it does so for the tolerant genotype.

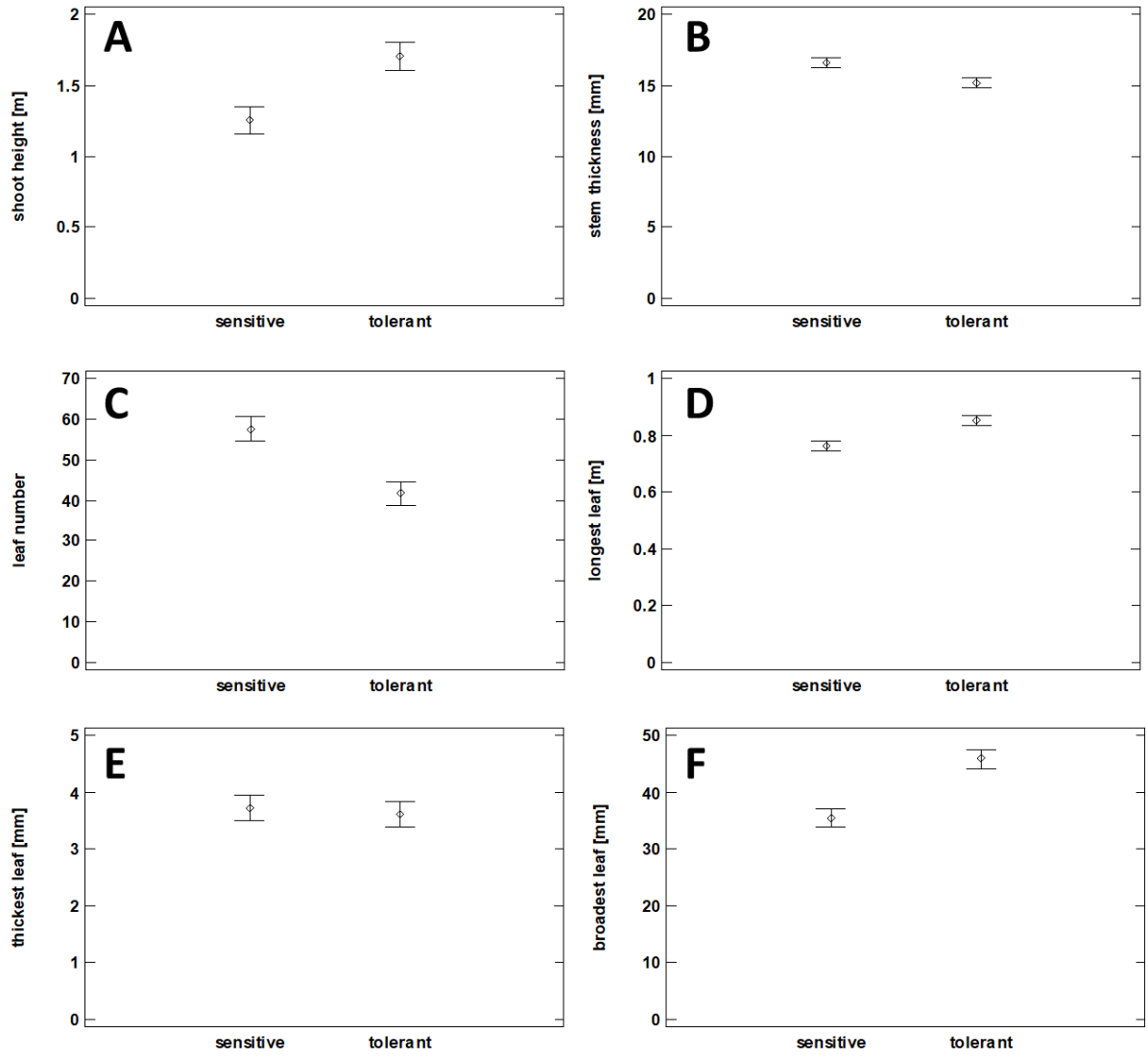

Figure 6: Biometrical measurements 42 DAS (days after sowing; i.e. 14 days before drought inducement, under well-watered conditions); comparison among drought sensitive genotype (843-22B) and tolerant genotype (ICTP8203); A: shoot height; B: stem thickness; C: leaf number; D: length of the longest leaf; E: thickness of the thickest leaf; F: breadth of the broadest leaf; EB = 95% LSD;

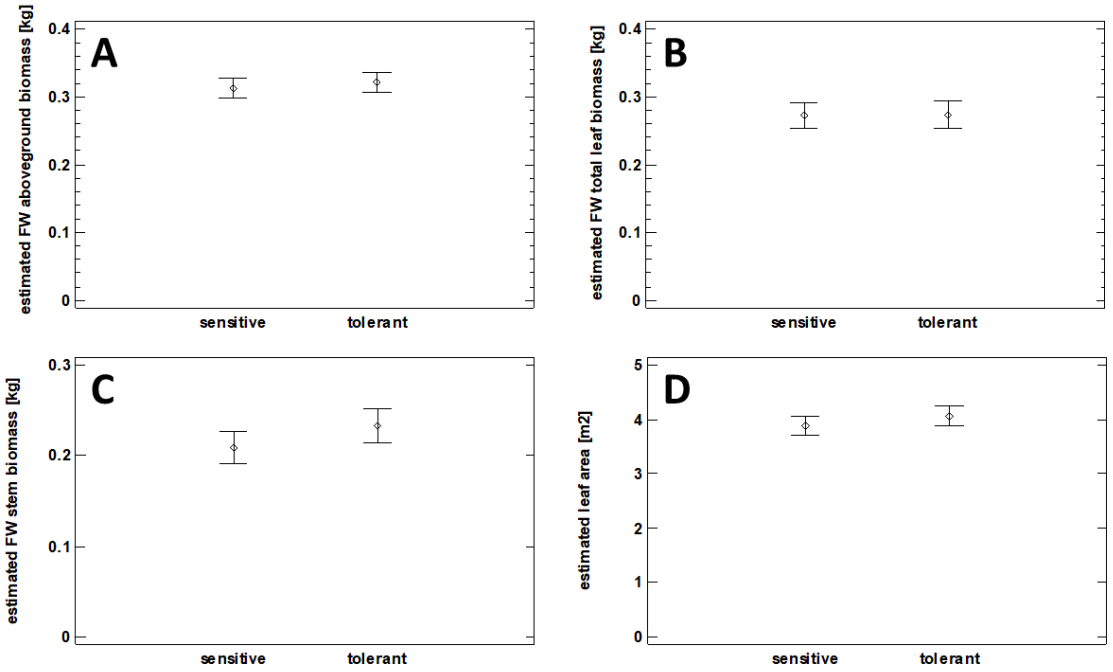

Figure 7: Estimated values based on biometrical measurements 42 DAS (days after sowing; see Fig. 6); calculations are described in chapter 3.6; A: estimated FW total aboveground biomass; B: estimated FW total leaf biomass; C: estimated FW stem biomass; D: estimated total leaf area per plant; EB = 95% LSD;

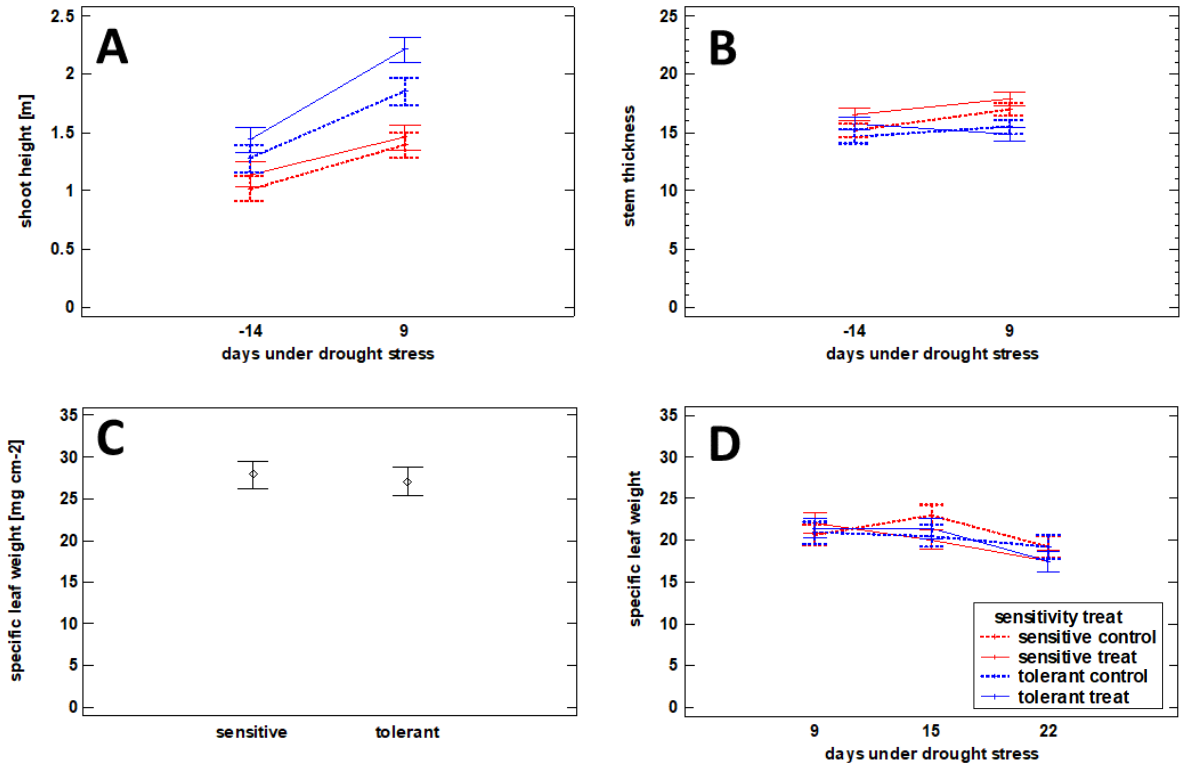

Figure 8: Change over time of biometrical values; -14 DUDS (days under drought stress) = 42 DAS (days after sowing); A: shoot height; B: stem thickness; C: estimated specific leaf weight by calculations based on biometrical measurements data 42 DAS; specific leaf weight yielded by leaf harvesting; EB = 95% LSD;

