# THE ROLE OF CHLOROPHYLL *b* IN BARLEY, *Hordeum vulgare*, AND THE CHLOROPHYLL *b*-LESS MUTANT, *Chlorina f2,* IN PHOTOSYNTHESIS AND PHOTOPROTECTION.

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KEY WORDS: Chlorophyll *b*, chlorophyll fluorescence, D1 protein, photodamage, photoinhibition, photoprotection, xanthophyll cycle

**ABSTRACT**

We hypothesized that if chlorophyll *b* is important for reducing light damage, then the *Chlorina f2 (Clo f2)* mutant lacking in chlorophyll *b* would fluoresce less in excess light and in recovery compared to the WT barley, *Hordeum vulgare,* due to accumulated damage from reactive oxygen species (ROS). High pressure liquid chromatography (HPLC) was used to determine the concentrations of photosynthetic pigments in both the WT and *clo f2* barley under three different treatments: control, photoinhibition and recovery. Chlorophyll fluorescence was used to calculate the photosynthetic efficiency of the different treatments of barley to assess the physiological consequences of diminished chlorophyll *b* levels in *Clo f2*. It was found that *Clo f2* contained no chlorophyll *b* and fluoresced significantly less than the WT, supporting the hypothesis that chlorophyll *b* plays an important role in dissipating excess energy to prevent the buildup of toxic ROS.

**INTRODUCTION**

Photosynthesis is a process in which $CO_2$, water and light energy is transformed into chemical energy in the form of ATP and the redox potential of NADPH. Photosynthesis requires photosynthetic pigments to capture light energy. This reaction occurs in the thylakoid membrane of chloroplasts, where photosynthetic pigments inside the light-harvesting complex (LHC) intercept light energy to transfer to the reaction center, photosystem II (PSII) (Taiz *et al*, 2015). Charge separation and the conversion of light energy into chemical energy takes place in PSII (Taiz *et al*, 2015).

The two main categories of photosynthetic pigments are chlorophylls and carotenoids (Taiz *et al*, 2015). Chlorophyll *a* and *b* make up the chlorophylls and they differ in the oxidation state of the side chain on ring 3; chlorophyll *a* has a methyl group while chlorophyll *b* has an aldehyde and they exist in a 3:1 ratio (Taiz *et al*, 2015). Chlorophyll *b* is formed from chlorophyll *a* when the enzyme chlorophyll *a* oxygenase oxidizes the methyl group to an aldehyde (Taiz *et al*, 2015). They can also be distinguished by their unique absorption spectra—chlorophyll *a* has strong absorption from 350 to 475 nm and 600 to 700 nm meanwhile chlorophyll *b* has strong absorption at 460 and 640 mn (Taiz *et al*, 2015). Both chlorophylls exist in the light harvesting complexes of photosystem I (PSI) and II (PSII), however “special pairs” of chlorophyll *a* form the reaction center of the photosystems (Taiz *et al*, 2015).

Carotenoids, the second category of photosynthetic pigments, include carotines (β-carotene) and xanthophylls (violaxanthin, antheraxanthin, zeaxanthin, lutein, etc.) (Taiz *et al*, 2015). Both are present in the light harvesting complexes of PSI and PSII and play a role in funneling light energy to reaction centers for chlorophyll *a* to use in photosynthesis (Taiz *et al*, 2015). Carotenoids also play a role in photoprotection in two ways. Lutein and β-carotene have a constitutive protection mechanism and xanthophylls also play a key role in dissipating excess energy though the xanthophyll cycle, which is light-regulated (Jahns *et al.,* 2008). The xanthophyll cycle allows a reversible switch for the photosynthetic LHCs to go from a light-harvesting state under low light to a light dissipation state under high light (Taiz et al, 2015).

The dissipation of excess energy is important as too much exposure to light leads to photoinhibition, in which photodamage to photosynthetic apparatus causes a decrease in photosynthetic rate (Jahns *et al.,* 2008). During periods of high light, light harvesting complexes absorb more energy than they can utilize for photosynthesis and the excess energy must be re-emitted as light or dissipated as toxic reactive oxygen species (ROS) may accumulate within the cells (Jahns *et al.,* 2008). Chlorophyll fluorescence measures the amount of energy that is re-emitted to gain insight about the amount of energy that was dissipated as well as the amount of energy that was actually used in photosynthesis (Maxwell and Johnson, 2000). This provides additional, quantitative information about the damage environmental stressors had on a plant's photosynthetic pigments (Maxwell and Johnson, 2000).

The *Chlorina f2* (*clo f2*) mutant of barley, *Hordeum vulgare*, is defective in the enzyme chlorophyll *a* oxygenase and thus, *clo f2* lacks chlorophyll *b* (Nezval *et al.,* 2017). Under "non-stress", normal light and temperature conditions *clo f2* plants function similar to wild type (WT) plants, however they are slower to grow and produce fewer and smaller seeds (Nezval *et al.,* 2017). While under light stress, the difference is more pronounced and *clo f2* plants appear pale and are stunted (Ghirardi *et al.,* 1986).

The objective of this laboratory experiment was to measure the differences in *clo f2* and WT barley to draw conclusions about how photosynthetic pigments, specifically chlorophyll *b*, function and to understand its role in photosynthesis. If chlorophyll *b* is important for reducing light damage in the leaf, then the *clo f2* mutant lacking in chlorophyll *b* would fluoresce less in excess light and in recovery compared to the WT barley due to accumulated damage from ROS.

Additionally, it was predicted that the lack of chlorophyll *b* in *Clo f2* would lead to lowered productivity and functional pigments after long periods of photoinhibition.

## MATERIALS AND METHODS

### GROWTH CONDITIONS

For this study 4 week old WT barley, *Hordeum vulgare*, and the mutant *Clo f2* were grown in Premier Pro-Mix general purpose growth medium in the Environmental Growth Chamber in Chagrin Falls, Ohio, USA. The normal light barley was grown in 16/8 hr photoperiod at 250 µmol quanta $m^{-2}$ $s^{-1}$ with a day/night temperature of 20 ℃/16 ℃. Hoagland's fertilizer solution was applied weekly to both treatments.

### TREATMENTS

For the photoinhibition treatment, the same growth conditions were used but the leaves were exposed to 1425 µmol quanta m-2 s-1 illumination for 180 minutes. The recovery from photoinhibition treatment plants underwent the same high light conditions for 180 minutes, and then allowed to recover under normal light (250 µmol quanta $m^{-2}$ $s^{-1}$) for an additional 180 minutes.

### PIGMENT EXTRACTION AND ISOLATION

Pigments were prepared for extraction by grinding up 5 to 6 cm of the WT barley leaf material with 0.5 mL acetone, a spatula tip of $CaCO_3$, and a spatula tip of sand in a 1.5 mL microfuge tube. The tube was then centrifuged for 5 minutes at 14 000 x *g* and the supernatant was transferred to a microfuge tube and placed on ice.

Individual pigments were separated using paper chromatography. The supernatant was applied to the chromatography paper (15 x 20 cm, Whatman # 3030917) and the paper was placed into a 1 L beaker containing 20 to 25 mL of solvent (petroleum ether-acetone, 9:1) for 25 minutes. Each band of separated pigments were cut out of the chromatography paper and transferred to individual 15 mL screw cap tubes and 2 mL of 95% ethanol was added to each tube in preparation for absorption spectroscopy.

