UNIVERSITÄT HOHENHEIM

Master's Thesis

Effects of drought, light intensity and leaf age on photosynthetic efficiency and pigment composition in sub-tropical bread wheat

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(490g)

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I. Abstract

(Sub)tropical wheat production is threatened by increasing, climate change -induced frequencies and intensities of soil water deficits. Leaves, and in particular their pigments, form the basis for assimilate accumulation in plants and, therefore, the source for yield building processes. Under water deficit, a high radiation intensity or a combination of both, leaves may adapt their pigment composition to mitigate negative impacts and maintain the integrity of the photosynthetic apparatus. However, little is known about the genetic variation in pigment composition and its potential for acclimation to stress in wheat. The aim of this study was to investigate the genotypic differences in photosynthetic efficiency (Φ PSII) and alterations in the pigment composition of wheat lines selected from CIMMYTs 10SATYN HEAT Panel, as well as their reaction to water deficit and an increased radiation intensity. It was shown that Φ PSII was non-significantly lower in older leaves compared to values measured in flag leaves of all lines. Concomitantly a decrease in pigment concentration with leaf age was observed, where the decrease in Chl was larger compared to the decrease in Cars. Only line 9410 had higher carotenoid concentration in older leaves compared to flag leaves. However, a systematic genotypic difference among the lines was not detectable. Likewise, when subjected to drought photosynthetic as well as pigment concentration decreased substantially but here a contrasting reaction to water deficit was observed, indicating that line 9410 was able to maintain the integrity of the photosynthetic apparatus to a greater extent compared to line 9412 due to a higher concentration in Car pigments. When exposed to a higher radiation intensity, photosynthetic quantum yield increased in line 9412 and decreased in 9410, even though pigment concentrations decreased in both lines. However, Chl concentrations decreased stronger in line 9410 compared to line 9412 offering an explanation for the contrasting reaction. Contrasting to the current scientific knowledge, a preferential synthesis of xanthophyll over Cars, or an increase in Car pigments in general under drought or increased radiation intensity, was not verified.

II. List of Figures

III. List of Tables

IV. List of abbreviations

Anthera	Antheraxanthin
Vio	Violaxanthin
Zea	Zeaxanthin
Neo	Neoxanthin
NPQ	Non-photochemical quenching
PSII	Photosystem II
Xanths	Xanthophylls
Cars	Carotenoids
Φ PSII	Photosynthetic quantum yield
LHCII	Light harvesting complex of PSII
HPLC	High-Performance-Liquid-Chromatography
Chl*a*	Chlorophyll a
Chl*b*	Chlorophyll b
Chl*a*+*b*	Chlorophyll a+b
Chl*a/b*	Ratio of Chlorophyll a to Chlorophyll b
L-NS-L	Lowest non-senescent leaf

V. List of Content

1. Introduction

Wheat is one of the world's most important staple crops, as about 21% of the world's food production is dependent on the annual wheat harvest (Enghiad et al. 2017). According to current estimates, the production of the world's basic staple foods, such as wheat, corn, rice and soybeans, will have to increase by up to 100% by 2050 in order to meet the growing human demand for food (Tilman and Clark 2015; Simkin et al. 2022). At the same time, the ongoing climate change is leading to increasingly adverse growing conditions, limiting today's crop yields, and further threatening the possibilities of increasing food production as drought alone accounts to 21% yield reduction in wheat on a global scale. At the same time, it is projected that wheat yields will further decline by 6% for each degree Celsius rise in temperature, also considering the possible increases in cooler regions (Fahad et al. 2017; Schewe et al. 2014; Mukherjee et al. 2018; Mendelsohn et al. 2006; Iizumi et al. 2018). Many approaches to increase the production of staple crops are needed to combat this dilemma. A possible solution to this problem lies in the further selection and breeding of stress-resistant, especially drought and heat-resistant, varieties. However, current approaches to increase grain yields in wheat are no longer delivering the necessary results to meet future demand, and new ways to increase the efficiency of wheat production are therefore needed (Ray et al. 2013; Jaleel, Manivannan, Wahid, Farooq, Somasundaram and Panneerselvam 2009).

Yield potential is characterized by three major factors: capacity to capture light, the radiation use efficiency (RUE) and finally the harvest index (HI), indicating the ratio of harvestable yield to total biomass (Reynolds et al. 2009). During the green revolution, breeders mainly focused on increasing the light harvesting capacity as well as components of the harvest index. However, improvements stagnated which resulted in a stronger focus on increasements of RUE in today's research (Simkin et al. 2022). Photosynthetic pigments, such as chlorophylls and carotenoids, are the most important component of light harvesting and RUE, as well as photoprotection of the photosynthetic apparatus when RUE decreases under adverse growing conditions and excess energy must be dissipated (Mirkovic et al. 2017; Lu and Lu 2004; Nyachiro et al. 2001). One key mechanism of plants to protect themselves when confronted with excess excitation energy is the energy dissipation via the xanthophyll cycle and its key pigments Vio, Anthera and Zea (Demmig-Adams and Adams 2018). When radiation energy exceeds the capacity of the photosynthetic apparatus the energy can be dissipated either via chlorophyll fluorescence or via a mechanism called non-photochemical quenching (NPQ). The

extent of this protection mechanism can be measured via the chlorophyll fluorescence of PSII as these mechanisms of energy conversions compete, a decrease in chlorophyll fluorescence can indicate a rise of NPQ. This relationship can be represented in a value, the photosynthetic quantum yield of PSII (Kalaji et al. 2017). As the xanthophyll cycle pigments are an essential part of NPQ, the leaf pigment composition and especially the concentrations of these pigments can provide valuable information on the plant's capacity to dissipate excess energy and maintain the integrity of the photosynthetic apparatus (Eskling et al. 1997). When plants are subjected to abiotic stresses such as water deficit or heat, excess light absorption is more likely and to prevent damages to the photosynthetic apparatus by reactive oxygen, this excess energy must be dissipated (Müller et al. 2001). Therefore, selecting wheat lines according to their leaf pigment composition to increase the photosynthetic and photoprotective capacity of a line provides a promising approach to increase the production efficiency of bread wheat especially when considering the adversities of crop production in the future on a background of increasing demand (Fahad et al. 2017; Simkin et al. 2022).

To determine which effect the pigment composition has on the photosynthetic efficiency of bread wheat, the genotypic and age-related differences in the pigment composition of wheat leaves from five Triticum aestivum L. lines selected from CIMMYT's 10SATYN Heat was investigated. Furthermore, two lines were selected for subsequent drought and light intensity trials to check how photosynthetic quantum yield and pigment composition of flag leaves changes upon induction of either of these stresses, as well as the relationship between Φ PSII and pigment composition.

2. Basic knowledge

2.1 *Triticum aestivum* L. and 10SATYN HEAT Panel

Global wheat production in 2022 is projected to increase by 1% to 787.2 million metric tonnes and these years forecasted utilization of 774 million tonnes is accounting for a fifth of the global caloric intake. While this highlights that consumption is still less than production, it is projected that demand will increase at a faster pace than production can be increased (FAO, 2022; Erenstein et al. 2022). When looked at agronomically, wheat is preferably cultivated in temperate climates as its tolerance to frost makes it an economically attractive crop in regions where temperatures fall below 0°C during the cultivation cycle. However, apart from Europe wheat is of particular importance as a dietary good in tropical regions such as central Africa and South-East Asia, where consumption per capita and year can exceed 100 kg of wheat and is expected to rise and as mentioned before, climatic conditions are becoming increasingly adverse as water availability will decrease and temperature extremes will occur more frequently (Erenstein et al. 2022). To combat this, international improvement facilities are working on breeding wheat varieties that express an increased resistance against abiotic stresses such as heat or drought stress, one of them is CIMMYT (International Maize and Wheat Improvement Center) who provided the seeds for this experiment. In regularly scheduled trials new lines are tested and exposed to certain stress factors to examine the performance of yield parameters under these circumstances. The lines in this experiment were from the 10SATYN HEAT panel, where 10SATYN is an acronym for 10^{th} Stress Adaptive Trait Yield Nursery. In these trials crosses are usually designed in a similar pattern with one parent expressing high performance in a source related trait while another parent usually shows high performance in at least one sink factor (e.g TGW) to create a resilient high yielding cross to ensure yield stability under unfavourable growing conditions (Reynolds et al. 2017).

2.1 Drought

In this study, drought always refers to agricultural drought, more precisely a drought stress, defined by Jaleel et al. (2009) as moderate to strong loss of water that results in closure of the stomata, inhibition of gas exchange, reduced leaf water potential, decrease in cell enlargement and growth and a disruption of the plants metabolism up to the complete cessation of photosynthesis. Plants generally cope with water shortage in two ways: (1) drought avoidance, which includes modification of the root system, water use efficiency and life cycle adaptions to efficiently use precipitation water, or (2) drought tolerance, which are physiological and

biochemical responses in the plant, depending on development stage, that allow to endure periods of water shortage without significant reductions in agronomical traits, and get reverted once water limitation is no longer an inhibiting factor (Nezhadahmadi et al. 2013; Rampino et al. 2006). It is important to note, that the plant's adaption to water limiting environments varies immensely in its time scale. While adaptions to phenology take a lot of time to develop, measures like the closure of stomata can be induced within seconds (Passioura 1996). Generally, drought results in a water stress for plants and under these circumstances, plants adapted in several ways to maintain photosynthetic integrity and ensure ongoing carbon assimilation (Fig. 1). Therefore, to adapt a crop like wheat to increasingly water limiting growing conditions, breeders can select for a plethora of traits that improve either the crops drought avoidance or tolerance (Nezhadahmadi et al. 2013).

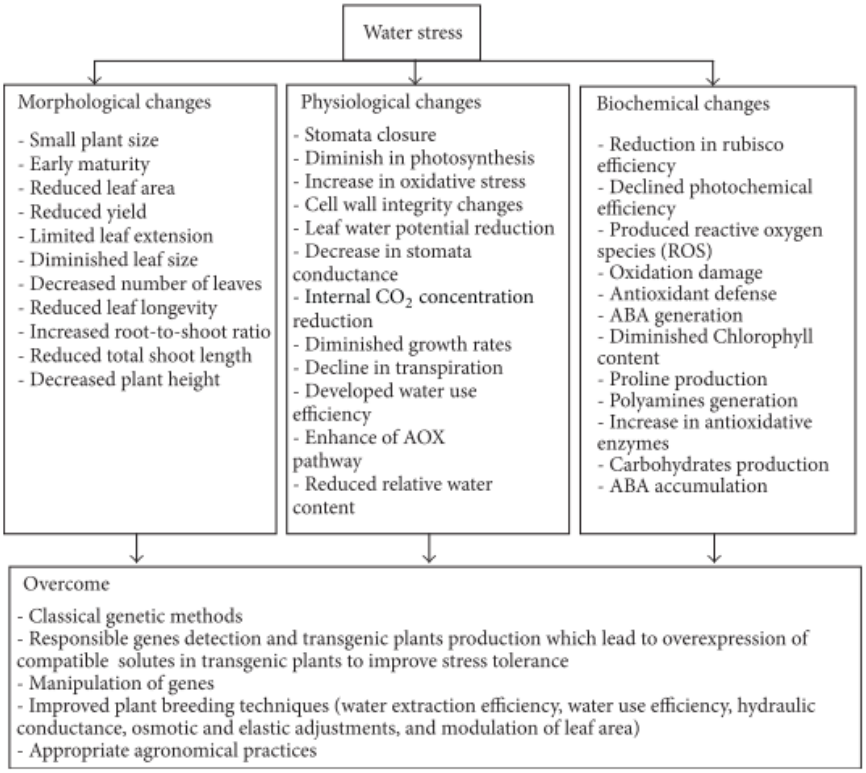

Figure 1: Morphological, physiological and biochemical reactions of wheat to drought stress. Taken from Nezhadahmadi et al. (2013)

2.2 Leaf pigments

2.2.1 Photosystem II and light harvesting

The photosystem II (PS II) is a pigment-protein complex located within the thylakoid membranes of photosynthetic active organisms and is mandatory for the light-energy dependent

splitting of water molecules (Horton and Ruban 2005; Vassiliev and Bruce 2008). To maximize the efficiency of PSII, it is associated with additional complexes, specifically designed to trap and forward the harvested energy towards the reaction centre inside the core complex of PSII, called light harvesting complex II (LHCII). These form in combination with the core complex of PSII the PSII-LHCII supercomplex that is responsible for the conversion of light energy into electro-chemical energy (Minagawa and Takahashi 2004; Chow 2003) (Fig.2).

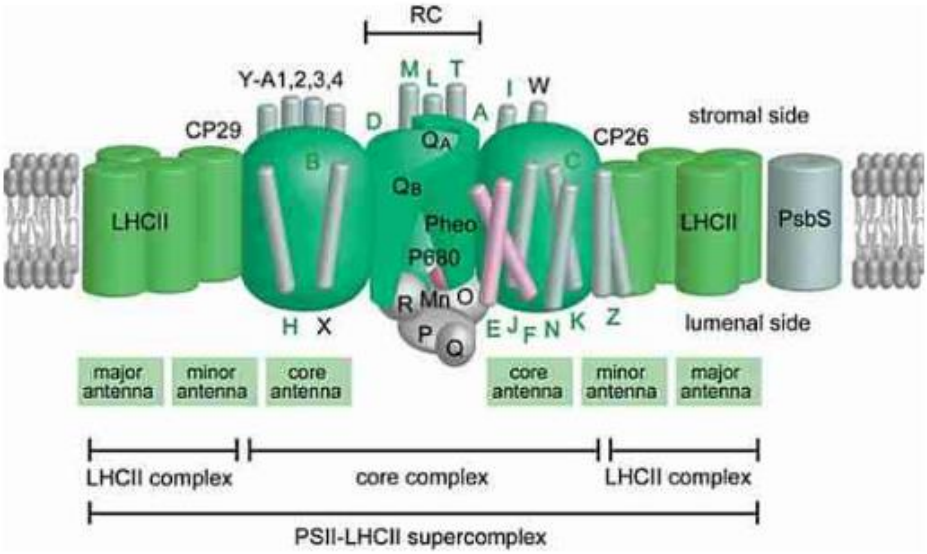

Figure 2: The PSII-LHCII supercomplex. Taken from Minagawa and Takahashi (2004)

The LHCII complex is also referred to as 'major antenna' and binds 8 Chlorophyll a (Chl*a*), 6 Chlorophyll b (Chl*b*), 2 luteins as well as one 9'-cis-neoxanthin. In total, the PSII-LHCII complex contains about 150 chlorophyll molecules with a ratio of Chl*a* to Chl*b* of approximately 2.5 (Vassiliev and Bruce 2008; van Amerongen and Croce 2013).