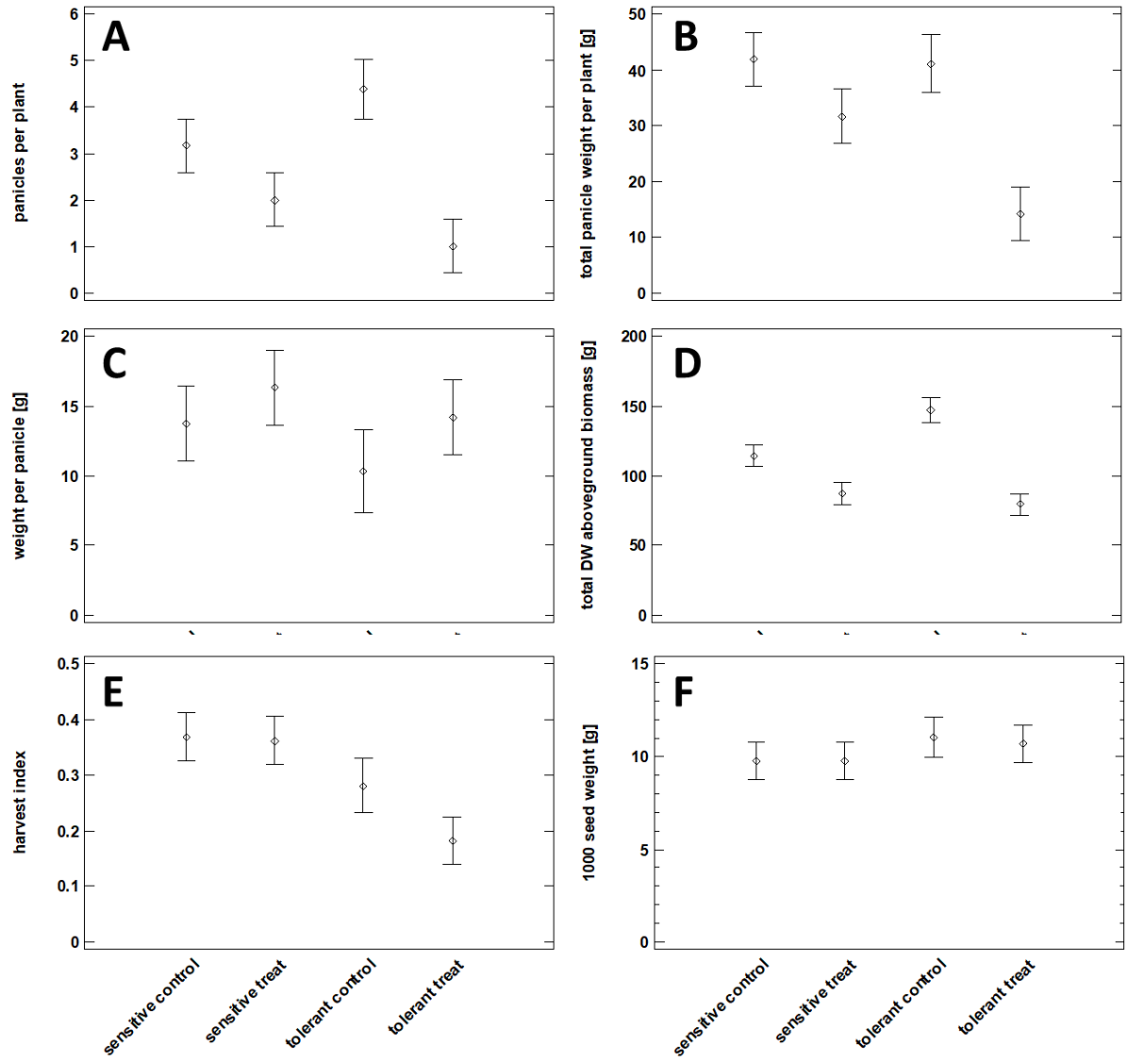

Figure 9: Physiological and phenotyping data 134 DAS (days after sowing); A: panicles per plant; B: total panicle weight per plant; C: weight per panicle; D: total DW (dry weight) aboveground biomass; E: harvest index (HI); EB = 95% LSD;

4.3. Photosynthesis

There are many physiological and especially photosynthesis parameters which may be measured in a non-destructive way, i.e. without harming plant tissue and therefore keep disturbance of the experiment low. These parameters can elucidate reactivity and health status of plants. The graphs x- axes are scaled by days after induction of drought stress i.e. at day 0 we started inducing drought stress by less watering, when plants were 56 days old.

Stomatal closure is the first response to water limitation and can happen within minutes. We used two methods which provide information about the average opening state of the stomata on a certain leaf surface: Stomatal conductance (i.e. diffusion conductance of leaf surfaces) measured by the AP4 Porometer (see chapter 3.5.) and leaf temp differential (i.e. temperature difference between leaf surface and ambient air) measured by the MultispeQ ™. In Fig. 10A we see a gradual increase of stomatal conductance for the control groups during their development and a decrease for the drought stressed plants, however this strong reaction to stomatal closure was observed only on day 7 after stress start. The long-term stomatal conductance remained virtually unchanged. Comparing genotypes, we observed a relevantly higher ($p<0.01$) stomatal conductance under drought conditions for the sensitive variety 843-22B. As a rule, leaf temp difference (Fig. 10B) correlates with the stomatal conductance, because higher stomatal conductance causes higher transpiration and higher transpiration causes a higher cooling effect of the leaf surface. However, this relation is inversely proportional, because leaf temp difference values are mostly negative. After 28 DUDS, the values of 843-22B did not differ any longer from those of ICTP8203. At that time point stomatal conductance values were already too low and accurate measurement was not possible anymore.

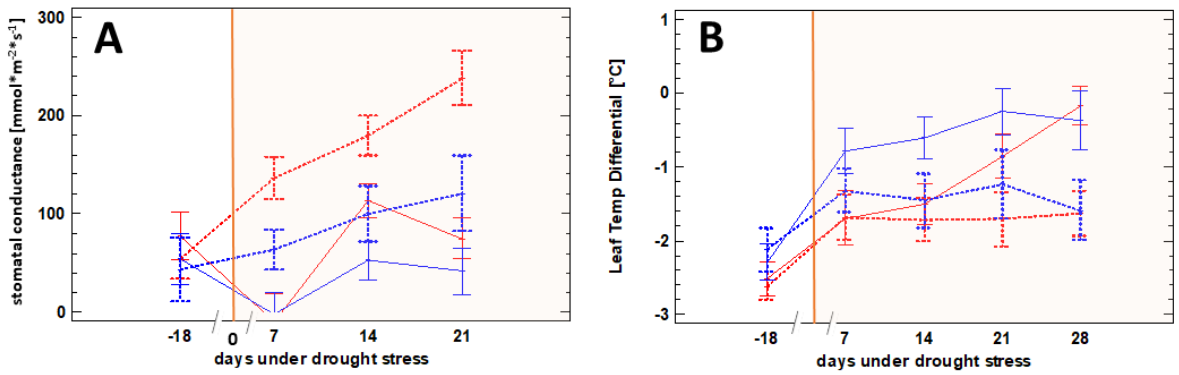

Figure 10: Water loss related trait measurements under progressive drought stress: A: stomatal conductance measured by AP4 porometer; B: leaf temp difference (temperature difference among leaf surface and ambient temperature); EB = 95% LSD;

Fig. 11 shows leaf chlorophyll contents measured by MultispeQ™ and Minolta™ SPAD502 devices. Fig. 11A+C shows chlorophyll content during drought stress. The overall leaf chlorophyll content (Fig. 11 B+D) is higher for the drought sensitive genotype (843-22B), although the difference is tiny (9.8%, p=0.0087 [SPAD], 2.4%, p=0.3875 [MultispeQ]). In both

genotypes, chlorophyll content gradually decreased under drought stress. However, ICTP8203 chlorophyll values decreased more rapidly.

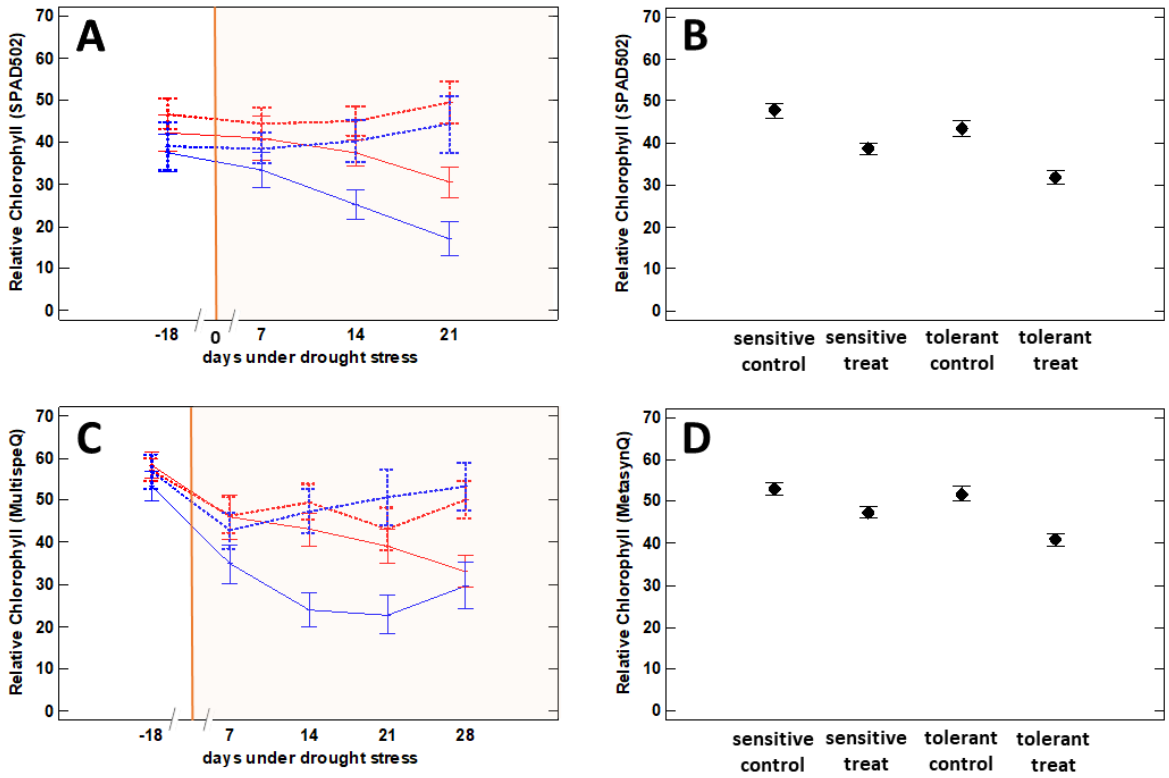

Figure 11: Relative chlorophyll content of the leaves, measured by two different devices; A: relative chlorophyll measured by SPAD502 device over time; B: relative chlorophyll measured by SPAD502 device for all measurement time points; C: relative chlorophyll measured by MultispeQ device over time; D: relative chlorophyll measured by MultispeQ device for all measurement time points; EB = 95% LSD;

At excessively high drought stress, light energy (above 400 µE) can damage the photosynthetic apparatus by the production of reactive oxygen species like singlet oxygen, superoxide and peroxide radicals. Therefore, plants developed mechanisms to dissipate this excess of light energy. The non-photochemical quenching is the sum of light energy which dissipates excess electrons by regulatory processes without damaging photosystems. Fig. 12 shows that the tolerant genotype (ICTP8203) reacts faster to drought by upregulation of non-photochemical quenching (PhiNPQ, Fig. 12D). This accompanies with the reduction of quantum yield of photosystem 2 (Phi2, Fig. 12B). PhiNO (Fig. 12C) which represents potential damage to the photosynthetic apparatus thus decreases. After 14 DUDS there are significant differences ($p<0.01$) between the two varieties for all parameters in Fig. 12. However, after 28

days under drought, values of sensitive genotype (843-22B) converged towards those of the tolerant genotype. At control conditions these parameters remained virtually unchanged without significant differences between the varieties. Fig. 12A shows that both control groups F_v/F_m remain at approx. 0.7. For the treatment groups, we may observe that the photosynthetic efficiency declined faster for ICTP8203. 843-22B genotype could stabilize their photosynthetic efficiency at least until 21 days under drought conditions. Only after 28 days F_v/F_m decreased to the same low level as ICTP8203 exhibited.

