

# Heat Priming Impacts on Root Morphology, Productivity and Photosynthesis of Temperate Vegetable Crops Grown in the Tropics

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**Abstract**—Many plants have an inherent basal thermotolerance and they are able to survive temperatures over the optimum for growth and development. This project aimed to investigate if heat stress priming at the root-zone (RZ) could be used to induce thermotolerance through the studies of root morphology, productivity and photosynthesis of temperate vegetables aeronically grown in the tropics. Two high valued temperate vegetable crops were used for this study: *Lactuca sativa* (cv. Canasta) and *Eruca sativa* (cv. Arugula). Heat stress at the RZ priming was studied by exposing the plants to three different root-zone temperature (RZT) treatments: (1) 25°C-RZT, (2) 25°C-RZT→42°C-RZT (defined as non-hardening) and (3) 25°C -RZT →38 °C-RZT→42°C-RZT (defined as hardening). It was found that RZ heat stress priming did not have any negative effects on the root morphology for both vegetable crops. Compared to Arugula, Canasta had heat hardening effects which increased productivity at high RZT of 42°C with better photosynthetic performance. Since RZ heat hardening stimulated shoot growth of Canasta, this finding has practical significance on using RZ heat stress priming to induce thermotolerance of certain temperate vegetable crops grown in the tropics that could enhance productivity at low production cost.

**Index Terms**—heat priming, photosynthesis, productivity, root morphology, temperate vegetable, tropics

## I. INTRODUCTION

Heat stress due to global warming is of major concern worldwide, causing a loss of crop yield including vegetable crops [1]. Plants are sessile organisms that cannot escape heat [2]. In nature, adverse conditions are frequently interminable or repeating. Therefore, plants have evolved a variety of strategies to cope with extreme temperatures to minimise the damage [3]. One of such strategy is known as priming. Plants can be primed by heat stress, which enable them to survive temperatures that are lethal to a plant [4]. It was reported that heat priming could effectively improve heat tolerance to the later recurred heat stress in winter wheat [5].

Singapore is hot, humid and relatively uniform throughout the year with average daily temperature ranges from 25-34°C. The highest ambient temperature in

the greenhouse can reach up to 42°C for 4 to 5 hours on sunny days. Furthermore, Singapore is high population density country with limited agricultural land. Thus, Singapore has to import all her needs in temperate crops. Temperate vegetables such as lettuce (*Lactuca sativa* L.) has been grown successfully in Singapore using our innovate aeroponic systems by cooling their root zoon (RZ) while shoots were exposed to hot fluctuating ambient temperatures ranging from 26 °C to 42 °C [6]-[8] in a tropical greenhouse. Today, in Singapore, all kinds of subtropical and temperate vegetables are grown all year-round by simply cooling their RZ while their aerial portions are subjected to hot fluctuating temperatures [9], [10]. High energy costs when cooling the RZ of the vegetable crops, however, is now much of a concern. Identifying thermotolerant temperate crops would be one of the means to minimize the cost of production. On the other hand, plants have the ability to acquire thermotolerance, induced by subjecting them to a sublethal high temperature (“hardening”). The acquisition of high level of thermotolerance protects plants from a subsequent lethal heat stress [11]. Moderate heat stress primes a plant to withstand high temperatures that are deadly to non-acclimatized plants [12]. The ability of plants to tolerate high temperatures, without hardening is referred to as basal thermotolerance, whereas acquired thermotolerance refers to the adaptive capacity to survive lethal high temperatures, after priming [11].

Heat stress adversely affects seed germinating, plant growth and development, photosynthesis, respiration, water relations and membrane stability [13], [14]. Although priming of the entire plants, seeds and cell lines have been reported, there is very little information available on the effects of RZ heat hardening and its effect on root morphology and photosynthetic performances are relatively unexplored. Thus, the objectives of this project were (1) to determine if a sublethal heat priming in the RZ could induce thermotolerance in temperate crops grown in the tropics and, (2) to evaluate the effects of heat priming on root morphology and photosynthesis. In this study, during the RZ heat hardening period, that was, from 11 to 20 days after transplanting (DAT), root morphology such as total root length, total root surface area, average root diameter and total number of root tip were first studied to

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investigate if RZ heat hardening had any negative impact on root development. At harvest (30 DAT), fresh weight (FW) and dry weight (DW) of root and shoot were measured to determine productivity. Midday chlorophyll (Chl) fluorescence  $F_v/F_m$  ratio, photosynthetic pigments and light- and  $\text{CO}_2$ -saturated photosynthetic  $\text{O}_2$  evolution ( $P_{\text{max}}$ ) were also measured to investigate the effects on photosynthesis.