In this pigment-protein complex the Chl molecules form a three-dimensional space that functions as an antenna to absorb light over a wide spectrum of wavelengths (Minagawa and Takahashi 2004; Horton and Ruban 2005). When excitation energy is absorbed by Chl, the absorbed photons turn the Chl molecules from the ground state to the first or second singlet excited state (Fig. 3).

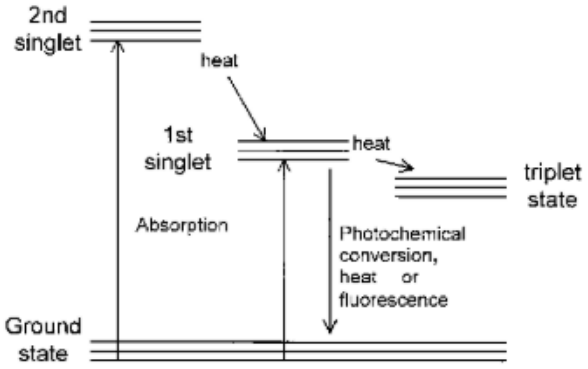

Figure 3: Energy levels of Chl molecules. Red light induces the 1st singlet stage, blue light the 2nd stage. From the 2nd stage Chl automatically converts to the 1st singlet by emission of heat. In the 1st state energy can be used photochemically but can also be dissipated via fluorescence or heat. Diagram taken from Chow (2003)

From the first singlet state, the excitation energy can be transferred to another Chl molecule by two mechanisms: (1) de-localized exciton coupling, where the energy oscillates between the respective donor/acceptor pigment, or (2) the Förster transfer where an acceptor gets excited, and the donor reverts to its ground state (Chow 2003). This way the Chl forms a kind of funnel that funnels the excitation energy of Chl molecules towards the reaction centre where the energy gets used to split water molecules (van Amerongen and Croce 2013). However, this energy of excited chlorophyll, when reaction centres are saturated with electrons, can be excessive and turn into the triplet state of chlorophyll which can, in combination with molecular oxygen, form singlet oxygen molecules that can permanently damage proteins, lipids and membranes of the plant's photosystem (Horton and Ruban 2005). For this case, plants have developed a protection mechanism relying on Cars that are bound to Chl*a* via Van der Waals contact. This protection mechanism is capable of scavenging over 95% of these triplet chlorophylls and is therefore one of the most important mechanisms in photoprotection (van Amerongen and Croce 2013; Minagawa and Takahashi 2004; Horton and Ruban 2005).

2.2.2 Carotenoids

Carotenoids are one of the most abundant class of pigments with around 750 occuring in nature, hence fulfilling a variety of different functions in both animals and plant life, but less than 50 of those play a role in light harvesting of photosynthetic organisms (Young 1991; Cazzonelli 2011). Most Cars are C_{40} lipophilic isoprenoids and occur naturally in the thylakoid membrane of the two photosystems of all photosynthetic organisms, where they are bound in a pigment-protein complex termed the LHCII trimeric complex (Cazzonelli 2011). These photosystems can be divided into a core complex (reaction centre) and the light-harvesting complex, with β-

carotene occurring predominantly in the reaction centre while the light-harvesting antenna are usually rich in Xanths (Young 1991). In these systems Cars are necessary for a manifold of key biological processes in plants like the assembly of photosystems, light harvesting antenna, where they extend the spectrum of absorbable light, and photoprotective processes such as scavenging of singlet oxygen and quenching of triplet state chlorophyll. (Young 1991; Nisar et al. 2015). The respective expression of these processes is fundamentally dependent on the Car composition within the leaf, which further underlines the importance of this pigment group for photosynthesis (Cazzonelli and Pogson 2010). Moreover, Cars are also precursor for two important plant hormones: ABA and Strigolactone (Nisar et al. 2015). It has been shown that genes that participate in carotenoid biosynthesis can be limiting for ABA synthesis and therefore impact the plants resistance to environmental stresses such as drought and oxidative stress as xantophyll and abscisic acid levels within the leaf are altered (Cazzonelli 2011; Cazzonelli and Pogson 2010). The general pathway of carotenoid synthesis is further illustrated in Figure 4.

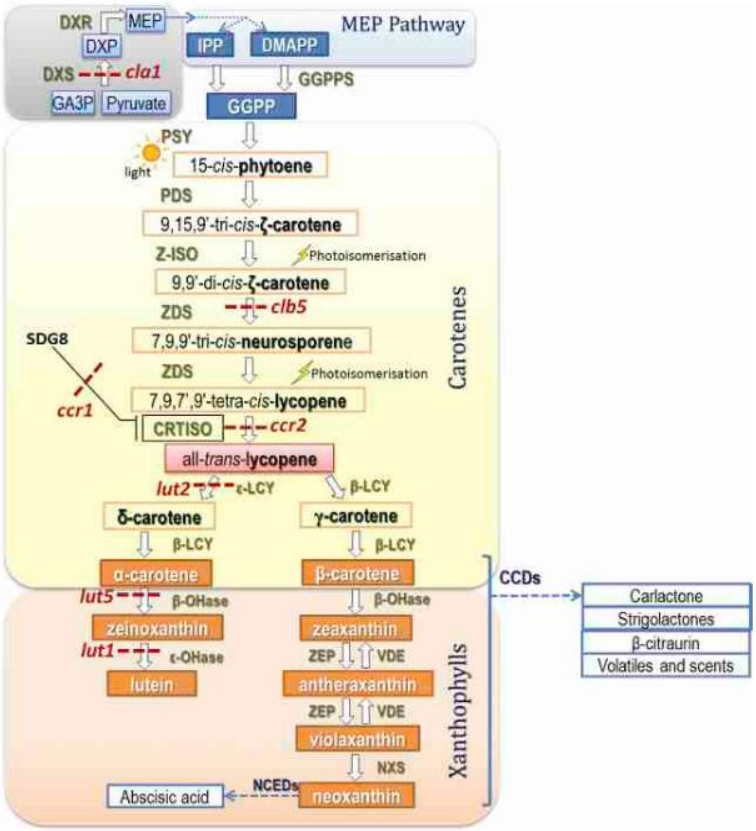

Figure 4: Biosynthesis pathway of carotenoids found in almost all plants. *Taken* from Nisar et al. (2015)

Important for this thesis is what happens after all-*trans*-lycopene where the pathway splits and lycopene is either converted via β-LCY and ε-LCY or β-LCY into α-carotene or β-carotene,

respectively. Afterwards these Cars can be further hydroxylated to form the Xanths (Nisar et al. 2015).

Usually, the most abundant Cars in leaf tissues are lutein, β-carotene, Vio and neo, however these abundancies can change dramatically under environmental stresses which offers the possibility to use Cars, and their composition within the leaf, as indicators of the plant's health status (Demmig-Adams and Adams 1996; Cazzonelli 2011; Nisar et al. 2015).

2.2.3 Xanthophyll Cycle

Although the photosynthesis of plants is impossible without light as its energy source, excess radiation that cannot be used in photosynthesis can pose a serious threat for the photosynthetic apparatus, especially in addition to already occurring stresses. To prevent the resulting damages, plants have developed a mechanism to safely dissipate the excess energy as heat through molecular vibrations (Demmig-Adams and Adams 1996a, 1996b; Demmig-Adams et al. 1999; Müller et al. 2001). This form of photoprotection is widespread in nature and occurs in the thylakoid membrane of all higher plants. The xanthophyll cycle related energy dissipation relies on three Xanths: Vio, Zea and their intermediate Anthera (Eskling et al. 1997). The extent of this heat dissipation can be measured as the non-photochemical quenching (NPQ) of PS II Chl*a*. This mechanism is regulated by the trans-thylakoid membrane pH gradient as well as the presence of these special xanthophyll cycle pigments (Gilmore 1997).

Under conditions in which light becomes excessive, the pH within the lumen lowers inducing a rapid de-epoxidation of Vio catalysed by violaxanthin de-epoxidase (VDE) to Zea with Anthera as an intermediate. Once the light is no longer excessive, Zea gets epoxidated back to Vio via Anthera by Zea epoxidase (Eskling et al. 1997). This mechanism is further illustrated in Figure 5.

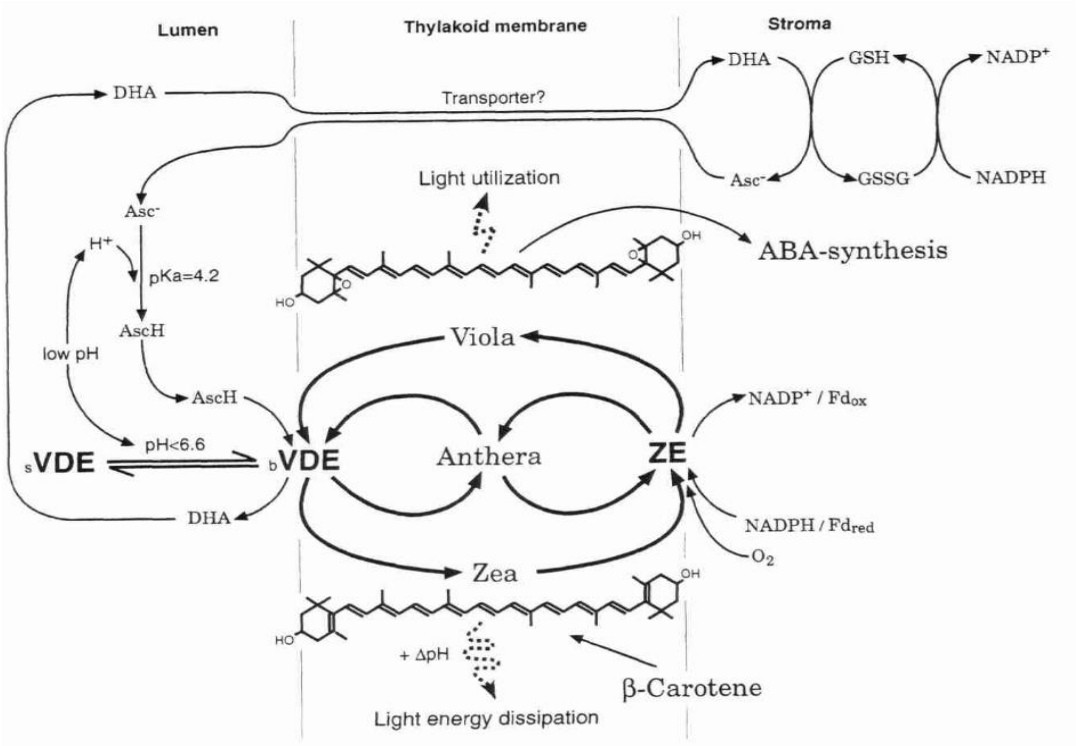

Figure 5 Model of the regulation of the xanthophyll cycle and its relation to ABA synthesis. sVDE, soluble VDE; bVDE, bound VDE; DHA, dehydroascorbate; Asc", ascorbate; AscH, ascorbic acid; GSH, glutathione; Viola, violaxanthin; Anthera, antheraxanthin; Zea, Zea; Fd, ferredoxin. Taken from Eskling et al. (1997).

2.3 Chlorophyll Fluorescence

Once light energy has been absorbed by the Chl in the plant's leaves, there are three ways in which energy is converted: (1) used in photochemistry, (2) dissipated as heat and (3) re-emmitted as light at 682-740 nm wavelength, the so-called chlorophyll fluorescence (Maxwell and Johnson 2000). This re-emitted light has a longer wavelength compared to the absorbed light, which makes it possible to simply measure the fluorescence, even though it only compromises of 1 – 2% of total light absorbed. Therefore, a leaf is subjected to a light source with a known wavelength and measure the amount of light at longer wavelength that is emitted by PS II (Maxwell and Johnson 2000). As the three mechanism of light energy conversion compete, any increase or decrease of chlorophyll fluorescence implies increases or decreases of one of the others and can therefore be used as an indicator for photosynthetic efficiency (Krause and Weis 1991). Changes in fluorescence yield occur for example when plants are transferred from darkness to light. Due to the plants being exposed to light after dark adaptation, the PSII receives electrons and the plastoquinone receptors are considered closed, as they cannot uptake more electrons, which in turn gives a rise in fluorescence yield. However, this increase is not permanent but that starts to decay shortly after. This process is called fluorescence quenching which can be divided into two mechanisms: either an increase in the

electron transport rate away from PS II, called photochemical quenching, or an increasing efficiency of energy dissipation as heat, termed non-photochemical quenching (Maxwell and Johnson 2000; Seaton and Walker 1990). The dynamic of chlorophyll fluorescence induction and its quenching mechanisms is further illustrated in Figure 6.