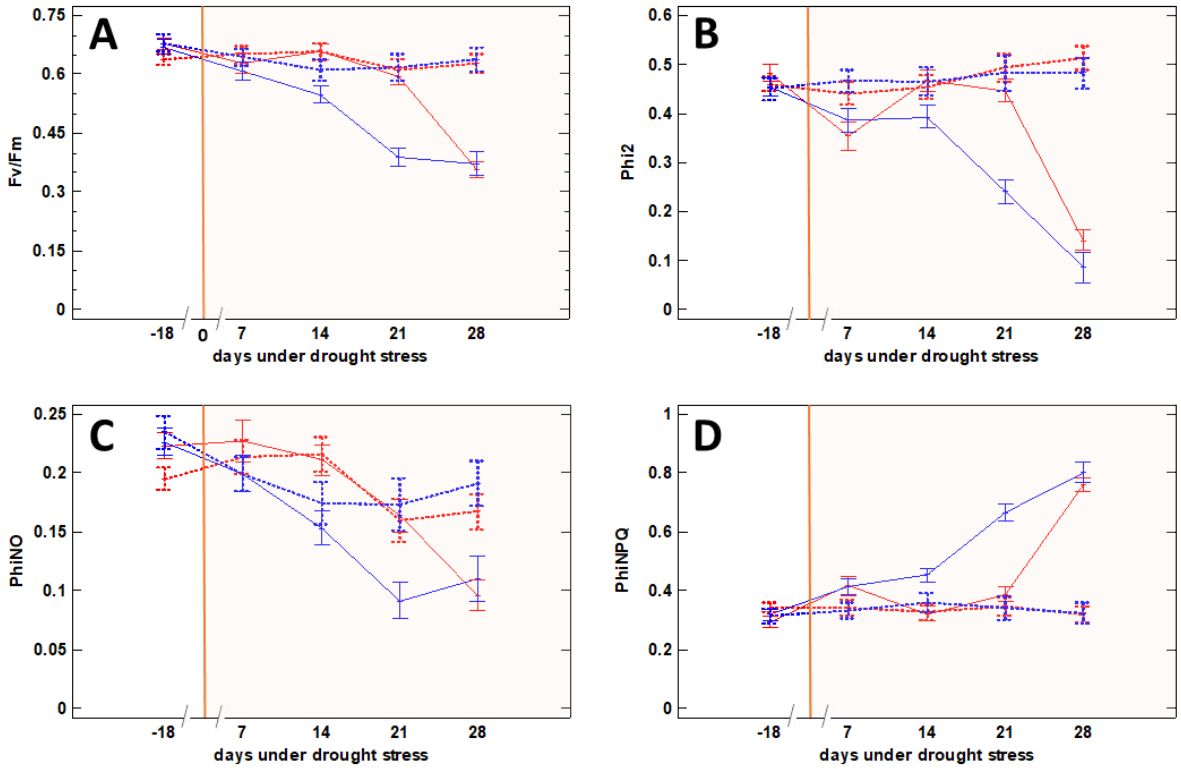

Figure 12: Photosynthesis related parameters measured by MultispeQ under progressive drought stress; A: F_v/F_m ratio; B: Phi2 = fraction of light energy captured by Photosystem II, converting light energy, producing ATP and NADPH; C: PhiNO = combination of a number of unregulated processes whose by-products can inhibit photosynthesis or be harmful to the plant; D: PhiNPQ = fraction of incoming light (excited electrons) that goes towards non-photochemical quenching, in order to protect from the adverse effects of high light intensity; EB = 95% LSD;

LEF (linear electron flow = number of electrons from absorbed photons which is used to oxidize H_2O at photosystem II; Fig. 13A) is directly related to Phi2 (LEF = Phi2 * light intensity PAR x 0.42) and thus closely correlates on it. Results measured by MINI PAM II (Fig. 13B-D) did not show relevant differences among genotype and drought stress because of the high

variance. Also, we analyzed only two biological replicates in each case with two technical measurement repetitions for practical reasons of time management, as all these measurements needed to be performed at comparable light conditions at approx. the same time of the day. A higher number of replicates is recommended for such analysis.

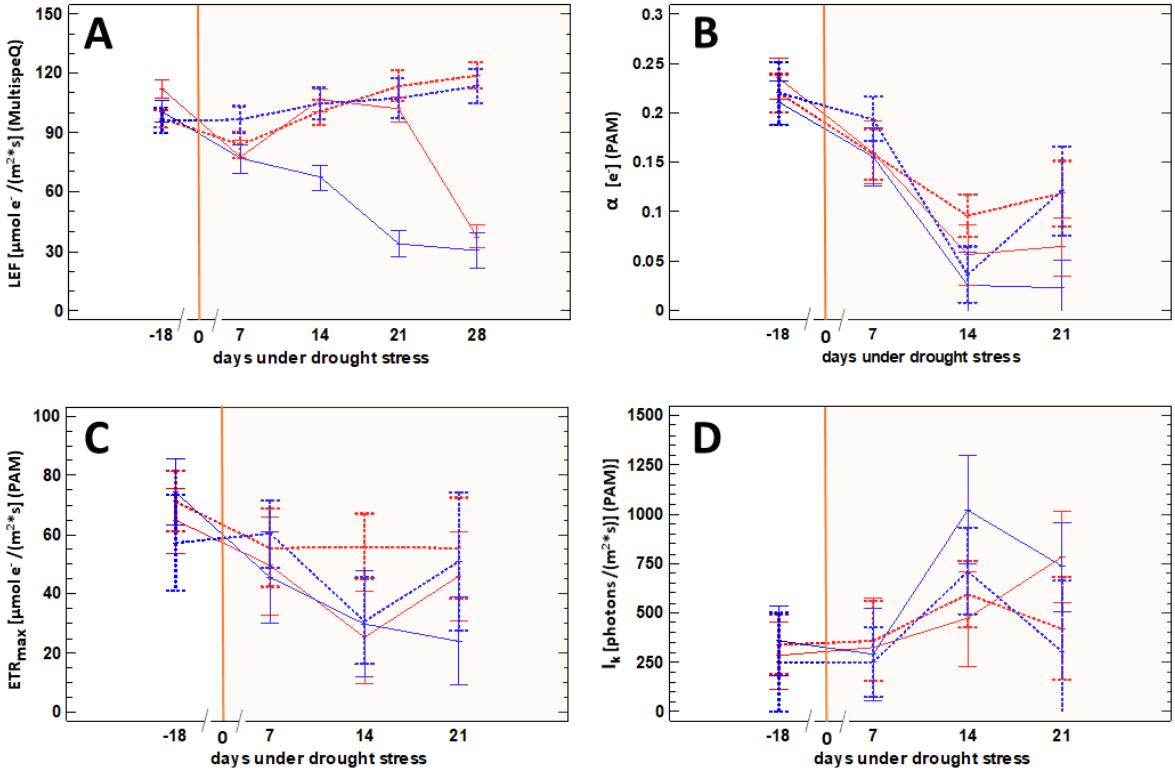

Figure 13: Photosynthesis related parameters measured by MultispeQ (A) and MINI PAM II (B-D) under progressive drought stress; A: LEF (linear electron flow); B: α = initial slope of light curve which is related to quantum efficiency of photosynthesis; C: ETR_{max} = maximum of electron transfer rate; D: I_k = minimum saturation irradiance; EB = 95% LSD;

Figure 14 shows results from WALZ GFS-3000 device. CO_2 assimilation rate (A; Fig. 14A), the total water vapor conductance (gH_2O; Fig 14B) and the water use efficiency (WUE; Fig 14C) which is defined as the ratio between A and gH_2O. At an earlier stage (18 days before drought stress inducement) drought tolerant genotype had a slightly higher assimilation rate and WUE. There was a wholesale decrease of all parameters over the time. However, there was a much stronger decrease for the drought stressed plants. Under control conditions we did not observe differences among genotypes. At high drought stress level, drought tolerant plants

recorded a higher reduction in A, gH_2O and WUE than drought sensitive plants. Certainly, WUE again increased from day 14 to day 21 under drought stress for drought tolerant genotype.

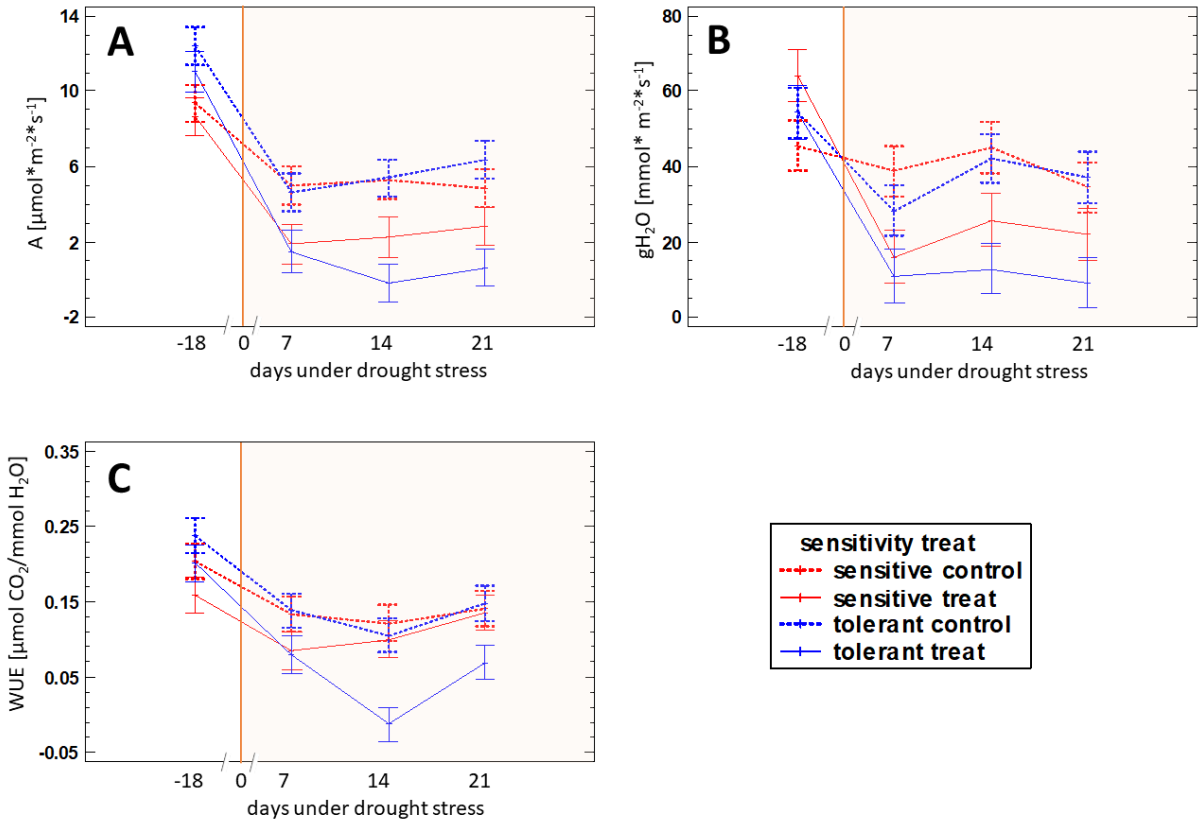

Figure 14: Secondary photosynthesis processes measured with WALZ GFS 3000; A: A = CO_2-assimilation, EB = 70% LSD; B: gH_2O = total water vapor conductance, EB = 70% LSD; C = WUE (water use efficiency), EB = 80% LSD;

4.4. Soil respiration

IRGA (infrared gas analyzation) of the soil was performed at two timepoints: once before the experiment (untreated soil) and other after the experiment. Basal soil respiration (BR) and substrate induced soil respiration (SIR; with 0.2 % glucose) was monitored (see chapter 3.5). Concerning basal respiration (BR; taken after at 2 hours of the measuring process; Fig. 15A), there are no significant differences among the groups to observe. Also, the untreated soil measured previous to the experiment is not relevantly different from the soil measured after the drought experiment. However, there is the trend that, after having been influenced by drought stressed plants, BR increased in the sensitive genotype, and decreased in the tolerant genotype. This pattern continues on for substrate induced respiration (SIR). SIR is monitored at 2 hours

(initial point of respiratory response; Fig. 15B) and 14 hours (maximum of induced soil respiration; Fig. 15C). In both cases the tolerant control group results are significantly higher ($p<0.05$) than the other groups. Fig 15D-E shows the respiratory quotient RESP in % of BR. SIR, monitored after 14 hours (SIR_{max}) may be more elucidating as far as preadaptation of the soil to previous glucose exudation by the plants is concerned, and we see that the sensitive SIR_{max} of the control groups is significantly higher ($p<0.05$) than SIR_{max} of the other groups. For the untreated soil SIR and respiratory quotient is much higher than for the soils which were dried during the experiment.