Samples were prepared for absorption spectroscopy by micro centrifuging at top speed for 5 minutes and then the supernatants were transferred into cuvettes. Absorption spectra were

recorded using a spectrophotometer (Ultrospec™ 2100 *pro* UV/Visible Spectrophotometer, Biochrom, England).

## QUANTITATIVE RECOVERY OF PIGMENTS USING HPLC

High Pressure Liquid Chromatography (HPLC) was used to quantitatively analyze the pigments in the control, photoinhibition and recovery treatments of the WT and *clo f2* barley. For each sample, 0.1 to 0.15 g of leaf material, 1 mL of acetone, a spatula tip of $CaCO_3$, and a spatula tip of sand was ground up in a 1.5 mL microfuge tube. The samples were then micro centrifuged for 5 minutes at 14 000 x *g* and the supernatant were transferred to an HPLC sample vial containing a glass micro volume insert and cap. The Agilent 1100 HPLC column used was C-18 (octadecyl) derived silica (5 µm), and an elution method modified from "Program 1" of Gilmore and Yamamoto (1991) was used to analyze the photosynthetic pigments.

## CHLOROPHYLL FLUORESCENCE

QUBIT SYSTEMS™ Chlorophyll Fluorescence Package (Kingston, ON) was used to measure chlorophyll fluorescence and calculate the photosynthetic efficiency of barley plants. Fo, F'o, Fm, F'm, Fv and Fs were measured and calculated by subjecting dark-adapted leaves of each treatment to a brief (0.8 s) pulse of saturating light (> 5000 µmol quanta $m^{-2}$ $s^{-1}$) to induce maximal fluorescence.

## GREEN GELS AND SDS PAGE

The isolation procedure performed was based on Komenda's 2000 study. The thylakoids were isolated for loading into the green gels by grinding 0.5 g of leaf tissue with 5 mL of cold isolation buffer (50 mM Tricine, 0.4 M Sorbitol, 10 mM NaCl, 5 mM $MgCl_2$) and a small amount of sand for each treatment. The homogenate was then filtered through 2 layers of wet Miracloth into a chilled 15 mL tube to be divided into 4 microfuge tubes. The tubes were then centrifuged for 5 minutes at 5000 x *g.* The supernatants were then discarded and the pellets were resuspended in a total volume of 1 mL wash buffer (50 mM Tricine, pH 8.0). 50 µL of the thylakoid suspension was extracted and added to 950 µL of acetone and spun in a microcentrifuge for two minutes at 14 000 *x* g. The supernatant was then removed and placed into a cuvette. Using the Ultrospec™ 2100 pro UV/Visible Spectrophotometer (Biochrom,

England), readings were obtained at 664 nm for chlorophyll *a* and at 654 nm for chlorophyll *b.* The thylakoid protein/pigment complexes were then separated using non-denaturing electrophoresis in 10% polyacrylamide gels.

The thylakoid protein/pigment complexes extracted were then separated using denaturing electrophoresis (SDS-PAGE) in 15% polyacrylamide gel, according to Laemmli's 1970 procedure. A *Running Gel* (15% acrylamide; 9 mL distilled water, 11.14 g urea, 16.60 mL 30% acrylamide/bis, 7.20 mL Laemmli Buffer (8x), 200 µL 20% SDS, 100 µL 10% APS solution, 60 µL TEMED) and a *Stacking Gel* (5% acrylamide; 2 mL 30% acrylamide/bis, 3 mL Laemmli Buffer (8x), 6.8 mL distilled water, 120 µL 20% SDS, 120 µL 10% APS solution, 16 µL TEMED) were loaded with 25 µL of the thylakoid extract and 5 µL of the low molecular weight protein markers. Electrophoresis was then performed at 30 mA per gel for 45 to 60 minutes. Afterwards, the gels were stained using InstantBlue™ from Expedeon (San Diego, CA) Coomassie staining.

### DATA ANALYSIS

ANOVA was used to compare the effects of treatment and genotype and Tukey's HSD test and least-squares means was used to identify where differences were among treatments and genotypes when there was a significant interaction.

All data analysis was performed with the software R (v. 4.1.2; R Development Core Team 2022).

## RESULTS

### FLUORESCENCE ANALYSIS

The maximal efficiency of photosynthesis, expressed by the parameter Fv/Fm, differed significantly among the treatment groups (Figure 1; ANOVA; $F_{2,146}=12.81$, $P<0.0001$). The different genotypes were not significantly different in Fv/Fm (ANOVA; $F_{1,146}=1.02$, $P=0.31$), however the maximal efficiency of photosynthesis were significantly different between the control and photoinhibition treatments (Tukey HSD; $P<0.0001$) as well as between the control and recovery treatment (Tukey HSD; $P=0.045$) and between the recovery and photoinhibition treatment (Tukey HSD; $P=0.021$).

The photochemical activity of the leaf (qP) did not differ significantly between the treatments (ANOVA; $F_{2,137}$=3.00, P=0.053) while the qP differed significantly among the two genotypes (Figure 2; ANOVA; $F_{1,137}$=10.949, P=0.0012).

The release of non-photochemical quenching (NPQ), primarily as heat or by fluorescence, differed significantly between the treatment groups (ANOVA; $F_{2,146}$=8.96, P=0.00021) and between the genotypes (Figure 2; ANOVA; $F_{1,146}$=14.75, P=0.00018). The NPQ of the genotypes were significantly different from each other (Tukey HSD; P=0.00018), as was the difference in NPQ between the control and photoinhibition treatments (Tukey HSD; P=0.00037) and between the control and recovery treatments (Tukey HSD; P=0.0033). The difference in NPQ between the recovery and photoinhibition treatments however, were not significant (Tukey HSD, P=0.81).

## HPLC ANALYSIS

The concentration of xanthophyll 1 was significantly different in both the genotypes (ANOVA; $F_{1,144}$=17.99, P<0.0001), the treatments (ANOVA: $F_{2,144}$=3.07, P=0.049) and the interaction between the genotypes and treatments (Figure 4; ANOVA; $F_{2,144}$=7.00, P=0.0013). In the *f2* mutant, there was a significant difference in xanthophyll 1 concentrations of the photoinhibition and recovery treatments (Least-squares mean; P=0.042). However the control and photoinhibition treatments (Least-squares mean; P=0.37) and the control and recovery treatments (Least-squares mean; P=0.53) did not show significant differences between treatments. In the WT barley, the photoinhibition treatment had a significantly decreased xanthophyll 1 concentration from the control (Least-squares mean; P= 0.00080) meanwhile there was no significant difference between the control and recovery treatments (Least-squares mean; P=0.054) and the photoinhibition and recovery treatments (Least-squares mean; P=0.35). The xanthophyll 1 concentration in the *f2* mutant was found to be significantly lower than in the WT in the control (Least-squares mean; P<0.0001) and recovery treatments (Least-squares mean; P=0.0004). The photoinhibition treatment's xanthophyll 1 concentration was not significantly different between the *f2* and WT barley (Least-squares mean; P=0.84).