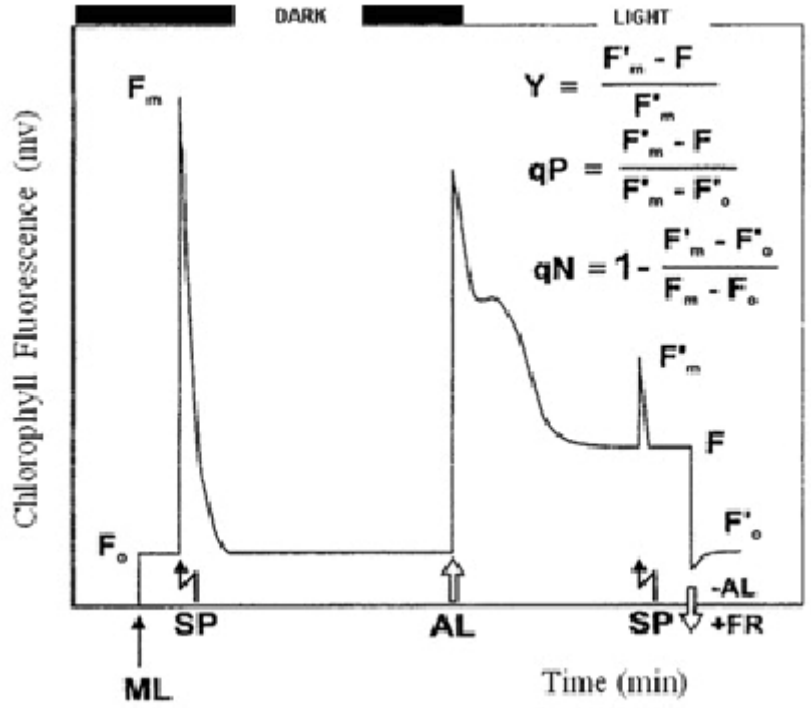

Figure 6: The dynamics of chlorophyll fluorescence and its related quenching mechanisms. Fo: fluorescence yield of dark-adapted sample, Fm: maximal fluorescence yield of dark-adapted sample, F: fluorescence yield observed at any time during illumination; Fm′: maximal fluorescence yield of illuminated sample, Fo′: minimal fluorescence yield observed shortly after darkening of illuminated sample and reoxidation of the PS II acceptor side, qP: coefficient of photochemical quenching, qN: coefficient of nonphotochemical quenching, ML: Measuring light, SP: Saturation light pulse, AL: Actinic light, FR: Far red light. Taken from Kalaji and Guo (2008)

3. Material and Methods

3.1 Line selection

Six wheat lines were selected from CIMMYT's Best physiological trait (Best PT) Panel, funded by the International Wheat Yield Partnership (IWYP) and the Foundation for Food and Agricultural Research FFAR. These lines showed contrasting reactions in their agronomic traits to heat and drought stress in field trials conducted in Obregón, Mexico in 2020.

Table 1: Agronomic traits of 5 wheat lines in a field experiment conducted by CIMMYT in Obregon, Mexico in 2020. Yield potential shows the plants traits under optimal growing conditions. Droughted and heat trial show traits when grown under water limiting conditions or under a high ambient temperature, respectively. GFR=grain filling rate; DTH= days to heading.

Yield Potential				
Line	Grain yield (t/ha)	GFR (g/m2/day)	Maturity (day)	DTH (days)
9402	6.62	16.5	109.5	64.5
9410	7.35	19.6	117.5	75
9412	7.76	21.6	116	75
9414	7.5	19.8	118.5	75.5
9432	6.58	17.8	110	78

Droughted				
Line	Grain yield (t/ha)	GFR (g/m2/day)	Maturity (day)	DTH (days)
9402	4.7	14.7	99	62
9410	5.28	15.8	105	67
9412	5.56	17.6	103	67
9414	5.07	15.9	105	68
9432	5.13	15.6	97	59

Heat				
Line	Grain yield (t/ha)	GFR (g/m2/day)	Maturity (day)	DTH (days)
9402	3.47	14.2	75	45.5
9410	2.98	12.4	85	56
9412	2.98	13.3	82.5	55
9414	3.1	14.1	83	56
9432	3.16	14.4	75	48

The grain yield under control conditions was the highest for line 9412, followed by 9412,9414 and 9410. 9432 had the lowest yield potential but reached maturity the fastest. Under drought conditions line yield decreased in all lines with a reduction of -32% in 9414, -29% in 9402, -28.35% in 9412, -28.16% in 9410 and -22% in 9432. 9412 and 9410. Additionally, all lines matured faster, and the time needed until heading was greatly reduced. When exposed to heat yield reduction was stronger with values ranging from -47.58% in 9402 up to a 61.60% reduction in 9412. Also, the time to reach maturity as well as the time to heading was even shorter than in the drought trial (Tab. 1).

3.2 Growing conditions

Eighty seeds of each line were pre-germinated in a water-soaked paper towels and placed in a plastic container inside the greenhouse. After radicle emergence, seedlings were transplanted into 1,5 l pots, containing a 1:1 volumetric mixture of loamy soil and commercial compost. The individual pots were each covered with a layer of gravel to minimise evaporation. A total of 360 seedlings were distributed over 120 pots, 24 pots for each line and 3 seedlings per pot. In addition to natural incident sunlight, artificial lighting was installed and set to a 14h photoperiod, with illumination starting at 06:00 AM.

Plants were kept well-watered until treatments started. In addition to the regular waterings the plants were fertilized with 50 ml of full-strength Yoshida Solution on the 36th days after transplant. The plants were then placed inside the chambers on the 40th DAT.

Chambers were equipped with humidifiers to keep the humidity in the desired range, however due to high ambient temperatures during the experiment growing conditions were not as desired initially. This resulted in a microclimate within the chambers as shown in Table 2:

Table 2: Mean value and standard deviation of temperature, rH and VPD within the growing chamber during the experiment.

	Temperature (°C)	**rH (%)**	**VPD (kPa)**	**Irradiation (µmol $m^{-2}s^{-1}$)**
Day	39.1± 5.8	44.7±15.6	3.89	400-500 µmol $m^{-2}s^{-1}$
Night	26.3±2.9	87.5±16.5	0.44	

The temperature as well as the relative humidity was measured in 10-minute intervals by Tinytag dataloggers (Gemini Data Loggers, United Kingdom) that were hung in the chambers.

3.2.1 Treatments

3.2.1.1 Drought treatment

On the 40th DAT watering was withheld from half of the plants of each line for 7 days, the other half of the plants was kept irrigated throughout. Droughted plants were added 50 mL of water on the 4th and 7th day of treatment to prevent a fast development of water deficit.

3.2.1.2 High Light Intensity Treatment (HL)

On the 47th DAT, well-watered plants of lines 9410 and 9412 were exposed to high light intensity (700-1000 µmol photons m^{-2} s^{-1}) for further 5 days.

3.3 Measurements

Quantum yield of photosystem II was measured 3 times: at the start of the treatment (leaf age) as well as 8 (water deficit treatment) and 12 (HL treatment) days after onset of treatment (DAOT). Using the fluorescence module of a WALZ's GFS-3000, actinic light was set at 1200 µmol m^{-2} s^{-1}. The flag and the oldest non-senescing leaves, ranging from the 6th to the 2nd leaf with the flag leaf being the first, of each plant were measured. For the water deficit treatment as well as the HLI treatment only flag leaves were measured. On 12 DAOT Fv/Fm was measured in plants exposed to HL treatment after dark adapting the flag leaf for 30 minutes.

SPAD measurements were taken with SPAD Meter 502Plus (Konica Minolta, Japan) and conducted 3 times for every tested leaf. After the non-destructive measurement, the respective sampled leaves were cut off, immediately frozen in liquid nitrogen, and then stored at -80°C until further analysis approximately 30 days after sampling.

3.4 Pigment analysis

The pigment analysis was done with high performance liquid chromatography (HPLC). Each leaf was first weighed, leaf weight ranged from 0.0115g to 0.208g, and then placed into Microtubes 2 ml (Sarstedt, Germany) containing 500 µl of filtered acetone and ceramic beads. The Microtubes were then placed in FastPrep -24 (MP Bio, Germany) for 5 min and afterwards centrifuged in the Biofuge fresco (Heraeus Instruments) at 13000 rpm and 3°C for 5 min to separate the extract from the residue. The extract was subsequently filtered with 0,45 µm Rotilabo Mini-Tip syringe filters (Carl Roth GmbH + Co. KG, Germany). This procedure was repeated until the residue was visibly clear of leaf pigments. Extracts were evaporated overnight in the fume hood and resuspended in 200 µl of acetone and stored at -18°C until analysis in the HPLC (BISCHOFF Analyse and Gerätetechnick GmbH, Germany). Therefore, 20 µl of the prepared sample was injected in the column and analysed at approximately 14 MPa for 20 min.

To determine the absolute quantity of pigments in the respective sample, calibration curves for each pigment were calculated using pure standards purchased from SIGMA-Aldrich.

Chl concentration was determined spectrophotometrically at 662 and 645 nm using a microplate reader Infinite M200 Pro (Tecan, Austria) in the cuvette mode. Tenfold diluted samples in 100% acetone were used. Calculations were performed using the equations published by Lichtenthaler and Wellburn (1983).

3.5 Statistics and graphs

Statistical analysis and creating of graphs were done with SIGMAPLOT 12.5 (SYSTAT Software Inc., USA). Two-way ANOVAs were used with either (1) line and leaf position, (2) line and water availability or (3) line and light intensity as sources of variation for the leaf position, water stress and HL trial, respectively. Statistically significant differences were assumed when $p \leq 0.05$, the Holm-Sidak's pairwise multiple comparison method was used as a *post hoc* test. Data was tested for normality using Shapiro Wilk's test for sample sizes <5000.

4. Results

4.1 Genotypic differences among the lines and leaf position

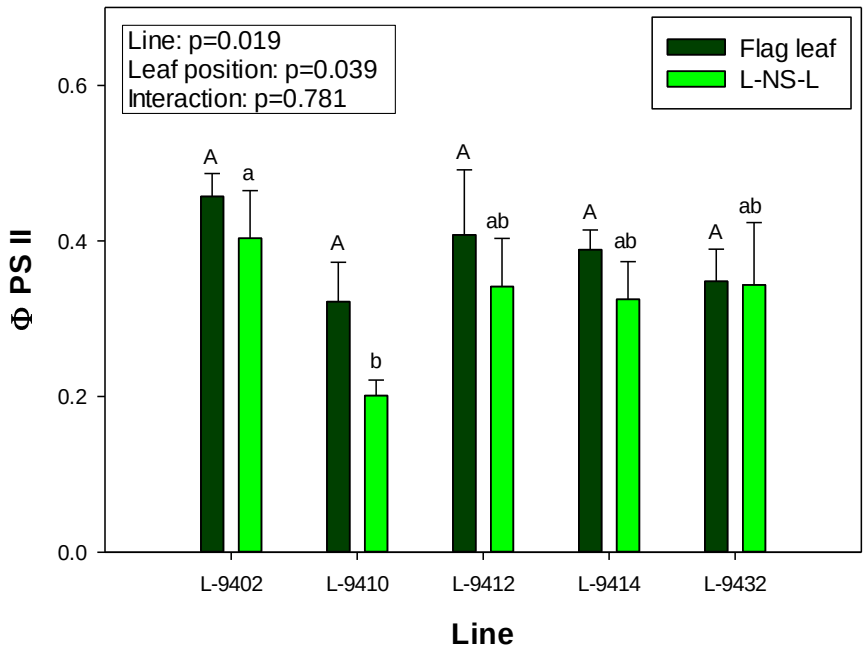

Figure 7: Φ PSII of 5 wheat lines at two different leaf position. L-NS-L=Lowest non-senescing leaf (ranging from leaf 2-6). Two-Way ANOVA was used to detect significant differences at α=0.05. Capital letters indicate significant differences among lines within the flag leaf. Lower case letters indicate differences among the lines within L-NS-L.

Significant differences for Φ PSII were detected between lines and leaf position. Despite that values of L-NS-L in all lines were lower compared to those measured in flag leaves, no differences were found when leaf positions of each line were compared individually. Although the values of flag leaf and L-NS-L varied in the same magnitude between the lines, significant differences were only found in the L-NS-L, where 9410 had a lower Φ PSII than 9402 (Fig. 7).

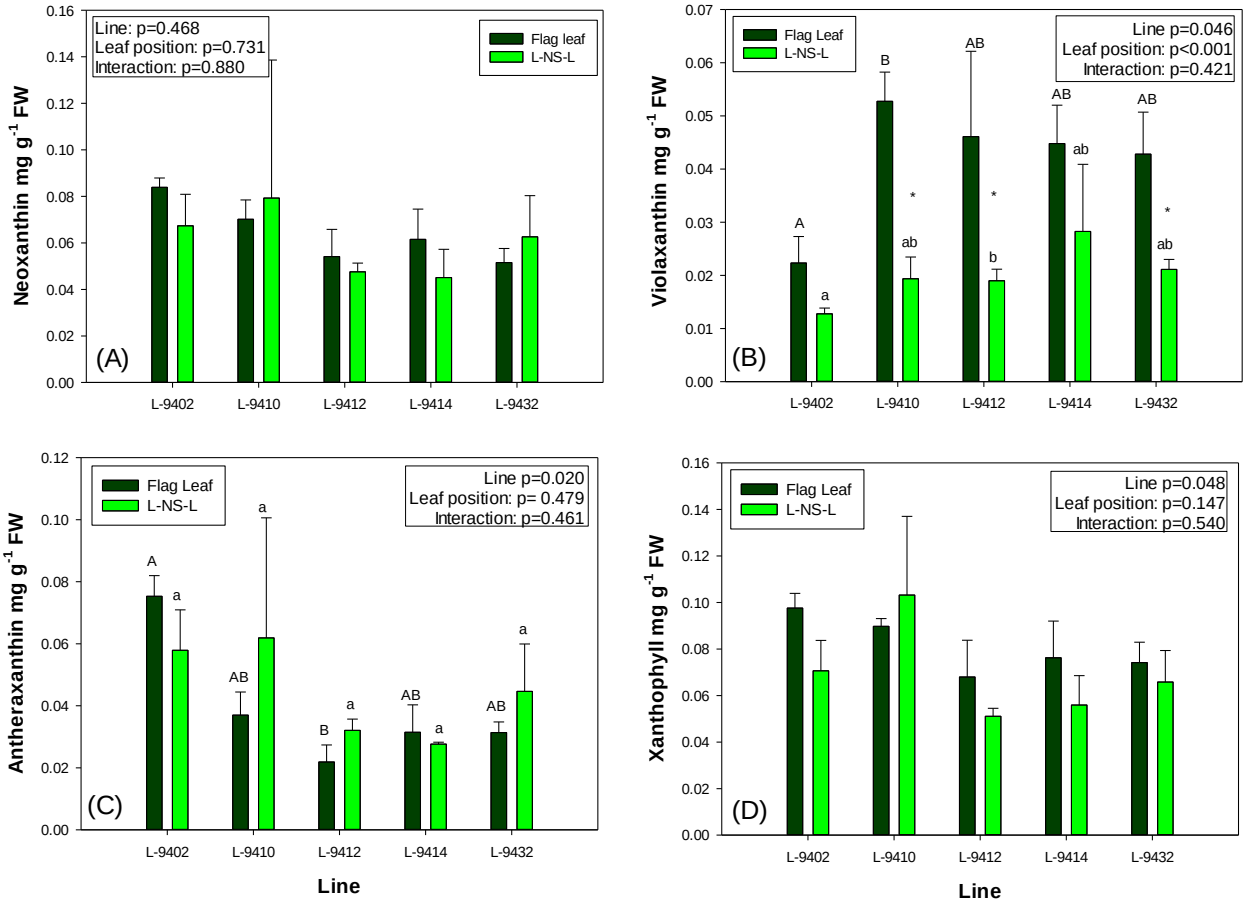

Figure 8: Concentration of xanthophyll Cycle pigments and total xanthophyll concentrations of 5 wheat lines at two different leaf position. L-NS-L=Lowest non-senescing leaf (ranging from leaf 2-6). Two-Way ANOVA was used to detect significant differences at α=0.05. Capital letters indicate significant differences among lines within the flag leaf. Lower case letters indicate differences among the lines within L-NS-L. Asterisks indicate significant differences between leaf level within a respective line.