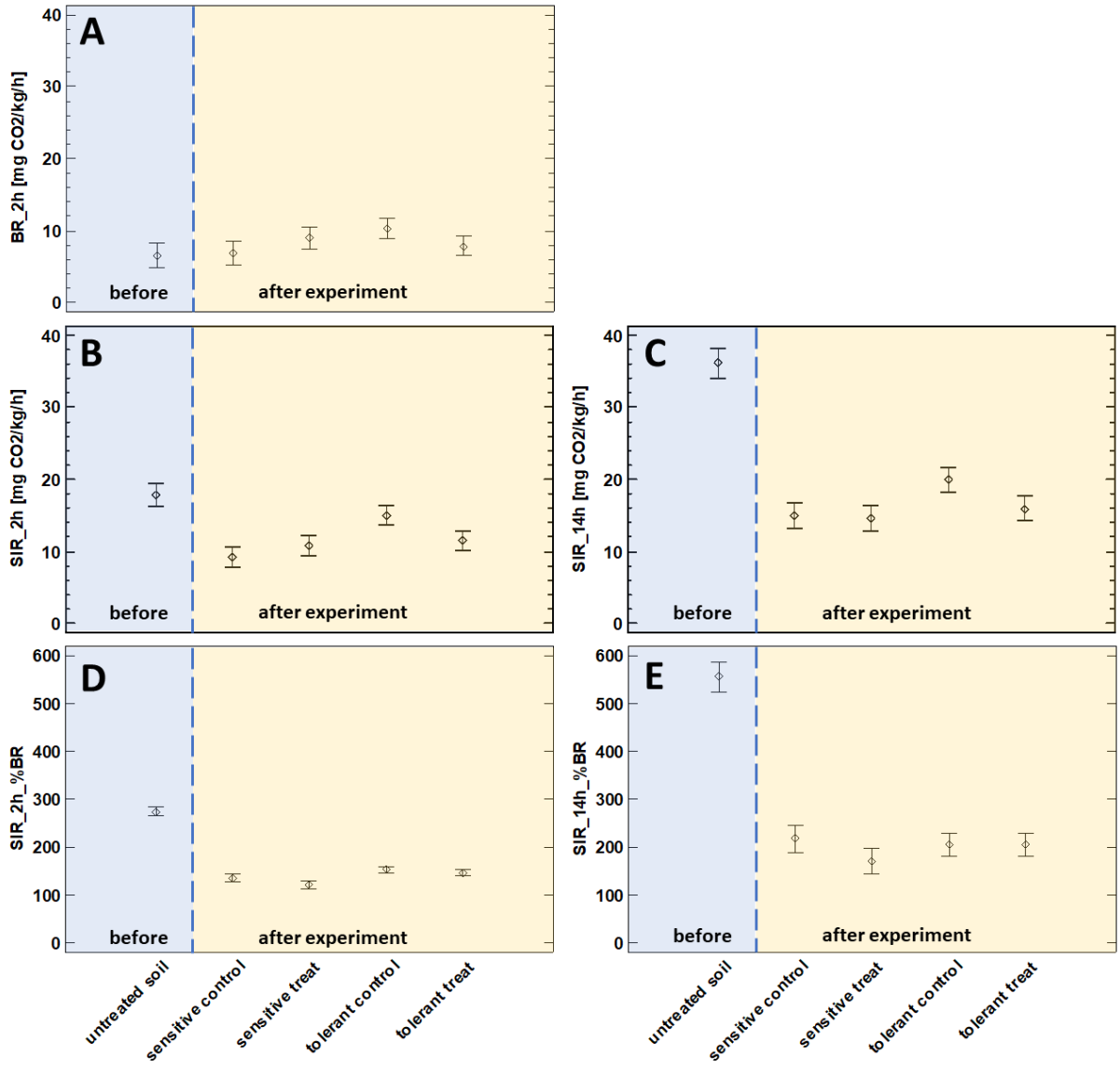

Figure 15: IRGA results: untreated soil before the experiment (blue, left side) and soil samples taken after the experiment (day 134, see Fig. 2) distinguished in the four groups (yellow, right side); A: BR (basal respiration) 2 hours after starting measuring IRGA (Infrared Gas Analyzer); B: SIR (substrate induced respiration) 2 hours after starting measuring IRGA; C: SIR 14 hours after starting measuring IRGA; D: SIR 2 hours after starting measuring IRGA in % of BR; E: SIR 14 hours after starting measuring IRGA in % of BR; EB = 95% LSD;

5. Discussion

The capability of pearl millet to cope with low water availability in comparison to other cereals is remarkable. Pearl millet is beside sorghum the most frequently cultivated millet variant. The physiological and molecular understanding of drought stress in plants, especially for crops, can support the management of agriculture in times of global warming and increased droughts (Wagner 1999; Tebaldi et al. 2006).

5.1. Monitoring of the environmental conditions

The median value of soil moisture among cultivars differed (see Fig. 4B), despite the, exactly similar irrigation regime. Therefore, water consumption of the drought tolerant genotype was approx. 20% higher at abundant water supply. However, in later stages (from day 72 after sowing) water consumption of sensitive genotype fell below water consumption of tolerant genotype. Caused by limited soil volume also control groups had to withstand some mild drought stress in later stages of the experiment. Under this mild drought stress ICTP 8203 reacts stronger to water limitation by reduced water consumption. The median CO_2 concentration was slightly higher (480 ppm) than global mean which, however, may appropriately simulate future scenarios with ongoing climate change (IPCC 2014). Air pressure (at around 1000 mbar) and relative humidity (at around 60 %) are comparable to actual growing areas. However, relative humidity is seasonally lower in semi-arid regions, where pearl millet is mainly cultivated. DLI (Daily light integral) and light intensity of PAR increased during the experiment; DLI close to 20 moles/day/m^2 and light intensity of PAR close to 800 µE at noon. This increase was caused by seasonal increasing sun azimuth angle and by longer bright hours and thus light radiation dosage from outside of the glasshouse. The values are of medium magnitude. DLI can go over 50 moles/day/m^2 and PAR over 2000 µE in regions of high light intensities (Torres and Lopez 2010). Some of the drought stress causing environmental parameters can be even more desiccation-promoting, however available soil volume for water storage is generally much greater than in our experiment. In the end, for such a drought stress experiment it is most important to regulate severity of stress depending on the plant species and on the time period of drought treatment. At this level of drought treatment, we could measure plant parameters during a gradual increase of drought stress for the plant over several weeks.

5.2. Growth traits

There are in general (i.e. without drought stress) some differences in growth among genotype (see Fig. 6). The drought tolerant genotype grew higher than the sensitive one, especially the difference in shoot height is remarkable. The results of the side-experiment in our outdoor garden (Fig. 21 [appendix]) support that there are general differences in shoot height and biomass. For ICTP 8203 cultivar (Rai et al. 1990) a shoot height of around 1.5 – 1.6 m is mentioned. In the main experiment ICTP 8203 plants reached a height of 2.2 meters in average under drought stress and 1.8 meters under controlled conditions and limited pot size. In the outdoor side-experiment ICTP 8203 plants grow up to approx. 3 meters. These even higher growth height is caused by more rooting space and good weather conditions with much light (up to 2000 micromoles h^{-1} m^{-2}) over the summer period in Vienna in the year 2019. 843-22B is known as a small-growing cultivar. Both groups (control and treat) reached a height of approx. 1.3 meters as well as the plants from the outdoor side experiment. In many drought stress studies of different plants an increased root/shoot ratio and/or a shift of the center of the root biomass to lower depth was observed (Zeid and Shedeed 2006; Kuster et al. 2013; Xu et al. 2015; Ghatak et al. 2016). Regarding the aboveground biomass, we observed a similar pattern (Fig. 9, Fig. 21): In the main-experiment aboveground biomass was higher for ICTP 8203 cultivar; drought stress decreases gain of biomass in general, but more so for ICTP 8203, and differences among cultivar in the outdoor side-experiment is more substantial.

In crop breeding, dwarfing by introducing dwarfing genes is often a main target and often leads to an increased harvest index resulting with an increased crop yield per hectare. For instance, the advantage of dwarf varieties in wheat was primarily the increased harvest index without any loss in total biomass (Austin et al. 1980). The introduction and utilization of dwarfing genes is responsible for much of the progress in breeding of wheat and rice (Mahalakshmi et al. 1991). However, this advantage cannot be maintained in dryer rainfed areas (McNeal et al. 1972; Laing and Fischer 1977; Gale, M. D. & Youssefian, S. 1985). Also, in pearl millet similar patterns are observed (Mahalakshmi et al. 1991). In contrast, in our experiment, harvest index of genotype ICTP 8203 (known as more drought tolerant) declined more under drought stress conditions than genotype 843-22B (known as more drought sensitive). Based on these results, it may be concluded that ICTP 8203 plants, known for deeper root layers (Chaturvedi, Palak et al. in prep.) were actually rather limited in soil volume than 843-22B for optimal growth and less regardless of their drought tolerance, as this would be contrasting the published knowledge of ICTP 8203 being the more drought tolerant cultivar.

For the results of Fig. 7A-B a correction factor was necessary for the calculation of the biomass (see chapter 3.6). The values of the estimated biomass, based on the calculation of leaves and stem as geometrical shapes and the usage of the density of water, were clearly to high. This was most probably due to an overestimation of the thickness of the leaves because of the non-planar shape of the leave because of the middle lamella.

5.3. Photosynthesis

Photosynthesis converts atmospheric CO_2 and H_2O into sugars and O_2 using light energy converted to chemical energy equivalents. This sugar is the basis for all physiological processes and growth in plants and therefore also for harvest in agriculture. Beside the number of photons (light energy), water is the most limiting factor for photosynthesis. In agriculture the basic goal is to keep growth dependent parameters close to their optimum, so that the crop can display their full genetic potential to gain high yields. Salt and drought stress are virtually affecting all aspects of plant physiology and metabolism (Zhu 2002). Hence, it makes sense to consider parameters closely related to processes of primary photosynthesis.