The concentration of xanthophyll 2 was significantly different in the genotypes (ANOVA; $F_{1,144}$=209.30, P<0.0001) but not the treatments (ANOVA: $F_{2,144}$=1.86, P=0.16). Additionally, there was a significant interaction between the genotypes and the treatments (Figure 5; ANOVA:

$F_{2,144}$=6.69, P=0.0017). In the *f2* mutant, there were no significant differences in xanthophyll 2 concentrations of the control and photoinhibition treatments (Least-squares mean; P=0.88), the control and recovery treatments (Least-squares mean; P=0.25) nor the photoinhibition and recovery treatments (Least-squares mean; P=0.50). In the WT barley however, there was a significant increase in xanthophyll 2 concentrations from the control to the photoinhibition treatments (Least-squares mean; P=0.0057) and between the control and recovery treatments (Least-squares mean; P=0.0025). There was no significant difference in xanthophyll 2 concentrations between the photoinhibition and recovery treatments of the WT barley (Least-squares mean; P=0.97). The xanthophyll 2 concentration in the *f2* mutant was found to be significantly lower than in the WT in the control (Least-squares mean; P<0.0001), photoinhibition (Least-squares mean; P<0.0001) and recovery treatments (Least-squares mean; P<0.0001).

The concentration of xanthophyll 3 was significantly different in the treatments (ANOVA; $F_{2,144}$=16.41, P<0.0001) but not the genotypes (Figure 6; ANOVA: $F_{1,144}$=2.99, P=0.086). Additionally, there was a significant interaction between the genotypes and the treatments (Figure 6; ANOVA: $F_{2,144}$=3.30, P=0.040). In the *f2* mutant, there was a significant decrease in xanthophyll 3 concentration from the control to photoinhibition treatment (Least-squares mean; P=0.01), and no significant difference between the control and recovery treatments (Least-squares mean; P=0.51) and the photoinhibition and recovery treatments (Least-squares mean; P=0.16). In the WT barley, there was a significant decrease in xanthophyll 2 concentrations from the control to the photoinhibition treatments (Least-squares mean; P=0.0005) and significant increase in xanthophyll 3 concentration between the photoinhibition and recovery treatments (Least-squares mean; P<0.0001). There was no significant difference in xanthophyll 3 concentrations between the control and recovery treatments of the WT barley (Least-squares mean; P=0.51). The xanthophyll 3 concentration in the *f2* mutant was found to be significantly lower than in the WT in the recovery treatment (Least-squares mean; P=0.0030) but not significantly different in the control (Least-squares mean; P=0.65) and photoinhibition (Least-squares mean; P=0.63) treatments.

The concentration of lutein was significantly different in both the treatments (ANOVA; $F_{2,146}$=5.56, P=0.0047) and the genotypes (Figure 7; ANOVA: $F_{1,146}$=51.46, P<0.0001). There was an overall significant difference between the photoinhibition and control treatments (Tukey

HSD; P=0.003), and no significant difference between the recovery and control treatments (Tukey HSD; P=0.12) and recovery and photoinhibition treatments (Tukey HSD; P=0.39).

The concentration of chlorophyll *b* was significantly different between genotypes (ANOVA; $F_{1,146}$=5613.46, P<0.0001) but not the treatments (Figure 7; ANOVA: $F_{2,146}$=0.92, P=0.4).

The concentration of chlorophyll *a* was significantly different between genotypes (ANOVA; $F_{1,146}$=20.54, P<0.0001) but not the treatments (Figure 7; ANOVA: $F_{2,146}$=0.32, P=0.73).

The concentration of β-carotene was significantly different between genotypes (ANOVA; $F_{1,146}$=8.41, P=0.0043) but not the treatments (Figure 7; ANOVA: $F_{2,146}$=0.75, P=0.48).

## DISCUSSION

### REDUCTION OF PHOTOPROTECTION

Photoinhibition is a decrease in photosynthetic rate at high levels of light due to photodamage to the D1 protein of PSII (Hopkins and Hüner, 2008). During these periods of high irradiance, plants absorb excess energy which must be dissipated as ROS can be accumulated (Jahns et al., 2008). Carotenoids minimize damage photodamage through photoprotection through constitutive protection using lutein and β-carotene and light-regulated protection through the xanthophyll cycles, which can be measured through the dissipation of excess energy in PSII (NPQ) (Jahns et al., 2008). The hypothesis that if chlorophyll *b* is important for reducing light damage in the leaf, then the *Clo f2* mutant would fluoresce less in excess light than its WT counterpart was supported. The *Clo f2* mutant had a significantly lower NPQ in the photoinhibition treatment than the WT barley. This supported the idea that due to the lack of chlorophyll *b, Clo f2* was more susceptible to ROS damage–the reduced NPQ shows that the light-regulated protection mechanism of dissipation of excess energy through the xanthophyll cycle was reduced in the mutant barley.

## REDUCTION OF PHOTOSYNTHETIC PIGMENTS

The results of the lack of chlorophyll *b* in the *Clo f2* mutant in our experiment was similar to the results of Ivanov *et al.* in their 2008 study. It was found that the *Clo f2* mutant contained no chlorophyll *b*, which also had an impact on the concentrations of other pigments (Ivanov *et al.,* 2008). The *Clo f2* barley had around half the amount of chlorophyll *a*, lutein and β-carotene compared to the WT barley (Ivanov *et al.,* 2008). Similarly, in our HPLC analysis we found that there was a significant decrease in the concentrations of lutein, chlorophyll *a* and β-carotene in the *Clo f2* mutant compared to the WT supporting the idea that protein complexes require chlorophyll *b* for assembly.

## UPREGULATION OF THE VIOLAXANTHIN CYCLE

Additionally like Ivanov's study, the concentration of some xanthophylls (1 and 2) were significantly lower in the control treatments of the *Clo f2* barley compared to the WT. However, Ivanov found that when under photoinhibition, *Clo f2* upregulates the violaxanthin cycle, causing zeaxanthin levels (the most dissipative pigment in the xanthophyll cycle) to rise from 0 to a significantly higher level than the WT's zeaxanthin levels. Similarly, we found that xanthophyll

3's concentrations in *Clo f2* significantly rose between the photoinhibition treatment and the recovery treatment, suggesting that xanthophyll 3 may represent zeaxanthin.

### UPREGULATION OF D1 PROTEIN PRODUCTION

Ivanof *et al.* (2008) also found that the decrease in LHCII and the antenna size of PSII in *Clo f2* was counterbalanced by an increased amount of D1 reaction centers. Our findings supported this claim as despite lacking chlorophyll *b* and having a significantly lower amount of chlorophyll a, lutein and β-carotene compared to the WT barley, the photochemical activity of the *Clo f2* mutant was not significantly different from the WT under control conditions. This could be attributed to the increased production of D1 reaction centers.

### CONCLUSIONS

It was supported that due to the lack of chlorophyll *b,* the *Clo f2* mutant contains less chlorophyll *a,* lutein, β-carotene, as well as some xanthophylls. However, the *Clo f2* mutants do not significantly differ in photochemical activity (qP) to the WT barley under normal light conditions as it produces an increased amount of D1 proteins for PSII to compensate for the lack of chlorophyll a, lutein, β-carotene (Ivanov *et al.,* 2008). Under photoinhibition and during recovery, however, *Clo f2* was unable to dissipate excess energy like WT barley. Additionally, as chlorophyll *b*-less *Clo f2* mutant has a significantly lower NPQ than the WT under photoinhibition, the hypothesis that chlorophyll *b* plays an important role in reducing ROS damage was supported.

