University of Nevada, Reno

Evaluation of ten genotypes for leaf physiological performance under a simulated heat wave

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Sciences

by
Ashley J. Eustis

Dr. Felipe Barrios Masias/Thesis Advisor

August, 2019

UNIVERSITY OF NEVADA RENO **THE GRADUATE SCHOOL**

We recommend that the thesis
prepared under our supervision by

ASHLEY J. EUSTIS

Entitled

Evaluation of ten genotypes for leaf physiological performance under a simulated heat wave

be accepted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Felipe Barrios Masias, Ph.D., Ph.D., Advisor

Melinda Yerka, Ph.D., Committee Member

Glenn Miller, Ph.D., Graduate School Representative

David W. Zeh, Ph.D., Dean, Graduate School

August 2019

Abstract

Quinoa (*Chenopodium quinoa* Willd.) sensitivity to high temperatures is an impediment to adoption in regions prone to heat waves, despite quinoa being a highly resilient crop to a wide range of abiotic stresses. Although reductions in yield due to heat are usually associated with pollen viability, the present study aimed to understand the effects of high temperature on the leaf and its capacity for carbon assimilation. Several trials were conducted with 10 quinoa genotypes classified as being either sensitive or tolerant to heat stress on a previous screening of 112 lines. Plants were grown in the greenhouse at normal temperatures (i.e., control), and at the sixth growth stage were exposed to temperature treatments in growth chambers. The heat treatment simulated heat waves of four consecutive days with temperatures higher during the day and night (Heat: 45/30 ˚C, and Control: 20/14 ˚C). Chlorophyll fluorescence (predawn and day), leaf gas exchange (day) and dark respiration (night) were measured during several experiments. In addition, leaf cell membrane stability was evaluated in the laboratory at temperatures of 47, 51 and 55 ˚C. Results show that most quinoa genotypes under the heat treatment increased their photosynthetic rates and stomatal conductance, resulting in a lower intrinsic water use efficiency. These results were partly corroborated by changes in the maximum quantum yield of photosystem II (F_v/F_m). Dark respiration decreased under the heat treatment in most genotypes. The cell membrane stability assays showed that temperatures of 51 ˚C or higher increased the percent injury to >70%, and a temperature of 47 ˚C may be a better screening temperature as injury was around 35%. These results suggest that heat stress does not affect carbon assimilation capacity, but higher transpiration and lower intrinsic water use efficiency may lead to water deficits and exacerbate plant stress responses, resulting in lower yields.

Acknowledgements

I would like to thank my advisor, Felipe Barrios, for his guidance and expertise. Thanks also to my committee members Melinda Yerka and Glenn Miller for their invaluable feedback, pep talks, and giving me the motivation to start and finish.

Thank you to my lab mate, Steven Bristow, and the undergraduate student assistants in our lab, Haylee Higgins, Rheena Am-Is and Graham Holton, I couldn't have gotten through this without their help and support. Thank you also to Leo Hernandez, the post-doc in our lab, who taught me how to use the Li-COR and helped me collect a ton of data.

Lastly, I would like to thank my boyfriend, Don Ho for all of his love and support; my parents for always having my back and listening to me talk about quinoa forever. I would also like to thank all my friends and family who provided essential support, I appreciate all of you more than you know!

Table of Contents

List of Tables

at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

Table 7. Afternoon F_v/F_m in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Vertical bars denote mean ±SE (n=15-32). *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

Table 8. Relative chlorophyll model output in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Each point represents a mean relative chlorophyll amount per genotype within treatment within that day (n=5-9). *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

List of Figures

at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

Figure 6. Afternoon F_v/F_m in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Vertical bars denote mean ±SE (n=15-32). *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

Figure 7A and B. Relative chlorophyll means by day with slope line, in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Each point represents a mean relative chlorophyll amount per genotype within treatment within that day (n=5-9). The lines indicate the slope calculated from the linear model where average relative chlorophyll is a response to day.

Figure 8. Relative chlorophyll means for day 4, in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Vertical bars denote mean ±SE (n=5-9). *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

Figure 9. Cell membrane injury (% CMI) in 10 quinoa genotypes exposed to a one hour heat treatment at 47°C, 51°C and 55°C. Vertical bars denote mean ±SE (n=3-8). *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level; n.s., not significant.

Figure 10. Aboveground biomass (seed and shoot, dried; g $plant^{-1}$) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and

.

Quinoa (*Chenopodium quinoa* Willd.) is a highly nutritious and stress tolerant crop that has gained recognition as a viable solution to food security issues due to global warming and population growth (Bazile, et al. 2016; Jacobsen, Mujica, & Jensen 2003). Native to the Andean region of South America, quinoa is divided into five globally recognized ecotypes, each adapted to unique conditions resulting in tolerance to many abiotic stressors (Bazile, et al. 2016; Murphy, Bazile, Kellogg, & Rahmanian 2016). Quinoa grows in environments with high irradiance, freezing temperatures, water deficit stress, and from sea level to >4,500 meter above sea level, which make quinoa a potential crop for growers facing climate change (Ruiz, et al. 2014,2015). The projected increase in global temperatures, approximately 1° and 3°C above the present value by 2025 and 2100, respectively, have increased the pressure to gain better understanding of plant responses to heat stress (Bita & Gerats 2013; Christensen & Christensen 2007; Porter & Semenov 2005). Quinoa sensitivity to high temperatures is an impediment to adoption in regions prone to heat waves, and understanding of leaf physiological responses to high temperature and their capacity for carbon assimilation can help advise novel management and future breeding efforts.

High temperature stress in plants is known to have a negative impact on physiological and biochemical processes, including gene regulatory pathways. Changes in various photosynthetic parameters under heat stress are considered good indicators of heat tolerance as they are positively correlated with growth (Wahid, Gelani, Ashraf, & Foolad 2007). The specific ways in which plants cope with heat stress varies among species and between genotypes. For example, heat stress has been reported as one of the most important causes of reduction in yield and dry matter production in many crops, including maize (*Zea mays*), pulse legumes (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), and quinoa (*Chenopodium quinoa*) (Giaveno & Ferrero 2003; Wahid, Gelani, Ashraf, & Foolad 2007). In general, a temporary rise in temperature of 10-

15° C above ambient is considered heat stress, but even plants exposed to temperatures 5° C higher than normal can lead to stress and reduce growth (Bita & Gerats 2013; Guy 1999).

Common plant heat-stress induced responses include: stomatal closure to reduce transpirational water loss, modifications to the photosynthetic machinery, and organizational changes in cellular structures to maintain membrane functions (Bita & Gerats 2013; Weis & Berry 1988; Zhang, et al. 2005). The initial effects of heat stress are structural changes in chloroplast protein complexes and photosynthetic machinery, with the chloroplast stroma and thylakoid membranes considered the primary sites of heat-induced damage. These changes are accompanied with a loss of grana stacking, reduced enzymatic activity and ion-leakage due to membrane damage (Ahmad, Diwan, & Abrol 2009; Allakhverdiev, et al. 2008; Karim, Fracheboud, & Stamp 1997; Wahid & Shabbir 2005; Wise, Olson, Schrader, & Sharkey 2004). This specific damage targets photosystem-II (PSII) and eads to a reduction of photosynthetic processes, or photoinhibition. This damage can be quantified by assessing gas exchange and chlorophyll fluorescence parameters, in particular F_v/F_m, which measures maximum quantum yield of PSII and has optimal values around 0.83 for most plant species. (Maxwell & Johnson 2000; Pastenes & Horton 1996). In addition, leaf respiration is also a sensitive process to high temperatures, and it may require up to half of the daily carbon gain from photosynthesis (REF) for general maintenance, without additional stress exposure. This expenditure is used for various protective and maintenance processes in the leaf, including protection of the photosynthetic apparatus and repair if damage occurs.

These perturbations of photosynthesis and respiration lead to reduced carbon net assimilation rates and plant growth, and diminished plant productivity (Barnabás, Jäger, & Fehér 2007; Wahid, Gelani, Ashraf, & Foolad 2007). The degree of plant susceptibility to high temperatures varies with developmental stage, and will modulate the degree of possible damages incurred

beyond the typical impacts of heat stress at all vegetative and reproductive stages (Barnabás, Jäger, & Fehér 2007; Bita et al, 2013; Sakata & Higashitani 2008; Hall 2010). For example, during vegetative stages, high day temperature can damage leaf gas exchange properties due to the structural modifications on PSII and chloroplasts. Additional decreases to efficiency can be due to photoinhibition or increased photorespiration (Wahid, Gelani, Ashraf, & Foolad 2007). Similarly, during reproduction, a short period of heat stress can cause major damage to floral development due to pollen impairment; which is an important factor contributing to decreased grain yield in many crops at moderate-to-high temperatures (Guilioni, Wery, & Tardieu 1997; Hinojosa, Matanguihan, & Murphy 2018; Willits & Peet 1998; Sato, et al. 2006; Young, Wilen, & Bonham-Smith 2004). However, a reduction in photosynthesis and net carbon assimilation will eventually result in limited resource availability for reproduction (Wahid, Gelani, Ashraf, & Foolad 2007; Sumesh, Sharma-Natu, & Ghildiyal 2008; Young, Wilen, & Bonham-Smith 2004).

The purpose of this study is to further elucidate the complexities of quinoa's response to summertime temperatures typical of North American environments, to support its development as a nutritious, high-value, climate-smart crop. While there is a large body of research on crop responses to heat stress (e.g., focused on pollen viability), there are limited studies available on leaf responses to extreme high temperatures in quinoa. The present study aimed to understand the effects of high temperature on the leaf and its capacity for carbon assimilation when quinoa plants were exposed to a four-day heat wave. We measured several parameters: leaf gas exchange, chlorophyll fluorescence, membrane stability, and yield. Trials were conducted with 10 quinoa genotypes classified as being either sensitive or tolerant to heat stress on a previous screening of 112 lines.

Materials and Methods

Experiments were conducted in a greenhouse and growth chambers at the Nevada Agricultural Experimental Station at University of Nevada, Reno. Ten quinoa genotypes were used, provided by Washington State University. Plants were grown in tree pots (Stuewe ID# CP413CH) with a 1:1 mix of sand (Commerical Grade Quikrete 30 grit) and soil medium (Sungro Fafard® 3B Mix, Metro-Mix® 830). Prior to planting, the soil mix was fully saturated, and seeds were planted ~1/4-in deep. Plants were irrigated by an automated system each morning, with irrigation time increasing as plants became larger. Plants were fertilized with a 20-20-20 (Jack's Fertilizer, J.R. Peters, Inc. Allentown, PA) 3 times a week, first at the lower rate of 5 grams per gallon (1 teaspoon) and then at the higher rate of 15 grams per gallon (1 tablespoon). This fertilizing regiment was modified for later experiments where slow releasing fertilizers were used. Osmocote (13-13-13) and Micromax Micronutrients were applied at approximately 15-18 grams (1/2 tablespoon), and 3-4 grams (1/2 teaspoon), respectively, per pot. Plants received a 14-hour photoperiod and supplemental light was used when needed. The greenhouse was kept at a temperature averaging 21°C ± 6° SD during the daytime, and 17°C ± 4° SD at night. Relative humidity fluctuated between 35 and 45%. Plants were exposed to a heat treatment at the sixth-leaf growth stage, approximately 6-8 weeks after planting, according to the BBCH scale (Sosa-Zuniga, Brito, Fuentes, & Steinfort 2017), where anthesis had just begun and anthers were extruding, a growth stage known to be especially susceptible to heat stress, resulting in reduced grain yield.

Conviron growth chambers (Model A1000 using the CMP6010 control system) were used to control temperature, humidity and light intensity for the duration of the simulated heat wave. Light intensity increased from 400 to 750 to 1050 mmol m^{-2} s^{-1} over the course of three hours to simulate sunrise and sunset in field conditions. Humidity was between 60-70% at night and 70-

80% during the day for the control treatment, and 30-40% at night and 60-70% during the day for the heat treatment. The temperature regime is shown in Supplemental Table 1.