As a first response to water limitation plants react by closing their stomata to reduce water loss (Austin 1991; Berry et al. 2010; Brodribb and Mcadam 2011; Clauw et al. 2015). The plant hormone ABA (abscisic acid) plays an important role in plant water regulation. After the imposition of water deficit in soil, root cells are producing more ABA by the plastidal 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway, which is then transported with the xylem sap up to the photosynthetically active tissues. Many drought stress studies also observed an alkalization of the xylem sap e.g. (Bahrun et al. 2002; Sobeih et al. 2004)). This xylem sap alkalization seems not to be a signal *per se* (Wilkinson and Davies 1997), however, it facilitates the mobilization and the transport of ABA caused by the Henderson-Hasselbalch kinetics (Sharp and Davies 2009). ABA is a weak organic acid. As pH increases, more ABA is loaded into the xylem sap and finally transported up to stem and leaves. However, there are several other factors determining ABA concentration in stomatal guard cells: ABA synthesis, recirculation and delivery rates (Sharp and Davies 2009). Mechanical opening and closing then takes place by ionic fluxes (K^+, Cl^-) and subsequent water flux between guard cells and subsidiary cells. These biochemical complexity of regulation makes it difficult to assess plant water management under drought stress, whereas measuring stomatal conductance or leaf temperature difference give direct information about transpiration rate and stomatal opening

state. Moreover, it is a non-invasive method and results are measured in real-time. Based on our results (Fig. 10), we can assume that stomatal conductance of 843-22B is in general slightly higher than for ICTP8203. Under drought stress, both genotypes react similarly with a strong downregulation of stomatal conductance as a first response to water limitation and a re-opening of stomata after 14 DUDS. Previous studies monitoring different species have shown that the control of leaf gas exchange can be divided in two different behavior types (Kholová et al. 2010): On the one hand, there are plants which are capable to sustain transpiration until the soil is fairly dry. On the other hand, there are plants that react already with a decline in leaf gas exchange at still relatively moist soil, e.g. maize (Ray and Sinclair 1997) and soybean (Vadez and Sinclair 2001; Hufstetler et al. 2007). The FTSW (fraction of transpirable soil water) is defined as the ratio between available soil water (ASW) at a given date and total transpirable soil water (TTSW) (Sinclair and Ludlow 1986). The TTSW is defined as the plant available water in the soil which can used for transpiration. This value can range between an upper and a lower limit. These limits are depending on soil characteristics but also on plant characteristics (Ritchie 1981; Lacape et al. 1998). ASW is the available soil water for a plant at a certain time point. FTSW can calculated as FTSW = (pot weight day n – final pot weight) / (initial pot weight – final pot weight) (Kholová et al. 2010). The use of FTSW as a threshold where transpiration declines is very helpful to understand genotypical differences in behavior under soil water deficit (Sadras and Milroy 1996). Depending on genotype, there is a certain value of FTSW when stomatal conductance starts to decrease quite rapidly. In the study of Kholová et al. (2010) they compared four different pearl millet genotypes on this behavior. They observed two important patterns: (i) threshold of FTSW is lower at the reproductive stage of the plants than the vegetative stage; (ii) threshold of FTSW is lower for more drought tolerant genotypes, however, these differences are only observed during vegetative stage. FTSW values represent the portion of remaining volumetric soil water available for transpiration (Kholová et al. 2010) and can be used as a stress indicator for drought (Ritchie 1981). FTSW is comparable to the volumetric water content of the soil, however, water content at the maximum field capacity is necessary to know for the comparison of the values. For the utilized soil with a maximum field capacity and a corresponding water content of approx. 50 %vol of total soil volume an FTSW of 0.2 corresponds to 10 %vol soil moisture. In our experiment more measuring time points of stomatal conductance would be necessary to determine these thresholds. However, what we get from our data is that still at less than 10 %vol soil moisture there is some gas exchange sustaining a marginal photosynthetic rate for grain-filling, facilitating the completion of the

plants life cycle. This pattern is similar for both genotypes, only stomatal conductance is slightly higher for the drought sensitive genotype (843-22B) as well as it is for control groups. Approx. 50 µmol*m^{-2}*s^{-1} for ICTP 8203 (tolerant) and 80 µmol*m^{-2}*s^{-1} for 843-22B (sensitive). That may hint at a higher capability to control gas exchange in ICTP8203.

Both chlorophyll a and b are sensitive to drought stress (Farooq et al. 2009). A decrease in chlorophyll content under drought stress is well documented in many crops e.g. *Triticum aestivum* (Bijanzadeh and Emam 2010), *Hordeum vulgare* (Anjum et al. 2003b), *Zea mays* (Khayatnezhad 2012), *Gossypium hirsutum* (Massacci et al. 2008) *Helianthus annus* (Poormohammad Kiani et al. 2008), *Vaccinium myrtillus* (Tahkokorpi et al. 2007) and *Pennisetum glaucum* (Toderich et al. 2018). Our results show a possibly higher chlorophyll content in the leaves for 843-22B genotype, however, only results from SPAD502 device (Fig. 11B) supply a significant difference ($p<0.05$). This difference was not observed on a relevant level by other monitoring methods (MultispeQ: mean of 47.8 for 843-22B and 43.6 for ICTP 8203). Indeed, the two genotypes reacted differently to drought stress. Drought tolerant genotype (ICTP 8203) reacts stronger to water limitation with a decline in chlorophyll content. For day 28 under drought stress (Fig. 11C), there seems to be an increase in chlorophyll compared to day 21, however, this might be biased by unintentional selection of the greener leaves for measuring.

The F_v/F_m ratios in pearl millet are not well documented and measured just in few of pearl millet studies. Toderich et al. (2018) measured values around 0.8 for unstressed plants at the seedling stage for genotype ‘HHVBC-Tall’ and ‘IP 19586’. Masojídek et al. (1991) also measured F_v/F_m values above 0.8 at 15 DAS. Therefore, as for most plants, we can assume an optimal F_v/F_m ratio for pearl millet at around 0.8. At the early response measurement (38 DAS, in Fig. 12A: 18 days before drought stress inducement) means of all groups were below a F_v/F_m of 0.7 and therefore below the optimum. However, this level could be retained for both control groups. Under water stressed conditions we observed relevant differences: Drought tolerant genotype (ICTP 8203) had a roughly continuous decline from start of drought stress inducement, whereas drought sensitive genotype (843-22B) maintained F_v/F_m values until day 21 under drought stress at the same level as control groups. After 28 DUDS sensitive genotype reached the same low level as tolerant one. Although the drought tolerant genotype had to cope with a more severe drought stress, based on the higher biomass, the drought sensitive genotype seems to have a higher ability to keep up a high leaf chlorophyll fluorescence quenching. This may be regarded as a stay-green effect/trait. The stay-green trait is a heritable delayed foliar

senescence trait of a model or crop plant species (Thomas and Ougham 2014). The stay-green physiotype is induced by a complex signaling cascade. It involves mutations suppressing ABA, ethylene, brassinosteroid and andstrigolactone signal transduction and cytokinin signaling (Serba and Yadav 2016). These signals are otherwise regulating leaf senescence (Kusaba et al. 2013). The onset and rate of leaf senescence in grain crops can be considered as the balance between N demand by the grain and N supply during grain filling (Borrell et al. 2001). During the grain filling period N is transported from vegetative tissues to the grains. This becomes especially important under conditions of N deficiency in the soil or drought stress (Ta and Weiland 1992). The stay-green phenomenon is described as a very well characterized response to drought adaptation in sorghum (Jordan et al. 2003; Harris et al. 2007; Kassahun et al. 2010). Also, in pearl millet putative functional markers for selecting genotypes with stay-green traits under drought stress were put forward (Sehgal et al. 2015; Debieu et al. 2018).

Even though, light is the energy base for photosynthesis, it can, in surplus, be harmful to the plant. At excessive/oversaturating light intensities inactivation of the electron transport and formation of ROS which cause damage to the reaction centre in particular to the D1 protein may occur (Aro et al. 1993). Therefore, mainly PS II is affected by this so called photoinhibition. Regulation processes to avoid photoinhibition are ranging from immediate local changes of the photosynthetic reaction centers or the light harvesting antennae, pH regulation of the cytochrome b6/f complex, altered chloroplast organization up to acclimation responses which are affecting gene expression (Lambrev et al. 2012). Especially persistent high-light conditions and/or the combination with other stressors cause photo-damage (Murata et al. 2007; Vass 2012). NPQ (non-photochemical quenching) is the most important short-term reversible photoprotective process. NPQ thermally de-excite singlet excited states in the PS II antenna and therefore reduces the excitation pressure on the reaction centre (Lambrev et al. 2012). Photochemical quenching at high light may, as PSII is already saturated, dissipates via unregulated processes and has a high potential to damage the photosynthetic apparatus. However, plants have developed rapid and efficient repair mechanisms to overcome damage by light (Aro et al. 1993; Dewez et al. 2009). Under well-watered conditions quantum yield of PS II (Phi2), NPQ and surplus photochemical quenching (PhiNO) remained constant during the experiment (Fig 12B-D) and were therefore adapted and acclimatized to the exposed light intensity (maximum daily light intensity close to 800 µE). In growing regions of pearl millet light intensity could be even higher (up to 2000 µE). Under drought stress conditions plants were not able to use the same amount of light energy for assimilation (Phi2). Though, both

genotypes were able to compensate this by NPQ without increased photochemical quenching (even slight decrease). Regarding genotypic differences, ICTP 8203 reacts faster to drought stress. This pattern can be correlated over the course of time to chlorophyll content and F_v/F_m ratio. This is standing to reason, since chlorophyll content is a major limiting factor for light capture towards the reaction centers for photosynthesis. NPQ can be seen as an indicator for excess light energy (Joshi et al. 1995; Ruban et al. 2002). A lowered value of F_v/F_m indicates that a proportion of the reaction centers of PS II is damaged or inactivated (Ashraf and Harris 2013). At the same amount of light energy but with lower chlorophyll content and F_v/F_m ratio it is to be expected that quantum yield decreases and non-photochemical quenching and/or photochemical quenching increase. This photoinhibition is commonly observed under abiotic stress like drought or cold (Baker and Rosenqvist 2004; Zhu et al. 2008; Zlatev 2009; Garbulsky et al. 2011; Vaz and Sharma 2011; Sukhova and Sukhov 2018).

5.4. Soil respiration

IRGA (Infrared Gas Analyzation) is a long-term established method for detecting CO_2 evolution in soils which is connected to heterotrophic microbiological activity. Growing plants have a strong impact on the soil. A considerable amount of a plants photosynthates are used for root exudation. However, quantity and quality of root exudates depend on plant species, age, abiotic and biotic environmental factors (Badri and Vivianco 2009). Root exudation is a main source of soil organic carbon (Hütsch et al. 2002). Root exudates mainly consists of carbon-based compounds (Bais et al. 2006) which are used as energy resource for microorganisms. Furthermore, root exudates are important in interacting with neighbouring plants and microorganisms (Bais et al. 2004; Weir et al. 2004; Bais et al. 2006; Broeckling et al. 2008). Based on our results we can make two assumptions: (i) drought stress decreases microbiological activity; (ii) drought tolerant genotype (ICTP 8203) had a more positive effect on the microbial community, because of the higher measured soil respiration. More symbiotic relationship of soil microorganisms to plants is a known part of the stay-green effect (Kusaba et al. 2013). Although drought stress can increase certain root exudates e.g. (Calvo et al. 2017), at long term drought stress the total amount of root exudates must decrease, because of the reduced photosynthetic assimilates transferred to the soil (van der Molen et al. 2011). This will in term also influence soil microbiome by decreased availability of energy-rich nutrients for heterotrophic organisms like fungi and many bacteria. The biological nitrification inhibition

(BNI) is defined as the ability to suppress the soil nitrification by the release of nitrification inhibitors from plant roots (Subbarao et al. 2013). Nitrification is the process which transfers low mobile NH_4^+ to highly mobile NO_3^-. This is due to the mainly negative partial charges of soil particles like clay minerals and humic substances which are not able to bind NO_3^-. This increased mobility of inorganic, plant available N causes higher loss due to leaching and/or gaseous emission (denitrification). This results in a low nitrogen use efficiency (NUE) and can cause environmental problems through nitrate pollution, eutrophication and emission of the greenhouse gas N_2O (Giles 2005; Subbarao et al. 2006; Galloway et al. 2008; Schlesinger 2009). This nitrification and denitrification processes are facilitated by a wide variety of soil organisms such as bacteria, fungi and archaea (Hayatsu et al. 2008). These organisms can be inhibited by biological nitrification inhibitors which are exudated by plant roots. Plants with BNI activity are widespread in tropical species e.g. *Bracharia* ssp. with intra- and interspecific differences (Subbarao et al. 2007), however, BNI activity was also observed in important cereals like sorghum, rice and pearl millet (Subbarao et al. 2007; Subbarao et al. 2013; Thivierge et al. 2015; Sun et al. 2016). I suggest that (i) higher root exudation and (ii) decreased N loss by BNI activity will increase soil respiration. Based on this, we hypothesize that the soil influenced by ICTP 8203 plants had a higher rate of BNI activity and therefore a lowered loss of N which is then available for plant uptake.