### FUTURE AREAS OF INTEREST

We lacked conclusive support for the notion that chlorophyll *b* is important for reducing ROS damage as the Fv/Fm and the chlorophyll *a* concentration between WT and *Clo f2* did not significantly differ in treatment. Due to this, we were unable to attribute significant decreases in Fv/Fm and chlorophyll *a* concentration to the lack of chlorophyll *b.* Future studies may include longer periods of photoinhibition for both the WT and *Clo f2* barley to further analyze the effects of chlorophyll *b* in photoprotection and to determine if a lack of chlorophyll *b* leads to reduced efficiency in photosynthesis due to ROS buildup.

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**FIGURES**

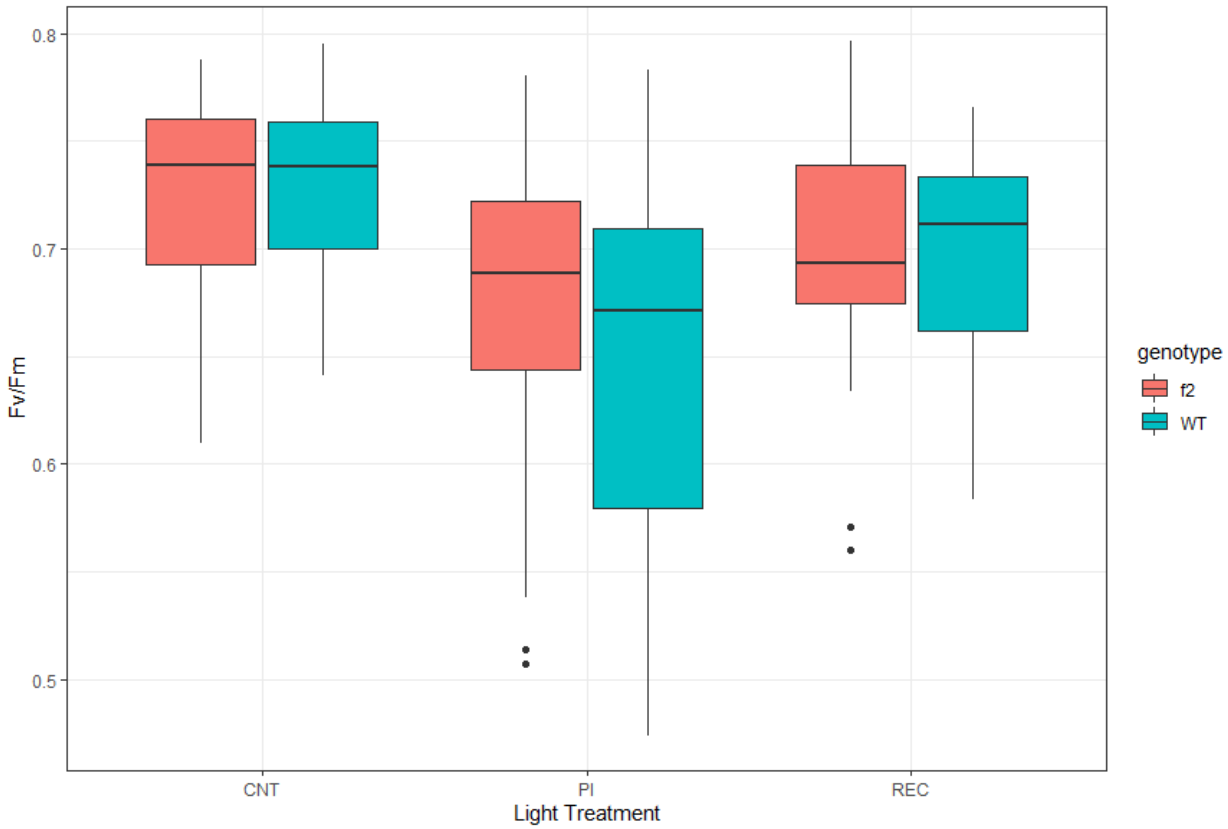


Figure 1. The Fv/Fm of WT barley, *Hordeum vulgare,* and the chlorophyll-b-less mutant, *Clorina f2,* under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination.

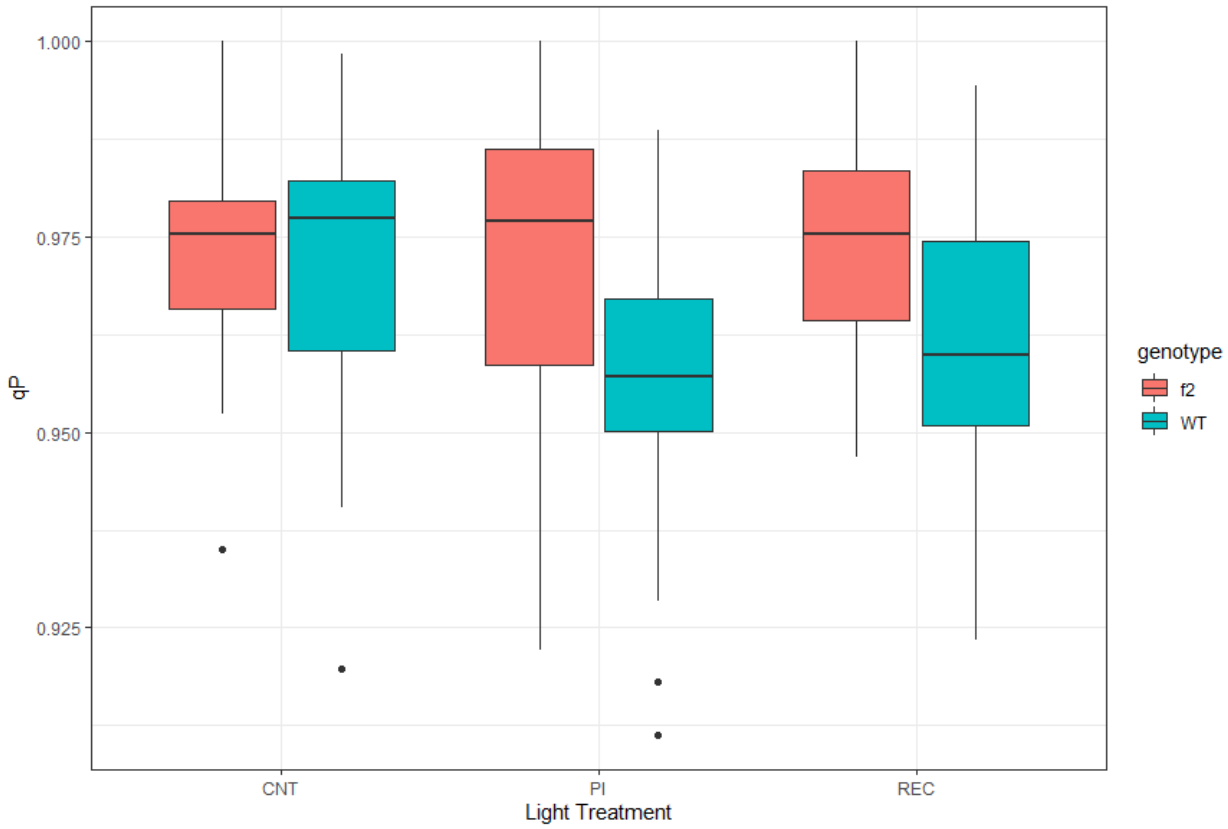


Figure 2. The qP of WT barley, *Hordeum vulgare,* and the chlorophyll-b-less mutant, *Clorina f2,* under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination.