The neo concentrations did not differ significantly between lines or leaf position. Contrarily to Neo, Vio concentrations showed significant differences between the lines and leaf position, where differences between leaf positions were highly significant in general and statistically significant differences were found in the post-hoc analysis in line 9410 9412 and 9432. Differences between the lines were found in flag leaves with 9402 having a significantly lower Vio concentrations compared to 9410 that showed the highest values among the tested lines. Although 9402 had substantially lower values compared to 9412,9414 and 9432, differences

were not significant (Fig. 8b). Similarly to Neo concentrations, Anthera concentrations did not differ significantly between leaf positions and showed non-significantly higher values in L-NS-L in 9432, 9412 and 9410. Interestingly, Anthera concentrations were highest in line 9402 and lowest in 9412, contrastingly to Vio concentrations, but likewise to Vio concentrations, differences among the lines were only significant in flag leaves (Fig. 8c). The combined Xanth concentrations, composed of neo-, viola-, and Anthera, did differ significantly between the lines, however, no significant differences were detected by the post-hoc analysis. Still, it is evident that except for line 9410 total Xanth concentrations were lower in L-NS-L compared to flag leaves (Fig. 8d). Neo was the most abundant of the three Xanths and Vio concentrations were higher than that of Anthera in flag leaves, but lower in L-NS-L.

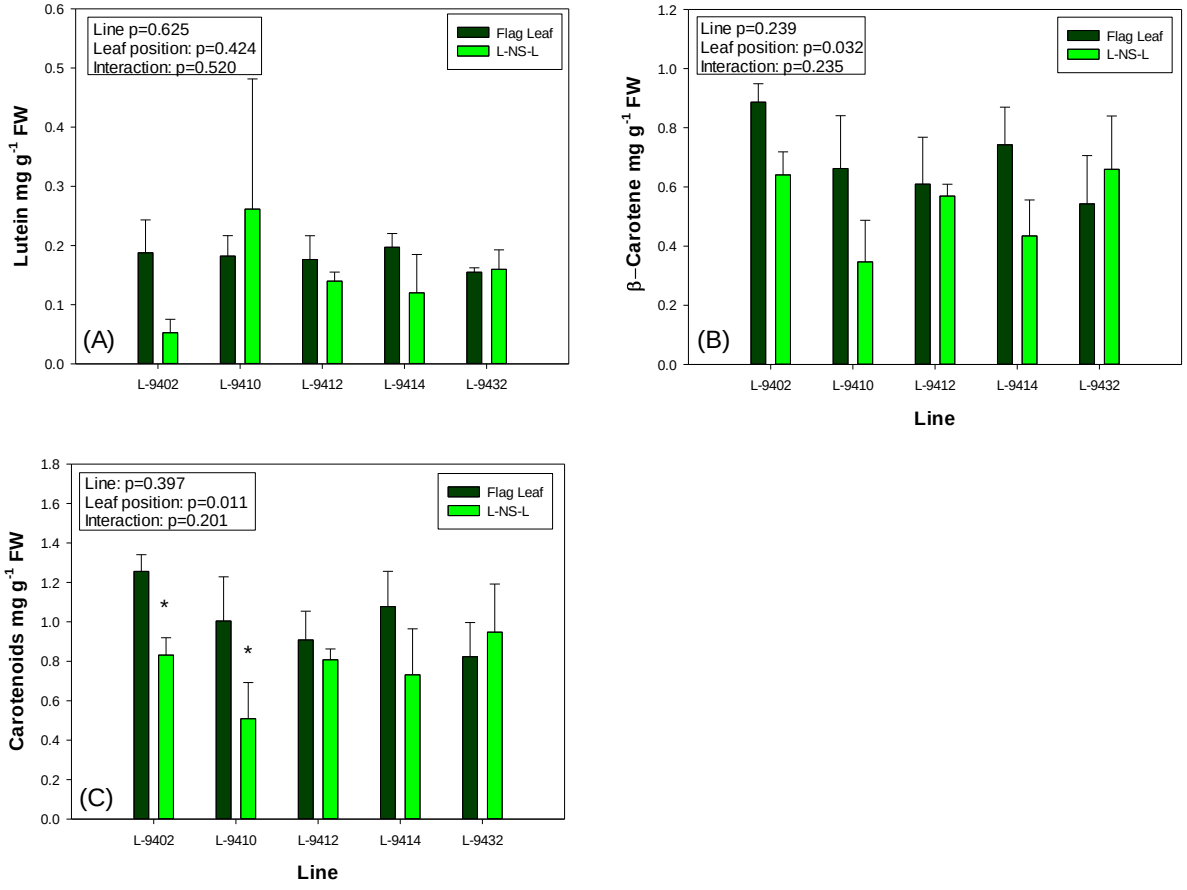

Figure 9: Concentrations of Carotenoid pigments and total Carotenoid concentrations of 5 wheat lines at two different leaf position. L-NS-L=Lowest non-senescing leaf (ranging from leaf 2-6). Two-Way ANOVA was used to detect significant differences at α=0.05. Asterisks indicate significant differences between leaf level within a respective line.

Lutein was the second most abundant Car found in the tested samples. While leaf position did not differ greatly among cultivars, L-NS-L concentrations showed greater variation, but no significant differences were observed for either line or leaf position. Similar to Neo and Anthera

concentrations, line 9410 showed non-significantly higher values in L-NS-L compared to the other lines (Fig.9a). The beta carotene concentrations did not differ among lines, but significant differences were detected between the flag leaf and L-NS-L. However, when the leaf positions within lines were tested in isolated multiple pairwise comparison, no significant differences were detected because of the low power of the test. It is worth mentioning that 9410 and 9414 had the highest differences between means of leaf position (Fig.9b). For the concentration of total Cars, significant differences were found for leaf position but not for line. Apart from 9432, Car concentration was higher in flag leaves but the difference to L-NS-L concentration was only significant in 9402 and 9410.

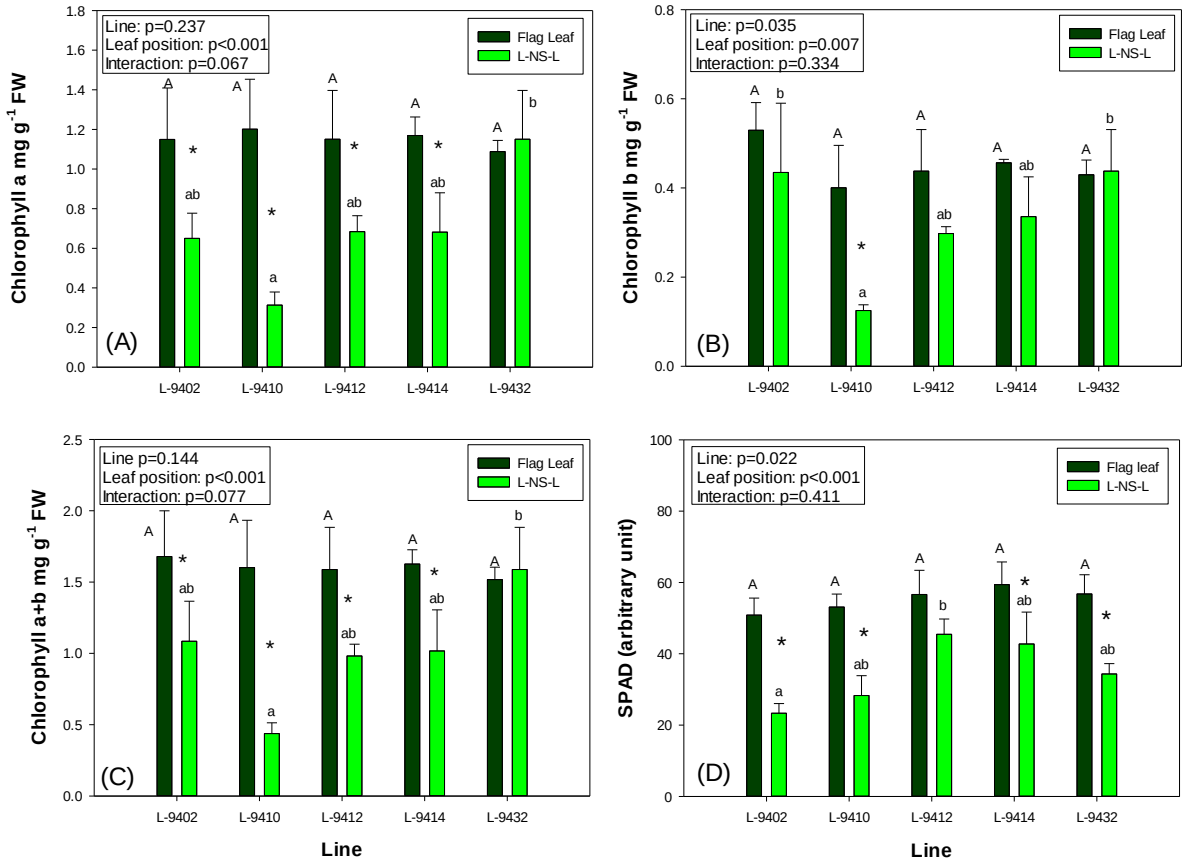

Figure 10*:* Concentrations of Chla, Chlb, Chla+b and SPAD values of 5 wheat lines at two different leaf position. L-NS-L=Lowest non-senescing leaf (ranging from leaf 2-6). Two-Way ANOVA was used to detect significant differences at α=0.05. Capital letters indicate significant differences among lines within the flag leaf. Lower case letters indicate differences among the lines within L-NS-L. Asterisks indicate significant differences between leaf level within a respective line.

Chl*a* concentrations, were highly significant due to leaf position in all lines but in 9432, while the largest difference was detected in 9410. Line 9410 also had the lowest Chl*a* concentration in L-NS-L that was significantly lower than 9432's but not different from 9402, 9412 and 9414, which still had twice the amount of Chl*a* compared with 9410. Even though the differences

were not significant, it is worth mentioning that in contrast to the other lines 9432 had a slightly higher Chl*a* concentration in L-NS-L compared to the flag leaf. Within the flag leaf, lines showed no significant differences (Fig. 10a). The difference of Chl*b* concentration between the leaf position were less pronounced compared to Chl*a*, with a significant difference only detected in line 9410. Similarly as with Chl*a*, the lines only varied significantly in their Chl*b* concentrations in the L-NS-L, but here, next to 9432, also 9402 had significantly higher values compared to 9410 (Fig. 10b). As for the total Chls, differences between leaf position were significant in all lines but in line 9432 while the other lines had a decrease in Chl*a*+*b* concentration. Line 9410 had the lowest concentration of total Chl in L-NS-L, being significantly lower than the concentration measured in line 9432. No differences were found in flag leaves concentrations of total Chl. SPAD values were highly significant due to leaf position, in all lines apart from 9412, and line within L-NS-L, where measured values were substantially lower in 9402 compared to 9412. No genotypic differences were found in the flag leaf of the tested lines.

Table 3*:* Leaf pigment ratios of 5 wheat lines at two different leaf position. L-NS-L=Lowest non-senescing leaf (ranging from leaf 2-6). Two-Way ANOVA was used to detect significant differences at α=0.05. Capital letters indicate differences within a

leaf level. Lower case letters indicate significant differences between the leaf level within a line. Two-Way ANOVA was used to detect significant differences at α=0.05

Line	Leaf level	Chl a/b	Chl/Car	Chl/Xanths	Xanths/Car	Anthera/Vio
9402	**Flag Leaf**	2.14±0.24	1.33±0.20	17.1±2.75 A a	0.08±0.01	0.26±0.05 A a
	L-NS-L	1.63±0.28	1.30±0.29	15.29±2.66 AB a	0.08±0.01	0.20±0.04 A a
9410	**Flag Leaf**	3.11±0.56	1.61±0.12	17.70±2.96 A a	0.09±0.02	0.78±0.17 A a
	L-NS-L	2.49±0.45	0.97±0.24	5.51±2.89 A b	0.28±0.20	0.50±0.31 A a
9412	**Flag Leaf**	2.74±0.76	1.86±0.56	26.73±11.03 A a	0.07±0.01	0.95±0.44 A a
	L-NS-L	2.31±0.30	1.22±0.10	19.22±1.11 AB a	0.06±0.00	0.41±0.07 A b
9414	**Flag Leaf**	2.56±0.16	1.55±0.19	22.25±3.68 A a	0.07±0.00	0.74±0.07 A a
	L-NS-L	2.02±0.60	1.62±0.23	17.92±1.02 AB a	0.08±0.02	0.58±0.12 A a
9432	**Flag Leaf**	2.54±0.10	1.99±0.55	21.04±3.70 A a	0.10±0.30	0.85±0.19 A a
	L-NS-L	2.74±0.76	1.86±0.56	24.32±2.27 B a	0.07±0.01	0.39±0.14 A a
Line		n.s.	n.s.	*	n.s.	n.s.
Position		n.s.	n.s.	n.s.	n.s.	*
Interaction		n.s.	n.s.	n.s.	n.s.	n.s.

Leaf pigment ratios did not differ significantly, except from Chl/Xanths and the ratio of Anthera to Vio, where statistical significance was ascertained for line and leaf position, respectively. Even though Anthera/Vio was consequently lower in the L-NS-L, statistical significance was only found in line 9412. Chl/Xanths differed significantly at L-NS-L position with 9410 having a lower ratio compared to 9432. Additionally, line 9410 had a lower ratio in L-NS-L compared to flag leaf that was detected in the post-hoc analysis, regardless of the missing significance of leaf position in general for this ratio. The ratio of Chl*a/b* aswell as Chl/Car both showed tendencies that in older leaves ratios decrease, but for neither of those ratios a statistical significance of these tendencies was detectable. It was also ascertained that neither leaf position nor line influenced Xanths/Car. However, it is worth mentioning that 9410 had an over 3 times higher ratio in L-NS-L compared to the other lines (Tab. 3).