## II. MATERIALS AND METHODS

### A. Plant Materials and Culture Method

*Lactuca sativa* (cv. Canasta) and *Eruca sativa* (cv. Arugula) were used in this study. It is hereby simply referred to as Canasta and Arugula. The seeds were germinated on moist filter paper lining the trays for 3 days. The germinated seedlings were inserted into sponges and they were then transferred to the greenhouse where the seedlings were allowed another 3 days of establishment before transplanting to aeroponics systems [15]. The top of each growing trough was insulated by polystyrofoam planks on which the experimental plants were anchored. The roots of the plants were maintained at  $25^\circ\text{C}$  within sealed trough while aerial parts were exposed to the diurnal fluctuating ambient temperatures ( $26^\circ\text{--}40^\circ\text{C}$ ). All plants were supplied with full strength Netherlands Standard Composition. The composition of full strength nutrient solution in  $\text{mg l}^{-1}$  was:  $\text{K}_2\text{HPO}_4$ , 187;  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 1237;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 609;  $\text{K}_2\text{SO}_4$ , 252;  $\text{KNO}_3$ , 293;  $\text{FeEDTA}$ , 61.56;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.06;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.06;  $\text{H}_3\text{BO}_3$ , 0.59;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.73;  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 0.75. The nutrient solution was supplied to plant roots in the form of a mist at a frequency of a spray for one min at every 5 min interval. The electrical conductivity and pH of the nutrient solution were checked daily and kept at  $2.0 \pm 0.2 \text{ mS cm}^{-1}$  and  $\text{pH } 6.5 \pm 0.5$ , respectively. The average maximal photosynthetic photon flux density (PPFD) was about  $600 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ .

### B. Root-Zone Temperature (RZT) Treatments

For the entire growth cycle, the RZ of both species were subjected to the following treatments: (1)  $25^\circ\text{C}$ -RZT: plants were remained at  $25^\circ\text{C}$ -RZT for 30 day; (2)  $25^\circ\text{C}$ -RZT $\rightarrow$  $42^\circ\text{C}$ -RZT: Plants were grown at  $25^\circ\text{C}$ -RZT for 20 days and then transferred to  $42^\circ\text{C}$ -RZT for 6 hours daily from 1100 to 1700 for 10 days (defined as non-hardening); and (3)  $25^\circ\text{C}$ -RZT  $\rightarrow$  $38^\circ\text{C}$ -RZT $\rightarrow$  $42^\circ\text{C}$ -RZT: Plants were grown at  $25^\circ\text{C}$ -RZT for 10 days, then given a  $38^\circ\text{C}$ -RZT heat shock hardening from 1100 to 1700 h for 6 hours daily from 11 to 20 DAT and then exposed to  $42^\circ\text{C}$ -RZT for 6 hours daily from 1100 to 1700 for another 10 days (defined as hardening).

### C. Determination of Root Parameters

Ten days after transplanting, root morphology was analysed with WIN MAC RHIZO V 3.9 programme during the hardening period. The root region of each test plant was detached from the shoot and placed in a tray of water. The water enables the roots to be spread out and

helps to keep the roots moist. The roots were first scanned with WIN MAC RHIZO scanner before the total root length, total number of root tips, total root surface area and average root diameter were determined by the programme.

### D. Measurement of Shoot and Root FW and DW

At 30 DAT, plants were harvested randomly from each treatment between 0900h to 1000h and the polyurethane cubes were carefully removed. Shoots and roots were separated for FW measurement. The roots of each plant were washed and dabbed dry before weighing. After recording the FW, all tissues were wrapped in aluminum foil that were preweighed and then dried at  $80^\circ\text{C}$  for 4 days and then reweighed.