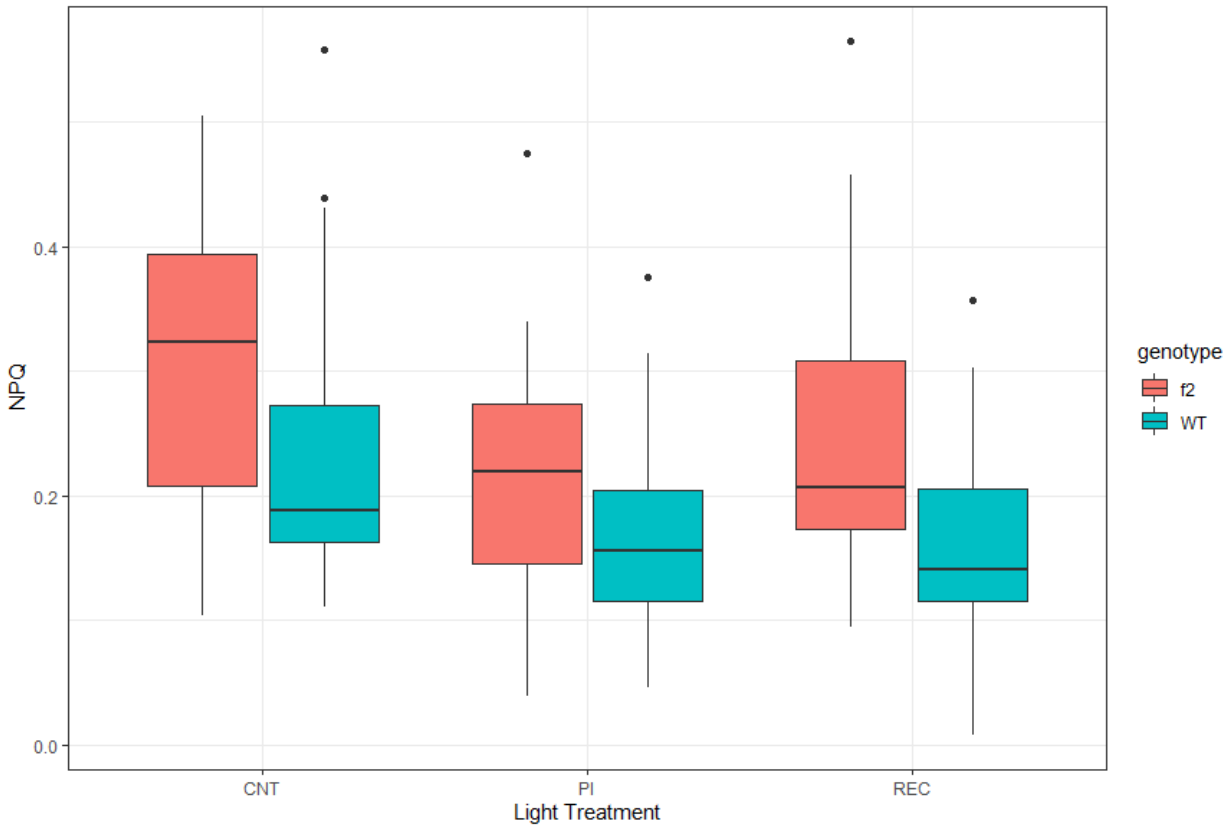


Figure 3. The NPQ of WT barley, *Hordeum vulgare,* and the chlorophyll-b-less mutant, *Clorina f2,* under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination.

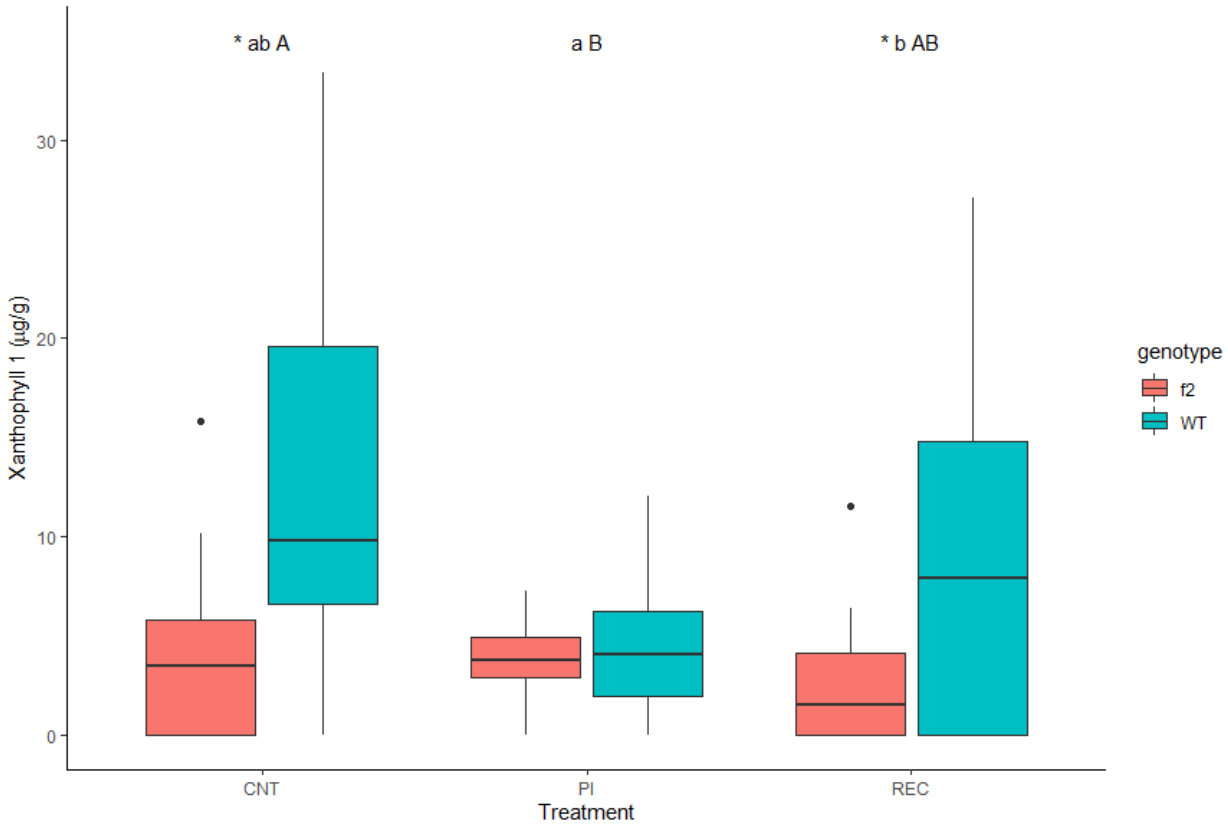


Figure 4. The concentration of xanthophyll 1 in WT barley, *Hordeum vulgare*, and the chlorophyll-b-less mutant, *Clorina f2*, under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination. * indicates significant differences between genotypes within treatments, lowercase letters indicate significant differences between treatments in the *f2* genotype and uppercase letters indicate significant differences between treatments in the WT genotype.

