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Physiological Performances of Temperate Vegetables with Response to Chronic and Acute Heat Stress

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Abstract

In face of climate change catastrophes, understanding the thermal limits and optimal physiological thermal window food crop is of particular urgency. This research aims to evaluate: 1) how physiological performances of plant will change with increasing chronic and acute heat stress; 2) if the examined parameters form a hierarchy in terms of thermal tolerance; and 3) the optimal thermal window and critical temperatures of the examined plants with response to chronic and acute heat stress. Six temperate vegetables were subjected to chronic and acute heat stress and a suite of physiological parameters were evaluated. Dose responses were observed in shoot fresh weight, photosynthetic gas exchange, photosynthetic oxygen evolution, electron transfer rate, photo- and non-photochemical quenching with significant drop in performance as early as 28˚C for selected species. Conversely, ratio of variable to maximum fluorescence (F_v/F_m) was not affected by heat stress until 46˚C in chronic heat stress. Examining the temperature at which a measured parameter's performance dropped by 50% compared to control (LT_{50}), a distinct hierarchy of the indices was observed for Canasta, recombinant inbred line 141, *Lactuca serriola* and *Lactuca sativa* (L. "Salinas"): shoot fresh weight, representing the highest integrated level of photosynthesis was the most sensitive to thermal stress (28˚C - 30˚C), followed by oxygen evolution (35˚C - 45˚C) while non-photochemical and photochemical quenching which is subcellular function of stress alleviation had a much higher capacity failure temperature (47˚C - 60˚C). It is expected that F_v/F_m ratio, a measurement of sub-cellular structural integrity, will approach that of non-photochemical and photochemical quenching, if not exceeding it. By examining the photosynthetic parameters via their hierarchy of biological organization, it can be inferred that plants like Arugula and recombinant inbred line 192 are already operating near their thermal limit and have less energetic investment into heat stress mediation whereas *L. serriola* prioritizes thermal tolerance at the expense of photosynthesis efficiency.

Keywords

Chlorophyll Fluorescence, Heat Stress, O_2 Evolution, Photosynthetic CO_2 Assimilation Rate, Stomatal Conductance, Thermotolerance

1. Introduction

Plant functions are highly sensitive to heat, where acute heat stress can result in negative effects on plant growth and survival [1]-[4]. Heat stress primarily affects thermolabile components, especially photosystem II (PSII) [1] [2] [5]-[7] and Rubisco activase in the Calvin cycle [8]-[10]. Physiological and photosynthetic functions could thus be significantly impaired by elevated heat stress.

It is hypothesized that, for a complex organism, a hierarchical series of tolerance prevails, ranging from systemic to cellular to molecular levels [11] [12]. In consequence, sensitivity levels of molecules, organelles, cells, tissues and the intact organism need to be distinguished to demarcate the optimum, pejus (pejus = getting worse) and pessimum (critical) ranges with respect to thermal stress, as adopted from the law of tolerance [13] [14] to elucidate the thermal tolerance of a species. From an ecological perspective, pejus rather than critical conditions are likely to reflect the upper and lower tolerance limits determining species distribution.

While there are many reports on the effects of heat stress on plants (e.g. general productivity drop [15]-[17]; cellular organization collapse [18]; enzyme inactivation and protein synthesis inhibition [19]), most of these workers focused on the manifestations of the stress effects and scientific reports on a dose-response of increasing heat stress on physiological parameters are relatively rare (e.g. [20]). In this study, a suite of stress indices of different biological hierarchy ranging from organismal growth to sub-cellular processes in temperate vegetables were evaluated for their response to acute and chronic heat stress. This study aims to establish a dose response of physiological parameters to increasing heat stress for background evaluation of the degree of stress experienced by the studied vegetables at each target temperature. In the context of global warming the examining of the sensitivities of these physiological parameters could help in understanding the hierarchy in photosynthesis impairment and dysfunction during stress and in determining the threshold of temperatures inducing cell dysfunction [21]-[23].

2. Materials and Methods

2.1. Plant Materials

A total of six cultivars and recombinant in-bred lines (RILs) of *Lactuca sativa* variants were used: *L. sativa* (cv. Canasta), *Eruca sativa* (cv. Arugula); two RILs of lettuce population obtained from crosses between lettuce cultivars: "Mother" (*L. sativa* L. "Salinas") which is thermosensitive and "Father" (*L. serriola* accession UC96US23; [24]) which is thermotolerant. Seeds of the two RILs and their parental plants were obtained from the

Michel more lab (Genome Centre, UC Davis, USA) while seeds for the two commercial cultivars Canasta and Arugula were obtained from a commercial seed supplier (Known-You Seed Co., Taiwan).

2.2. Growth Conditions and Harvest Samples

After germination, seedlings that sprout were then inserted into water soaked sponge blocks and left in natural light to acclimate for 4 - 5 days before transplanting onto aeroponic systems. Full-strength Netherlands Standard Composition [25] nutrient solution (w/v%: K_2HPO_4 = 0.019; $MgSO_4$ = 0.061; K_2SO_4 = 0.025; KNO_3 = 0.029; $Ca(NO_3)_2{\cdot}4H_2O$ = 0.124; $[CH_2N(CH_2COO)_2]_2FeNa$ = 0.006; trace amount (<0.001 w/v%) of $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, H_3BO_3, $MnSO_4{\cdot}H_2O$, and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$; conductivity = 2.0 ± 0.2 mS and pH = 6.0 ± 0.5) maintained at target temperature ±1˚C was used [26]. Plants were grown in natural light with maximal photosynthetic photon flux density (PPFD) of 600 to 800 photon $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. While root zones temperatures were kept constant (see below), environmental temperatures experienced by the shoots ranged between 25˚C - 35˚C (min-max) while relative humidity ranged between 65% - 85% (min-max).