### E. Measurement of Midday Chlorophyll (Chl)

#### Fluorescence $F_v/F_m$ Ratio

Midday Chl fluorescence  $F_v/F_m$  ratios were measured between 1230h to 1330h from the attached leaves using the Plant Efficiency Analyser (Hansatech Instruments, UK) after 15 min of dark adaptation using the leaf clips. Dark-adapted leaves were placed under the light pipe and irradiated with the pulsed lower intensity-measuring beam to measure  $F_0$ , initial Chl fluorescence.  $F_m$ , maximum Chl fluorescence was assessed by 0.8 s of saturated pulse ( $>6000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). The variable fluorescence yield,  $F_v$ , was determined by  $F_m - F_0$ . The efficiency of excitation energy captured by open photosystem II (PS II) reaction centres in dark-adapted plant samples was estimated by the fluorescence  $F_v/F_m$  ratio.

### F. Measurements of Light- and $\text{CO}_2$ -Saturated Photosynthetic $\text{O}_2$ Evolution ( $P_{\text{max}}$ )

Photosynthetic  $\text{O}_2$  evolution rates were determined using a leaf disc  $\text{O}_2$  electrode (CB1D, Hansatech, King's Lynn, Norfolk, UK). Newly fully expanded leaves were harvested at 1000h. A leaf section was placed in saturating  $\text{CO}_2$  condition (1%  $\text{CO}_2$  from 1M carbonate/bicarbonate buffer, pH 9) as described by Ball *et al.* [16]. Leaf disc was illuminated at a PPFD of  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  at  $25^\circ\text{C}$  in the laboratory.

### G. Measurements of Chl and Carotenoids (Car)

Fresh leaf discs of 1 cm diameter were cut, and placed in an Eppendorf tube before freezing at  $-20^\circ\text{C}$  until analysis. Organic solvent, N,N-dimethylformamide (N,N-DMF, sigma chemical co.) of 1.5 ml was added to each Eppendorf tube with the single leaf disc and incubated in the dark for 48 hours at  $4^\circ\text{C}$  to extract the pigment. Absorbance was read at 647 nm, 664 nm and 480 nm to determine the amount of Chl a, Chl b and Car. The pigment contents were calculated according to the method of Welburn [17].

## III. RESULTS AND DISCUSSION

### A. Root Morphology

During the hardening period, changes of root morphology were analyzed from 11 to 20 DAT. In

general, total root length increased gradually in both vegetable species. On day 8 of hardening, there were no significant difference in total root length of both species between 25°C-RZT plants and 25°C-RZT→38°C-RZT plants (hardening plants) (Fig. 1A, Fig. 1B).

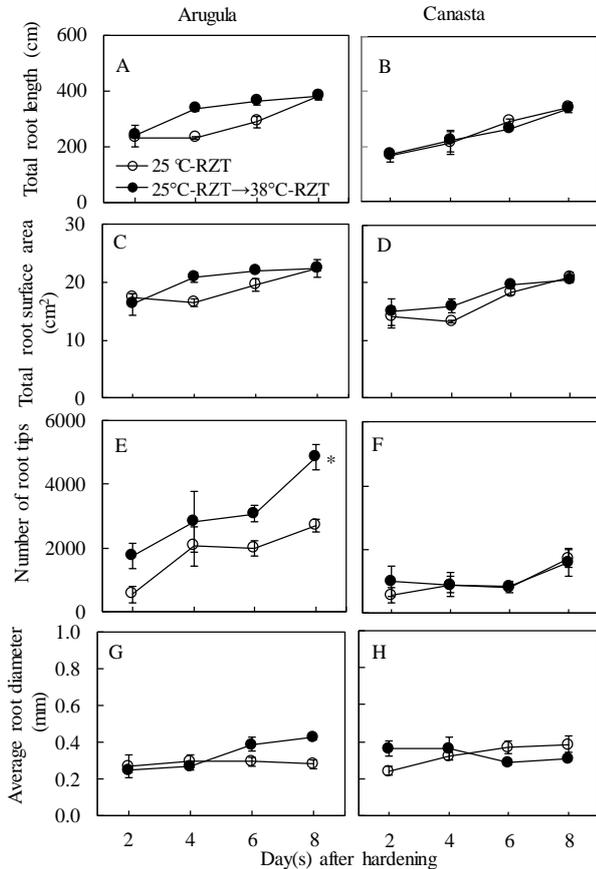


Figure 1. Changes of total root length (A, B), total root surface area (C, D), total number of root tips (E, F) and average root diameter (G, H) of Arugula (A, C, E, G) and Canasta, (B, D, F, H) during heat hardening period. Each point is mean ± SE (n = 3). Asterisk \* denote significant differences between treatment groups as determined by the Two-way ANOVA. Each species was analysed separately.