5.5. Conclusion

Pearl millet can generally cope with very sparse soil humidity as compared to other cereals. Genotype ICTP 8203 (drought tolerant) grow very high (over 3 m in the field). At limited soil volume (as it was in main experiment) genotype ICTP 8203 still grows high (control: 2.2 m; drought stressed: 1.8 m) but then tends to etiolate, while the genetic potential in growth height of genotype 843-22B could already be exploited in the plant pots. Under low-stress conditions (>60 %RH and 25 %vol soil humidity) differences in harvest are very low, although ICTP 8203 had a slightly higher total aboveground biomass and therefore a slightly lower HI. At high drought stress conditions ICTP 8203 could not take advantage of its higher drought tolerance caused by to less soil volume. In comparison, 843-22B did not get that hampered by this limitation, since growth height and biomass was lower than for ICTP 8203. It could be shown that both genotypes require soil depths above 50 cm for optimal growth, however, genotype ICTP 8203 benefits more from even deeper soils to exploit more water from deeper parts of the

soil. The drought sensitive genotype invested a priori more into primary photosynthetic processes and therefore had a higher demand in water and N. Additionally, the drought tolerant genotype could probably build up a higher BNI activity by a higher root exudation which corresponds to measured soil respiration.

Due to its deeper root layers of ICTP 8203 compared to 843-22B, further drought experiments on this genotype should include higher soil volumes for a better simulation of field conditions, where soils are in general deeper. Genotype ICTP 8203 can then exploit better its full potential and allows a better comparison to smaller growing pearl millet genotypes like 843-22B. In this work I did not analyze any quality or quantity of root exudates nor perform any microbial analyses. Furthermore, for the time being, just a few BNI active compounds are verified particularly for pearl millet. However, these are pressing topics and a lot of research is going on in this field. More research on this line, would be necessary to prove the hypothesis that ICTP 8203 exudates more than 843-22B and can also build up a higher BNI activity.

These experiments will help to increase Water use efficiency (WUE) and Nitrogen use efficiency (NUE) in cereal crops and breeding programs adapted by the breeders all over the world. Pearl millet though one of the very important and nutritional crops underutilized commercially, therefore these experiments build a foundation stone for more screening of different geographically distributed cultivars for more efficient breeding and also provide climate resilient crops for future.

6. References

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7. Appendix

7.1. Zusammenfassung (German)

Die Aufrechterhaltung der Ernährungssicherheit und die weitere Reduzierung des Welthungers gehören zu den größten Herausforderungen, die der Klimawandel und die wachsende Weltbevölkerung mit sich bringen. Neben der Erhöhung der Jahresmitteltemperatur, resultiert der Klimawandel in der Erhöhung der Wetterextreme. Trockenheit führt weltweit gesehen zu den größten Ernteeinbußen. Dies legt nahe, dass Kulturpflanzen mit hoher Toleranz gegenüber Trockenheit in Zukunft an Bedeutung gewinnen werden. C_4 Pflanzen sind grundsätzlich besser an limitierte Wasserressourcen adaptiert und weisen eine höhere Wassernutzungseffizienz (WUE) auf. Die Perlhirse (*Pennisetum glaucum* [L.] R. Br.) ist bekannt für ihre hohe Toleranz gegenüber Trockenheit und Salzstress. In dieser Arbeit wurden die beiden Kultivare ‚ICTP 8203' (trockenheitstolerant) und ‚843-22B' (trockenheitssensitiv), zur Verfügung gestellt vom ‚International Crops Research Institute for the Semi-Arid Tropics' (ICRISAT) auf deren physiologischen und wachstumsstrategischen Unterschiede unter Trockenheitsstress untersucht. Dies wurde unter kontrollierten Glashausbedingungen, wo die Pflanzen in Polyethylen-Rohren mit definiertem Bewässerungs-Regime kultiviert wurden, und im Freilandexperiment, durchgeführt. Unsere Untersuchungen zeigten, dass Kultivar ‚ICTP 8203' eine deutlich höhere Wuchshöhe und auch höhere oberirdische Biomasse erreicht gegenüber ‚843-22B'. Diese starken Unterschiede führten dazu, dass das vorhandene Bodenvolumen in den Polyethylen-Rohren limitierender für ‚ICTP 8203' waren und dessen höhere Trockenheitstoleranz gegenüber ‚843-22B' nicht ausspielen konnte. Basierend auf der Messung der Bodenatmung und Untersuchungen der Rhizosphäre (Chaturvedi et al. in prep.) kann ‚ICTP 8203' vermutlich eine höhere BNI-Aktivität (biologische Nitrifikations-Inhibition) durch Wurzelexudation aufbauen. Dies zu überprüfen erfordert allerdings weitere, detailliertere Untersuchungen in diesem Feld. Des Weiteren wäre ein Experiment mit höherem Bodenvolumen, welches die Feldbedingungen besser repräsentiert in Erwägung zu ziehen.

7.2. Preliminary experiment

In a preliminary experiment I did some physiological measurements to get some experience with the devices and characteristics of the two different genotypes. Figure 16 shows soil humidity, light intensity, ambient humidity and ambient temperature conditions during the preliminary experiment. All plants were well-watered during the experiment, however, there was a mild drought stress measured at day 14, where soil humidity declined down to approx. 15-20 % (Fig. 16A). Furthermore, there was a slightly increased ambient temperature (Fig. 16C) and a decreased ambient humidity (Fig. 16B). At day 0 light intensity was quite low, caused by outside weather conditions.

Figure 17 shows stomatal conductance (A-B) and leaf temperature difference (C-D). Over time fluctuations are too high to get significant differences among genotype, if there are some. However, overall measurements (Fig. B+D) indicate, that drought sensitive genotype (843-22B) may transpire little more than drought tolerant genotype (ICTP8203).

In chlorophyll content (Fig. 18), measured by SPAD502-device and MultispeQ-device, drought sensitive genotype (843-22B) has a slightly higher leaf chlorophyll content than tolerant genotype (ICTP8203). However, difference is only significant ($p<0.05$) measured by MultispeQ-device (Fig. 18D), for SPAD502 measurements $p=0.11$. Further, difference is rather slight than substantial.

Parameters in Fig. 19 remained more or less unchanged over time. However, at day 14 with slightly different environmental conditions (see Fig. 16) we observe a completely different reaction to drought stress. Phi2 (fraction of light energy captured by PS II) decreases for the tolerant genotype but increases for sensitive one, both significantly to each other and to 7 days before and 7 days after. In reverse, PhiNPQ (fraction of light energy captured by PS II lost by unregulated processes that can damage photosynthetic apparatus) increases for tolerant genotype and decreases for sensitive one. Also, F_v/F_m ratio (Fig. 19A) and LEF (linear electron flow, Fig. 20A) follow the same pattern. PhiNO remained unchanged without difference among genotype. Measurements from MINI-PAM-device cannot give any significant difference among genotypes, however, we measured just one biological replicate per genotype (5 measurements per plant and measuring day).

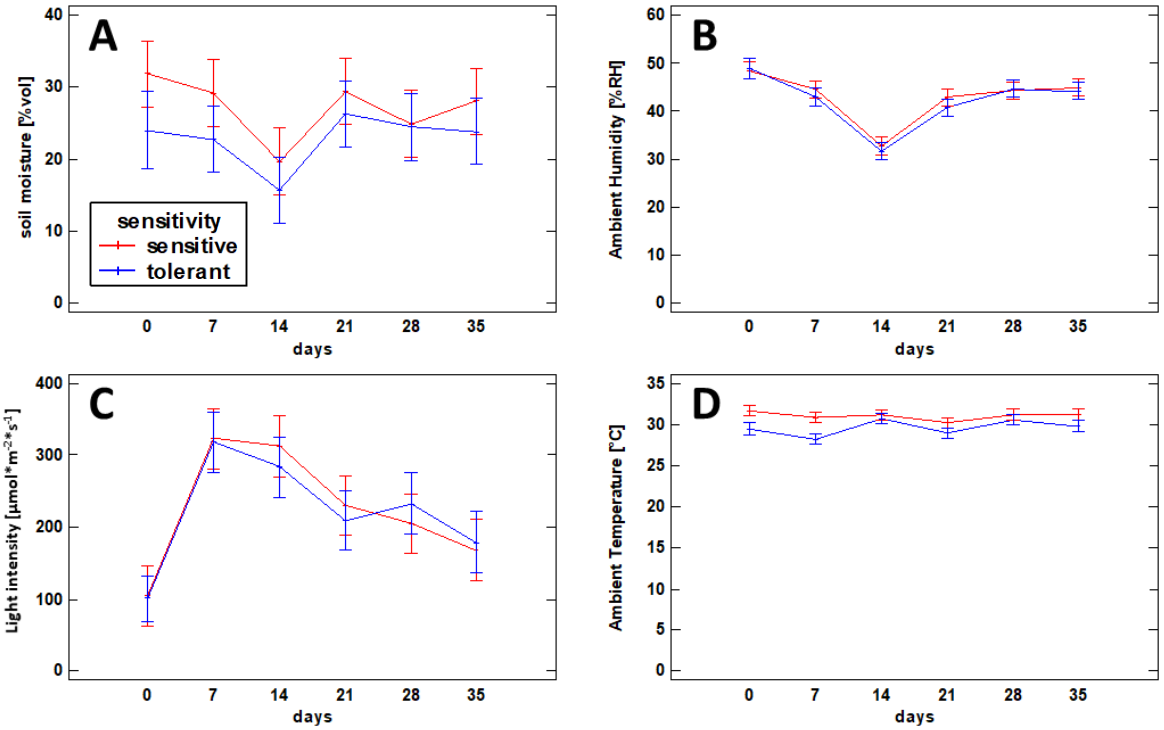

Figure 16: Environmental parameters of the pre-experiment, measured once per week at the timepoint of measuring the photosynthetic parameters: A: soil moisture in %vol; B: ambient humidity in % relative humidity; C: light intensity in µmol*m^{-2}*s^{-1}; D: ambient temperature in °C; EB = 95% LSD;