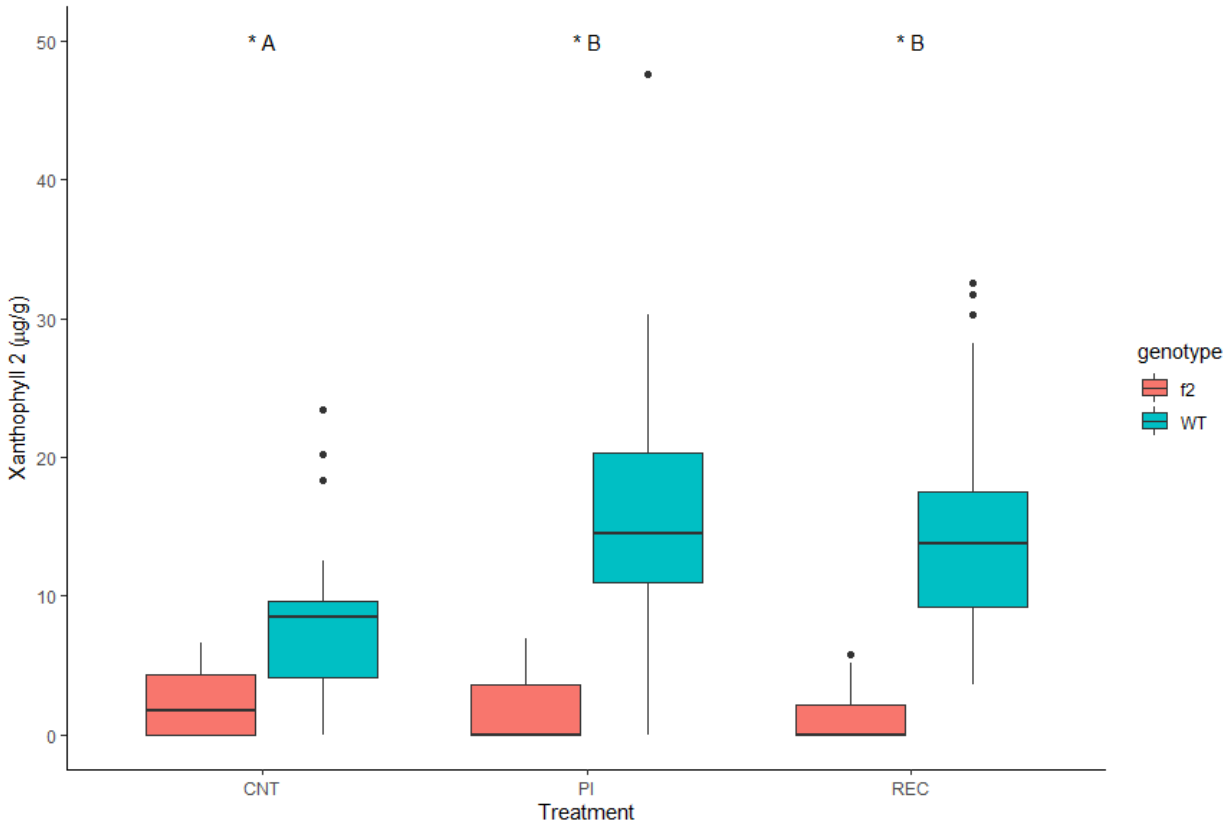


Figure 5. The concentration of xanthophyll 2 in WT barley, *Hordeum vulgare*, and the chlorophyll-b-less mutant, *Clorina f2*, under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination. * indicates significant differences between genotypes within treatments and uppercase letters indicate significant differences between treatments in the WT genotype. There was no significant difference in xanthophyll 2 concentrations between the treatments for the *f2* mutant.

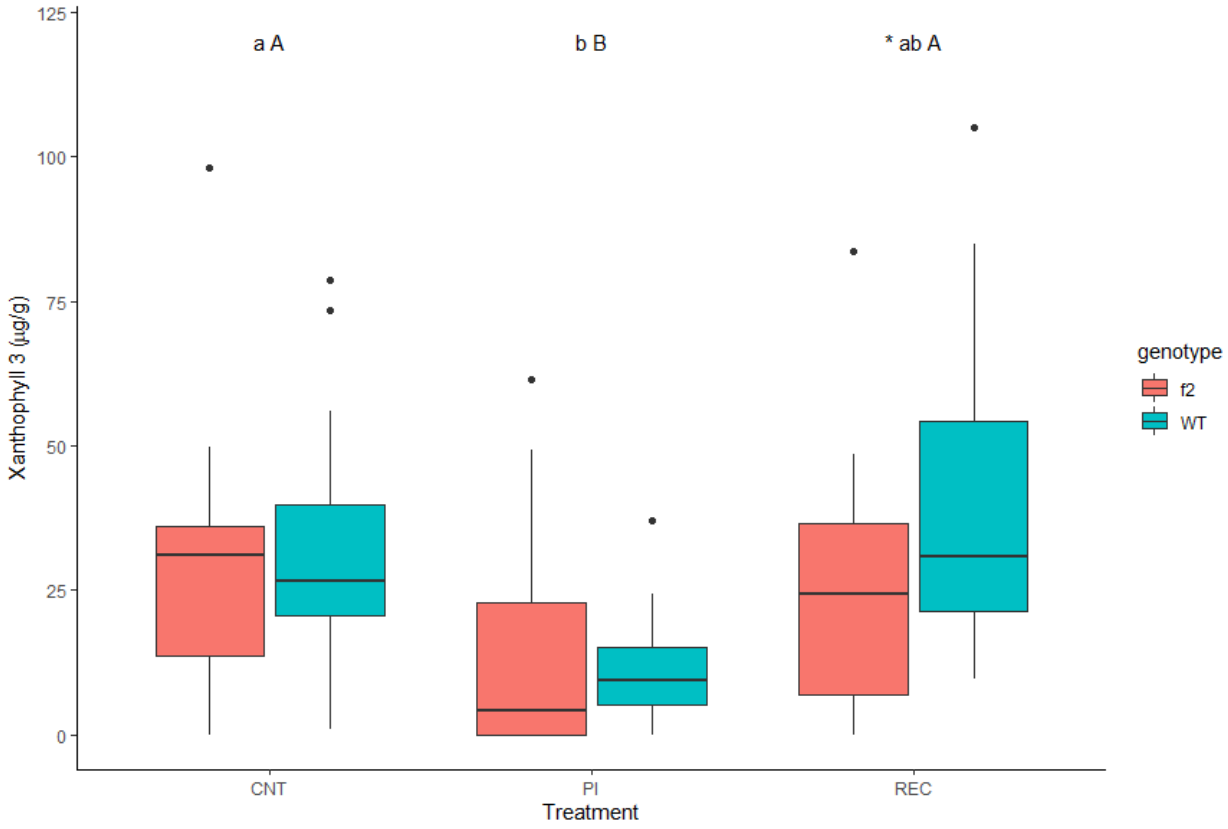


Figure 6. The concentration of xanthophyll 3 in WT barley, *Hordeum vulgare*, and the chlorophyll-b-less mutant, *Clorina f2*, under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination. * indicates significant differences between genotypes within treatments, lowercase letters indicate significant differences between treatments in the *f2* genotype and uppercase letters indicate significant differences between treatments in the WT genotype.