Three youngest mature leavers were harvested on day 25 from three different plants per treatment, except for productivity analysis where five whole plants were used per treatment group per species. All samples were harvested between 0700 hrs - 0900 hrs.

2.3. Chronic and Acute Heat Stress Manipulation

For chronic heat stress evaluation, four RZTs of 25˚C, 28˚C, 32˚C and 36˚C ± 1˚C were maintained after transplanting till parameter assessment. Chronic heat stress plants were examined for their shoot and root productivity, total reduced nitrogen (TRN), rate of O_2 evolution, light-saturated photosynthetic CO_2 assimilation rate (A_{sat}), stomatal conductance ($g_{s\ sat}$), transpiration and chlorophyll fluorescence parameters electron transfer rate (ETR), non-photochemical quenching (NPQ), photochemical quenching (qP) and F_v/F_m ratio.

For acute heat stress experiments, leaf discs of 1cm diameter were excised from each leaf and subjected to acute heat stress of 26˚C (control), 30˚C, 34˚C, 38˚C, 42˚C and 46˚C for one hr. Leaf discs were placed in petri dishes laid with two pieces of filter paper: the bottom layer was lightly infused with a 1M carbonate/bicarbonate buffer (pH 9) to provide saturating CO_2 conditions while the top layer was lightly dampened with deionized water upon which leaf discs were placed. The petri dish was then covered with a transparent plastic film and partially submerged into a water bath pre-heated to the target temperature under PPFD of 1000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. One hour after heat stress, O_2 evolution, ETR, NPQ, qP and F_v/F_m ratio were examined. For photosynthetic O_2 evolution, an extra set of leaf discs was incubated at 44˚C. These parameters were chosen for they would likely respond to a short term stress.

2.4. Shoot and Root Productivity

Shoot and roots were separated for fresh weight (FW) measurement. The roots of each plant were washed and dabbed dry before weighing.

2.5. Measurement of TRN

TRN was assessed via the Kjeldahl method. In gist, fresh leaf samples were dried in an over at 80˚C for 3 days after which 0.05g of dry sample were digested in 5 ml H_2SO_4 with the Kjeldahl tablet. The resultant mixture was then analysed for total N content with a Kjeltec auto 2300 analyser.

2.6. Measurements of A_{sat}, $g_{s\,sat}$ and Transpiration

Readings were taken between 0900 h to 1200 h in the greenhouse with an open infrared gas analysis system with a 6 cm^2 chamber (LI-6400, Biosciences, US). LED light source, which supplied 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PPFD were used. The light source emitted in the wavelength ranged between 420 to 510 nm and 610 nm to 730 nm. The spectral output of the light source has one peak centred at about 465 nm and second peak centred at about 670 nm. Average ambient [CO_2] and relative humidity in the chamber were 400 ± 3.5 $\mu mol \cdot mol^{-1}$ and 50% respectively. Measurements were recorded when both A_{sat} and $g_{s\,sat}$ were stable.

2.7. Measurements of Photosynthetic O_2 Evolution

Leaves harvested for O_2 evolution analysis were kept in a covered petri dish on top of a moist filter paper and left under a PPFD of 1350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ prior to analysis. Rates of maximum photosynthetic O_2 exchange were determined with a leaf disc O_2 electrode (Hansatech, King's Lynn, UK) under a PPFD of 1350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 25˚C at saturating CO_2 conditions (1% CO_2 from a 1 M carbonate/bicarbonate buffer, pH9) as described by [26].

2.8. Measurement of Chlorophyll Fluorescence F_v/F_m Ratio

F_v/F_m ratio was read off using a Plant Efficiency Analyser, PEA (Hansatech Instruments Ltd., England) after 15 minutes of dark adaptation.

2.9. Measurement of ETR, qP and NPQ

Leaf discs (1 cm diameter) were punctured and placed on moist filter papers in Petri dishes, pre-darkened for 15 min prior to measurements. Via the Imaging-PAM ChlFluorometer (Walz, Effeltrich, Germany), images of fluorescence emission were digitized within the camera and transmitted via a Firewire interface (400 megabits/s) (Firewire-1394, Austin, TX, USA) to a personal computer for storage and analysis. Measurements and calculations of qP, qN and ETR were determined as described by [27].

2.10. Statistical Analysis

For each physiological index obtained, each species was analysed separately with one-way analysis of variance and posthoc SNK tests. LT_{50}, or temperature at which index performance changed by 50% compared to that of control plants at 26˚C was calculated

by probit analysis. Significant difference in LT_{50} for each parameter of the six vegetables was scored if there was no overlap in the 95% confidence intervals. As F_v/F_m only started to show a decrease in observed value at 46˚C probit analysis could not be performed on this parameter.

3. Results

3.1. Chronic Heat Stress Responses

There were significant differences in shoot FW (Figure 1(A)) and root FW (Figure 1(B)) across all six species with increasing heat stress. A drastic drop in shoot FW was observed as early as 28˚C for Arugula ($F_{(3,19)} = 45.0$, $p < 0.001$), Canasta ($F_{(3,19)} = 24.4$, $p < 0.001$), RIL 141 ($F_{(3,19)} = 24.6$, $p < 0.001$), RIL 192 ($F_{(3,19)} = 59.9$, $p < 0.001$), "Father" (*L. serriola* accession UC96US23; $F_{(3,19)} = 72.3$, $p < 0.001$) and "Mother" (*Lactuca sativa* L. "Salinas"; $F_{(3,19)} = 80.9$, $p < 0.001$). A similar trend was observed in root FW of Canasta ($F_{(3,19)} = 12.2$, $p < 0.001$), RIL 141 ($F_{(3,19)} = 16.1$, $p < 0.001$), RIL 192 ($F_{(3,19)} = 30.1$, $p < 0.001$), "Father" ($F_{(3,19)} = 20.5$, $p < 0.001$) and "Mother" ($F_{(3,19)} = 36.2$, $p < 0.001$) where significant difference was detected at 28˚C, but for Arugula ($F_{(3,19)} = 19.4$, $p < 0.001$) this was only observed at 32˚C.