4.2 Water deficit trial

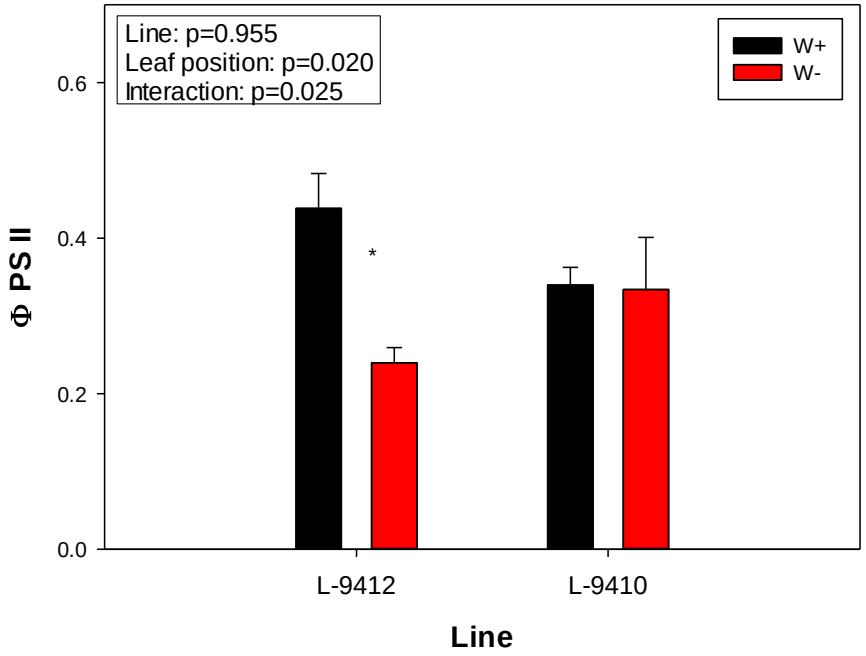

Figure 11: Φ PSII of two wheat lines subjected to either regular irrigation (W+) or water deficit (W-). Two-Way ANOVA was used to detect differences at α=0.05. Capital letters indicate differences between lines in the W+ treatment, lower case letters indicate differences in the W- treatment. Asterisks indicate significant differences between treatments within a line.

Photosynthetic quantum yield decreased in line 9412 significantly when subjected to water deficit, while it remained unaffected in line 9410, which led to a statistically significant interaction. Genotypic differences were not found in either treatment (Fig.11).

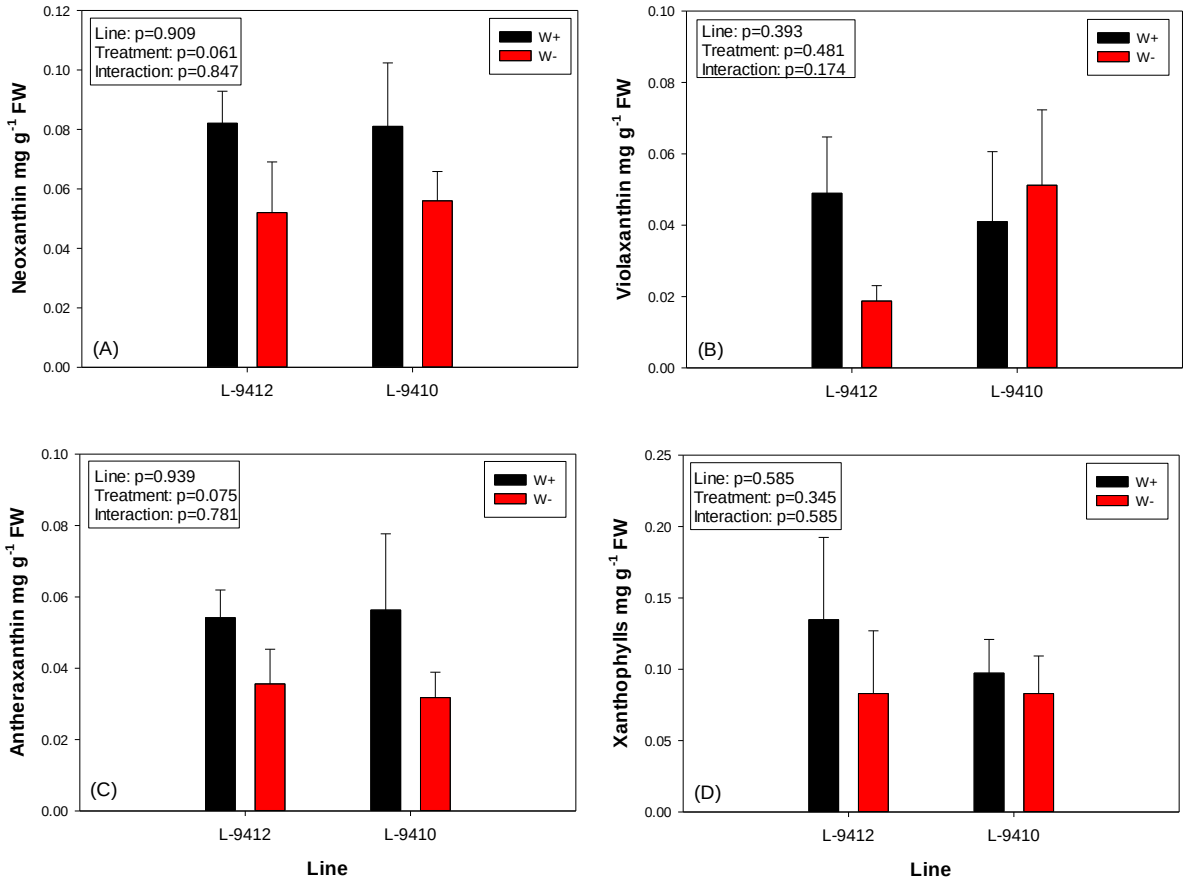

Figure 12: Concentration of xanthophyll cycle pigments of two wheat lines subjected to either regular irrigation (W+) or water deficit (W-). Two-Way ANOVA was used to detect differences at α=0.05.

The Xanth composition was not significantly affected by water deficit in neither genotype. Regardless of that, a clear trend, especially for Neo and Anthera concentrations, that a withdrawal of water decreases the concentration of these pigments. (Fig. 12a,c). Interestingly, Vio concentrations decreased under water deficit in 9412 but increased slightly in 9410, although differences were, as mentioned before, not statistically significant (Fig. 12b). Considering the total Xanth concentrations, it is noteworthy that the reduction in xanthophylls due to water deficit in 9412 was nevertheless substantially more important (38.52%), compared to the reduction of 14.7% in 9410.

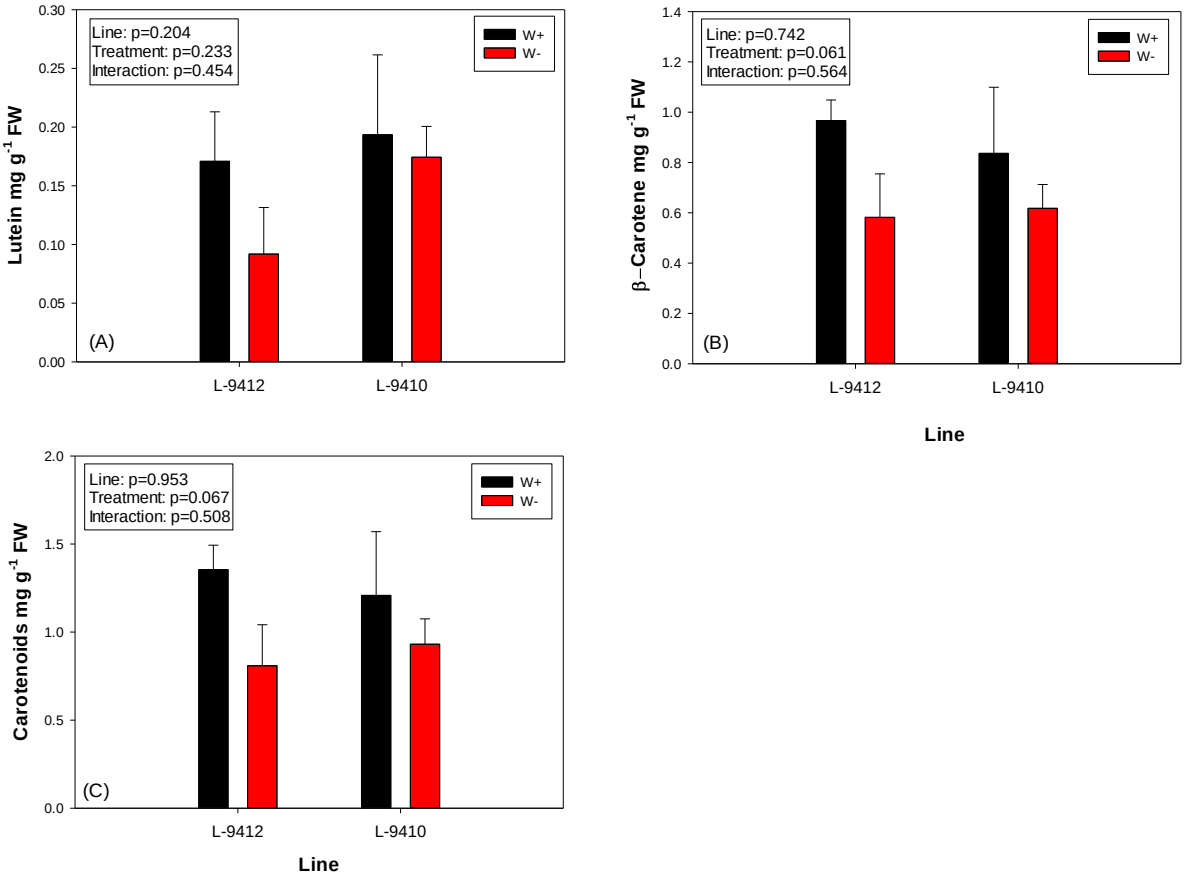

Figure 13: Concentrations of lutein, *β*-carotene and total carotenoids of two wheat lines subjected to either regular irrigation (W+) or water deficit (W-). Two-Way ANOVA was used to detect differences at α=0.05

The concentrations of lutein and beta carotene as well as the total Car concentration, did not exhibit significant differences due to either genotype or treatment. Despite the lack of statistical significance, droughted plants showed a reduction in pigment concentration compared to well-watered ones. Furthermore, there was also a tendency for this non-significant reduction to be smaller in line 9410 than in 9412, similar to the results shown in Figure 12.

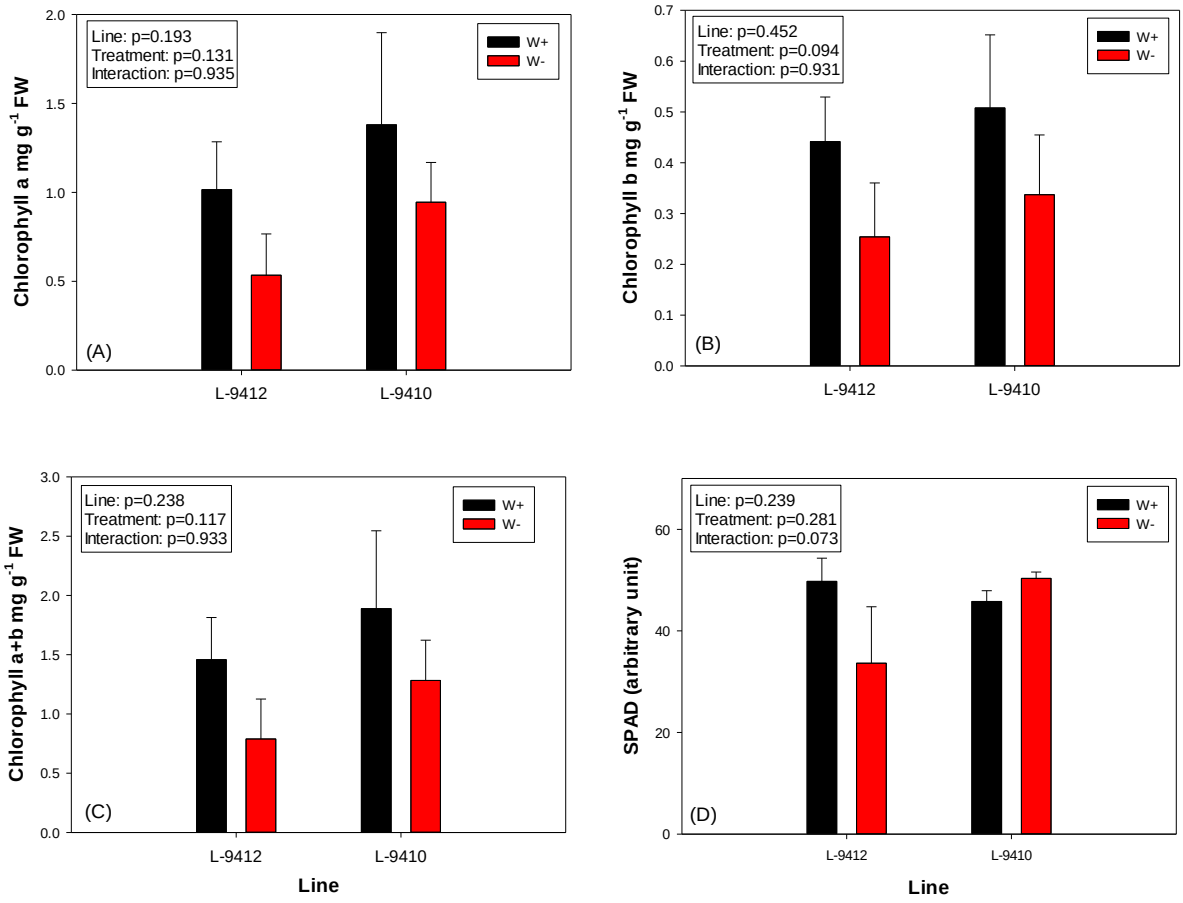

Figure 14: Concentrations of Chl and SPAD measurements of two wheat lines subjected to either regular irrigation (W+) or water deficit (W-). Two-Way ANOVA was used to detect differences at α=0.05.