Time	Heat Treatment (°C)	Control Treatment (°C)
00:00	30	14
06:00	32	16
08:00	35	18
10:00	40	18
12:00	45	20
16:00	40	18
18:00	35	16
20:00	32	16
22:00	30	14

Leaf-level photosynthesis was measured using two LI-6400 portable photosynthesis systems (LI-COR Inc., Lincoln, NE) between the hours of 14:00 and 17:00. Photosynthesis was measured for four consecutive days, and the same fully mature leaf (towards the apical end of the plant) was used for each measurement, to minimize any potentially confounding effects of changing photosynthetic capacity in the tissues sampled. The area of the chamber was set to 6 cm^2 and the middle portion of the leaf was used for measurement. Measurements were taken between 14:00 and 17:00 each day, corresponding to times of maximum heat and sunlight under field conditions. While taking day time measurements the photosynthetic photon flux density (PPFD) was set to 2000 mmol m^{-2} s^{-1}, the CO_2 concentration in the leaf cuvette was set to 400 µmol, the flow was set to 500 µmol s^{-1}, and the temperature was set to 20°C and 40°C depending on whether the plant was subjected to an ambient temperature or heated temperature treatment. Genotypes and treatments were evenly split between the two LI-6400s. The parameters of interest were, photosynthetic rate (P_n), stomatal conductance (g_s) and intrinsic water use efficiency (WUE_i), calculated as P_n/g_s.

Nighttime measurements to quantify dark respiration were taken two hours after dark began. The LI-6400 had a similar set up as described above with the exception that the photosynthetic photon

flux density (PPFD) was set to 0 mmol m^{-2} s^{-1}, and, the flow was reduced to 300 µmol s^{-1} or less, and the block temperature was set to <15°C or <35°C depending on whether the plant was subjected to an ambient temperature or heated temperature treatment. Nighttime respiration, R_N, was measured as the absolute value of P_n.

Chlorophyll fluorescence measurements were taken at pre-dawn, between 04:30 and 06:30, as well as in the afternoon between 14:00 and 16:00 using a modulated fluorometer (MultispeQ v1.0). As above, the same leaves were used for each measurement, ensuring they were fully mature leaves towards the apical end of the plant.

Cell Membrane Stability and Injury (CMS and CMI)

Cell membrane stability was measured following the methods in Blum and Ebercon (1981). Using a leaf borer, four leaf disks were taken from 1-3 fully mature, apical leaves . In total 7.76 cm^2 leaf area was taken for each sample. Leaf discs were placed in pre-moistened Falcon tubes and then rinsed two times with deionized water. For the heat treatment, tubes were loosely covered and placed in a water bath for one hour at the predetermined temperature (47, 51, and 55°C treatments), ensuring that the water level was above the leaf discs in the tubes. The control tubes were maintained at room temperature (20°C). After the heat treatment, 9 mL of deionized water was added to each tube and tubes were incubated in a refrigerator at 7°C for approximately 18 hours. After the incubation period, tubes were equilibrated to room temperature, and the initial electric conductivity was measured using a Orion Star A215 pH/Conductivity Meter. Samples were then autoclaved, left to equilibrate to room temperature, and the final electric conductivity taken. Cell membrane stability and injury were calculated using the following equations (Blum and Ebercon 1981):

$$CMS\ (\%) = [1 - (T1/T2)/1 - (C1/C2)] \times 100 \qquad \text{[Equation 1]}$$

$$CMI\ (\%) = 1 - [1 - (T1/T2)/1 - (C1/C2)] \times 100 \qquad \text{[Equation 2]}$$

where T1 and T2 are treatment conductivities before and after autoclaving, respectively; and C1 and C2 are the respective control conductivities. The calculations were performed so that each T value was calculated against the average of all C values for the given genotype.

Data Analysis

All experiments were conducted in a randomized complete block design and analyzed using R version 3.5.1 (R Core Team, 2018). Genotypes were randomly divided among treatments, and they were randomly assigned to growth chambers. A mixed effects modelling approach with REML was used, including *lme4* (*v.1.20*; Bates et al., 2015), *nlme* (Pinheiro et al., 2018) and *emmeans* (*v1.3.2*; Lenth, 2019) packages. Genotype, Treatment, and their interaction (GxT) were considered fixed effects, whereas Chamber, Block, Device, Experiment ,and Day, were considered random effects. Model selection was performed on models that did not fail to converge using Akaike Information Criteria (AIC) values. Data was evaluated for normality based on visual inspection of residuals, and for homogeneity of variance using Levene's Test in the *car* package (Fox and Weisberg, 2011). In some cases outliers were removed using Mahalanobis's distance using the *mvoutlier* (Filzmoser and Gschwandtner, 2018). The data was transformed when ANOVA assumptions were not met. Significance was assessed at $p < 0.05$. Genotype and treatment means were compared using Tukey's post hoc test with the *emmeans* (*v1.3.2*; Lenth, 2019) package. Data was organized and visualized using the *dplyr* package (Wickham et al., 2019) and *ggplot2* (Wickham, 2016).

Results

The P_n in quinoa showed an interaction effect between the temperature treatments and genotypes. Overall, the P_n was higher in the heat treatment compared to the control for all genotypes except for QQ065 and the Japanese Strain (Fig. 1 and Table 2). In the heat treatment, the Japanese strain was only similar to QQ065, and had at least a 22% lower P_n than the other genotypes; the P_n of all other genotypes was similar and range between 22 and 25.6 μmol CO_2 m^{-2} s^{-1}. Within genotype, the highest increase in P_n was 44% for 3UISE, with a rate of 17.8 ± 1.34 SE μmol CO_2 m^{-2} s^{-1} in the control and 25.6 ± 1.39 SE μmol CO_2 m^{-2} s^{-1} in the heat treatment. The lowest increase in P_n was 12% for Kaslaea (22.0 ± 0.91 SE and 24.6 ± 1.39 SE μmol CO_2 m^{-2} s^{-1} for control and heat treatments, respectively), but within the control treatment, this genotype had one of the highest P_n rates. In the control treatment, Kaslaea P_n was 30%, 28%, and 27% higher than that of QQ74, Titicaca and the Japanese strain, respectively. No other differences were observed.

The g_s showed an interaction between genotype and temperature similar to observations for P_n. Overall, g_s was higher in the heat treatment compared to the control for all genotypes except Kaslaea, which had one of the highest g_s under the control treatment (Fig. 2 and Table 3). Under the heat treatment, 3UISE, 17GR, QQ74 and Titicaca had over a 100% increase in g_s compared to the control treatment, while UDEC-1, QQ065, Quinhua, Pison and the Japanese strain showed increased g_s values ranging from 45% to 95% relative to the control. Within the heat treatment, 17GR had one of the highest g_s values (0.81 ± 0.56 SE mol H_2O m^{-2} s^{-1}), which was 40%, 37% and 31% higher than Titicaca, the Japanese strain and Pison, respectively. The latter three genotypes had the lowest g_s values (0.58 ± 0.50 SE, 0.59 ± 0.06 SE and 0.62 ± 0.53 SE mol H_2O m^{-2} s^{-1}, respectively). Within the control treatment, Kaslaea and Quinhua had some of the highest g_s values (0.57 ± 0.06 SE and 0.46 ± 0.06 SE mol H_2O m^{-2} s^{-1}; respectively), which were 104%

and 64% higher than g_s values for QQ74, 3UISE and Titicaca, which had the lowest g_s values (0.25 ± 0.02 SE, 0.27 ± 0.03 SE and 0.28 ± 0.04 SE mol H_2O m^{-2} s^{-1}, respectively).

WUE_i data also reflected an interaction between genotype and temperature. WUE_i decreased in the heat treatment for most genotypes except 3UISE, QQ065, Quinhua and Kaslaea, which showed no change compared to the control treatment (Fig. 3 and Table 4). In the heat treatment, WUE_i was similar among genotypes, and it ranged from 33.2 ± 1.78 SE (UDEC-1) to 43.0 ± 3.06 SE (Pison). In the control, WUE_i rates ranged between 46.4 ± 3.08 (Kaslaea) and 68.1 ± 3.78 SE (QQ74). QQ74 and Titicaca had some of the highest WUE_i, with rates of 68.1 ± 3.78 SE and 65.2 ± 3.94 SE, respectively. Kaslaea had one of the lowest WUE_i (46.4 ± 3.08 SE), and was 31%, 40% and 46% lower than Pison (60.9 ± 3.36 SE), Titicaca (65.2 ± 3.94 SE) and QQ74 (68.1 ± 3.78 SE). Within genotypes, the WUE_i decreased from 29% to 49% in the heat treatment compared to the control for QQ74, UDEC-1, 17GR, Pison, Titicaca and the Japanese strain.

Dark respiration data also reflected a significant interaction between treatment and genotype, as with the other leaf gas exchange parameters. For most genotypes, R_N decreased under the heat treatment compared to the control, except for UDEC-1, 17GR, Pison and Titicaca (Fig. 4 and Table 5). The decrease in R_N for the heat treatment compared to the control ranged from 19% and 31%. R_N in the heat treatment was between 1.19 ± 0.14 SE μmol CO_2 m^{-2} s^{-1} (Japanese strain) and 1.85 ± 0.18 SE μmol CO_2 m^{-2} s^{-1} (Pison). Within the heat treatment, Pison and 17GR (1.83 ± 0.11 SE μmol CO_2 m^{-2} s^{-1}) had some of the highest R_N, 35% and 36% higher, respectively than QQ065 (1.36 ± 0.08 SE μmol CO_2 m^{-2} s^{-1}) and 54% and 55% higher than the Japanese strain (1.19 ± 0.14 SE μmol CO_2 m^{-2} s^{-1}), which had the some of the lowest R_N. Within the control treatment, 3UISE and Kaslaea had some of the highest R_N (2.34 ± 0.12 SE and 2.17 ± 0.09 SE μmol CO_2 m^{-2} s^{-1}, respectively), and were between 27% and 41% higher than UDEC-1 (1.71 ± 0.14 SE μmol CO_2

m^{-2} s^{1}) and the Japanese strain (1.66 ± 0.08 SE μmol CO_2 m^{-2} s^{-1}), respectively, which were the lowest.

Pre-dawn F_v/F_m also showed an interaction between treatment and genotype. Although small changes in pre-dawn F_v/F_m were observed, most genotypes in the heat treatment had higher pre-dawn F_v/F_m than the control except 3UISE and QQ065 (Fig. 5 and Table 6). The heat treatment F_v/F_m ranged between 0.721 ± 0.006 SE and 0.763 ± 0.005 SE across all genotypes. The highest F_v/F_m were observed in UDEC-1, QQ74, Quinhua and Kaslaea (0.757 ± 0.003 SE, 0.759 ± 0.003 SE, 0.760 ± 0.003 SE, 0.763 ± 0.005 SE, respectively), while the lowest value was observed in QQ065 (0.721 ± 0.006 SE). Within the control treatment, F_v/F_m ranged between 0.7215 ± 0.006 SE and 0.747 ± 0.003 SE across genotypes. Quinhua (0.747 ± 0.003 SE) was higher than all other genotypes, except UDEC-1 (0.742 ± 0.003 SE), and Kaslaea (0.736 ± 0.004 SE). The Japanese strain (0.715 ± 0.006 SE) was lower than all genotypes except 17GR (0.717 ± 0.004 SE) and Pison (0.731 ± 0.005 SE).

Afternoon F_v/F_m also showed an interaction effect between genotype and treatment. Afternoon F_v/F_m were higher for the heat treatment in 3UISE, 17GR, QQ74, Titicaca and the Japanese strain than the control (Fig. 6 and Table 7). 17GR, QQ74 and 3UISE showed a 20%, 14% and 11% increase, respectively, in the heat treatment compared to the control. Within the heat treatment, the Japanese strain (0.663 ± 0.022 SE) was between 8.3% and 10.6% lower than all other genotypes except Pison (0.667 ± 0.022 SE), 3UISE (0.707 ± 0.014 SE), and Kaslaea (0.725 ± 0.014 SE). Within the control, 17GR and the Japanese strain (0.614 ± 0.018 SE and 0.622 ± 0.018 SE, respectively) had one of the lowest F_v/F_m. 17GR was between 11.6% and 14%, and the Japanese strain between 10% and 12.7% lower than UDEC-1, QQ065, Quinhua, Titicaca and Kaslaea (F_v/F_m ranged from 0.693 ± 0.014 SE to 0.711 ± 0.014 SE).