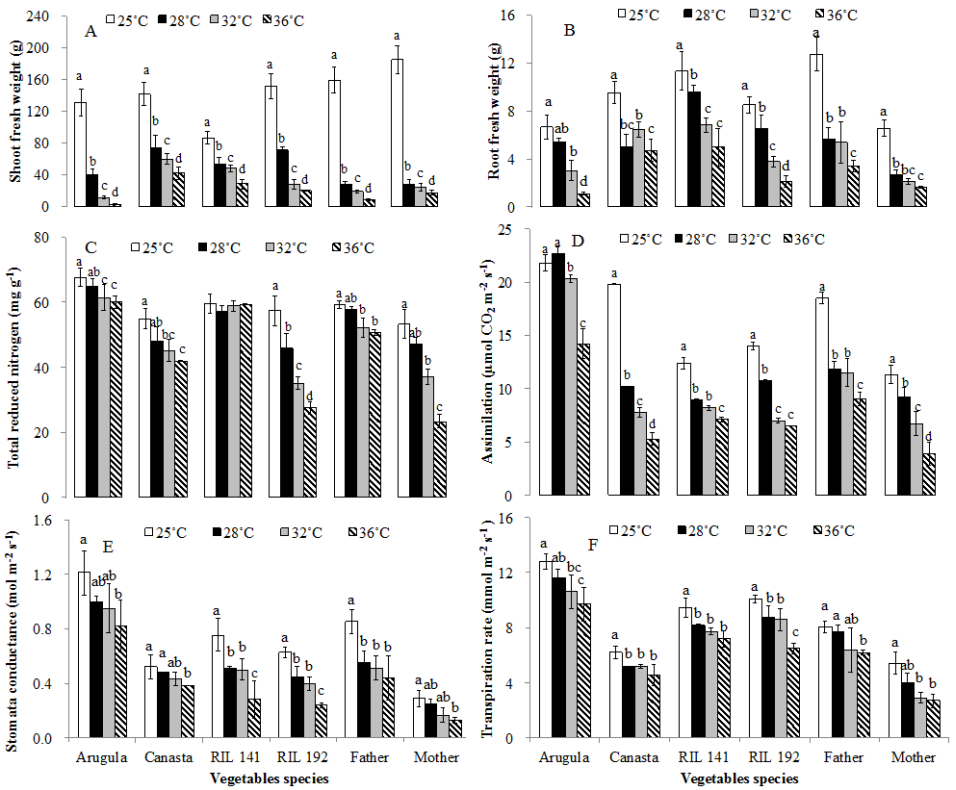

Figure 1. Mean ± SE plot of physiological response for the six vegetables with response to chronic heat stress. (A) Shoot fresh weight; (B) Root fresh weight; (C) Total reduced nitrogen; (D) Light-saturated photosynthetic CO_2 assimilation rate; (E) Stomatal conductance; (F) Transpiration rate. Different letter groups denote significant differences between treatment groups. Each species was analysed separately.

There was also a trend of decreasing TRN with increasing RZT (Figure 1(C)) for most of the examined vegetables. RIL 141 was the exception where no significant difference was observed for all heat treatments ($F_{(3,11)} = 2.61$, $p = 0.153$). Canasta ($F_{(3,11)} = 3.31$, $p = 0.027$), RIL 192 ($F_{(3,11)} = 5.901$, $p = 0.038$) and "Mother"($F_{(3,11)} = 232$, $p = 0.017$) had the most distinct dose response TRN decrease with heat. Conversely, although significant drop in TRN was observed in 32˚C Arugula ($F_{(3,11)} = 2.726$, $p = 0.044$) and "Father"($F_{(3,11)} = 7.88$, $p = 0.047$) relatively high TRN was still maintained at 36˚C compared to 25˚C plants.

There was a decrease in A_{sat} with response to increasing RZTs (Figure 1(D)). For Canasta ($F_{(3,11)} = 771.04$, $p < 0.001$), RIL 141 ($F_{(3,11)} = 46.3$, $p < 0.001$), RIL 192 ($F_{(3,11)} = 229.9$, $p < 0.001$), "Father" ($F_{(3,11)} = 22.8$, $p < 0.001$) and "Mother" ($F_{(3,11)} = 10.9$, $p < 0.001$) a significant decrease was observed in 28˚C RZT plants, but for Arugula ($F_{(3,11)} = 2.61$, $p < 0.001$), significant decrease in A_{sat} was only observed at 32˚C.

Similarly $g_{s\ sat}$ generally decreased with increasing RZTs (Figure 1(E)), but the decrease was only statistically significant for RIL 141 ($F_{(3,11)} = 3.55$, $p = 0.047$), RIL 192 ($F_{(3,11)} = 9.01$, $p = 0.006$) and "Father" ($F_{(3,11)} = 2.76$, $p = 0.012$).

Transpiration rate also generally decreased with increasing RZTs (Figure 1(F)). The decrease was, however, only statistically significant for Arugula ($F_{(3,11)} = 6.09$, $p = 0.018$), Canasta ($F_{(3,11)} = 4.13$, $p = 0.048$), RIL 141 ($F_{(3,11)} = 6.00p = 0.019$), RIL 192 ($F_{(3,11)} = 5.82$, $p = 0.021$), but not for the parental plants "Father" ($F_{(3,11)} = 1.18$, $p = 0.37$) and "Mother" ($F_{(3,11)} = 3.8$, $p = 0.057$).