Similar trends were observed in total root surface area for both species (Fig. 1C, Fig. 1D). The number of root tips also increased gradually for Arugula with significant higher reading in 25°C-RZT→38°C-RZT than in 25°C-RZT plants (Fig. 1E). However, a very slight increases of the number of roots tip were also for Canata during the hardening period. However there was no significant differences in the total number of root tip in Canasta (Fig. 1F). The root diameter remained constant over the 10-day hardening period in both species (Fig. 1G, Fig. 1H).

The results shown in Fig. 1 imply that heat priming did not have a detrimental on the root morphology of both species. Our previous results showed that temperate lettuce of different cultivars grown under hot ambient-RZT had much smaller roots compared to those growth under cool-RZT [6], [7], [18], [19]. However, in this study, RZ heat hardening at 38°C during the hottest part of the day did not result in any detrimental effects compared to those grown at 25°C-RZT of both lettuce (*L. sativa* cv. Canasta) and *E. sativa* (cv. Arugula). It is

assumed that root growth may recover and even faster for RZ heat hardening plants during the night when the temperature was cooler. This was supported by the fact that the number of root tips for Arugula were greater in 25°C-RZT→38°C-RZT than in 25°C-RZT plants after heat priming for 8 days (Fig. 1E). Active root growth normally takes place during the night [20], [21]. It was reported that in the study with two cultivars of *Salvia splendens*, “Vista Red” and “Sizzler Red” subjected to short duration heat preconditioning for 3 hours a day for 3 weeks before subjecting to two high temperature conditions for another 3 weeks, hardening promoted root growth [22]. The heat tolerant “Vista Red” had greater root growth than the non-heat tolerant “Sizzler Red” [21]. Our studies with lettuce plants [18], [19], [23] and others with creeping bentgrass [24] showed that extensive root systems help to increase transpiration cooling by facilitating the movement of water, therefore, results in lower leaf temperature and hence heat tolerance .

B. Shoot and Root Productivity

Hardening treatment (25°C-RZT →38°C-RZT→42°C-RZT) resulted in higher shoot FW (Fig. 2A), higher shoot DW (Fig. 2B), higher shoot/root ratio FW (Fig. 2E) and higher shoot/root ratio DW (Fig. 2F) for Canasta compared to those of non-hardening Canasta plants (25°C-RZT→42°C-RZT). However, there were no significant differences in root FW (Fig. 2C) and root DW (Fig. 2D) for both species among the different RZT treatments. For Arugula, statistically, all plants had similar values of growth parameters regardless of RZT treatments.

□ 25°C-RZT ■ 25°C-RZT→42°C-RZT ▨ 25°C-RZT→38°C-RZT→42°C-RZT

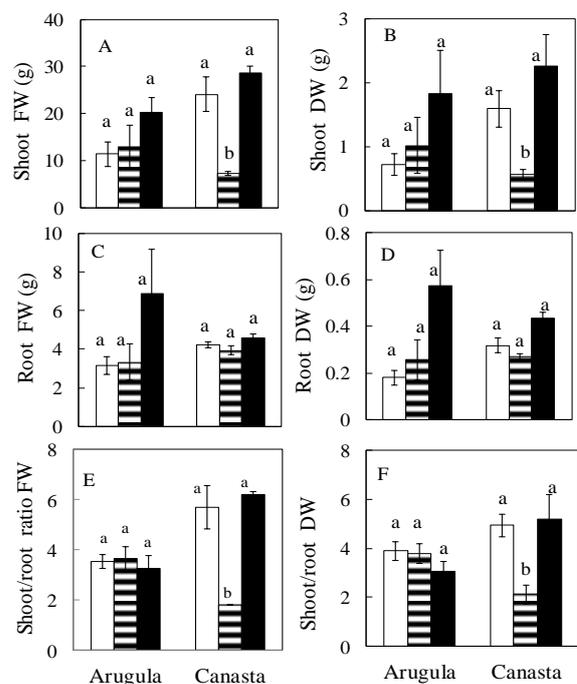


Figure 2. Shoot FW (A), shoot DW (B), root FW (C), root DW (D), shoot/root ratio FW (E) and shoot/root ratio DW (F) at harvest (30 DAT). Each point is mean ± SE (n = 3). Mean with different letters above the column are statistically different (P < 0.05) as determined by Tukey’s multiple comparison test.