No significant effect of line or treatment were detectable for Chl*a*, Chl*b* or both combined, but as previous findings of this study, values measured in water stressed plants were lower than the regularly irrigated control. Contrastingly to Car concentration, that was non-significantly higher in 9412 compared to 9410, Chl*a* and Chl*b* concentrations were both higher in 9410 than in 9412 (Fig. 14).

Table 4: Ratios of leaf pigments of two wheat lines subjected to either regular irrigation (W+) or water deficit (W-). Two-Way ANOVA was used to detect differences at α=0.05. Capital letters indicate differences between lines in the W+ treatment, lower case letters indicate differences in the W- treatment. Lower case letters indicate significant differences between treatments within a line.

Line	Treatment	Chl a/b	Chl/Car	Chl/Xanths	Xanths/Car	Anthera/Vio
9410	**W+**	2.64±0.34 A a	1.57±0.22 A a	18.93±2.87	0.08±0.01	1.83±0.86
	W-	2.94±0.30 A a	1.36±0.21 A a	17.10±5.91	0.09±0.02	0.70±0.22
9412	**W+**	2.26±0.29 A a	1.09±0.26 A a	13.89±5.87	0.10±0.03	1.32±0.55
	W-	2.08±0.15 B a	0.90±0.21 A a	11.21±6.22	0.10±0.04	2.04±0.68
Line		*	*	n.s.	n.s.	n.s.
Treat-ment		n.s.	n.s.	n.s.	n.s.	n.s.
Interac-tion		n.s.	n.s.	n.s.	n.s.	n.s.

Significant differences between the lines were detected for Chl*a/b* (p=0.011) and Chl/Car. While distinctions were still detectable in the *post-hoc* comparison for Chl*a/b*, the detected variation for Chl/Car was no longer detectable in the post-hoc analysis. Similar to the results in table 3, Xanths/Car was unaffected by line or treatment. Chl/Xanths showed a slight decrease in both lines when subjected to water deficit, but differences were not statistically significant. Interestingly, the ratio of Anthera to Vio showed different tendencies in 9410 compared to 9412 when subjected to water deficit. While a reduction in plant available water led to a decrease of 61.75% in Anthera/Vio in 9410, values measured in 9412 increased by 51.52%, nevertheless findings lacked statistical significance (Tab. 4).

4.2 High Light Intensity Treatment

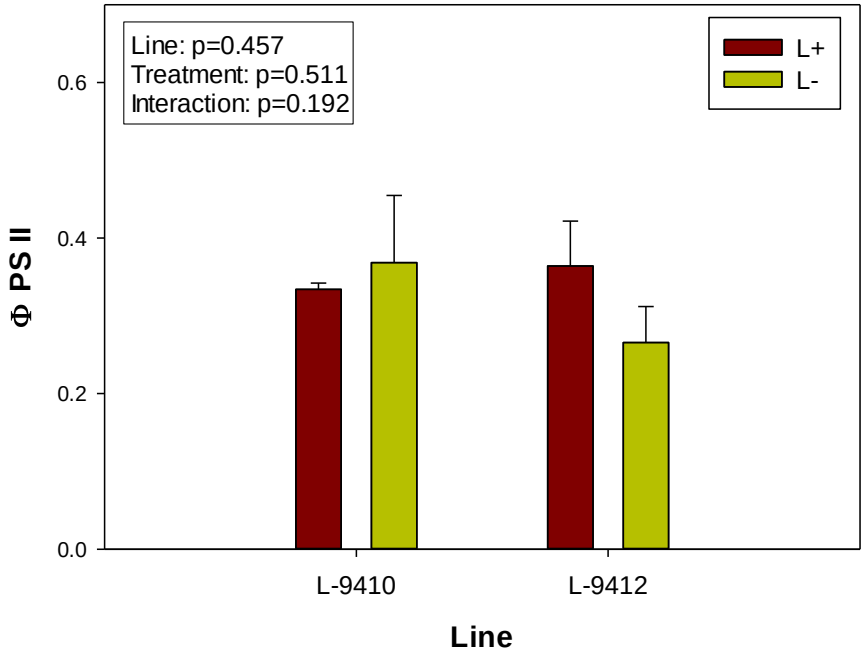

Figure 15: Φ PSII of two wheat lines subjected to either 400-500 µmol m-2 s-1 (L-) or 800-1000 µmol m-2 s-1 (L+) light intensity. Two-Way ANOVA was used to detect differences at α=0.05.

After exposure to high light intensity (1000 µmol m-2 s-1) for 5 days, Φ PSII of the two lines expressed varying trends: while 9412 showed a slight decrease when exposed to a high light, 9410 on the other hand slightly increased, but findings were not statistically significant (Fig. 15).

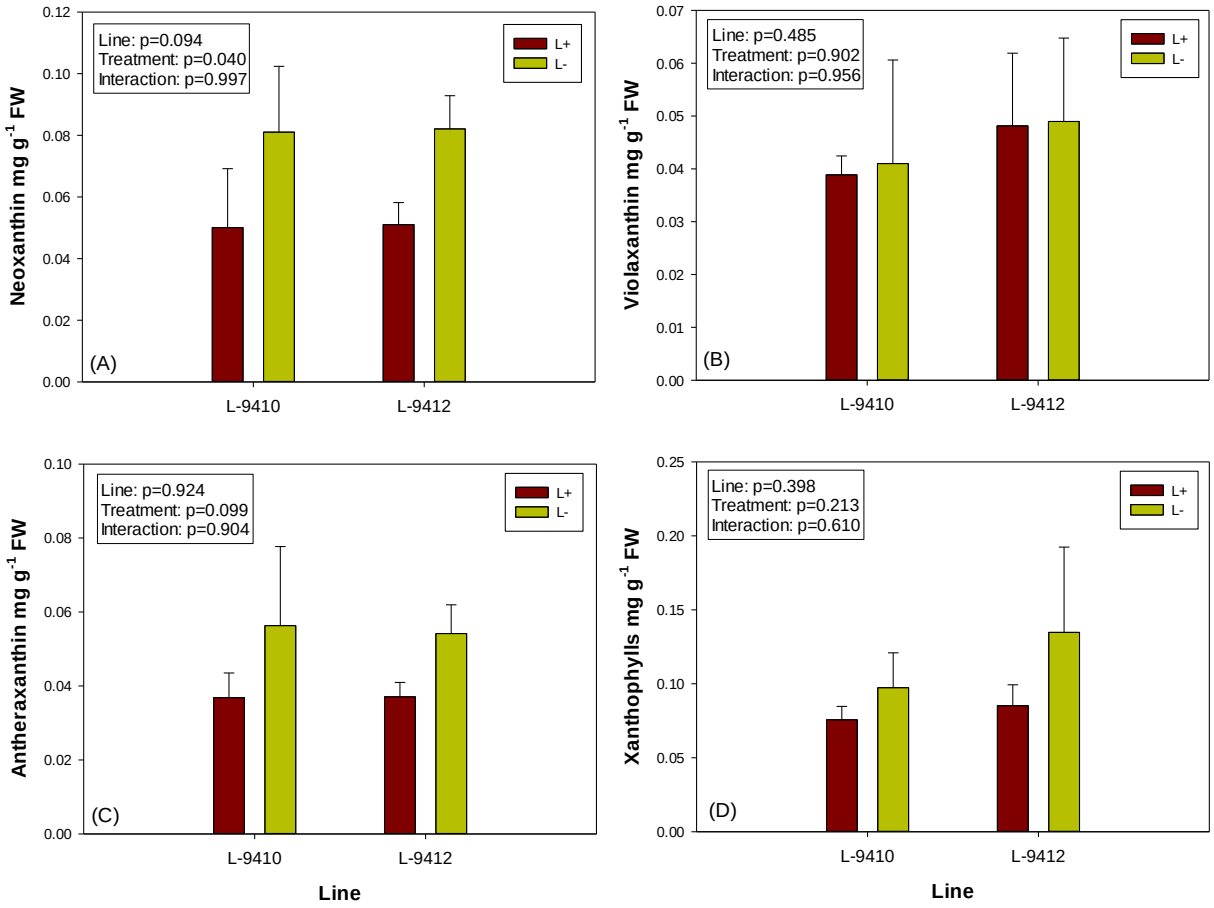

Figure 16: Xanthophyll cycle pigments and total amount of xanthophylls of two wheat lines subjected to either 400-500 µmol m^{-2} s^{-1} (L-) or 800-1000 µmol m^{-2} s^{-1} (L+) light intensity. Two-Way ANOVA was used to detect differences at α=0.05.

The concentrations of the Xanths, were not significantly affected by exposure to high light intensity. Nevertheless, a striking tendency was also found in this case: an increase in light intensity reduced Xanth concentrations, whereby the reduction in Anthera and Neo was higher than in Vio. No significant differences due the genotype could be detected. (Fig. 16).

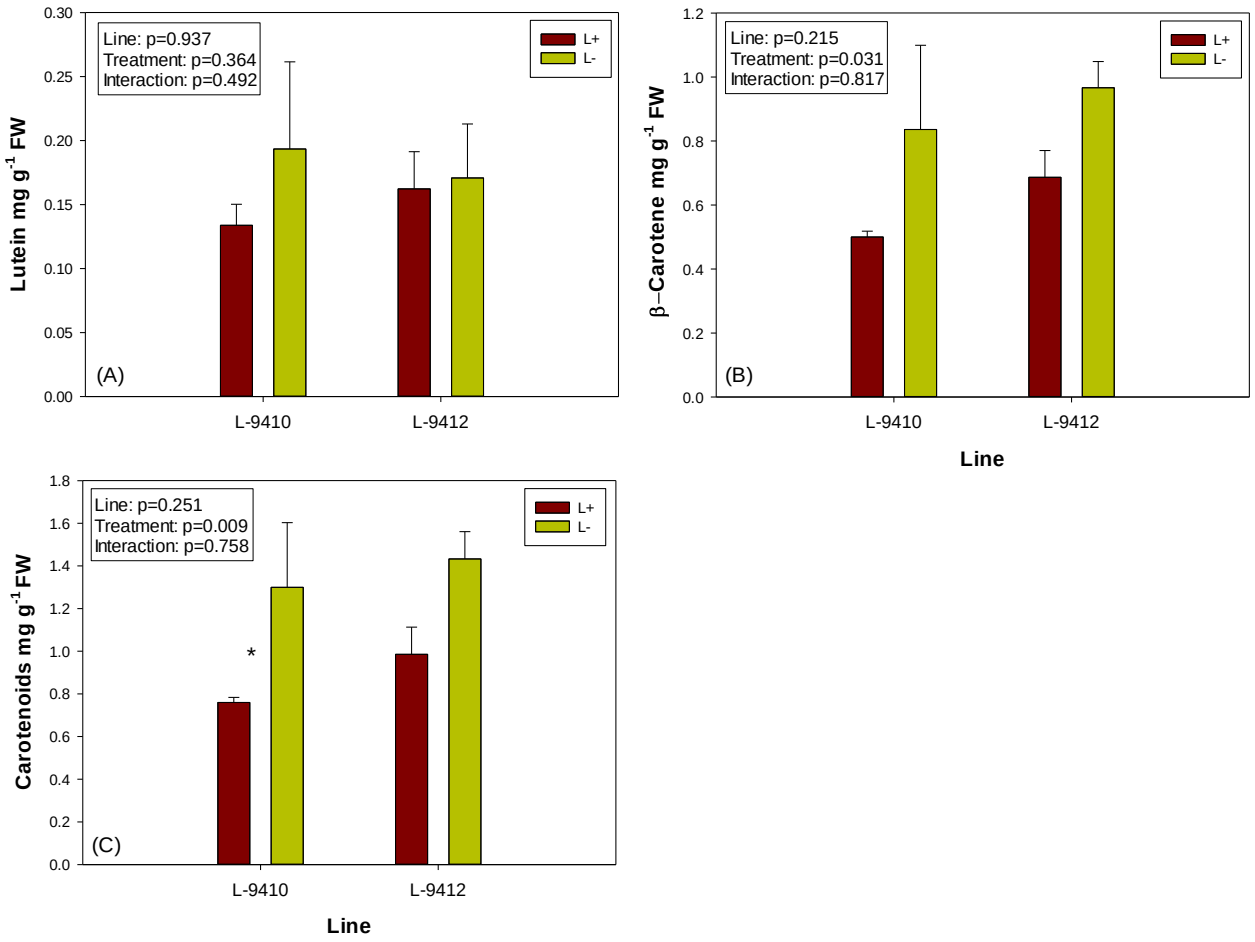

Figure 17: Concentration of lutein, ß-Carotene and total carotenoid concentration of two wheat lines subjected to either 400-500 µmol m^{-2} s^{-1} (L-) or 800-1000 µmol m^{-2} s^{-1} (L+) light intensity. Two-Way ANOVA was used to detect differences at α=0.05. Asterisks indicate differences between treatments within a line.

The lutein concentration of the leaves was not influenced by the line or the treatment. In contrast, light intensity had a significant effect on β-carotene concentration. 9412 showed slightly higher values than 9410 over both treatments, a statistical significance was not detectable. When all Cars were combined, 9410 showed a significant reduction in carotene concentration as a result of increased irradiance. Analogous to the previous findings, the trend here was that an increase in radiation intensity resulted in a reduction in Cars. However, statistical confirmation of this also remained elusive here (Fig. 17).