3.2. Chronic vs Acute Heat Stress Responses

There were significant decreases in photosynthetic O_2 evolution across all six species with increasing heat stress for both the chronic treatment (Figure 2(A)) and acute treatment (Figure 2(B)). For chronic heat stressed plants, Arugula ($F_{(3,11)} = 9.74$, $p = 0..013$), "Father" ($F_{(3,11)} = 18$, $p = 0.003$) and "Mother" ($F_{(3,11)} = 1.59$, $p = 0..028$) all showed significant decrease in O_2 evolution at 28˚C RZT while Canasta ($F_{(3,11)} = 3.57$, $p = 0.035$), RIL 141 ($F_{(3,11)} = 5.3$, $p = 0.047$) and RIL 192 ($F_{(3,11)} = 3.455$, $p = 0.01$) only showed significant decrease of O_2 evolution at 32˚C RZT. For acute heat stressed plants, Canasta and the parental plants "Father" and "Mother" all showed a significant decrease in O_2 evolution compared to control plants (26˚C) at 34˚C (Canasta: $F_{(6,20)} = 87.8$, $p < 0.001$; "Father": $F_{(6,20)} = 24.9$, $p < 0.001$; "Mother": $F_{(6,20)} = 112.86$, $p < 0.001$). Photosynthetic O_2 production dropped significantly for RIL 192 at 38˚C ($F_{(6,20)} = 25.7$, $p < 0.001$) while Arugula (*Erucasativa*) had a significant decrease of O_2 evolution compared to control plants (26˚C) at 42˚C ($F_{(6,20)} = 17.4$, $p < 0.001$). Photosynthetic O_2 production dropped significantly at the high temperature of 44˚C for RIL 141 compared to the other five vegetables ($F_{(6,20)} = 60.1$, $p < 0.001$) while showing no significant differences in O_2 production from 26˚C to 42˚C.

No significant difference was detected for all chronic heat stress treatment groups across the six species for F_v/F_m ratio (Figure 2(C)). For acute stressed plants, F_v/F_m ratio for all six vegetables at 46˚C were all significantly lower compared to values obtained

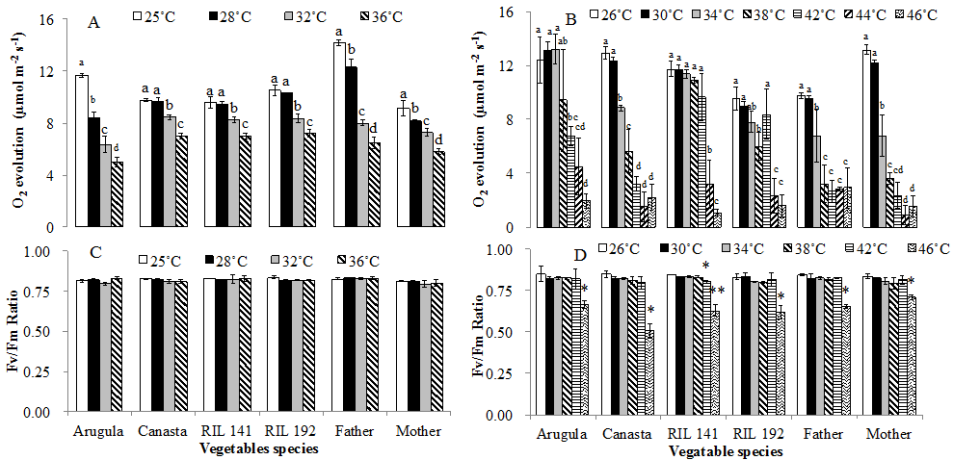

Figure 2. Mean ± SE plot of physiological response for the six vegetables with response to heat stress. (A) Photosynthetic O_2 evolution with chronic heat stress; (B) Photosynthetic O_2 evolution with acute heat stress; (C) F_v/F_m ratio for chronic heat stress; (D) F_v/F_m ratio for acute heat stress. Different letter groups (asterisk * for (D)) denote significant differences between treatment groups. Each species was analysed separately.

for control plants (**Figure 2(D)**, Arugula: $F_{(5,17)} = 12.5$, $p < 0.001$; Canasta: $F_{(5,17)} = 66.8$, $p < 0.001$; RIL 141: $F_{(5,17)} = 96.2$, $p < 0.001$; RIL 192: $F_{(5,17)} = 29.4$, $p < 0.001$; "Father": $F_{(5,17)} = 94.7$, $p < 0.001$; "Mother": $F_{(5,17)} = 12.8$, $p < 0.001$). Although there was also a significant drop in F_v/F_m ratio for RIL 141 at 42˚C compared to the control plants, the value obtained were still above the optimal value of 8.0.

All six vegetables showed a general decrease in ETR (**Figure 3**) with increasing heat stress.A similar trend was observed in NPQ (**Figure 4**) and qP (**Figure 5**), although the decrease in qP were more subtle for the 26˚C - 42˚C plants. Interestingly, for Arugula (**Figure 5(A)**) and Canasta (**Figure 5(B)**) at 46˚C, qP was markedly decreased, with qP being not detected at all in Arugula in spite of increasing light intensity. Conversely, qP of Canasta exposed to 46˚C heat stress approached zero only at light intensity above 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For RIL 141 and RIL 192, treatment groups at 46˚C had the lowest ETR and qP with increasing light intensity, but performance of both parameters were not significantly different from the 42˚C treatment groups for the two respective vegetables. NPQ for both vegetables, conversely, were significantly lower at 46˚C compared to the other treatment groups. There were almost no significant differences in ETR, NPQ and qP of "Father" among all the treatment groups (**Figure 3(E)**, **Figure 4(E)** and **Figure 5(E)**). ETR, NPQ and qP generally all decreased with increased heat stress for "Mother", and a flat lining of NPQ (**Figure 4(F)**) for vegetables exposed to 46˚C heat stress was prominent. A similar trend was observed for chronic stressed plants (data not presented).