Relative chlorophyll was affected through the course of the temperature treatment as indicated by the slope of the regression fitted between day 1 and 4 for all measurements within genotype (Fig. 7). Genotype and the GxT interaction between genotype and treatment were not significant effects. There were differences in the slopes between the heat treatment and control for 17GR, Piso and Titicaca; all genotypes had positive slopes in the heat treatment and negative slopes in the control. In the heat treatment, the Japanese strain showed a change between Day 1 (35.18 ± 5.21 SE) and Day 4 (31.71 ± 5.61 SE), a 9.8% decrease. It was also one of two genotypes that showed a decrease in relative chlorophyll in the heat treatment. In the control treatment, 17GR showed a change between Day 1 (38.64 ± 2.51 SE) and 4 (34.80 ± 4.31 SE), a 9.9% decrease. In the heat treatment, the Japanese strain had a lower slope than 17GR. Within the control treatment, no differences between slopes were observed among genotypes.

By the end of the temperature treatment (Day 4), relative chlorophyll was affected by temperature treatment and genotype, but there was no GxT interaction effect (Fig. 8). Both 17GR and Kaslaea increased in relative chlorophyll content in the heat treatment compared to the control. 17GR increased its relative chlorophyll from 34.8 ± 4.31 SE in the control to 43.4 ± 2.65 SE in the heat treatment (a 24.7% change). Kaslaea showed a 47.5% increase in relative chlorophyll content with 38.3 ± 3.37 SE in the control and 56.6 ± 6.98 SE in the heat treatment. Within the heat treatment, the Japanese strain had the lowest relative chlorophyll content (31.7 ± 5.61 SE), and was at least 52% lower than 3UISE (48.3 ± 5.66 SE), Quinhua (48.5 ± 4.38 SE), Titicaca (50.3 ± 2.46 SE) and Kaslaea (56.6 ± 6.98 SE), which were the highest. Within the control treatment, no significant differences were found among genotypes, but the higher relative chlorophyll content of UDEC-1 compared to the Japanese strain was of borderline significance ($p = 0.06$).

Percent injury (CMI) was effected by both genotype and treatment. The percent of cell membrane injury at the 47°C treatment was lower than at temperatures of 51°C and 55°C (Fig. 9) across genotypes. Within the 47°C treatment, Titicaca and QQ065 had the lowest cell membrane injuries (25% and 27 %, respectively), compared to Quinhua and 17GR (39% and 30%, respectively). Within the 51°C and 55°C treatments there were no differences between genotypes: cell membrane injury ranged from 77% (UDEC-1) to 84% (17GR) for the 55°C treatment, and 73% (the Japanese strain) to 81% (17GR) for the 51°C treatment.

Seed biomass was not affected by the temperature treatment, but differences were observed among genotypes (Fig. 11). There was no interaction between genotype and treatment. Quinhua was the only genotype to demonstrate a trend of borderline significance towards lower seed biomass in the heat treatment compared to the control (7.95 ± 0.85 SE and 5.62 ± 1.05 SE grams plant^{-1}, respectively; p=0.06). Within the heat treatment, Kaslaea and QQ74 had the highest seed biomass (7.83 ± 0.58 SE and 7.07 ± 0.90 SE grams plant^{-1}, respectively). This was 148% and 124% higher than QQ065 (3.16 ± 1.01 SE grams plant^{-1}). The higher seed biomass in UDEC-1 than QQ065 was of borderline significance (p=0.07). Within the control treatment, no differences were observed among genotypes. Seed biomass ranged between 4.40 to 7.90 grams plant^{-1} for the control treatment, and 3.20 to 7.8 grams for the heat treatment. Aboveground biomass was not affected by genotype or temperature treatment, and biomass was on average 14.4 ± 0.5 SE grams plants^{-1} for the control and 14.9 ± 0.5 SE grams plant^{-1} for the heat treatment (Fig. 10).

Discussion

This study shows that quinoa plants can withstand exposures to a simulated heat wave with high temperatures of 45°C for a period of four days when no other confounding stressors such as drought are present. Leaf gas exchange in quinoa was not sensitive to high temperatures and P_n and g_s increased in most genotypes. This response may have resulted from the well-watered conditions that plants were kept in during the simulated heat wave, which resulted in a decrease in WUE_i. Quinoa's enhanced response in leaf gas exchange was supported by an increased efficiency in the quantum yield of PSII (i.e., F_v/F_m) under the heat treatment. The latter may be associated with the observed increase in relative chlorophyll content during the period that plants were exposed to elevated temperatures. Interestingly, R_N decreased under the heat treatment although the plants had increased their C assimilation capacity. In addition, total aboveground biomass (shoot and seed) and total seed weight was unaffected by the temperature treatments. It has been assumed that the cultivation of quinoa in the northern hemisphere has been constrained by its sensitivity to heat stress (REF), similar to the impact of other seed producing crops (e.g sorghum) (Johnson 1990; Prasad, Pisipati, Mutava, & Tuinstra 2008). Our study shows that for most of the 10 genotypes evaluated, high temperature improved quinoa's capacity for carbon assimilation and had no negative effect on seed production.

Leaf gas exchange responses of quinoa to elevated temperatures have been shown to be similar under temperatures of 40°C compared to the control treatment (Hinojosa, Matanguihan, & Murphy 2018), and lowered when exposed to confounding stressors such as drought (Yang, Akhtar, Amjad, Iqbal & Jacobsen 2016) and salinity (Becker, et al. 2017). Many other crops such as soybean (*Glycine max*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), tobacco (*Nicotiana tabacum*) and grapevine (*Vitis vinifera*), show reductions in photosynthetic parameters when exposed to elevated temperatures (at least 5°C above optimum)

(Bita & Gerats 2013; Hall 2010; Hasanuzzaman, Nahar, Alam, Roychowdhury, & Fujita 2013; Wahid, Gelani, Ashraf, & Foolad 2007). These reductions in C assimilation capacity may result from damage to the photosynthetic apparatus, particularly the thylakoid membranes, where PSII is located (Wahid, Gelani, Ashraf, & Foolad 2007). Additionally, P_n and g_s can be inhibited due to decreases in the activation state of Rubisco as a result of heat stress (Bita & Gerats 2013; Hall, 1992; Wahid, Gelani, Ashraf, & Foolad 2007). Our data suggests that quinoa is tolerant to high temperatures and it can even improve its photosynthetic capacity, which implies no damage to PSII even at temperatures of 45°C .

Under high temperatures, soil water availability and increased g_s can maintain evaporative cooling (Becker, et al. 2017), but this may result in lower WUE_i when changes in P_n are proportionally smaller than fluctuations in g_s. For instance, cotton under elevated temperatures increased g_s and allowed for lower leaf temperatures (Salvucci & Crafts-Brandner 2004). In this study, the heat treatment generally lowered WUE_i with the exception of some genotypes including Quinhua, which increased both P_n and g_s; and Kaslaea, which had a high g_s under the control temperature and one of the lowest WUE_i under both temperature treatments. These differences in g_s and the resulting WUE_i are important traits to consider when selecting genotypes for water-limited environments.

The observed increases in leaf gas exchange were accompanied by increases in the quantum yield (i.e., F_v/F_m) under high temperatures. Decreases in F_v/F_m compared to non-stressed conditions usually indicate impaired capacity for electron transport in the photosynthetic machinery that can result in photoinhibition (Maxwell & Johnson 2000). F_v/F_m decreased in heat sensitive cultivars of wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*) and beans (*Phaseolus* vulgaris) exposed to high temperature; whereas heat tolerant cultivars of the same species showed no

decrease in F_v/F_m, or quickly recovered after being exposed to a high temperature period (Camejo, et al. 2005; Pastenes & Horton 1996; Petkova, Denev, Cholakov, & Porjazov 2007; Sharma, Andersen, Ottosen, & Rosenqvist 2012;Willits & Peet 2001). In quinoa, F_v/F_m decreased under drought stress (Fghire, et al. 2015), but it did not under heat stress (40˚C; Hinojosa, Matanguihan, & Murphy 2018). This increase in F_v/F_m has been reported before for quinoa under high heat and drought exposure with F_v/F_m increasing at higher temperatures (Yang, Akhtar, Amjad, Iqbal & Jacobsen 2016). Within our experiment, the Japanese strain had one of the lowest F_v/F_m values in both the heat treatment and control, whereas Quinhua had one of the highest. Kaslaea had a higher F_v/F_m in the control, but a lower one in the heat treatment. It is usually assumed that non-stressed F_v/F_m values are about 0.83 for many species, but for the 10 genotypes evaluated in this study, the mean F_v/F_m under control conditions was 0.734 ± 0.001 SE. The increase in F_v/F_m we observed in plants exposed to the heat treatment indicates that a higher quantum efficiency of PSII electron transport is followed by a subsequent increase in gas exchange at higher temperatures (Pastenes & Horton 1996). This supports the conclusion that no heat induced damage occurred that could cause chronic photoinhibition which would result in a sustained lower F_v/F_m rate (Lambers 2010). Although F_v/F_m were higher at pre-dawn than in the afternoon, both showed a consistent pattern, and this provides evidence that damage to or impairment of PSII was, at most, transient. The higher F_v/F_m reflects an increased capacity for electron transport, which was also supported by an increase in the relative chlorophyll in this study and others (e.g., Buttery & Buzzell 1977; Sharma, Andersen, Ottosen, & Rosenqvist 2014). Additionally, there is a negative correlation between chlorophyll content and thylakoid membrane damage, thus further supporting the conclusion that the increased efficiency we observed in P_n and F_v/F_m is due to the maintained integrity of PSII as a result of increased chlorophyll content (Hinojosa, Matanguihan, & Murphy 2018; Ristic, Bukovnik, and Prasad, 2007).

The increase in C assimilation of quinoa under elevated temperatures implies a higher demand in resources for maintenance of the photosynthetic apparatus. Our findings suggest that the changes in relative chlorophyll, and an assumed increase in leaf N content could result in heightened daytime respiratory activities (Cowling & Sage 1998). In leaves, the metabolic processes associated with nitrogen assimilation and amino acid synthesis are mostly performed during the day, resulting in higher demand for assimilates and R_d. Nighttime respiration is typically associated with maintenance and repair, although these respiratory processes are not evenly distributed through the day and night (Mohammed & Tarpley 2009; O'Leary, et al. 2017). In our study we observed that nighttime respiration (R_N) had lower rates than the control. This is in contrast to other plants which show increases in dark respiration as a stress response. Studies involving rice (*Oryza sativa*) show an increase in R_N due to increased maintenance respiration (Mohammed and Tarpley, 2009). However, quinoa exposed to heat and salinity stress showed no difference in R_N (Becker, et al. 2017). A possible explanation for the reduced R_N we observed in the heat treatment plants, is lower assimilate availability for respiratory processes (Prange, Mcrae, Midmore, & Deng 1990), or a capacity of quinoa to acclimate its R_N, although R_N is generally thought to be temperature sensitive (Becker, et al. 2017; Wright, et al. 2005).

Damage to photosynthetic processes is also indicated by membrane damage (Blum & Ebercon 1981; Camejo, et al. 2005). Our findings indicate that membrane damage was significant (above 70%) after exposure to temperatures of 51°C or higher. At a temperature of 47°C damage ranged from 26 to 43%. This indicates that there is a crucial temperature at which cell membrane injury becomes severe. In studies done with various beans, 15 minutes of exposure to 45°C led to injuries ranging from 39 to 80% (Srinivasan, Takeda, & Senboku 1996). In rice, an elevated nighttime temperature of 32°C led to cell membrane injury above 60% (Mohammed & Tarpley

2009). These studies suggest that quinoa does not experience significant membrane damage until temperatures are very elevated (>51°C).

Our study showed that high temperature also did not affect shoot and seed biomass per plant, supporting our conclusion that a heat wave by itself is not a major stressor for quinoa in the long term. However, under field conditions, confounding factors such as drought, usually accompany high heat. Our study suggests that water availability may help plants cope with a heat wave, and that management practices to minimize these confounding factors (e.g., supplemental irrigation) may prevent decreases in yield. We observed differences in seed biomass among genotypes, and together with leaf physiological traits evaluated in this study, this data could support selection and breeding efforts for improved performance of quinoa cultivars in field settings.