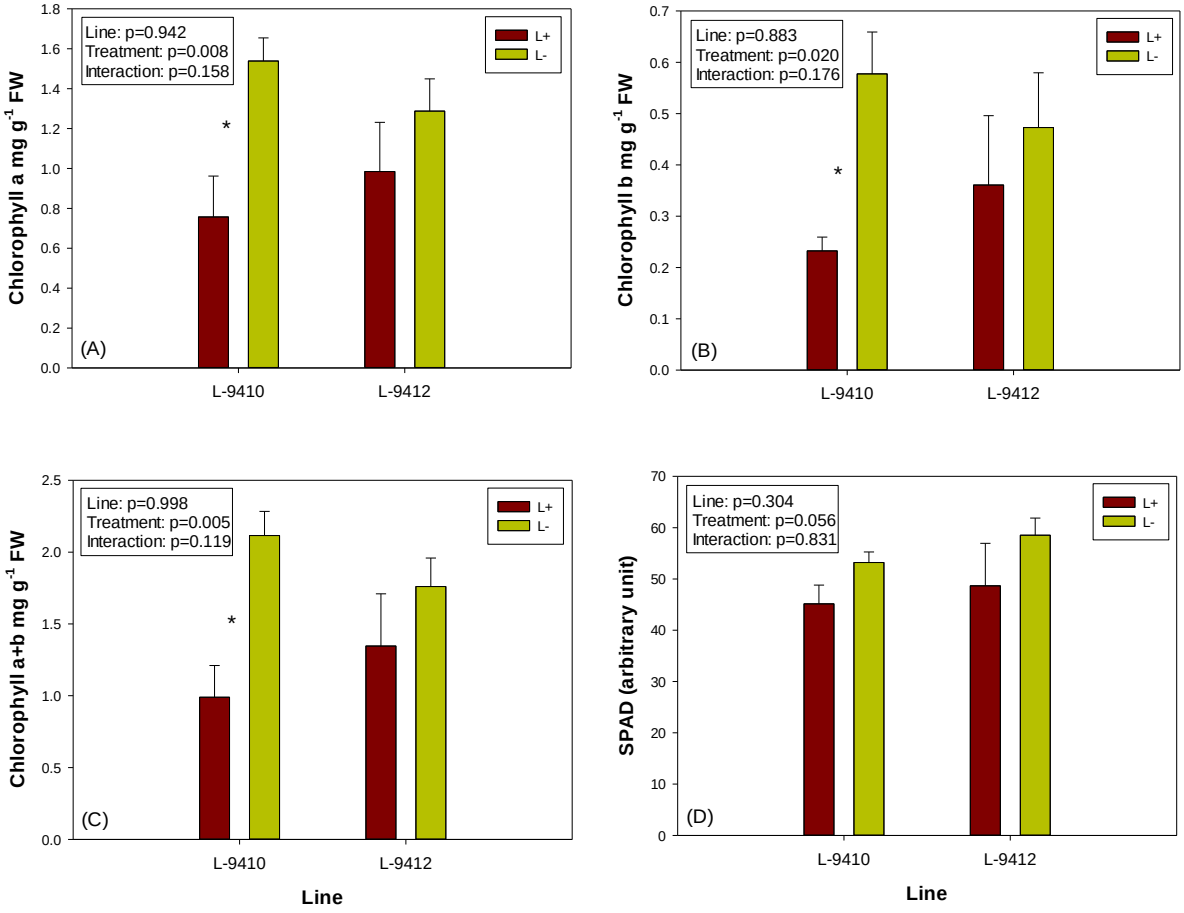

Figure 18: Chl concentrations and SPAD measurements of two wheat lines subjected to either 400-500 µmol m^{-2} s^{-1} (L-) or 800-1000 µmol m^{-2} s^{-1} (L+) light intensity. Two-Way ANOVA was used to detect differences at α=0.05. Asterisks indicate differences between treatments in a line.

Similar to the Cars, Chl concentrations measured in both lines also decreased when exposed to a high irradiance. For Chl*a*, Chl*b* and Chl*a*+*b*, a significant effect of light intensity could be detected in line 9410, with a reduction of 50.81%, 59.79% and 53.26%, respectively. In line 9412, however, the reduction was smaller, and the effect was not significant. SPAD values partially reflected these results, because in contrast to the Chl concentration, the spad values decreased less as a result of the treatment and no statistical significance was detectable (Fig. 18).

Table 5: Leaf pigment ratios of two wheat lines subjected to either 400-500 µmol m^{-2} s^{-1} (L-) or 800-1000 µmol m^{-2} s^{-1} (L+) light intensity. Two-Way ANOVA was used to detect differences at α=0.05.

Line	Treatment	Chl a/b	Chl/Car	Chl/Xanths	Xanths/Car	Anthera/Vio
9410	**L+**	3.23±0.73	1.30±0.29	13.33±3.60	0.10±0.01	1.83±0.86
	L-	2.72±0.34	1.71±0.27	24.66±9.03	0.09±0.04	0.95±0.14
9412	**L+**	2.89±0.61	1.24±0.19	15.64±5.15	0.10±0.05	1.32±0.55
	L-	2.92±0.54	1.33±0.22	15.59±3.11	0.09±0.01	0.90±0.36
Line		n.s.	n.s.	n.s.	n.s.	n.s.
Treatment		n.s.	n.s.	n.s.	n.s.	n.s.
Interaction		n.s.	n.s.	n.s.	n.s.	n.s.

No significant effect of line or treatment could be detected the leaf pigment ratios. Again, it is interesting to note that Chl/Car, Chl/Xanths, Xanths/Car and Anthera/Vio responded similarly to an increased radiation. However, there was no statistical significance. Similar to the water deficit experiment, the ratio of Xanths/Car was almost identical in both lines and remained unchanged by increasing light intensity.

4.4 SPAD and Chl concentration

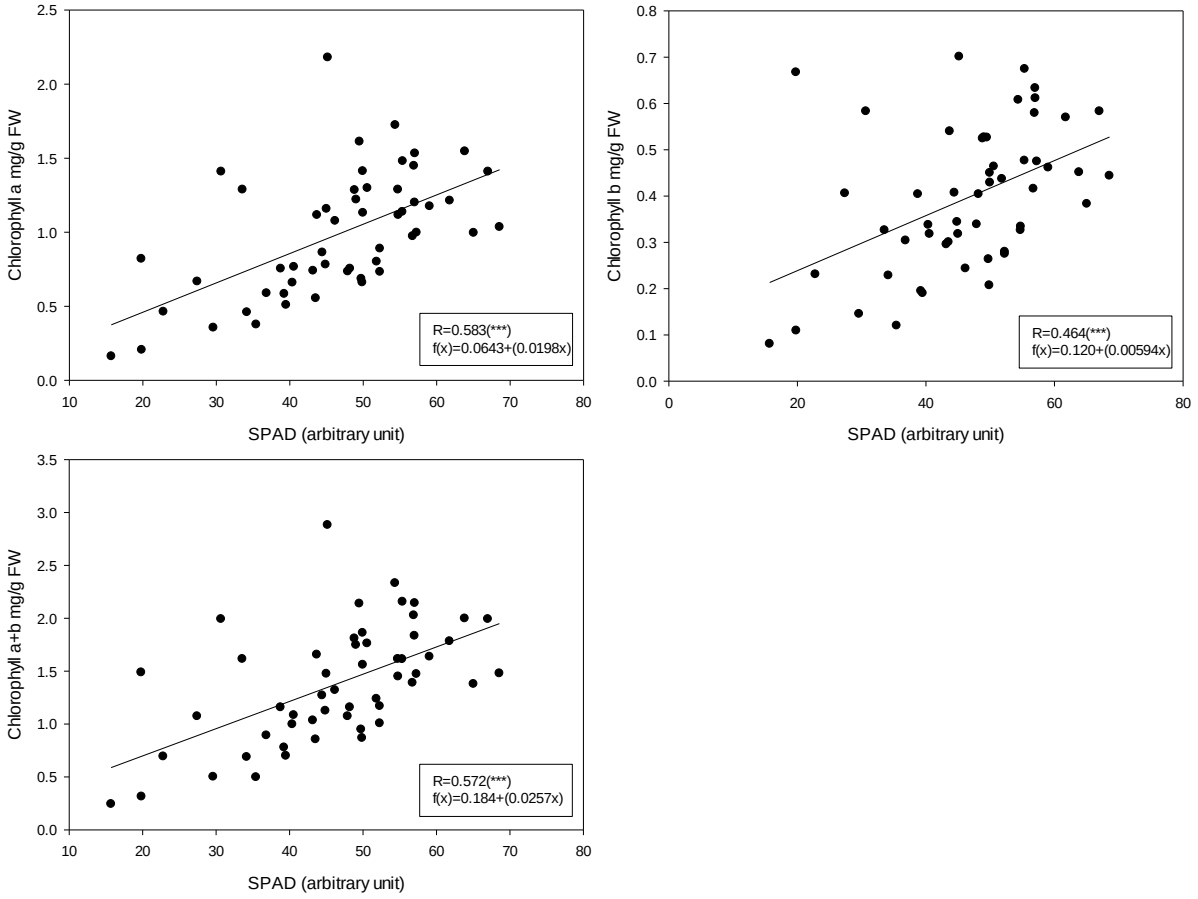

Figure 19: Linear regression of SPAD measurements to Chla , Chlb and Chla+b values of 5 wheat lines. Level of significance a=0.05, n=53.

To test whether spad measurements can be used as a valid indicator of Chl concentrations, correlations of Chl*a*, Chl*b* and Chl*a+b* to spad measurements were calculated. Correlations were highly significant throughout, with Chl*a* showing the highest correlation (r=0.583) (Fig. 19a). Correlation of Chl*b* was lower at r=0.464 and when combined with Chl*a*, the correlation coefficient was slightly lower than Chl*a* alone (r=0.572) (Fig. 19b,c). However, it is worth mentioning that the test for normality failed for all three parameters.

5. Discussion

5.1 Leaf position and genotypic differences

The quantum yield Φ PSII declined in all lines with leaf age, and even though significant differences were not detectable in pair-wise comparisons, this finding is in line with the results of previous studies such as Bauerle et al. (2020) who found that photosynthetic capacity decreased in leaves of *Cannabis sativa L.* as they aged, regardless of crown position, light intensity, or visible greenness. Furthermore, Lu et al. (2001) found that Φ PSII decreased substantially in wheat plants as leaf age progressed and that such a mechanism was significantly more pronounced at midday when plants were subjected to a higher light intensity of 700-1400 µmol $m^{-2}s^{-1}$ compared to the morning when light intensities were low (250 µmol $m^{-2}s^{-1}$). The authors concluded that this reversible downregulation of PSII is a mechanism to prevent damages in the photosynthetic apparatus as they concomitantly found an increase in NPQ and an associated increase in the de-epoxidation state of the xanthophyll cycle. Even though Φ PSII was non-significantly lower in L-NS-L, no concomitant increase in Anthera or its precursor Vio was found in this study. On the contrary, in this experiment the Vio concentration was substantially, and above all statistically significantly, lower in L-NS-L leaves in almost all lines than in the flag leaves. It is also worth mentioning that contrarily to previous findings, Anthera to Vio ratio (Anthera/Vio) did not increase but decreased significantly in L-NS-L compared to flag leaves, indicating that the photoprotective process of heat dissipation via Anthera and Zea might be of a lower magnitude with progressing leaf age (Table 3). However, as Zea was not detectable by the HPLC in this study, this hypothesis needs further validation (Lu et al. 2001). However, the Vio concentrations of 9402 were substantially lower at both leaf positions, in L-NS-L even statistically significant, while Anthera and Neo concentrations were non-significantly higher than in 9410, indicating that a stronger interconversion of Vio to either Neo or Anthera might have taken place, although one has to keep in mind the difference in the speed of conversion, as a a conversion from Vio to Anthera is much faster than Vio to Neo (Demmig-Adams et al. 1999; Yamamoto et al. 1999). Nevertheless, the photosynthetic quantum yield of 9410 was significantly lower in L-NS-L than in 9402 and substantially but not significantly lower than that of the other lines. A closer look at the Xanth concentrations revealed that 9410 had higher concentrations of Anthera and Neo, total Xanths as well as an increased ratio of Xanths over total Cars, indicating a higher potential of NPQ reflected in a lower Φ PSII (Demmig-Adams and Adams 1996; Hong et al. 1999; Genty et al. 1989). Additionally, the lutein concentration was higher in the older leaves of line 9410 compared to the other lines.

Recently, using computational simulations including fully atomistic dynamics and multiscale quantum descriptions of protein complexes, Cupellini et al. (2020) discovered that lutein, more precisely the fraction of lutein within the *a612*/Lut1 dimer of LHCII, can quench >90% of excitation energy of the entire LHCII by changing the energy level of the CT state of Chl*a612*. Thus, we could assume that the increased lutein might be a significant factor for energy dissipation in the LHCII-PSII supercomplex. Furthermore, it has been shown in *Arabidiopsis thaliana* npq1 mutants, a mutant unable to synthesise Zea and exhibits low concentrations of Vio, Anthera and Neo, that lutein is able to take the place of Zea and can directly quench singlet-excited chlorophyll by formation of lutein radical cations. This replacement mechanism has so far been proven in a variety of plants species (Li et al. 2009; Förster et al. 2011; Leuenberger et al. 2017; Duffy et al. 2013). Interestingly total Car concentrations did not differ significantly between the lines but in 9402 and 9410 values of L-NS-L were significantly lower when compared to flag leaf values, even though the total amount of xanthophyll cycle pigments was higher in L-NS-L in line 9410, reflected also in the photosynthetic quantum yield. This is because in these lines leaves, β-carotene made up around 65% of total Cars and its impact on Φ PSII is lower compared to that of Xanths (Xu et al. 2020). It has been shown in *Arabidopsis thaliana* that a mutant, that has a decreased activity in lycopene-β-cyclase and therefore only contained about 30% of carotene compared to the wildtype, showed reduced chlorophyll fluorescence quenching, higher singlet oxygen release and significantly stronger photoinhibition in PSI compared to PSII, indicating that the effect of β-carotene could be of less importance in PSII than in PSI (Cazzaniga et al. 2012). It has been shown that the main role of β-carotene in photoprotection is the scavenging of singlet oxygen, while the Xanths formed from β-carotene are primarily responsible for energy dissipation. Since the oxidation potential of PSII is significantly higher than that of PSI, the ability of carotene to scavenge singlet oxygen formed by triplet P680 is severely limited compared to PSI (Xu et al. 2020). It is also worth noting that, contrary to the relevant scientific knowledge that lutein is the most abundant carotene in leaf tissues, lutein concentrations were very low and most importantly substantially lower than those of (Nisar et al. 2015).

As illustrated in Fig.: 3, the carotenoid synthesis pathway splits after *all-trans*-lycopene. Afterwards it can either be converted by

(1) ε-LCY via δ-carotene to α-carotene, from which lutein is derived
    or
(2) β-LCY via γ-carotene to β-carotene, from which the xanthophyll cycle pigments are formed.