Data for ETR, NPQ and qP at PPFD of 835 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was extracted and analysed. For chronic heat stressed plants, no significant difference was detected in ETR of "Father" for all treatment groups ($F_{(3,59)} = 2.084$, $p = 0.113$) and qP of "Mother" for all treatment groups ($F_{(3,59)} = 2.026$, $p = 0.121$). All other parameters for all treatment

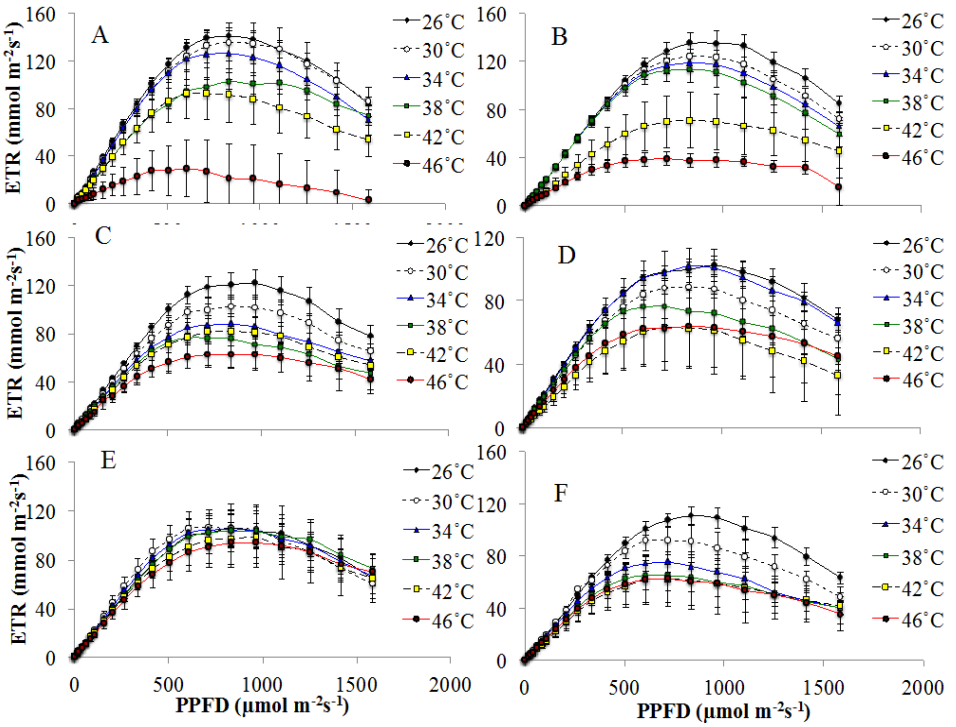

Figure 3. Mean ± SE plot of ETR light responsive curve. (A) Arugula; (B) Canasta; (C) RIL 141; (D) RIL 192; (E) "Father"; (F) "Mother".

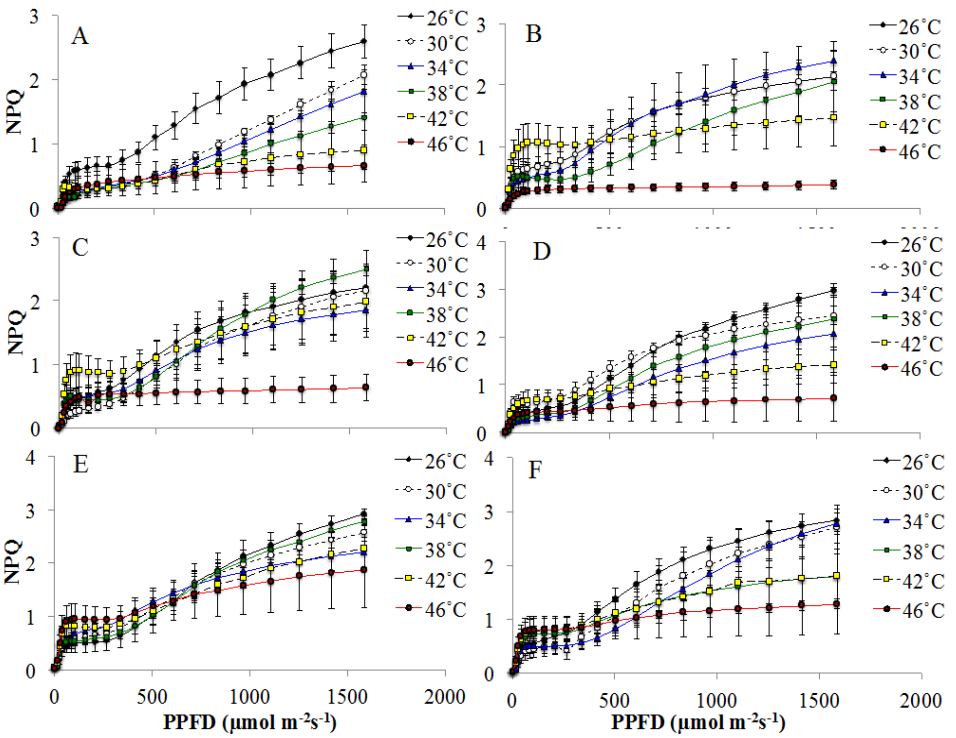

Figure 4. Mean ± SE plot of NPQ light responsive curve. (A) Arugula; (B) Canasta; (C) RIL 141; (D) RIL 192; (E) "Father"; (F) "Mother".