To our knowledge, this is the first study to expose quinoa to temperatures of 45°C for a four-day period to evaluate leaf physiological performance and effects on aboveground biomass and seed yield. Other studies combined high heat with other stressors (e.g., drought), whereas ours looked solely at high temperatures, which allowed us to conclude that high temperatures (typical of North American summers) are not an impediment to the growth of quinoa. Yet, under field conditions, it is common for plants to experience more than one stress at a time; underlining the importance of identifying appropriate management techniques (e.g. precision irrigation) that could help mitigate the negative effects of compounded drought and heat.

Ahmad, Altaf, et al. “Global Climate Change, Stress and Plant Productivity.” *Abiotic Stress Adaptation in Plants*, 2009, pp. 503–521., doi:10.1007/978-90-481-3112-9_23.

Allakhverdiev, Suleyman I., et al. “Heat Stress: an Overview of Molecular Responses in Photosynthesis.” *Photosynthesis Research*, vol. 98, no. 1-3, 2008, pp. 541–550., doi:10.1007/s11120-008-9331-0.

Barnabás, Beáta, et al. “The Effect of Drought and Heat Stress on Reproductive Processes in Cereals.” *Plant, Cell & Environment*, vol. 31, no. 1, 17 Sept. 2007, pp. 11–38., doi:10.1111/j.1365-3040.2007.01727.x.

Bazile, Didier, et al. “The Global Expansion of Quinoa: Trends and Limits.” *Frontiers in Plant Science*, vol. 7, 9 May 2016, doi:10.3389/fpls.2016.00622.

Bazile, Didier, et al. “Worldwide Evaluations of Quinoa: Preliminary Results from Post International Year of Quinoa FAO Projects in Nine Countries.” *Frontiers in Plant Science*, vol. 7, 2016, doi:10.3389/fpls.2016.00850.

Becker, Verena I., et al. “Combined Effects of Soil Salinity and High Temperature on Photosynthesis and Growth of Quinoa Plants (Chenopodium Quinoa).” *Functional Plant Biology*, vol. 44, no. 7, 3 May 2017, p. 665., doi:10.1071/fp16370.

Bita, Craita E., and Tom Gerats. “Plant Tolerance to High Temperature in a Changing Environment: Scientific Fundamentals and Production of Heat Stress-Tolerant Crops.” *Frontiers in Plant Science*, vol. 4, 31 July 2013, doi:10.3389/fpls.2013.00273.

Blum, A., and Adelina Ebercon. “Cell Membrane Stability as a Measure of Drought and Heat Tolerance in Wheat1.” *Crop Science*, vol. 21, no. 1, 1981, p. 43., doi:10.2135/cropsci1981.0011183x002100010013x.

Buttery, B. R., and R. I. Buzzell. “The Relationship Between Chlorophyll Content And Rate Of Photosynthesis In Soybeans.” *Canadian Journal of Plant Science*, vol. 57, no. 1, 1977, pp. 1–5., doi:10.4141/cjps77-001.

Camejo, Daymi, et al. “High Temperature Effects on Photosynthetic Activity of Two Tomato Cultivars with Different Heat Susceptibility.” *Journal of Plant Physiology*, vol. 162, no. 3, 2005, pp. 281–289., doi:10.1016/j.jplph.2004.07.014.

Christensen, Jens Hesselbjerg, and Ole Bøssing Christensen. “A Summary of the PRUDENCE Model Projections of Changes in European Climate by the End of This Century.” *Climatic Change*, vol. 81, no. S1, 2007, pp. 7–30., doi:10.1007/s10584-006-9210-7.

Cowling, S. A., and R. F. Sage. “Interactive Effects of Low Atmospheric CO2 and Elevated Temperature on Growth, Photosynthesis and Respiration in Phaseolus Vulgaris.” *Plant, Cell and Environment*, vol. 21, no. 4, 1998, pp. 427–435., doi:10.1046/j.1365-3040.1998.00290.x.

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Fghire, Rachid, et al. “Physiological and Photosynthetic Response of Quinoa to Drought Stress.” *Chilean Journal of Agricultural Research*, vol. 75, no. 2, 2015, pp. 174–183., doi:10.4067/s0718-58392015000200006.

Giaveno, C., and J. Ferrero. “Introduction of Tropical Maize Genotypes to Increase Silage Production in the Central Area of Santa Fe, Argentina.” *Cropp Breeding and Applied Biotechnology*, vol. 3, no. 2, 2003, pp. 89–94., doi:10.12702/1984-7033.v03n02a01.

Guilioni, Lydie, et al. “Heat Stress-Induced Abortion of Buds and Flowers in Pea: Is Sensitivity Linked to Organ Age or to Relations between Reproductive Organs?” *Annals of Botany*, vol. 80, no. 2, 1 Aug. 1997, pp. 159–168., doi:https://doi.org/10.1006/anbo.1997.0425.

Guy, C. “Molecular responses of plants to cold shock and cold acclimation.” *Journal of Molecular Microbiology and Biotechnology*, vol 1, 1999, pp 231-242.

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2019). dplyr: A Grammar of Data Manipulation. R package version 0.8.3. https://CRAN.R-project.org/package=dplyr

Hall, Anthony E. “Breeding for Heat Tolerance.” *Plant Breeding Reviews*, 2010, pp. 129–168., doi:10.1002/9780470650011.ch5.

Hasanuzzaman, Mirza, et al. “Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants.” *International Journal of Molecular Sciences*, vol. 14, no. 5, 2013, pp. 9643–9684., doi:10.3390/ijms14059643.
Hinojosa, Leonardo, et al. “Effect of High Temperature on Pollen Morphology, Plant Growth and Seed Yield in Quinoa (Chenopodium Quinoa Willd.).” *Journal of Agronomy and Crop Science*, vol. 205, no. 1, 2018, pp. 33–45., doi:10.1111/jac.12302.

Jacobsen, S.-E., et al. “The Resistance of Quinoa (Chenopodium Quinoa Willd.) to Adverse Abiotic Factors.” *Food Reviews International*, vol. 19, no. 1-2, 2003, pp. 99–109., doi:10.1081/fri-120018872.

John Fox and Sanford Weisberg (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

John Fox and Sanford Weisberg (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Johnson, D.L., "New grains and pseudograins." *Advances in New Crops*, 1990, p. 122-127. In: J. Janick and J.E. Simon (eds.),Timber Press, Portland, OR.

Karim, M A, et al. "Heat Tolerance of Maize with Reference to Some Physiological Characteristics." *Annals of Bangladesh Agriculture*, vol. 71, no. 1, 1997, pp. 27–33.

Lambers, Hans. *Plant Physiological Ecology*. Springer 2010.

Lesjak, Jurka, and Daniel F. Calderini. "Increased Night Temperature Negatively Affects Grain Yield, Biomass and Grain Number in Chilean Quinoa." *Frontiers in Plant Science*, vol. 8, 23 Mar. 2017, doi:10.3389/fpls.2017.00352.

Maxwell, Kate, and Giles N. Johnson. "Chlorophyll Fluorescence—a Practical Guide." *Journal of Experimental Botany*, vol. 51, no. 345, 2000, pp. 659–668., doi:10.1093/jxb/51.345.659.

Mohammed, Abdul-Razack, and Lee Tarpley. "Impact of High Nighttime Temperature on Respiration, Membrane Stability, Antioxidant Capacity, and Yield of Rice Plants." *Crop Science*, vol. 49, no. 1, 2009, p. 313., doi:10.2135/cropsci2008.03.0161.

Murphy, Kevin M., et al. "Development of a Worldwide Consortium on Evolutionary Participatory Breeding in Quinoa." *Frontiers in Plant Science*, vol. 7, 9 May 2016, doi:10.3389/fpls.2016.00608.

O'Leary, Brendan M., et al. "Variation in Leaf Respiration Rates at Night Correlates with Carbohydrate and Amino Acid Supply." *Plant Physiology*, vol. 174, no. 4, 14 Aug. 2017, pp. 2261–2273., doi:10.1104/pp.17.00610.

Pastenes, C., and P. Horton. "Effect of High Temperature on Photosynthesis in Beans (I. Oxygen Evolution and Chlorophyll Fluorescence)." *Plant Physiology*, vol. 112, no. 3, 1996, pp. 1245–1251., doi:10.1104/pp.112.3.1245.

Peter Filzmoser and Moritz Gschwandtner (2018). mvoutlier: Multivariate Outlier Detection Based on Robust Methods. R package version 2.0.9. https://CRAN.R-project.org/package=mvoutlier

Petkova, Valentina, et al. “Field Screening for Heat Tolerant Common Bean Cultivars (Phaseolus Vulgaris L.) by Measuring of Chlorophyll Fluorescence Induction Parameters.” *Scientia Horticulturae*, vol. 111, no. 2, 2007, pp. 101–106., doi:10.1016/j.scienta.2006.10.005.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2018). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-137, URL: https://CRAN.R-project.org/package=nlme>.

Porter, John R, and Mikhail A Semenov. “Crop Responses to Climatic Variation.” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 360, no. 1463, 2005, pp. 2021–2035., doi:10.1098/rstb.2005.1752.

Prange, Robert K., et al. “Reduction in Potato Growth at High Temperature: Role of Photosynthesis and Dark Respiration.” *American Potato Journal*, vol. 67, no. 6, 1990, pp. 357–369., doi:10.1007/bf02987277.

Prasad, P. V. V., et al. “Sensitivity of Grain Sorghum to High Temperature Stress during Reproductive Development.” *Crop Science*, vol. 48, no. 5, Sept. 2008, p. 1911., doi:10.2135/cropsci2008.01.0036.

Ristic, Zoran, et al. “Correlation between Heat Stability of Thylakoid Membranes and Loss of Chlorophyll in Winter Wheat under Heat Stress.” *Crop Science*, vol. 47, no. 5, Sept. 2007, p. 2067., doi:10.2135/cropsci2006.10.0674.

Ruiz, K. B., et al. “Quinoa – a Model Crop for Understanding Salt-Tolerance Mechanisms in Halophytes.” *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, vol. 150, no. 2, 2015, pp. 357–371., doi:10.1080/11263504.2015.1027317.

Ruiz, Karina B., et al. “Quinoa Biodiversity and Sustainability for Food Security under Climate Change. A Review.” *Agronomy for Sustainable Development*, vol. 34, no. 2, 2014, pp. 349–359., doi:10.1007/s13593-013-0195-0.

Russell Lenth (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.2.https://CRAN.R-project.org/package=emmeans

Sakata, Tadashi, and Atsushi Higashitani. “Male Sterility Accompanied with Abnormal Anther Development in Plants – Genes and Environmental Stresses with Special Reference to High Temperature Injury .” *International Journal of Plant Developmental Biology* , vol. 2, 2008, pp. 42–51.

Salvucci, Michael E., and Steven J. Crafts-Brandner. “Inhibition of Photosynthesis by Heat Stress: the Activation State of Rubisco as a Limiting Factor in Photosynthesis.” *Physiologia Plantarum*, vol. 120, no. 2, 2004, pp. 179–186., doi:10.1111/j.0031-9317.2004.0173.x.

Sato, S., et al. “Moderate Increase of Mean Daily Temperature Adversely Affects Fruit Set of Lycopersicon Esculentum by Disrupting Specific Physiological Processes in Male Reproductive Development.” *Annals of Botany*, vol. 97, no. 5, 2006, pp. 731–738., doi:10.1093/aob/mcl037.

Sharma, Dew Kumari, et al. “Phenotyping of Wheat Cultivars for Heat Tolerance Using Chlorophyll a Fluorescence.” *Functional Plant Biology*, vol. 39, no. 11, 17 Sept. 2012, p. 936., doi:10.1071/fp12100.

Sharma, Dew Kumari, et al. “Wheat Cultivars Selected for High Fv/Fm under Heat Stress Maintain High Photosynthesis, Total Chlorophyll, Stomatal Conductance, Transpiration and Dry Matter.” *Physiologia Plantarum*, vol. 153, no. 2, 2014, pp. 284–298., doi:10.1111/ppl.12245.

Sinsawat, Veerana, et al. “Effect of Heat Stress on the Photosynthetic Apparatus in Maize (Zea Mays L.) Grown at Control or High Temperature.” *Environmental and Experimental Botany*, vol. 52, no. 2, 2004, pp. 123–129., doi:10.1016/j.envexpbot.2004.01.010.