It was reported that heat hardening improved the heat tolerance in *Salvia* cultivars [22]. Heat priming also enhanced the growth of creeping bentgrass, especially under salinity and high temperature [24]. It was reported that some plants produced heat shock proteins (HSP) in response to temperature that are not lethal to repair denatured proteins to maintain cellular functions and processes. It was observed that HSPs play important roles in heat tolerance, also known as thermotolerance [11]. The results of this study showed that acquiring thermotolerance helps to increase shoot and root productivity of Canasta (Fig. 2). Like other organism, it is well-known that plants have the ability to acquire thermotolerance within hours or days to survive lethal high temperatures [11]. However, effects of RZ heat hardening on productivity was not observed for Arugula (Fig. 2). This implies that the RZ heat hardening effects depends on species. The ability of plants to cope with the extreme temperature is a complicated process and is determined by the environmental factors as well as the genetic capability of the plant [11], [14].

### C. Photosynthesis

Studies of photosynthetic performances of the plants after different RZT treatments were carried out at harvest on 30 DAT. Although there were statistical differences observed in PS II activity measured by midday Chl fluoresces  $F_v/F_m$  ratio for both species (Fig. 3A), all plants had  $F_v/F_m$  ratio closer or greater than 0.8. Therefore, no photoinhibition had occurred in any plants grown in the greenhouse during the hottest part of the day [23], [25]. Furthermore, no significant differences were detected for  $P_{max}$  in laboratory among the treatment groups for both species due to the large standard errors (Fig. 3B).

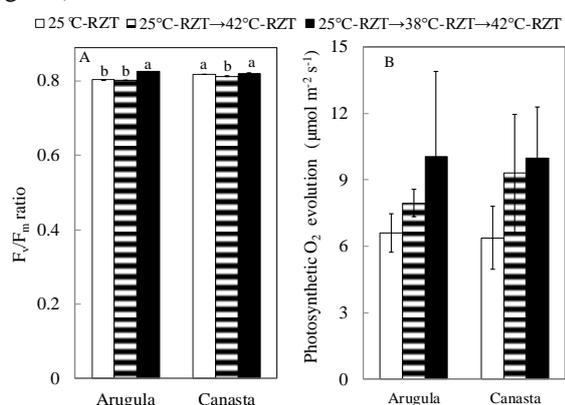


Figure 3.  $F_v/F_m$  ratio (A) and  $P_{max}$  (B) at 30 DAT. Each point is mean  $\pm$  SE (n = 3). No significant differences in both  $F_v/F_m$  ratio and  $P_{max}$  among the different RZT treatments ( $P > 0.05$ ) as determined by Tukey's multiple comparison test.

Photosynthetic processes are sensitive to heat stress [26]-[29]. At optimal physiological temperatures fluorescence originates mainly from Chl a of PS II that is an important tool for assessing photochemical efficiency of PS II. Chl fluorescence  $F_v/F_m$  ratio has been shown to correlate with heat tolerance. High temperatures influence thermotolerance of PS II [28]. In this study, the effect of

RZT temperature on the functioning of the thylakoid membrane was assessed by Chl fluorescence  $F_v/F_m$  ratio together with the determination of maximal  $O_2$  evolution,  $P_{max}$  after the different RZT treatments. In this study, high RZT of 42°C did not seem to have significant impact on  $F_v/F_m$  ratio (Fig. 3A) and  $P_{max}$  (Fig. 3B) in the late development stage from 20 DAT. However, based on the results shown in Fig. 3, the means of  $F_v/F_m$  ratio and  $P_{max}$  were slightly higher in RZ heat hardened plants of species. It was previously found by our research team that when the lettuce plants were transferred from hot ambient-RZT to cool-RZT,  $P_{max}$  increased significantly compared to that of hot ambient-RZT [23]. Similar results were reported in another study with subtropical vegetable, Chinese broccoli [25]. However, in this study all the plants had  $F_v/F_m$  ratio above 0.8 and hence no photoinhibition occurred in any plants. Results from this study also differed from previous results done by Lai and He [30] in which there were significant decreases in  $F_v/F_m$  ratio (Fig. 3A) and  $P_{max}$  for all the six temperate crops including the same species, Canasta and Arugula with increasing heat stress. In another study, we also found that the growth of *E. sativa* (Arugula) plants was adversely affected by hot ambient-RZT in a tropical greenhouse. Hot Ambient-RZT resulted in poor growth, stomatal and non-stomatal limitation of photosynthesis compared to 20°C-RZT [31]. An explanation could be that the plants undergone heat hardening increased photosynthesis to resist the effects of heat stress and compensate for effects from high RZT, such as respiration in the roots to resist the effects of heat stress. On the other hand, it could also be due to the fact that the effects of RZT on photosynthesis and growth of temperate and subtropical vegetable studied previously were different cultivars and the light intensity was also much higher in the previous studies compared to PPFD inside the greenhouse in the present study.