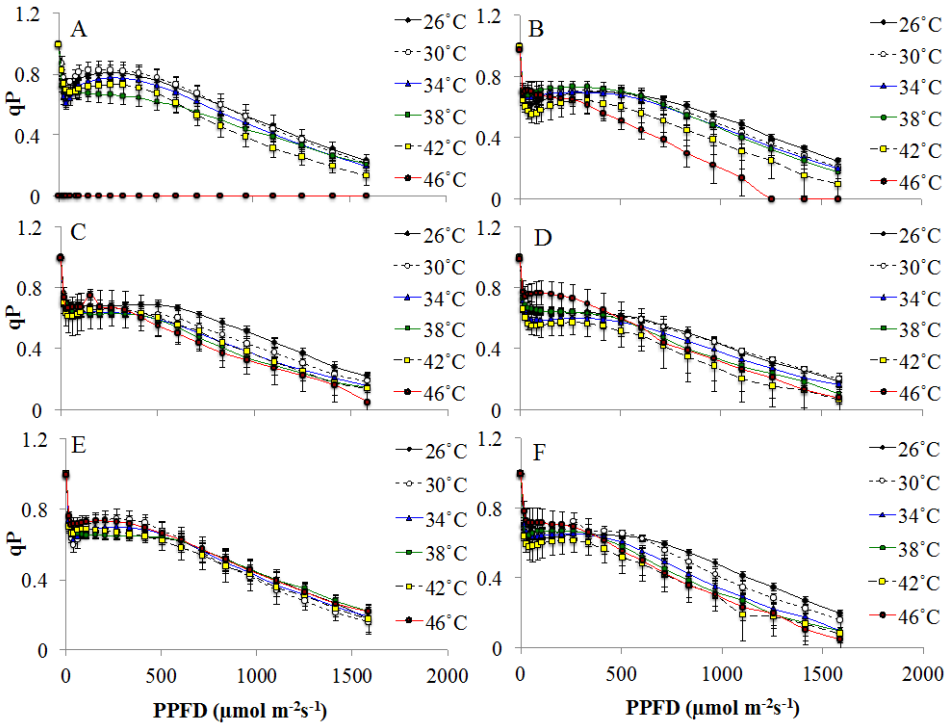

Figure 5. Mean ± SE plot ofqP light responsive curve. (A) Arugula; (B) Canasta; (C) RIL 141; (D) RIL 192; (E) "Father"; (F) "Mother".

groups showed significant decrease in ETR (Figure 6(A)), NPQ (Figure 6(B)) and qP (Figure 6(C)). For acute heat stressed plants, except for "Father", performance drops in ETR, NPQ and qP were observed with increasing heat stress for the other five vegetables (Figure 6(B), Figure 6(D) and Figure 6(F) respectively). There were no significant differences between "Father" treatment groups for ETR ($F_{(5,89)} = 1.8$, $p = 0.129$) and qP ($F_{(5,89)} = 2.5$, $p = 0.36$), but a significant drop in NPQ was observed for the 46˚C treatment group compared to control plants ($F_{(5,89)} = 3.2$, $p = 0.01$).

LT_{50}

For chronic heat stressed plants, shoot FW was significantly lower compared to all the other parameters (Figure 7(A)). LT_{50} of TRN for RIL 141 could not be resolved for there was no significant difference between the four treatment groups. Transpiration rates and TRN (except RIL 141) were significantly higher compared to the other parameters. In comparing O_2 evolution and chlorophyll fluorescence, chronic stressed plants tend to have lower LT_{50} values compared to acute stressed plants for the same temperature stress (Figure 7(B)). For acute heat stressed plants, while Arugula and RIL 192 had similar LT_{50} values for all four parameters, Canasta, RIL 192 and the two parental plant "Father" and "Mother" all had significantly higher NPQ and qP LT_{50} values compared to their respective O_2 evolution LT_{50} values (Figure 7(C)).

4. Discussion

This study reports the changes in physiological parameters of six temperate vegetables

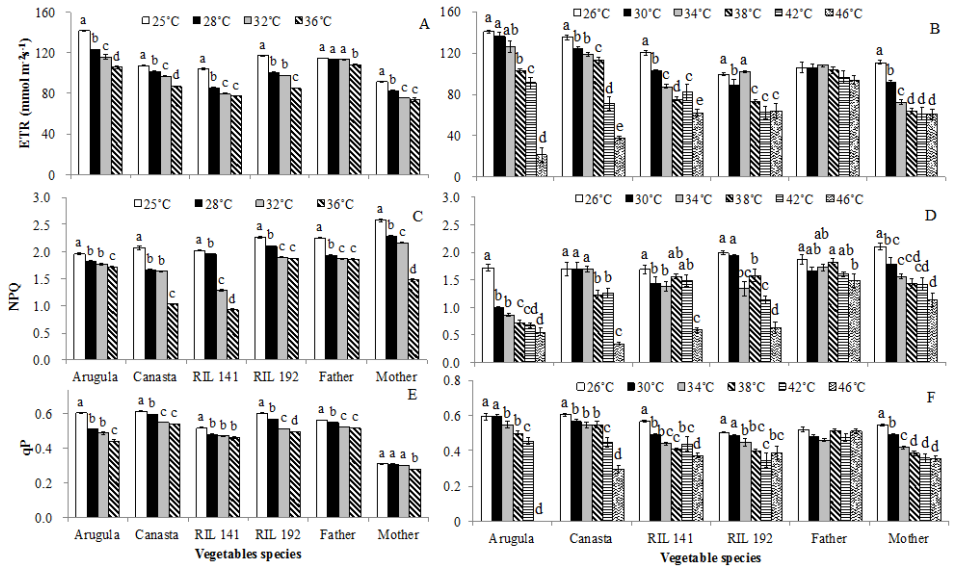

Figure 6. Mean ± SE plot of chlorophyll fluorescence for the six vegetables at 835 PPFD with response to heat stress. (A) ETR with chronic heat stress; (B) ETR with acute heat stress; (C) NPQ for chronic heat stress; (D) NPQ for acute heat stress; (E) qP for chronic heat stress; (F) qP for acute heat stress. Different letter groups denote significant differences between treatment groups. Each species was analysed separately.