Sosa-Zuniga, V., et al. “Phenological Growth Stages of Quinoa (Chenopodium Quinoa) Based on the BBCH Scale.” *Annals of Applied Biology*, vol. 171, no. 1, 2017, pp. 117–124., doi:10.1111/aab.12358.

Srinivasan, Ancha, et al. “Heat Tolerance in Food Legumes as Evaluated by Cell Membrane Thermostability and Chlorophyll Fluorescence Techniques.” *Euphytica*, vol. 88, no. 1, 1996, pp. 35–45., doi:10.1007/bf00029263.

Sumesh, K. V., et al. “Starch Synthase Activity and Heat Shock Protein in Relation to Thermal Tolerance of Developing Wheat Grains.” *Biologia Plantarum*, vol. 52, no. 4, 2008, pp. 749–753., doi:10.1007/s10535-008-0145-x.

Wahid, A, et al. “Heat Tolerance in Plants: An Overview.” *Environmental and Experimental Botany*, vol. 61, no. 3, 2007, pp. 199–223., doi:10.1016/j.envexpbot.2007.05.011.Wahid, Abdul, and Asma Shabbir. “Induction of Heat Stress Tolerance in Barley Seedlings by Pre-Sowing Seed Treatment with Glycinebetaine.” *Plant Growth Regulation*, vol. 46, no. 2, 2005, pp. 133–141., doi:10.1007/s10725-005-8379-5.

Weis, E. and Berry, A.J. “Plants and High Temperature Stress.” *Symposia of the Society for Experimental Biology* , vol. 42, 1 Jan. 1988, pp. 329–346.

Willits, D.H, and Peet, M.M. “The Effect of Night Temperature on Greenhouse Grown Tomato Yields in Warm Climates.” *Agricultural and Forest Meteorology*, vol. 92, no. 3, 1998, pp. 191–202., doi:10.1016/s0168-1923(98)00089-6.

Willits, D.H., and Peet, M.M. “Measurement of Chlorophyll Fluorescence as a Heat Stress Indicator in Tomato: Laboratory and Greenhouse Comparisons.” *Journal of the American Society for Horticultural Science*, vol. 126, no. 2, 2001, pp. 188–194., doi:10.21273/jashs.126.2.188.

Wise, R. R., et al. “Electron Transport Is the Functional Limitation of Photosynthesis in Field-Grown Pima Cotton Plants at High Temperature.” *Plant, Cell and Environment*, vol. 27, no. 6, 2004, pp. 717–724., doi:10.1111/j.1365-3040.2004.01171.x.

Wright, Ian J., et al. “Irradiance, Temperature and Rainfall Influence Leaf Dark Respiration in Woody Plants: Evidence from Comparisons across 20 Sites.” *New Phytologist*, vol. 169, no. 2, 2005, pp. 309–319., doi:10.1111/j.1469-8137.2005.01590.x.

Yang, A., et al. “Growth and Physiological Responses of Quinoa to Drought and Temperature Stress.” *Journal of Agronomy and Crop Science*, vol. 202, no. 6, 2016, pp. 445–453., doi:10.1111/jac.12167.

Young, L. W., et al. “High Temperature Stress of Brassica Napus during Flowering Reduces Micro- and Megagametophyte Fertility, Induces Fruit Abortion, and Disrupts Seed Production.” *Journal of Experimental Botany*, vol. 55, no. 396, 2004, pp. 485–495., doi:10.1093/jxb/erh038.

Zhang, Meng, et al. “Modulated Fatty Acid Desaturation via Overexpression of Two Distinct ω-3 Desaturases Differentially Alters Tolerance to Various Abiotic Stresses in Transgenic Tobacco Cells and Plants.” *The Plant Journal*, vol. 44, no. 3, 2005, pp. 361–371., doi:10.1111/j.1365-313x.2005.02536.x.

PI #	Genotype	Location
AMES 13756	3UISE	New Mexico, USA
PI 634923	UDEC-1	Bucalemu, Chile
PI 614880	QQ065	Los Lagos, Chile
NA	Quinhua	Chile
AMES 13735	17GR	New Mexico, USA
PI 614886	QQ74	Maule, Chile
AMES 13746	Pison	New Mexico, USA
NA	Titicaca	Denmark
AMES 13745	Kaslaea	New Mexico, USA
PI 677100	Japanese strain	WSU, USA

Table 1. Accession information for the quinoa genotypes used in this study.

Genotype	Control	Heat	Significance
3UISE	17.83 ± 1.34	25.56 ± 1.39	***
UDEC-1	18.61 ± 0.83	23.81 ± 1.28	*
QQ065	18.91 ± 1.19	21.67 ± 1.16	n.s.
Quinhua	20.82 ± 1.29	24.81 ± 1.25	*
17GR	17.69 ± 1.21	23.95 ± 0.95	***
QQ74	16.8 ± 0.84	22.23 ± 0.96	***
Pison	18.45 ± 0.92	22.96 ± 1.13	**
Titicaca	17.32 ± 1.05	21.95 ± 1.27	**
Kaslaea	22.02 ± 0.91	24.56 ± 1.39	*
Japanese strain	17.23 ± 0.85	17.01 ± 1.21	n.s.

Table 2. Photosynthetic rate (P_n; µmol CO_2 m^{-2} s^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:30 and 17:00. Vertical bars denote mean ±SE (n=32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

Genotype	Control	Heat	Significance
3UISE	0.27 ± 0.03	0.65 ± 0.06	***
UDEC-1	0.37 ± 0.04	0.72 ± 0.05	***
QQ065	0.44 ± 0.05	0.64 ± 0.06	*
Quinhua	0.46 ± 0.06	0.70 ± 0.61	**
17GR	0.35 ± 0.04	0.81 ± 0.56	***
QQ74	0.25 ± 0.02	0.70 ± 0.06	***
Pison	0.32 ± 0.03	0.62 ± 0.53	***
Titicaca	0.28 ± 0.04	0.58 ± 0.50	***
Kaslaea	0.57 ± 0.06	0.67 ± 0.61	n.s.
Japanese strain	0.35 ± 0.05	0.59 ± 0.06	***

Table 3. Stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:30 and 17:00. Vertical bars denote mean ±SE (n=32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

Genotype	Control	Heat	Significance
3UISE	54.02 ± 4.76	42.86 ± 2.69	n.s.
UDEC-1	52.21 ± 3.96	33.24 ± 1.78	**
QQ065	46.76 ± 3.89	39.26 ± 3.01	n.s.
Quinhua	50.02 ± 3.35	44.50 ± 3.83	n.s.
17GR	55.65 ± 3.18	33.25 ± 2.69	**
QQ74	68.07 ± 3.78	34.67 ± 3.10	***
Piso	60.89 ± 3.36	42.99 ± 3.06	**
Titicaca	65.22 ± 3.94	39.99 ± 2.68	**
Kaslaea	46.44 ± 3.08	39.14 ± 2.99	n.s.
Japanese strain	57.34 ± 4.27	35.52 ± 3.95	**

Table 4. Intrinsic water use efficiency (WUE_i; calculated as P_n/g_s) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:30 and 17:00. Vertical bars denote mean ±SE (n=23-32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

Genotype	Control	Heat	Significance
3UISE	2.34 ± 0.12	1.67 ± 0.10	***
UDEC-1	1.71 ± 0.14	1.60 ± 0.10	n.s.
QQ065	1.96 ± 0.10	1.36 ± 0.08	***
Quinhua	2.00 ± 0.14	1.63 ± 0.13	*
17GR	1.92 ± 0.14	1.83 ± 0.11	n.s.
QQ74	1.88 ± 0.08	1.45 ± 0.16	*
Pison	2.02 ± 0.10	1.85 ± 0.18	n.s.
Titicaca	1.90 ± 0.12	1.65 ± 0.11	n.s.
Kaslaea	2.17 ± 0.09	1.54 ± 0.16	**
Japanese strain	1.66 ± 0.08	1.19 ± 0.14	**

Table 5. Dark respiration (R_N; μmol CO_2 m^{-2} s^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and a control treatment (20°C/14°C); day and night, respectively. Measurements were taken in full darkness between 21:30 and 23:00. Vertical bars denote mean ±SE (n=18-28).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

Genotype	Control	Heat	Significance
3UISE	0.738 ± 0.008	0.726 ± 0.007	n.s.
UDEC-1	0.742 ± 0.003	0.757 ± 0.003	***
QQ065	0.725 ± 0.005	0.721 ± 0.006	n.s.
Quinhua	0.747 ± 0.003	0.760 ± 0.003	***
17GR	0.717 ± 0.004	0.740 ± 0.003	***
QQ74	0.726 ± 0.005	0.759 ± 0.003	***
Pison	0.731 ± 0.005	0.733 ± 0.006	**
Titicaca	0.733 ± 0.005	0.742 ± 0.003	***
Kaslaea	0.736 ± 0.004	0.763 ± 0.005	***
Japanese strain	0.715 ± 0.006	0.737 ± 0.009	**

Table 6. Pre-dawn F_v/F_m in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken pre-dawn between 05:00 and 06:30. Vertical bars denote mean ±SE (n=15-32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

Genotype	UTC	HT	Significance
3UISE	0.638 ± 0.022	0.707 ± 0.014	**
UDEC-1	0.693 ± 0.014	0.730 ± 0.012	n.s.
QQ065	0.709 ± 0.013	0.721 ± 0.012	n.s.
Quinhua	0.711 ± 0.014	0.733 ± 0.009	n.s.
17GR	0.614 ± 0.018	0.726 ± 0.009	***
QQ74	0.644 ± 0.022	0.732 ± 0.012	***
Piso	0.659 ± 0.016	0.667 ± 0.022	n.s.
Titicaca	0.696 ± 0.022	0.728 ± 0.010	*
Kaslaea	0.696 ± 0.013	0.725 ± 0.014	n.s.
Japanese strain	0.622 ± 0.018	0.663 ± 0.022	n.s.

Table 7. Afternoon F_v/F_m in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Vertical bars denote mean ±SE (n=15-32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

Treatment	Genotype	Estimate	Standard Error	df	P-value	Significance
Control	3UISE	-2.047	1.142	107.0	0.076	n.s.
	UDEC-1	0.567	1.614	107.0	0.726	n.s.
	QQ065	0.421	1.583	107.0	0.791	n.s.
	Quinhua	-0.089	1.751	107.0	0.960	n.s.
	17GR	-3.379	1.683	107.1	0.047	*
	QQ74	1.039	1.614	107.0	0.521	n.s.
	Piso	-1.424	1.645	107.0	0.389	n.s.
	Titicaca	-1.094	1.618	107.2	0.500	n.s.
	Kaslaea	0.443	1.614	107.0	0.785	n.s.
	JPN	2.164	1.583	107.0	0.175	n.s.
Heat	3UISE	0.934	0.892	11.35	0.317	n.s.
	UDEC-1	0.280	1.142	107.0	0.807	n.s.
	QQ065	0.416	1.097	107.0	0.705	n.s.
	Quinhua	0.911	1.142	107.0	0.427	n.s.
	17GR	1.780	1.035	107.0	0.088	n.s.
	QQ74	0.694	1.098	107.0	0.528	n.s.
	Piso	1.407	1.142	107.1	0.221	n.s.
	Titicaca	0.728	1.098	107.1	0.509	n.s.
	Kaslaea	-0.195	1.142	107.0	0.865	n.s.
	JPN	-2.236	1.097	107.0	0.044	*

Table 8. Relative chlorophyll model output in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Each point represents a mean relative chlorophyll amount per genotype within treatment within that day (n=5-9).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