Since lutein concentrations are low and β-carotene concentrations relatively high, it is therefore reasonable to assume that the biosynthesis of the Cars has been altered in all lines so that more β-carotenes and the Xanths formed from them are produced, also reflected in the ratio of Xanths/Car (Cazzonelli 2011).

Like Car and Xanth concentration, Chl concentrations decreased significantly with leaf position in all lines except 9432 that showed a slight increase. It is visible, that Chl*a* concentrations decreased stronger than Chl*b*. A concomitant decrease of Chl*a/b* leaves the suggestion that a relatively stronger loss of PSI than PSII may be the reason for that as PSI has a larger Chl*a/b* ratio (Dinç et al. 2012). Additionally, it has been shown in previous research that the Chla/b ratio declines significantly with leaf age (Hikosaka 1996). The assumption that PSI might be stronger affected than PSII is further backed by the low ratio of Xanths/Cars as Fiore et al. (2012) linked a low ratio of PSI to PSII.

Another interesting aspect of line 9410 is the large decrease in Chl and the at the concomitantly occurring substantial increase in Xanths leading to the significantly lower Chl/Xanths in L-NS-L of 9410, also suggesting the high capacity for NPQ in this line. It has been stated by Dai et al. (2004) that in senescent plants under high light intensity (500 µmol m-2s-1) NPQ by xanthophyll cycle was not sufficient to protect the leaves against photooxidation, proposing an explanation on why, despite the high concentrations of xanthophyll cycle pigments, the efficiency of PSII was low. They concluded that there might be a different photoprotection mechanism activated in severely senescent leaves. However, line 9432, which had the smallest change in Φ PSII between leaf positions, was also the line in which pigment concentrations varied the least, underlining the effect that leaf pigments have on photosynthetic efficiency (Bauerle et al. 2020; Lu et al. 2001).

5.2 The effect of water deficit

When exposed to water limiting conditions, Φ PSII showed a different tendency for line 9410 than 9412. While Φ PSII in 9412 decreased significantly when exposed to water deficit, 9410 remained almost unaffected. This is in line with the research of Flagella et al. (1995) who tested the flag leaves of 25 *Triticum durum* cultivars subjected to drought and found that Φ PSII decreases on average by about 20%, but values ranged among the cultivars from 45% to up to 114% of control values. Interestingly, the authors stated that no interaction between genotype and treatment was found despite the large variation. So far it has been proven in many of studies, that Φ PSII decreases significantly under water deficit with a concomitant decrease in Car concentrations (Kalaji et al. 2017; Agric et al. 2010). In this study it has been shown as well that Car concentrations declined due to water deficit. Even though statistical significance of this effect was not detected, it is still apparent that the decrease observed in line 9412 was of a larger magnitude than the reduction in 9410. When looking at the total Xanth concentrations and the single Cars in general, it becomes apparent, that Vio, total Xanths and lutein concentrations are the ones that vary the most between these two lines. While Vio concentrations in 9412 decreased substantially, 9410 had slightly increased concentrations compared to W+ treatment but almost 2.5 times more Vio than 9412 under water deficit. At the same time Anth concentrations were slightly higher in 9412 W-, indicating that the xanthophyll cycle pool in 9412 could be in a higher de-epoxidation state compared to 9410 and therefor had a higher NPQ capacity reflected in the lower Φ PSII in 9412 as well as the Anthera/Vio ratio (Tab. 3) (Ahmad et al. 2018; Flagella et al. 1995). Also interesting is the fact that the decrease in total xanthophyll cycle pigments was much higher in 9412 (-38.52%) than in 9410 (-14.7%), providing the assumption that 9410 has a higher capacity of photoprotection than 9412 (Liu et al. 2006).

It may has become apparent that Zea, the most important Xanth for energy dissipation, concentrations were not displayed in this thesis as they were only detectable in 3 samples by the HPLC (Demmig-Adams and Adams 1996). While no definite answer to why this was the case can be given, two hypothesises can be considered:

(1) The peak for Zea was masked by the lutein peak in the HPLC chromatogram

(2) Zea was degraded due to the high ambient temperature

(3) A combination of 1 and 2

While possibility (1) could be a viable answer, as lutein is in much higher concentrations compared to Zea, it is unlikely since well distinguishable peaks were found when combined lutein and Zea standards were analysed. Zhang et al. (2011) showed that in *Tobacco* and *Arabidopsis* plants grown at 40°C, Zea concentrations decreased significantly after 45-min of illumination compared to plants grown at 23°C. In this study, average ambient temperature in the grow chambers was close to 40°C, with temperature reaching over 50°C due to heat waves that occurred during the experiments reported here. In combination with the fact that Cars concentrations decrease generally under water deficit or heat stress, it can be hypothesized that Zea might have been degraded and was therefore not detectable in this study (Kalaji et al. 2017; Agric et al. 2010). However, this is a possible hypothesis and further research on Zea synthesis and degradation under high temperature is needed to have a clearer view.

Furthermore, Vio is a precursor of Neo which ultimately forms abscisic acid (ABA) and concentration of Vio and Neo could thus provide information on the rate of ABA synthesis from Neo or Vio (Dall'Osto et al. 2007; Agric et al. 2010). However, the increase in Vio observed in line 9410 is not reflected in the concentrations of Neo, implying that Neo synthesis is not fully dependent on Vio concentrations. It has been proposed that Neo is not actively involved in fluorescence quenching but rather in protection of PSII/PSI? from photooxidation by reactive oxygen species similarly to β-carotene (Dall'Osto et al. 2007). Contrarily to the previous observation that lutein concentrations are considerably higher in the droughted plants of 9410 compared to 9412, indicating a higher potential for NPQ but here Φ PSII does not decrease as previously described. This suggests that relationship between processes (Φ PSII and NPQ) might be different in the two lines and that 9410 might have a higher potential of keeping the integrity of PSII. However, one must keep in mind that next to the thermal energy dissipation *via* the xanthophyll cycle there exist more quenching processes in NPQ which are independent of Xanths. A decrease of Φ PSII can also be the result of photoinhibitory quenching (qI) as a result non-functional PSII, or the newly termed sustained antenna quenching (qH). The latter has been shown to be a part of the slow relaxing qI and, even though its mechanisms could be the same as of qE or qZ, they are dependent on different molecular factors and can thus provide an adaption for the large abundancy of growing conditions that plants endure (Malnoë 2018).

When looking at the Chl concentrations it is noticeable that 9410 had non-significantly but considerably higher Chl concentrations than 9412 under well-watered conditions. Furthermore, Chl/Xanth and Chl/Car ratios were both higher in 9410 than in line 9412, indicating a larger

potential for protection of Chl from photoxidation and a higher capacity to utilize excitation energy (Eberhard et al. 2008; Horton and Ruban 2005). Additionally, the Chl*a/b* ratio can be used as an indicator for the ratio of PSII-core-complexes/LHCII as LHCII contains the most of Chl*b* and has a lower Chl*a/b* ratio than PSII. A decrease in the Chl*a/b* ratio could thus imply a stronger loss of PSII-core-complexes and result in a reduction of Φ PSII as described above (Kitajima and Hogan 2003). Here an increase of Chl*a/b* ratio in 9410 under water deficit was observed while it significantly decreased in line 9412 compared to line 9410, further underlining the hypothesis that 9410's capacity to maintain the integrity of PSII is higher under water stress compared to 9412, providing a higher drought resistance (Sharifi and Mohammadkhani 2016; Behera et al. 2002). However, information of the effect of water deficit on the pigment composition and moreover the function of each pigment of the xanthophyll cycle is still very scarce and further research in this field is necessary.

5.3 The effect of light intensity

A higher radiation intensity provoked a different response in the tested wheat lines. While in 9412 high light intensity resulted in a noticeable but not significant increase in Φ PSII, 9410 expressed slightly lower values. Despite that, Car concentrations decreased noticeably upon an increasing light intensity and pigment concentrations were similar across both lines. This is congruent with the research of Behera and Choudhury (2003) who also found a decrease of all Cars and Chls in primary wheat leaves when plants were subjected to 250W m^{-2} (1150 µmol $m^{-2}s^{-1}$), a radiation intensity slightly higher than the one used in this study, for 5 days. Concomitantly they observed a decrease in Φ PSII, whose extent significantly increased with increasing radiation intensity, and an increase in NPQ. Also, an increase in NPQ and decrease of Φ PSII was found by Chen et al. (2011) in wheat hybrids exposed to 1500 µmol $m^{-2}s^{-1}$ for 6 h. However, contrarily to Behera and Choudhury (2003) they observed an increase in Anth and Zea that could explain the increase in NPQ. In this study the only factor indicating a higher NPQ, and higher de-epoxidation state of the xanthophyll cycle is that Anthera/Vio increased substantially but not significantly under increased irradiance in both lines, proposing a possible explanation for the slight decrease of Φ PSII in line 9410. Additionally, lutein concentrations were less affected by a change in light intensity in 9412 compared to 9410. In combination with the increased Anthera/Vio this could mean a higher protection capacity of the photosynthetic apparatus resulting in the higher quantum yield of PSII (Cupellini et al. 2020; Förster et al. 2011). Another approach at explaining the differing response to light intensity lies on the concentration of Chls. In 9410 the decrease in Chls concentrations is much larger and compared

to values measured in leaves grown under lower irradiance, significantly lower. This suggests that an increase in radiation intensity leads to a higher Chl degradation in 9410 and because of that to a lower Φ PSII due to qI (Kitajima and Hogan 2003; Malnoë 2018). This is further supported by the decrease in Chl/Car and Chl/Xanths. On the contrary however, the Chl*a/b* ratio was substantially increased as the decrease of Chl*b* in 9410 was far more pronounced that of Chl*a*. As mentioned before, the majority of Chl*b* is in the LHCII and an increase in Chl*a/b* could thus suggest that LHCII was more strongly affected by photooxidation than the core complexes of PSII, further supported by the larger decrease of lutein in 9410 (Kitajima and Hogan 2003; van Amerongen and Croce 2013; Li et al. 2009). This is further supported by the work of Fiore et al. (2012) who demonstrated that Xanths/Cars is directly linked to the ratio of LHCII/PSII core-complexes and that an increase of the first is associated with an increase of the latter. As Xanths/Cars was very low in this study, it can therefore be concluded that LHCII was more strongly inhibited than PSII core-complexes.

At this point it must be mentioned that the effect of aging processes cannot be ruled out as sources of variation as the measurements of L+ were conducted 5 days after L-. This was because of the low number of plants that were still measurable at this point. It has been shown in previous studies, and in the chapters above, that leaf age significantly affects the pigment composition as well as Φ PSII (Lu et al. 2003). Results of this section therefore must be interpreted with care.

5.4 Heat

As ambient temperatures during the time of this experiment were extraordinarily high, temperatures inside the growing chambers did reach almost 40°C on average with temperature spikes of up to 52°C during the midday. It is reported that PSII is directly impaired when leaf temperatures exceed 40°C (Kalaji et al. 2017). Haque et al. (2014) also showed that in addition to a decrease in Φ PSII and other fluorescence parameters, Chl concentrations declined significantly, and Feng et al. (2014) showed that this was due to inhibition of PSII and a decline in RuBisco activity. Furthermore, it has been shown that heat combined with water deficit or high irradiation intensity can critically amplify the already existing stress condition and lead to a faster damage of the plant (Zahra et al. 2021; Chen et al. 2017). In addition to that, Abdelhakim et al. (2021) were able to show that a combination of heat and drought stress resulted in a significantly lower concentration in all pigments compared to either stress in isolation. Furthermore, when looking at the results of the heat and drought trial by CIMMYT,

it is easily noticeable that all lines expressed greater reduction in yield parameters when subjected to heat stress compared to drought. Given the relationship between biomass, yield and photosynthetic activity, particularly under stress conditions, it can therefore be assumed that heat has a significant impact on the photosynthetic efficiency of the tested lines and that when interpreting the results of this study.

5.5 SPAD and Chlorophyll

Just briefly it shall be mentioned that, congruently with the existing scientific knowledge, SPAD readings were highly significantly correlated with Chl concentration while the correlation coefficient R^2 was higher for Chl *a* (0.583; $p<0.001$) than Chl*b* (0.464; $p<0.001$). These values were substantially lower than those described by Uddling et al. (2007) who found $R^2=0.9$ for wheat plants. However, Ke-Qiang Yu et al. (2016) found that with increasing senescence, correlation between SPAD readings and Chl concentrations declined, implying that SPAD measurements should be interpreted with care when dealing with senescent leaves. However, this also underlines the assumption that plants in this trial were severely senescent and that this might have had an important impact on the results of this trial.

6. Consideration for a re-run

As the climatic conditions in central Europe are changing towards higher temperatures earlier in the year, one should consider conducting greenhouse experiments, especially when conducted in greenhouses without sufficient options to maintain stable temperatures, during the late autumn/winter or late winter/early spring to prevent an overheating inside the growing chamber. Additionally, a bigger pot size as well as a different growing medium and a more frequent fertilization should be considered. The experimental conditions were far from perfect as a general effect of senescence of the severely stressed plants as well as an effect of heat as co-stress cannot be ruled out and a re-run should be considered to double check and validate these findings. Additionally, the sample size of n=3 for each combination of line and treatment, is too low and might have contributed to the lack of statistical significance despite large differences in average values among lines and treatments.

7. Conclusion

The genotypic differences in the photosynthetic efficiency, pigment composition as well as the acclimation potential to water or light stress of different wheat lines were examined in this study. Leaf age/position, water availability as well as light intensity had a measurable impact on Φ PSII as well as on the pigment composition. In general, Φ PSII as well as Car and Chl concentration were reduced in lower (older) leaves compared to the youngest fully expanded leaf and, apart from line 9410, no direct effect of xanthophyll cycle pigments on Φ PSII was detectable in this study. It has also become apparent, that the decrease in Car concentrations was less than that of Chl in all treatments. It has been shown that 9410 shows signs that can lead to the conclusion that this line might be more resistant to water deficit while 9412 expressed signs of a higher resilience against higher radiation intensities. However, it must be mentioned that 9410 had a low photosynthetic quantum yield compared to the other lines but was also able to maintain that level throughout the experiments.

In general, plenty of research can be found about Φ PSII and NPQ but information on how leaf pigments composition changes upon water-, light- and heat stress or a combination of those in wheat is scarce and further research towards this topic is needed and can be useful for breeders to combat the future adversities of wheat cultivation and ensure the food security under these conditions.

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