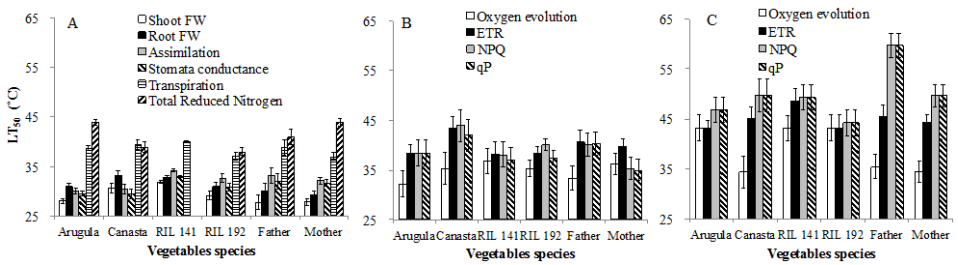

Figure 7. Mean ± 95% confidence interval LT_{50} of physiological parameters of the six vegetables with response to heat stress. (A) Chronic heat stress; (B) Chronic heat stress; (C) Acute heat stress. LT_{50} of each parameter is significantly different if the 95% confidence intervals do not overlap.

with response to chronic and acute heat stress. Except for F_v/F_m ratios for both the chronic and acute heat stressed plants, all parameters observed showed a general dose response decrease in functionality with response to increasing heat stress.

4.1. Chronic Stress Response

Generally plants grown in higher RZTs had lower shoot and root fresh weight, which has previously been reported by our team [26]-[31]. Productivity in the shoot FW was highly likely influenced by the decrease in A_{sat} with increasing RZT, which in turn was influenced by the decrease in $g_{s\ sat}$. Decrease in $g_{s\ sat}$ indicates a CO_2 decrease at the chloroplast level due to stomatal closure which could result in stomatal limitation of photosynthesis (e.g. [27] [31]-[33]). As such, this translates to reduced plant growth due to impairment of photosynthesis. Conversely, although TRN generally decreased

with increased RZT, the change was not as drastic in plants like Arugula, Canasta and "Father", although shoot FW were significantly reduced for these plants. These likely indicate an energy expenditure on maintaining TRN production for these plants despite the increased RZTs.

4.2. Chronic Stress Response vs Acute Stress Response

As shoot FW and TRN are unlikely to change with response to acute heat stress (<24 hrs) and excised leaf discs were too small to have their A_{sat} and $g_{s\,sat}$ measured, only O_2 evolution, F_v/F_m ratio, ETR, NPQ and qP were assessed for both chronic and acute heat stress responses. There was no significant difference in F_v/F_m ratio for either acute or chronic heat stressed plants across all six vegetables, showing that dynamic PSII photoinhibition was rather mild [27]. This is either due to the lower solar irradiation in the greenhouse (maximum PPFD *circa* 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for chronic stressed plants or that the one hr incubation period for acute stressed plants was not long enough to induce PSII photoinhibition for incubation temperatures below 42˚C. A significant decrease in F_v/F_m ratio was observed at 46˚C acute heat stress for all six vegetables. This photoinhibition was likely the reason for the flat line observed in NPQ 46˚C of Arugula, Canasta, RIL 141 and RIL 192 (**Figure 4**) and qP 46˚C of Arugula and Canasta (**Figure 5**).

O_2 evolution generally decreased at a lower temperature for chronic stressed plants compared to acute stressed plants. This agrees with the concept that effects of chronic stresses tend to be more complex than acute stress responses where strategies of energy metabolism and energy use efficiency are integrated into the time function for plant survival and daily maintenance of physiological processes [34]-[36]. This decrease in O_2 evolution was highly likely a function of the decrease in ETR for the six vegetables with increasing heat stress: ETR was significantly lower at 28˚C for chronic stressed plants for all six vegetables while acute heat stressed Arugula and RIL 192 only showed significantly lower ETR at 38˚C. While NPQ helps to protect damage to PSII [37]-[39], the decreasing NPQ with heat stress observed here represents a systemic failure of the plant to function optimally. The relative indifference in ETR, NPQ and qP responses of "Father" to increasing heat stress suggests a possible coping mechanism [40] although this might come at a cost to the plant [6] [7], for despite the TRN, ETR, NPQ and qP profiles staying relatively the same, O_2 evolution and shoot FW were all significantly reduced with higher RZTs (chronic) or incubation temperatures (acute).

4.3. Hierarchy of Stress Indices

It is proposed that thermal sensitivity is defined by capacity limitations at the highest level of organizational complexity [41], meaning that higher levels of functional integrations like growth will be disturbed or ceased under duress (pejus = getting worse thresholds, [13]) before cellular, molecular functions and metabolic complexes will become disturbed [41]. At more extreme temperatures, survival of organisms becomes a passive temporal function of energy budget reserve of the individual organism and a time function of protection of molecular functions by heat shock proteins and anti-

oxidative molecules as a last line of defence [42]. While much of that symmorphosis concept was based off the animal kingdom where oxygen limitation is the unifying principle for tolerance [43], it is evidential that a parallel could be drawn in defining thermotolerance of plants in this study.