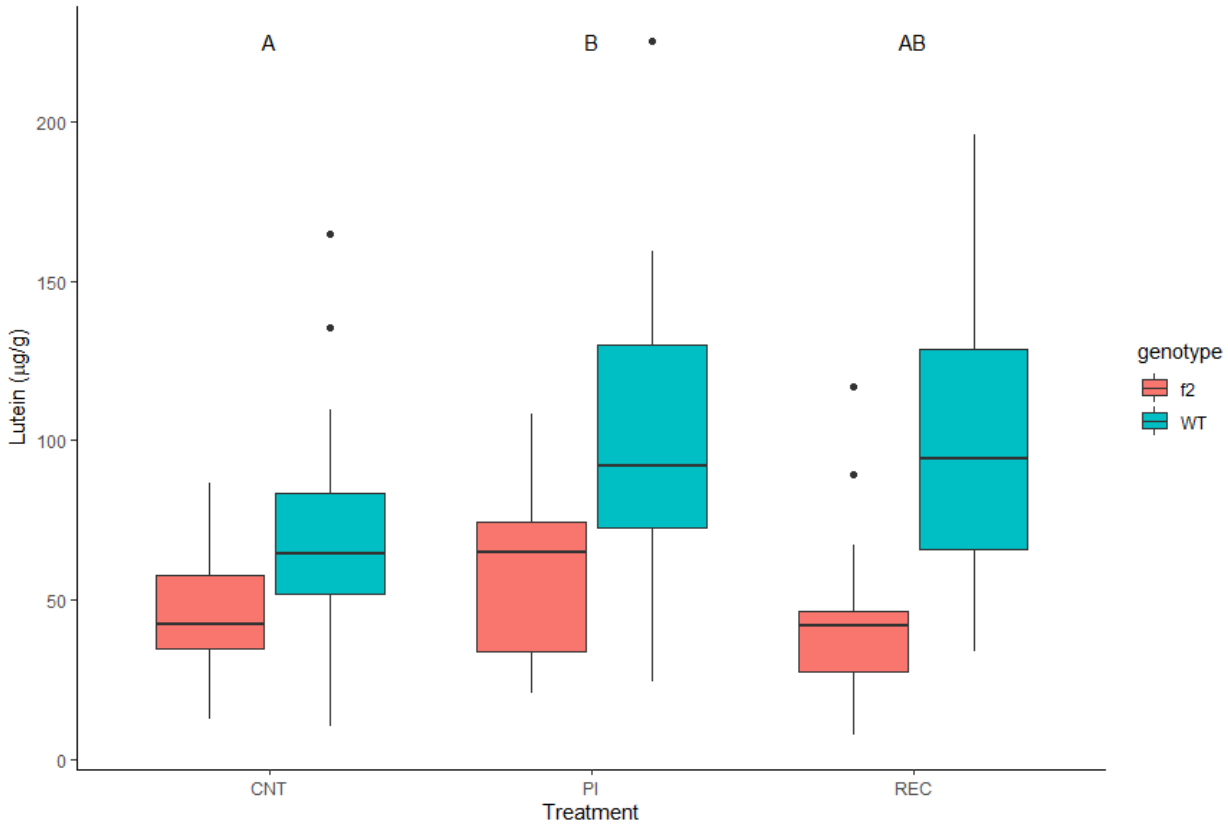


Figure 7. The concentration of lutein in WT barley, *Hordeum vulgare*, and the chlorophyll-b-less mutant, *Clorina f2*, under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination. There was a significant difference in lutein concentrations between the genotypes and uppercase letters indicate significant differences between treatments overall.

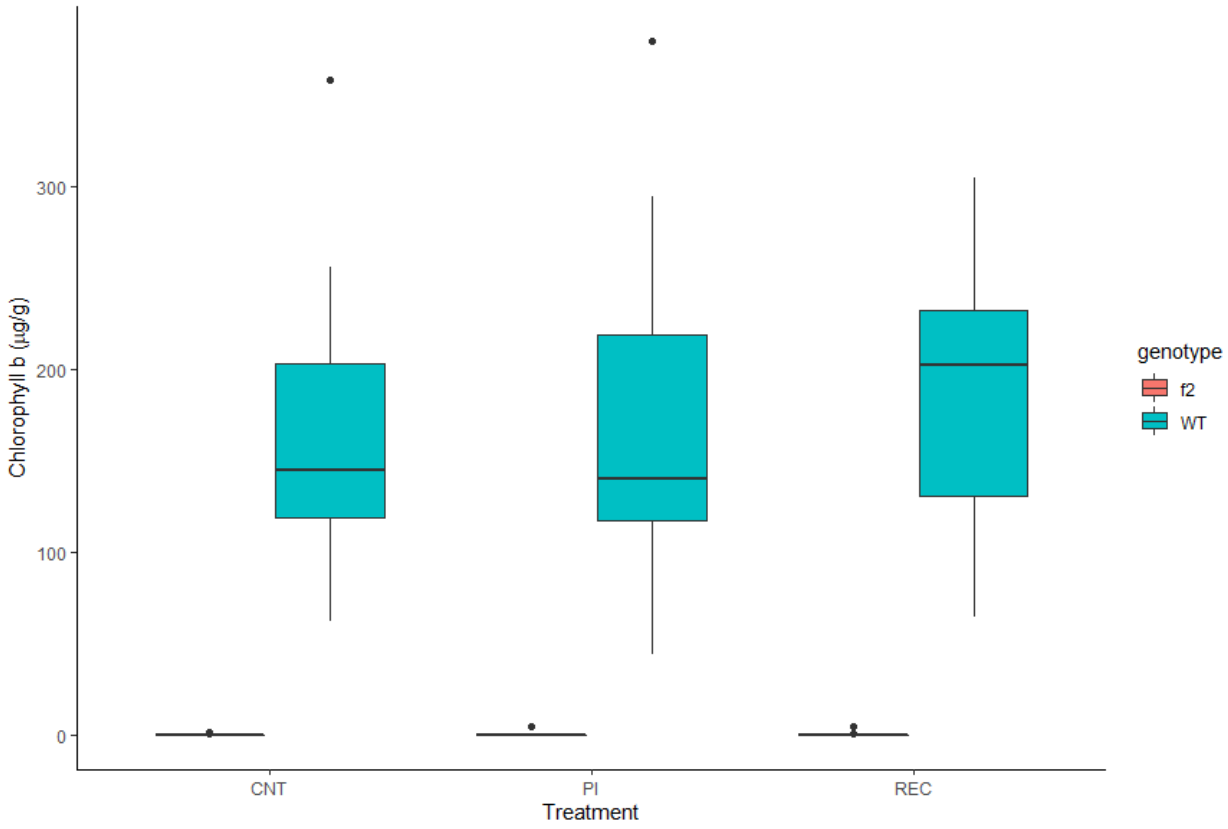


Figure 8. The concentration of chlorophyll *b* in WT barley, *Hordeum vulgare,* and the chlorophyll-b-less mutant, *Clorina f2,* under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination.