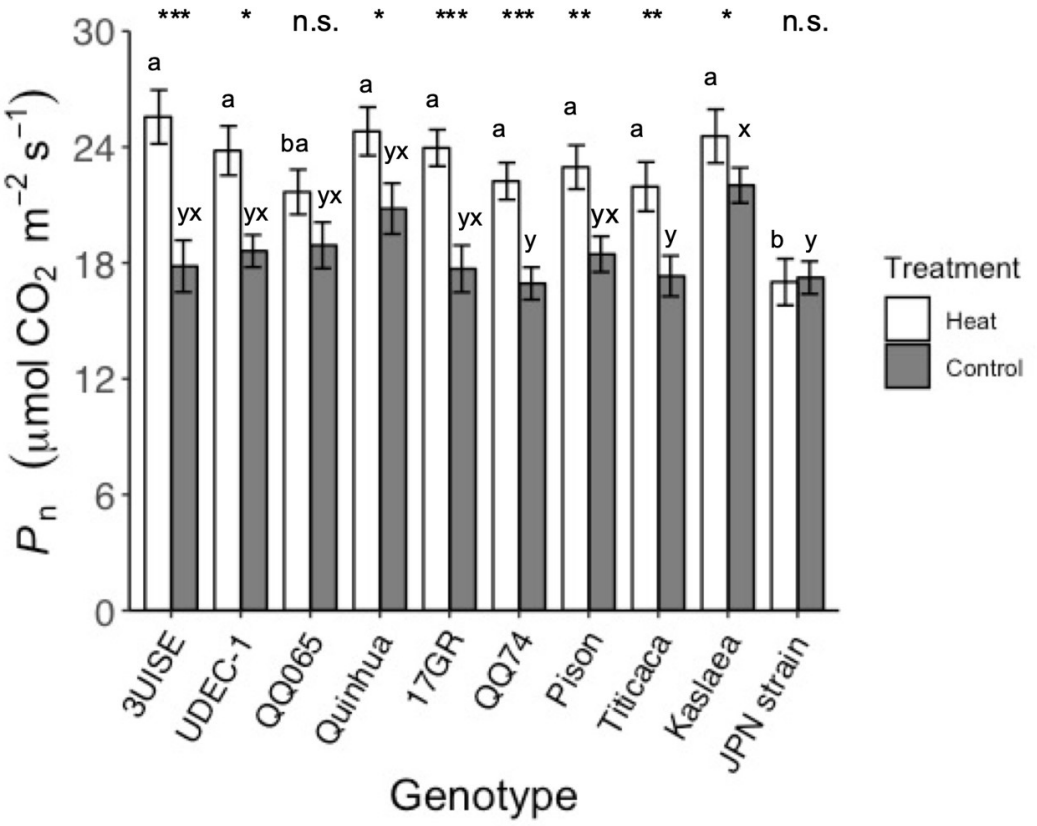

Figure 1. Photosynthetic rate (P_n; µmol CO_2 m^{-2} s^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:30 and 17:00. Vertical bars denote mean ±SE (n=32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

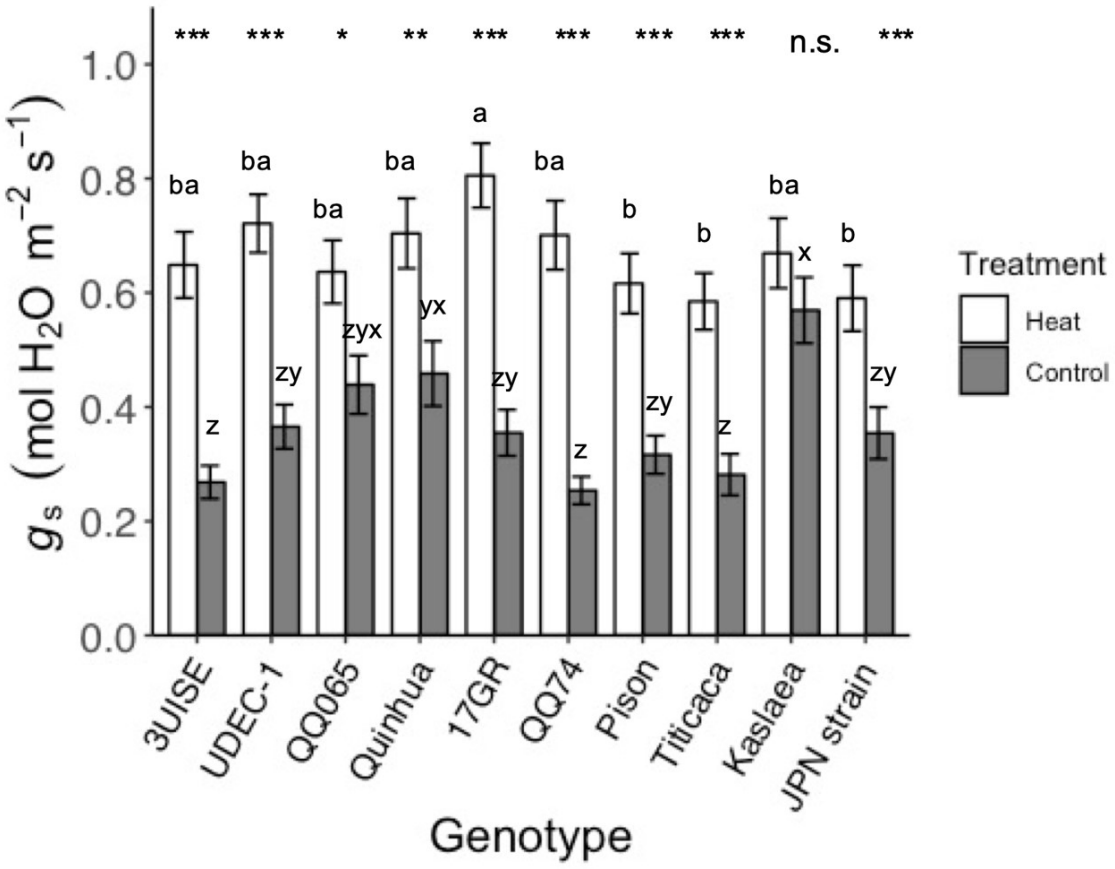

Figure 2. Stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:30 and 17:00. Vertical bars denote mean ±SE (n=32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

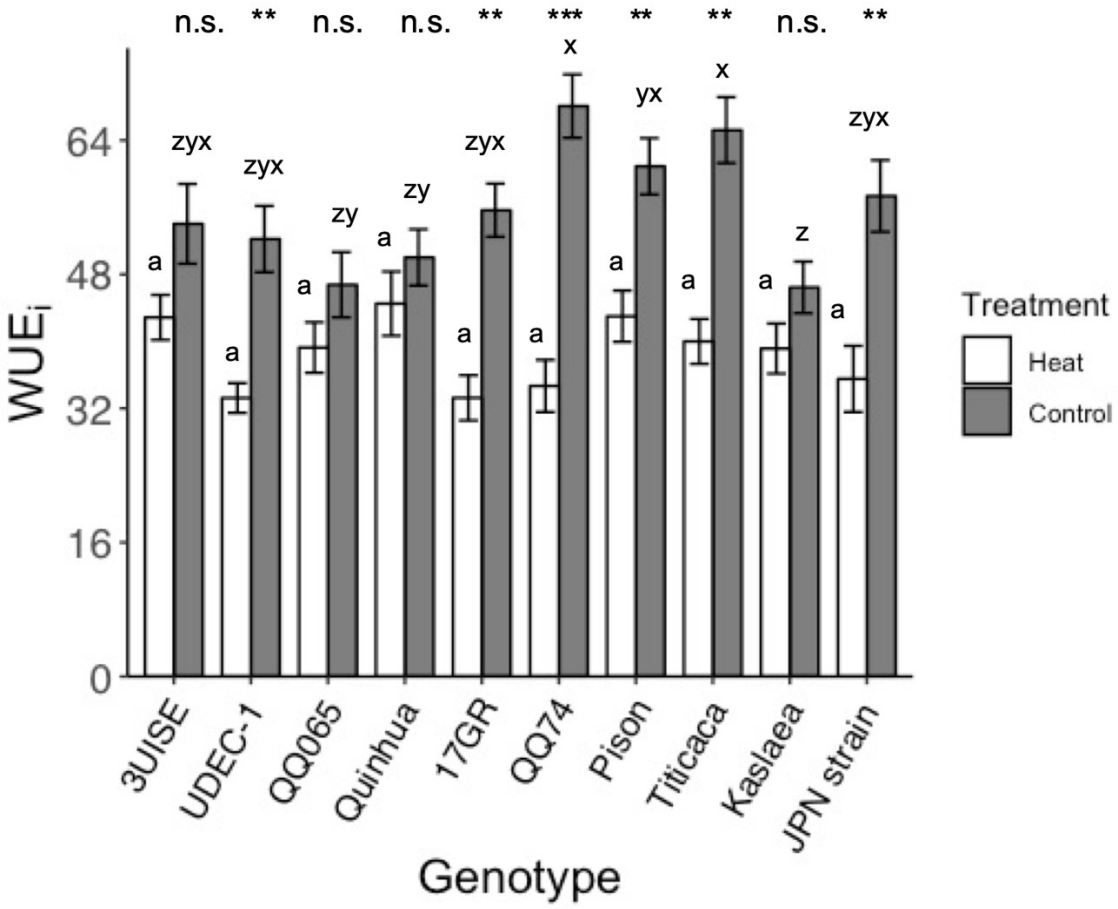

Figure 3. Intrinsic water use efficiency (WUE_i; calculated as P_n/g_s) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:30 and 17:00. Vertical bars denote mean ±SE (n=23-32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

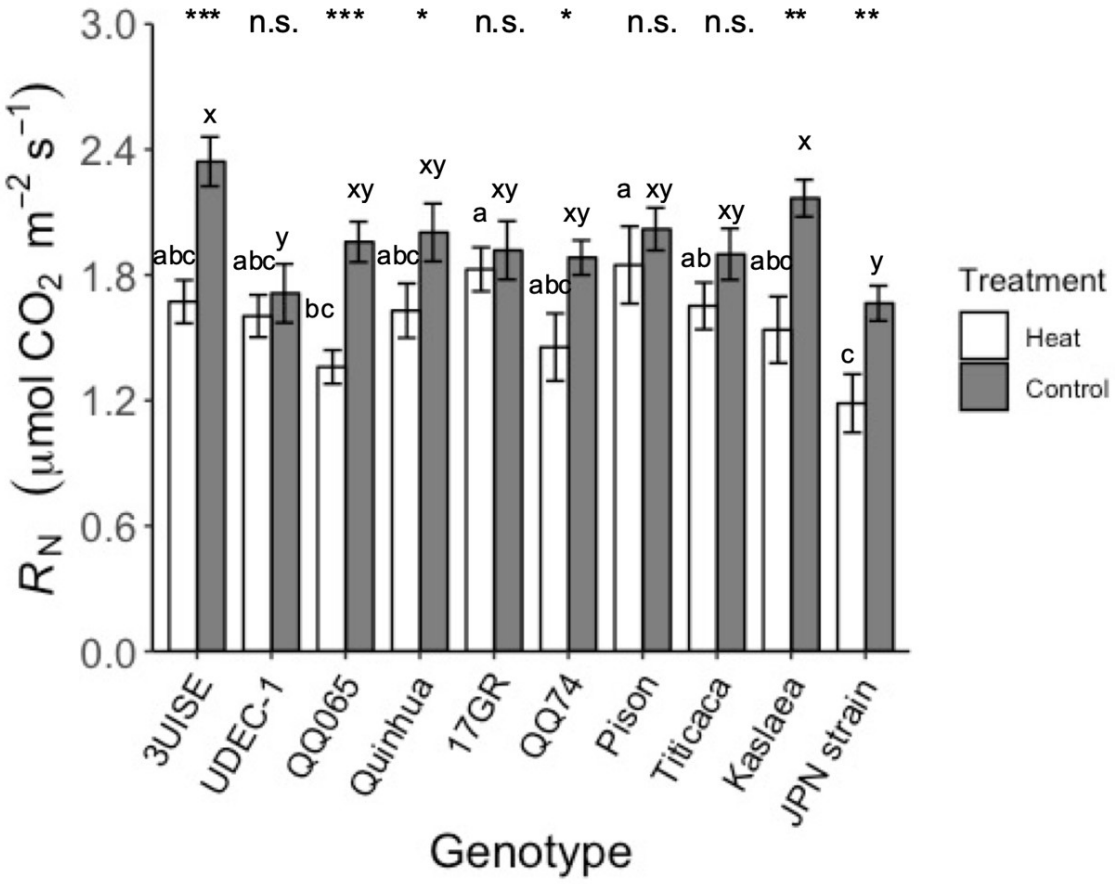

Figure 4. Dark respiration (R_N; µmol CO_2 m^{-2} s^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and a control treatment (20°C/14°C); day and night, respectively. Measurements were taken in full darkness between 21:30 and 23:00. Vertical bars denote mean ±SE (n=18-28).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