Some of the parameters examined here have a definite causative and manifestation effect: shoot FW productivity is highly likely influenced by A_{sat}, which in turn is influenced by $g_{s\,sat}$. Looking at LT_{50} values of these parameters, except for Canasta, the other vegetables all had much lower LT_{50} for shoot FW than A_{sat}, and $g_{s\,sat}$, which conversely were very similar for all six vegetables. This suggests that while A_{sat}, and $g_{s\,sat}$ are very much related and on the same level of biological hierarchy, shoot FW represents a higher, more complex and integrated biological level, if not the highest in the hierarchy of plant stress indices. TRN and transpiration were both much lower in biological organization and thus had much higher LT_{50} values.

Generally LT_{50} values of chronic stressed plants were significantly lower than that obtained for the acute stressed plants. While acute stressed plants may activate "hardening" or coping mechanism (e.g. heat shock protein production) with response to sudden heat shock, chronic stressed plants would have a more normalized response to the long term heat stress taking into account available environmental resources and internal energy budgets for chaperon molecules and anti-oxidant defence activation [21] [41].

Interestingly, although significant decreases in ETR, NPQ and qP generally manifested at lower temperatures (28˚C - 38˚C) compared to O_2 evolution (34˚C - 44˚C), LT_{50} values of ETR, NPQ and qP were either similar to that of O_2 evolution or significantly higher for both chronic and acute heat stressed plants. Given that F_v/F_m ratios were only significantly lower than the other treatment groups at 46˚C (~10% drop) for acute stressed plants, it would be logical to predict that LT_{50} values of F_v/F_m ratios will approach that of NPQ and qP if not exceeding.

Among the photosynthetic parameters (chronic vs acute heat stress) measured in this study, photosynthetic O_2 evolution represents the highest level of integrated function for it measures the end production of oxygen produced as a result of photosynthesis. F_v/F_m ratio represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centres were open, and is thus essentially a measure of structural integrity of sub-cellular organelles. ETR, NPQ and qP measures functionalities of plant light collection structures where ETR and qP are measures of photosynthetic efficiency while NPQ measures the efficiency of heat dissipation [44]. In this setup where light intensity was kept at 835 $\mu mol \cdot m^{-2} \cdot s^{-1}$, decrease in ETR, NPQ and qP with increasing heat stress represents a capacity failure of PSII processes.

In this study, LT_{50} values of photosynthetic O_2 evolution is generally the lowest, with LT_{50} values of ETR, NPQ and qP in the intermediate range, followed by a hypothetical highest value of F_v/F_m ratio. This finding is in accordance with the heat tolerance principle put forth by [42] where it is hypothesized that capacity failure will first occur at the highest level of biological organization (photosynthetic O_2 evolution) followed by

cellular and sub-cellular processes (ETR, NPQ and qP), and structural integrity will be maintained as a last line of defence of organismal survivability (F_v/F_m ratio). Although it has always been advocated that the choice of the measuring parameter is important for a specific type of plant stress (e.g. [20] [45]), it is proposed here that considering the biological hierarchy of the index used might provide a bigger picture of heat response and energy budgeting of the studied plant in stress analysis.

4.4. Critical Temperatures and Thermal Windows

Species distribution are very much dependent on physical and biological limits where organismal fitness and physiological performance will be compromised near the edges of optimum living conditions [46]. Understanding how the factors define species ranges is crucial to our understanding of the response and fate of food crops in the face of climate change where food security and productivity would be adversely affected.

Arguably, while organismal mortality, pessimum (least favourable conditions, usually the switch from aerobic to anaerobic respiration) and pejus (=getting worse; compromise of physiological performances) may be difficult to pinpoint in the study of plant stress response (c.f. [47] [48]), it is proposed in this study here that for photosynthetic parameters, F_v/F_m ratio approaches the pessimum (CT_{max}: critical thermal maxima, see [49] [50]) if not the upper lethal limit of organismal survival while LT_{50} values of photosynthetic O_2 evolution could represent the onset of photosynthetic compromises (pejus) due to the increasing heat stress.

Arugula and RIL 192 had very similar LT_{50} values for all four acute heat stress parameters. It is likely that the optimal thermal windows for these two vegetable species are very close to their pessimum or upper thermal limit, and that these plants do not invest heavily into "hardening" strategies that allow for better chance of survivability in harsh living conditions. The fact that qP for Arugula at 46˚C was not detected throughout the light responsive curve further validates the possibility of pessimum conditions setting in at 46˚C or slightly beyond.

"Father" *Lactuca serriola is* on the other extreme: photosynthetic O_2 was affected at a very low temperature of around 35˚C, but NPQ and qP were maintained way beyond 50˚C. Generally described as a thermotolerant plant [24], this plant strategizes a lot more energy allocation into survival traits and process maintenance at the expense of somatic growth (photosynthesis) as evidential from the very similar light response curves of ETR, NPQ and qP with increasing heat stress in contrast with the significant drop in O_2 evolution.

5. Conclusion

It is a fundamental paradigm within thermal physiology that ectotherms have an optimal physiological temperature range [21]. In the context of global warming, knowing the pejus thermal threshold of an organism is ecologically more relevant than critical temperatures for this temperature demarcates the boundary between optimal physiological functioning and a compromise of Darwinian fitness. Arguably, survival beyond

the point of CT_{max} is a temporal function of the individual organism's energy store and physiological status prior to heat stress [40], illustrated here in the drop of F_v/F_m ratio signifying a total capability failure in photosynthesis and cessation of energy uptake. By defining the optimal, pejus and pessimum thermal threshold of the vegetable crops studied here, it is hoped that sub-lethal (hardening) heat shock regimes could be better designed to prime these vegetables to better tolerate heat stress with the imminent threat of a global climate change.

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