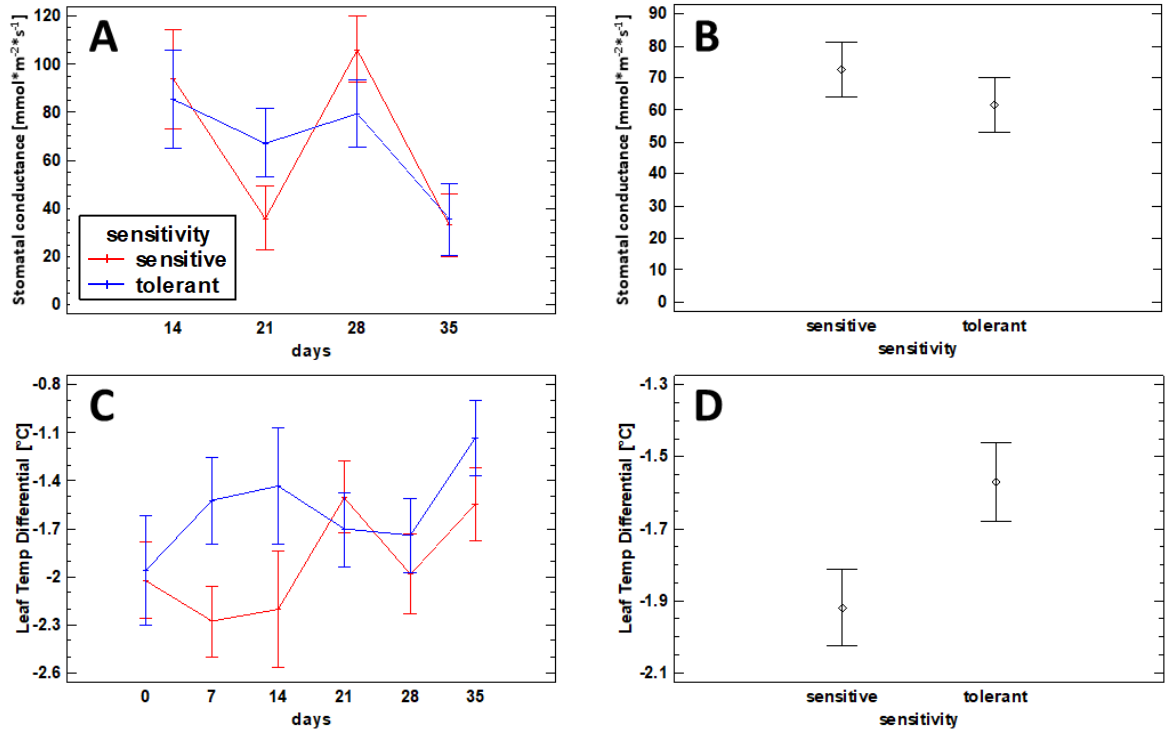

Figure 17: Water loss related measurements of the pre-experiment: A: stomatal conductance in µmol*m^{-2}*s^{-1} over time; B: stomatal conductance overall timepoints; C: leaf temperature differential in °C over time; D: leaf temp differential overall timepoints; EB = 95% LSD;

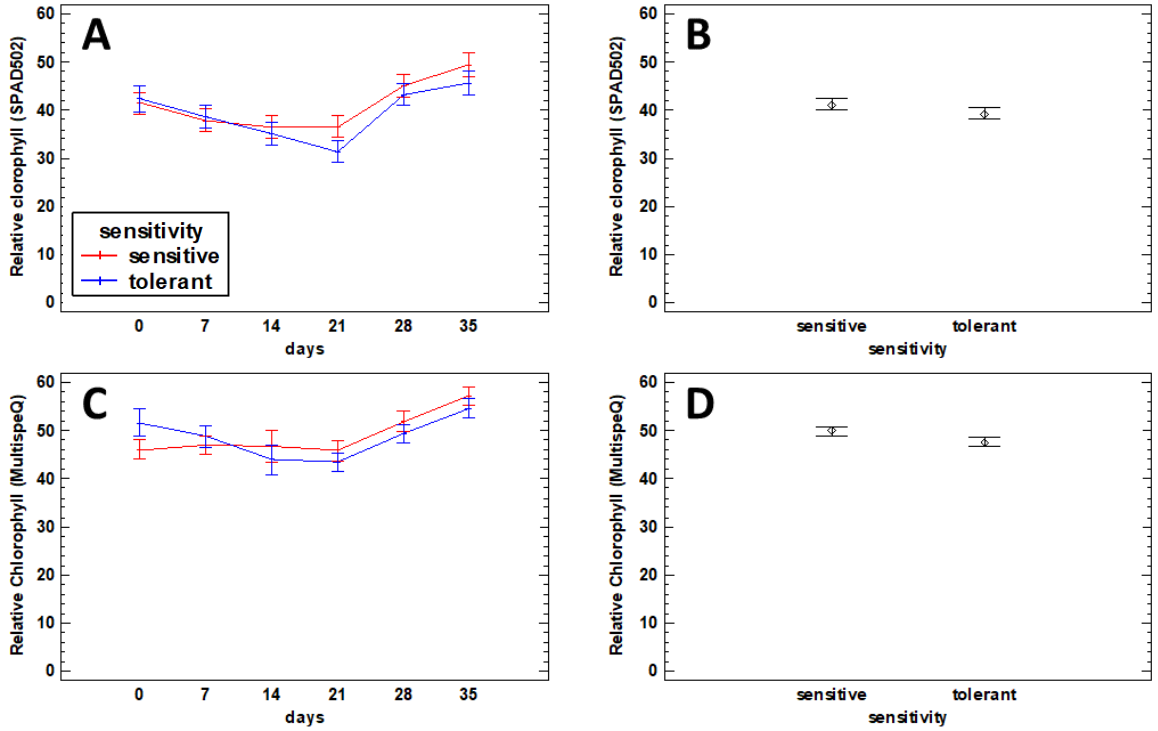

Figure 18: Relative leaf chlorophyll contents of the pre-experiment: A: relative chlorophyll content measured by SPAD503 over time; B: relative chlorophyll content measured by SPAD503 overall timepoints; C: relative chlorophyll content measured by MultispeQ over time; D: relative chlorophyll content measured by MultispeQ overall timepoints; EB = 95% LSD;

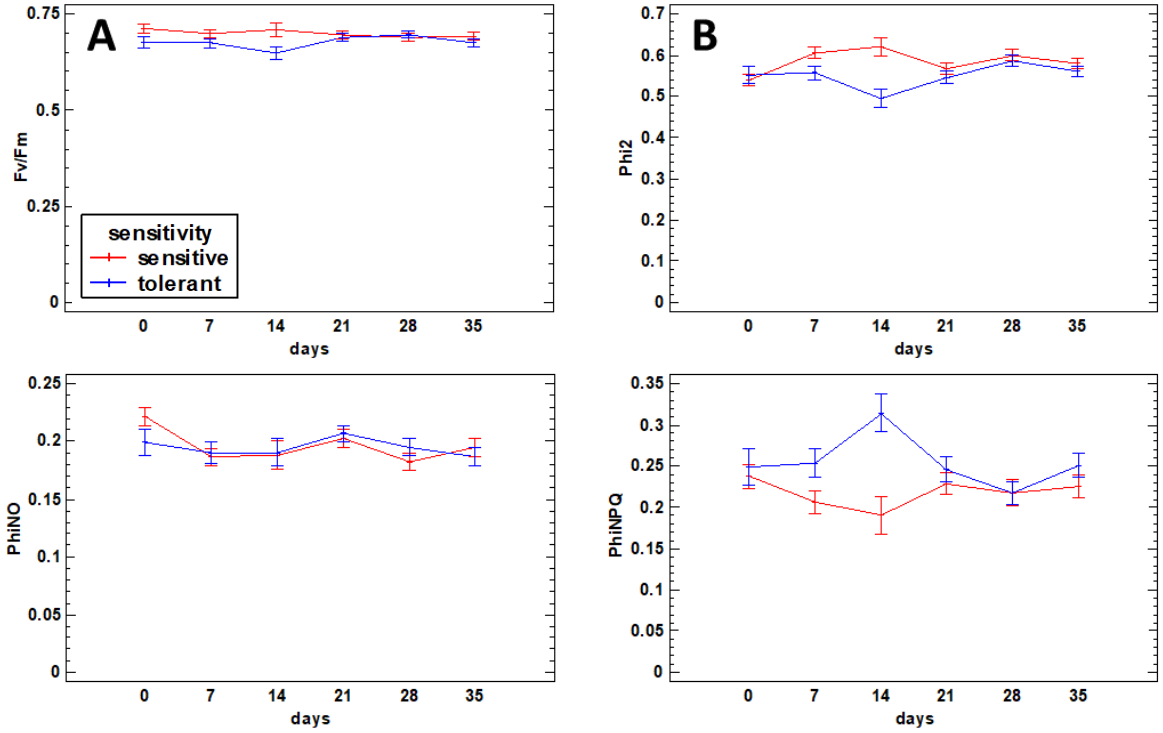

Figure 19: Photosynthesis related parameters measured by MultispeQ for the pre-experiment: A: F_v/F_m ratio; B: Phi2 = fraction of light energy captured by Photosystem II, converting light energy, producing ATP and NADPH; C: PhiNO = combination of a number of unregulated processes whose by-products can inhibit photosynthesis or be harmful to the plant; D: PhiNPQ = fraction of incoming light (excited electrons) that goes towards non-photochemical quenching, in order to protect from the adverse effects of high light intensity; EB = 95% LSD;

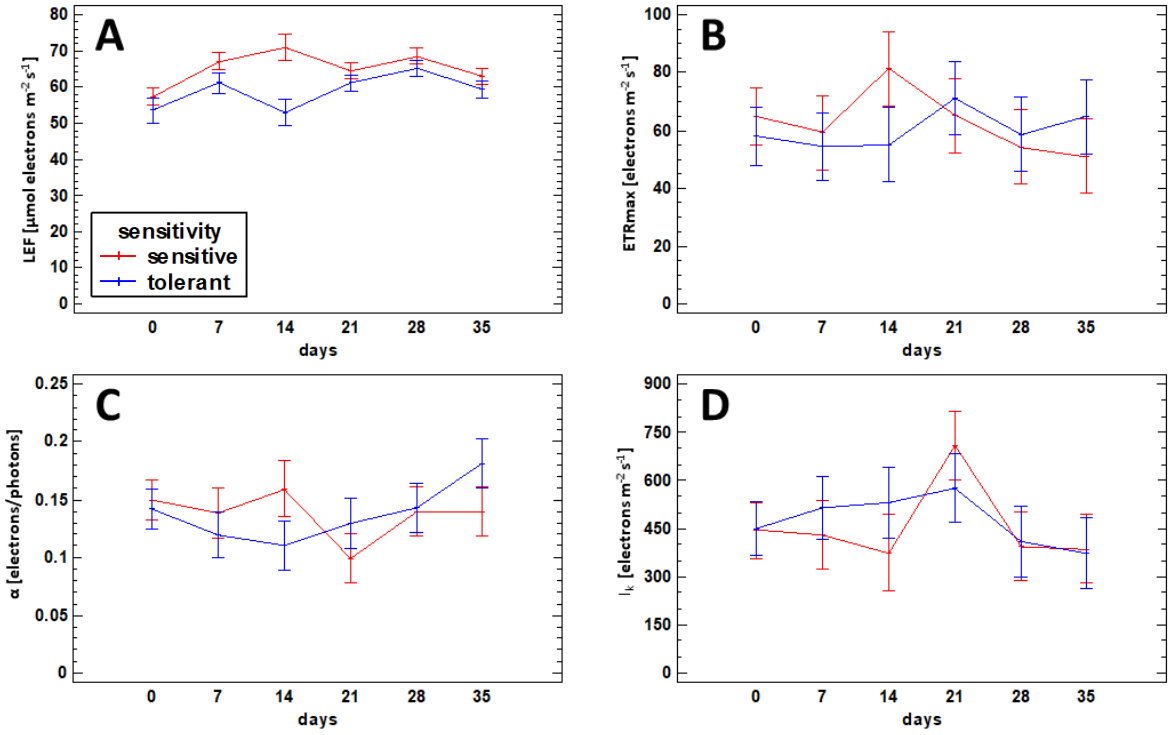

Figure 20: Photosynthesis related parameters of the pre-experiment measured by MultispeQ (A) and MINI PAM II (B-D) under progressive drought stress; A: LEF (linear electron flow); B: α = initial slope of light curve which is related to quantum efficiency of photosynthesis; C: ETR_{max} = maximum of electron transfer rate; D: I_k = minimum saturation irradiance; EB = 95% LSD;