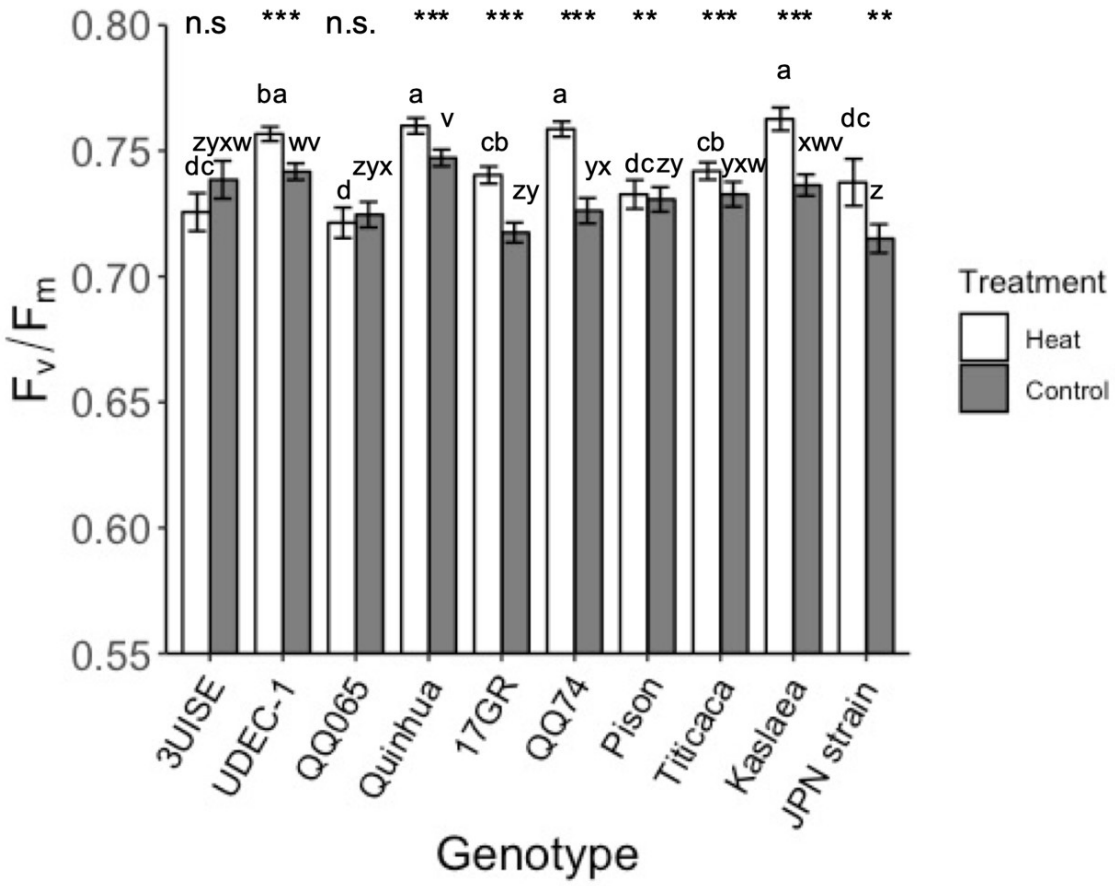

Figure 5. Pre-dawn F_v/F_m in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken pre-dawn between 05:00 and 06:30. Vertical bars denote mean ±SE (n=15-32).

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

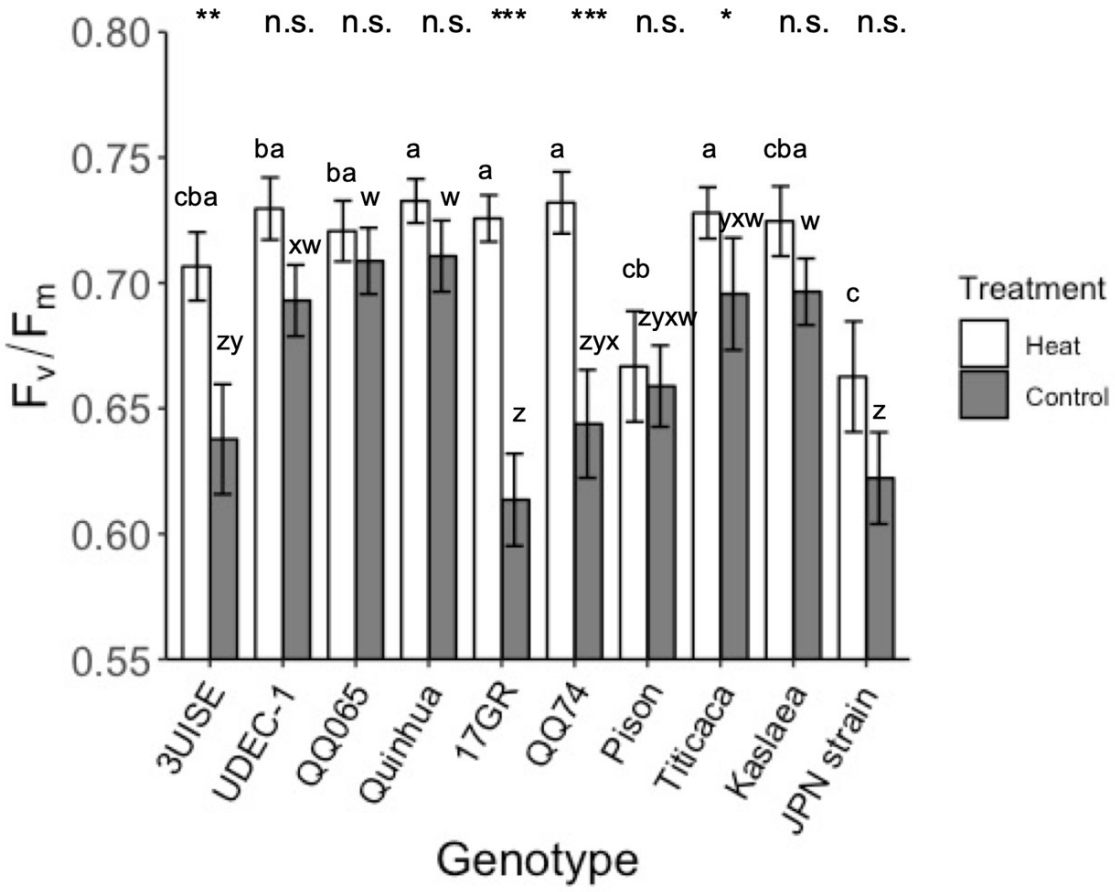

Figure 6. Afternoon F_v/F_m in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30.Vertical bars denote mean ±SE (n=15-32).

**Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

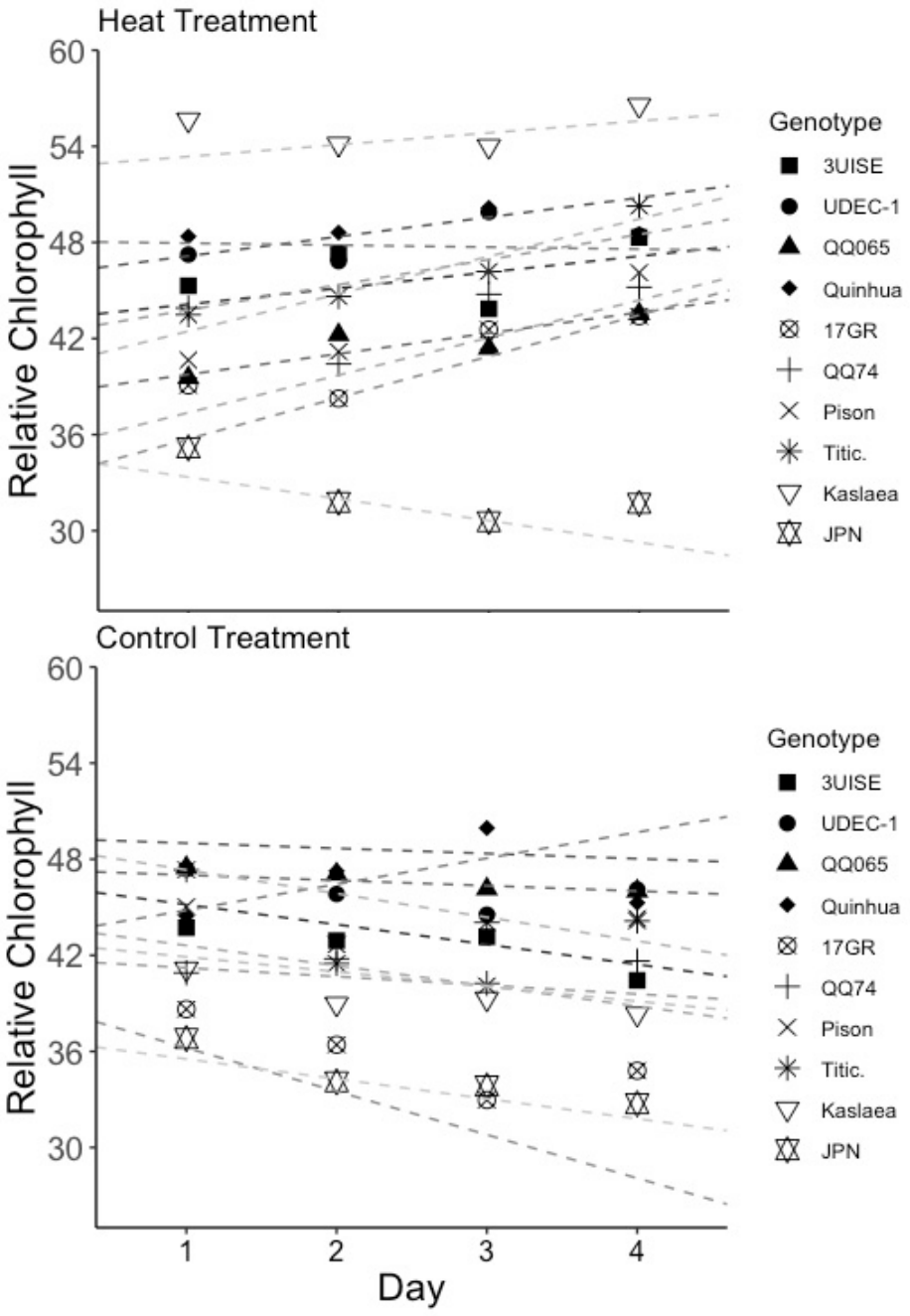

Figure 7A and B. Relative chlorophyll means by day with slope line, in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Each point represents a mean relative chlorophyll amount per genotype within treatment within that day (n=5-9). The lines indicate the slope calculated from the linear model where average relative chlorophyll is a response to day.

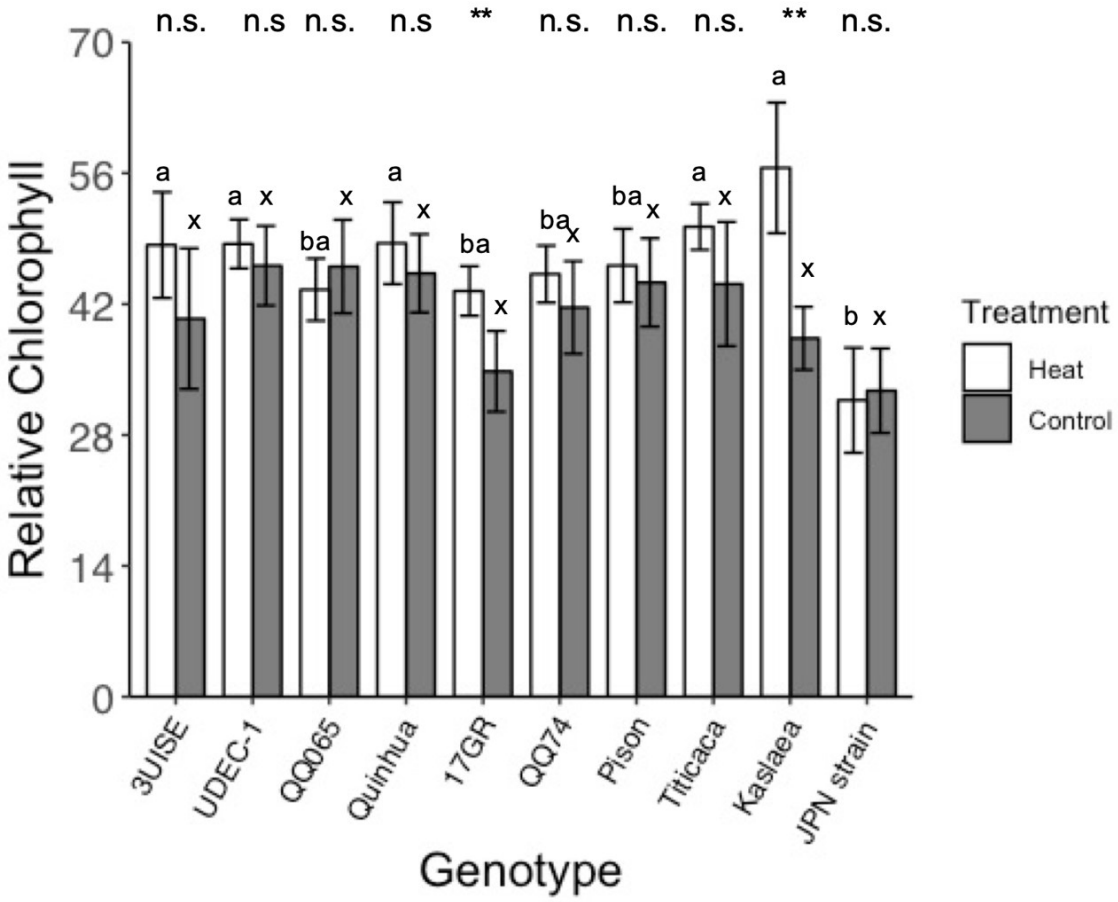

Figure 8. Relative chlorophyll means for day 4, in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Measurements were taken midafternoon between 14:00 and 15:30. Vertical bars denote mean ±SE (n=5-9).

**Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

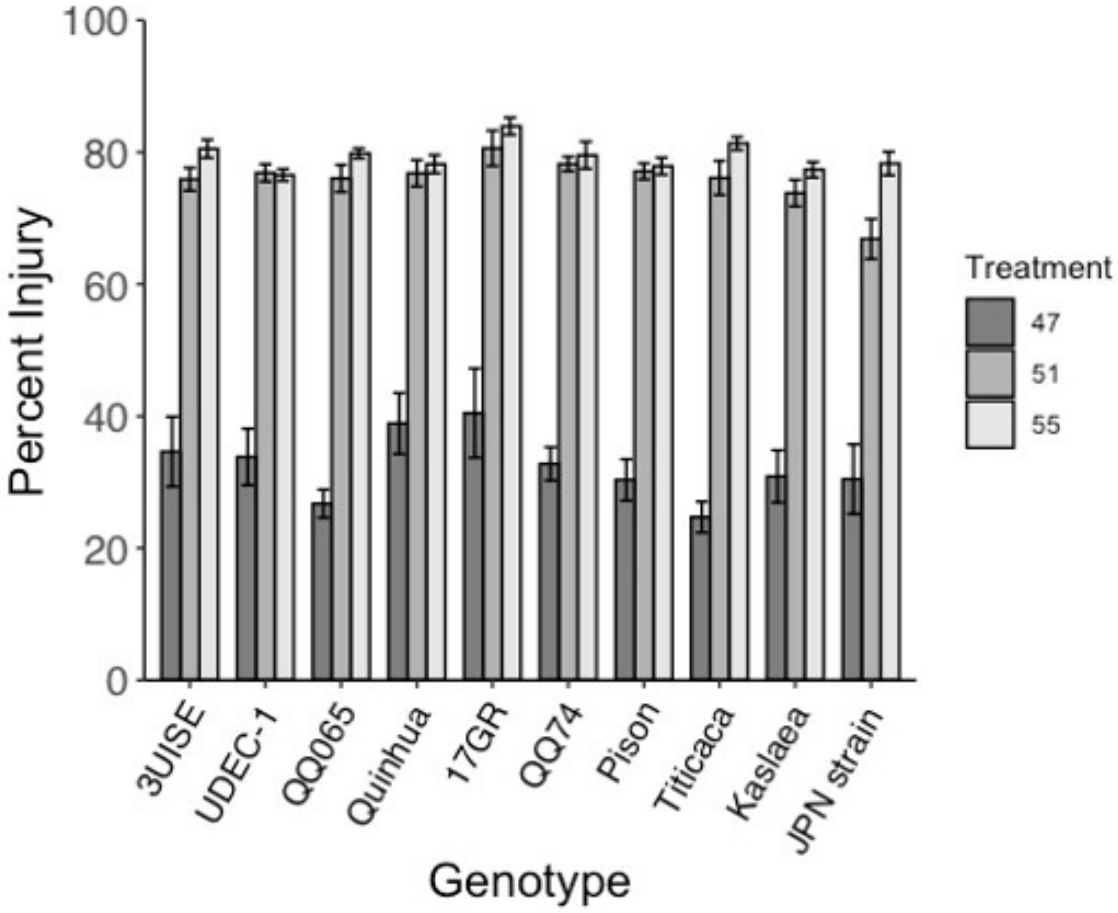

Figure 9. Cell membrane injury (% CMI) in 10 quinoa genotypes exposed to a one hour heat treatment at 47°C, 51°C and 55°C. Vertical bars denote mean ±SE (n=3-8).

**Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
n.s., not significant.

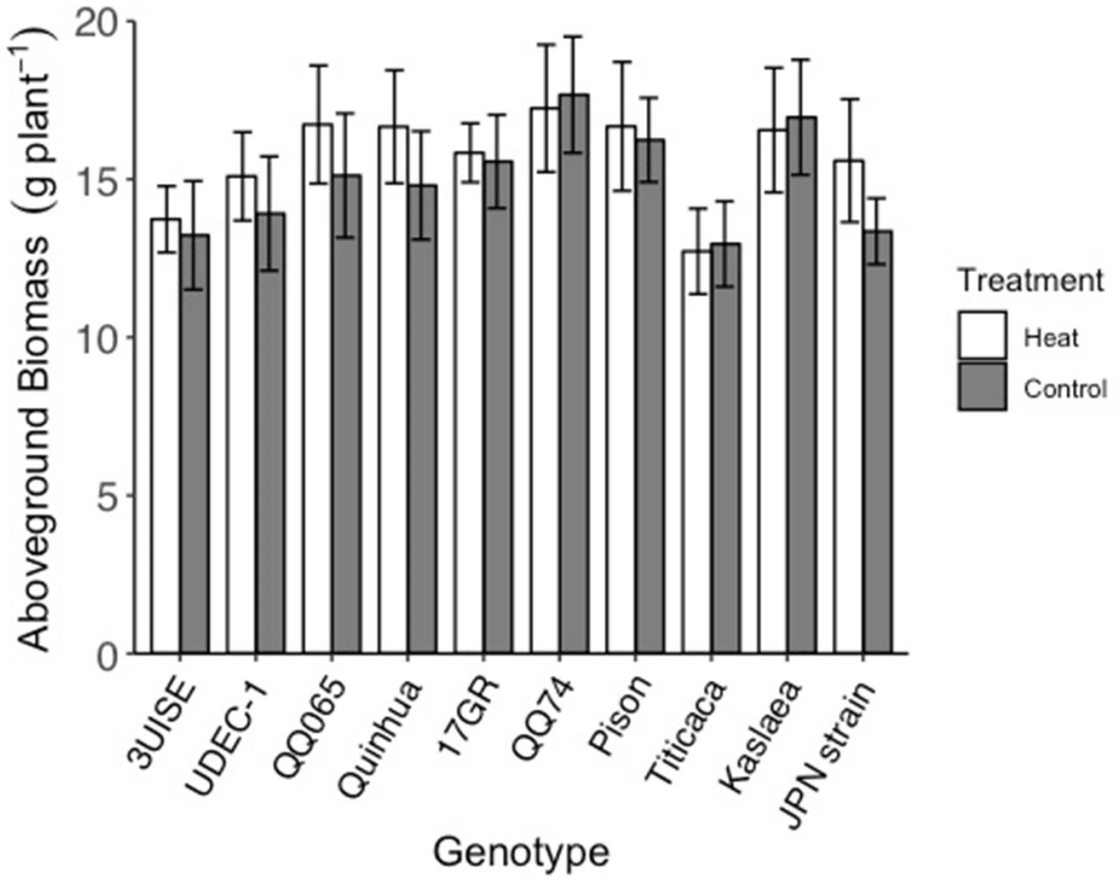

Figure 10. Aboveground biomass (seed and shoot, dried; g plant^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Vertical bars denote mean ±SE (n=10-13). No differences were observed between genotypes or treatments.

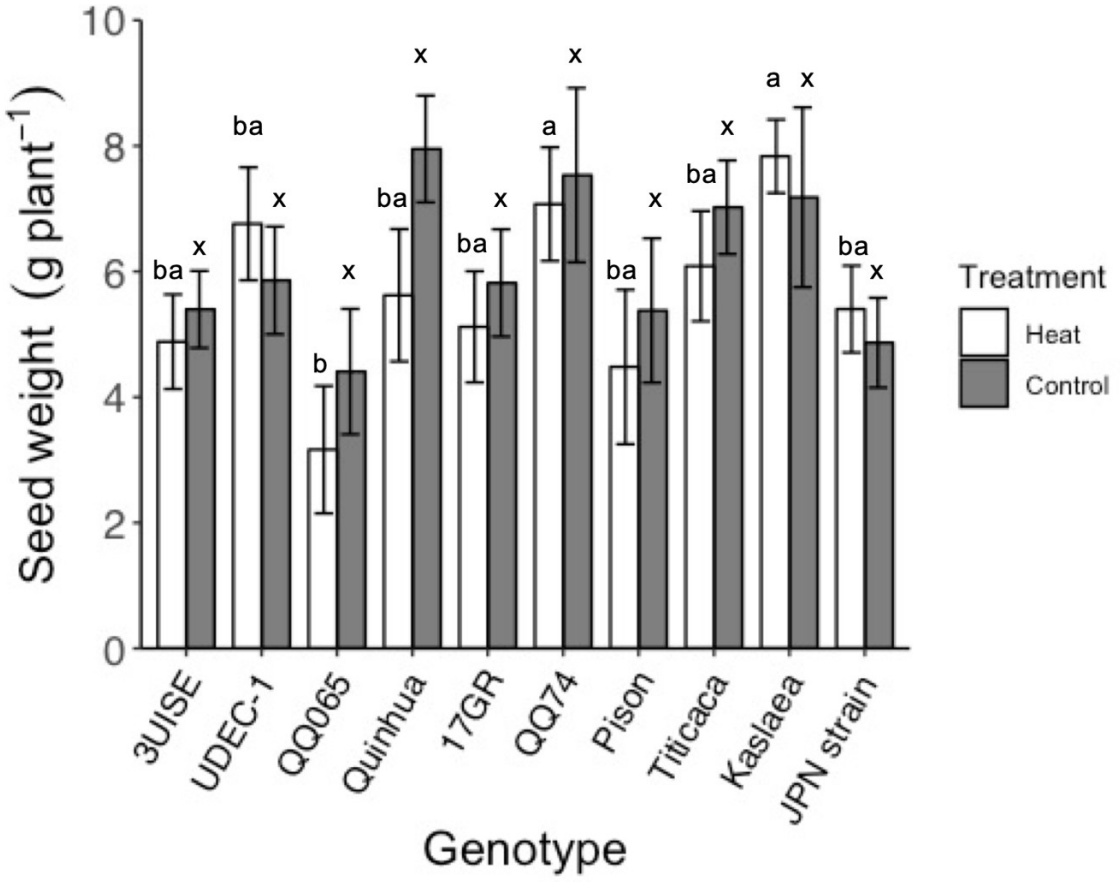

Figure 11. Seed weight (g plant^{-1}) in 10 quinoa genotypes exposed to a four-day heat treatment (45°C/30°C) and control treatment (20°C/14°C); day and night, respectively. Vertical bars denote ±SE of mean (n=10-12).

Time	Heat Treatment (°C)	Control Treatment (°C)
00:00	30	14
06:00	32	16
08:00	35	18
10:00	40	18
12:00	45	20
16:00	40	18
18:00	35	16
20:00	32	16
22:00	30	14

Supplemental Table 1. Time table showing times and temperatures for the heat treatment and control treatment 10 quinoa genotypes were exposed to for four-days.