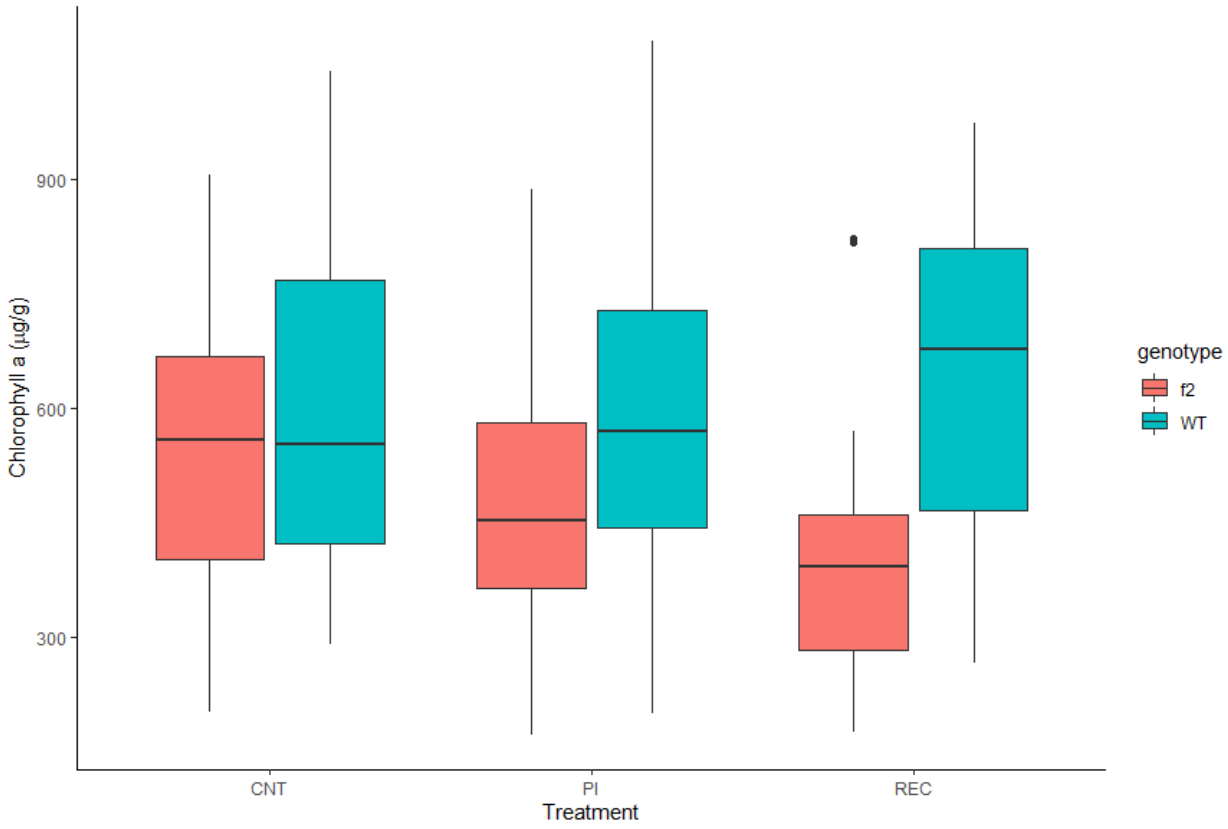


Figure 8. The concentration of chlorophyll *a* in WT barley, *Hordeum vulgare,* and the chlorophyll-b-less mutant, *Clorina f2,* under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination.

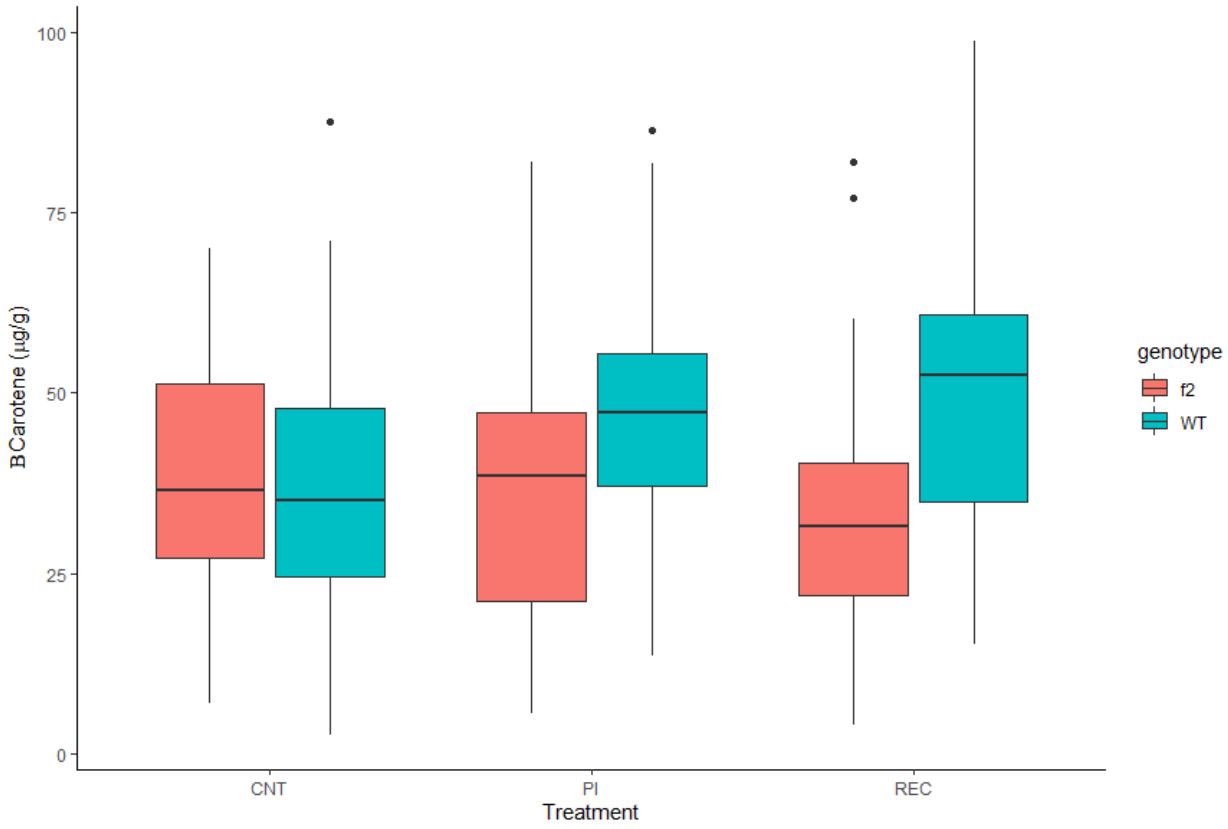


Figure 10. The concentration of β-carotene in WT barley, *Hordeum vulgare,* and the chlorophyll-b-less mutant, *Clorina f2,* under control, high light and recovery conditions. Data presented are median and interquartile ranges, N=25 for each treatment and genotype combination.