7.3. Outdoor garden experiment

Simultaneous to the main-experiment we did an outdoor garden side experiment to get a first impression of growth potential in temperate climate like in Vienna and to check potential differences and similarities to the main-experiment in the glasshouse. Figure 21 shows the measured basal growth parameters. Plants of drought tolerant genotype (ICTP 8203) grew up to 3 meters in height, whereas drought sensitive genotype (843-22B) stay small at around 1.2 meters. The fresh weight (FW) (Fig. 21B) is likewise higher for tolerant genotype than for sensitive one. Also, the number of panicles per plant (Fig. 21C) is three-times higher for tolerant genotype.

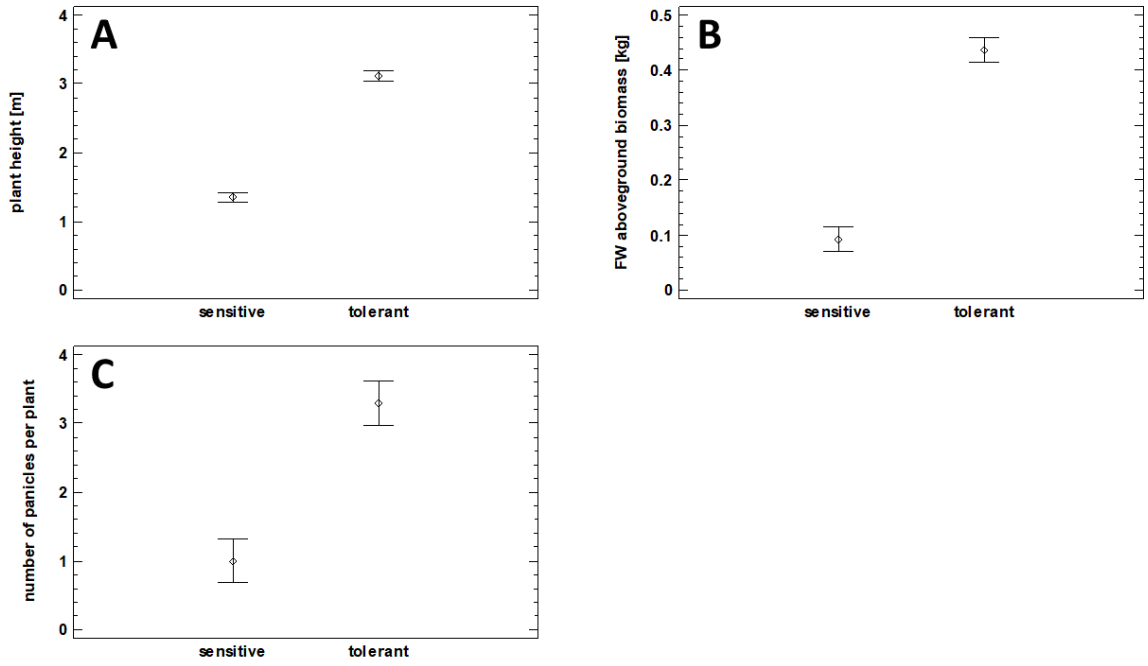

Figure 21: Results from the outdoor garden experiment: A: plant height in meters; B: FW (fresh weight) of the aboveground biomass in kg; C: number of panicles per plant; EB = 95% LSD;

7.4. Secondary photosynthesis processes: A/c_i-curves and assimilation rates

Figure 22 shows A/c_i curves at an early response measurement of the main experiment (between 38-42 DAS). WALZ GFS-3000 device can simulate certain CO_2 concentrations within a cuvette enclosing the measured leaf and is therefore able to generate data for a A/c_i-curve. Drought tolerant genotype (ICTP 8203) is featuring lower CO_2 assimilation rates compared to drought sensitive genotype (843-22B) but has an earlier saturation at lower internal CO_2 concentrations (c_i).

In Figure 23 records from the early response measurements (42 DAS) are shown. This is how some raw data from the WALZ GFS-3000 look like. Based on such data, graphs like A/c_i-curves (see Fig. 22) can be generated.

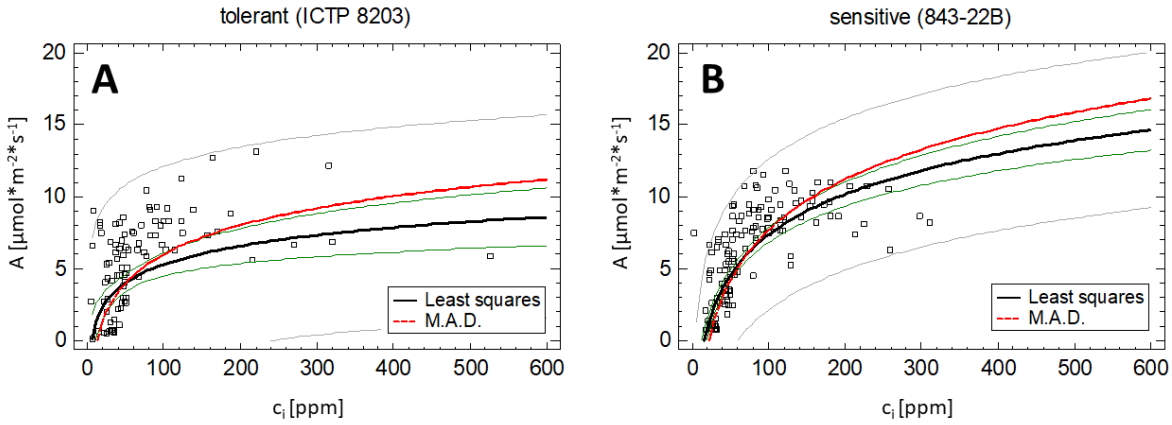

Figure 22: A/c_i-curves (assimilation rates at saturating internal CO_2 concentrations) at an early response measurement of main experiment (between day 38-42 after sowing); A, B: simple regression with least squares and M.A.D. (median absolute deviation); A = assimilation rate; c_i = intercellular CO_2 concentration; A: A/c_i curve of drought tolerant genotype; B: A/c_i curve of drought sensitive genotype;

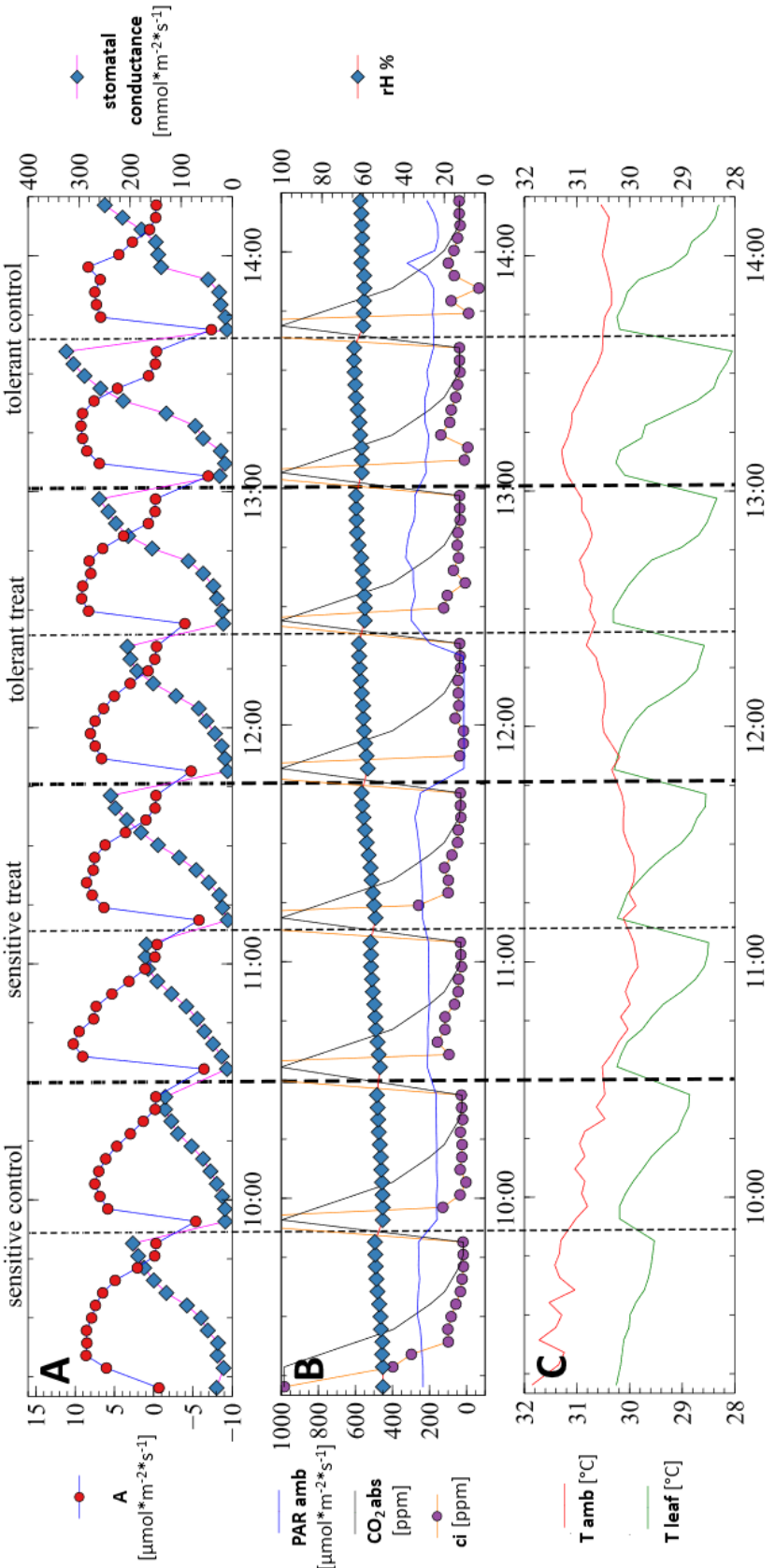

Figure 23: Data from WALZ GFS 3000 device at an early response measurement of main experiment on day 42 after sowing; A: A = assimilation rate, stomatal conductance = total water vapor conductance; B: PAR amb = ambient photosynthetic active radiation, CO_2 abs = CO_2 mole fraction in reference cell of analyzer (equal to ambient CO_2 concentration), ci = intercellular CO_2 mole fraction, rH % = relative ambient humidity; C: T amb = ambient temperature, T leaf = leaf temperature;