### D. Photosynthetic Pigment

Total Chl content was significant higher for the 25°C-RZT plants in Arugula compared to other RZT treatments. However, such a trend was not observed for Canasta. There were no significant differences in total Chl content among the treatments for Canasta (Fig. 4A). Similar trends were observed for total Car content (Fig. 4C). There were no significant differences in Chl a/b ratio (Fig. 4B) and Chl/Car ratio (Fig. 4D) among the different treatments for both species.

Under high temperatures, damages of Chl a and Chl b were more pronounced in developed leaves compared to developing leaves [32]. Such effects on photosynthetic apparatus were associated with the production of active oxygen species [33]. In this study, subjecting Arugula plants to high RZT of 42°C for 10 days during the late growth stage resulted in reduced Chl and Car contents compared to those plants grown under 25°C for the entire growth cycle (Fig. 4A, Fig. 4C.). However, decreases of Chl and Car contents under high RZT were not observed in Canasta. In tomato genotypes differing in their capacity for thermotolerance, an increased Chl a/b ratio

and a decreased Chl/Car ratio were observed in the tolerant genotypes under high temperatures, indicating that these changes were related to thermotolerance of tomato [34]. However, there were no differences in Chl a/b ratio and Chl/Car ratio of both species in the present study (Fig. 4B, Fig. 4D).

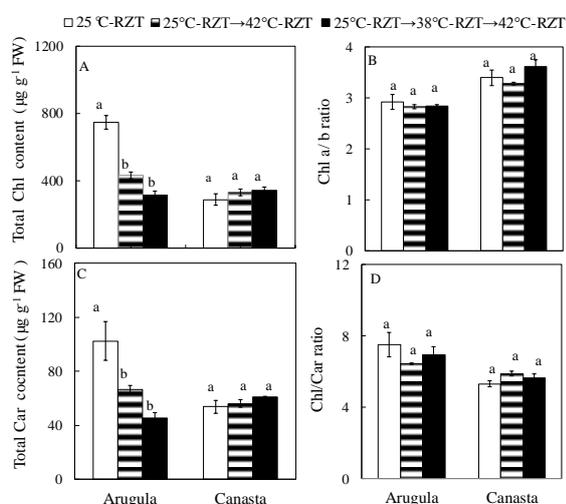


Figure 4. Total Chl content (A); Chl a/b ratio (B); total Car content (C) and Chl/Car ratio (D) of Arugula and Canasta at harvest. Each point is mean  $\pm$  SE (n = 3). Mean with different letters above the column are statistically different ( $P < 0.05$ ) as determined by Tukey's multiple comparison test.

The causes of RZ heat hardening of Canasta in this study is likely due to production of the HSPs after subjecting plants to sublethal high RZT (unpublished data). HSPs under heat stress is an adaptive defence mechanism which protects plants from injury and facilitate recovery and survival. Adaptive mechanisms which protect the cells from injury effects of heat stress are imperative in acquiring thermotolerance [35]. Another possible reason is that stress proteins is an important acclimation to cope with the temperature stress. A large portion of the stress proteins are solvent in water and in this way, contribute to stress tolerance by means of hydration of cellular structures [14]. Although HSPs are exclusively involved in heat-stress responses, other proteins are also additionally included. Moreover, the heat tolerant traits were enhanced with heat hardening concur with the study of *Salvia splendens* [22] where the higher tolerance levels of the heat resistant "Vista Red" increased its productivity. It is hinted that the Canasta therefore attained higher biomass accumulation to compensate for the heat stress loss, therefore being able to increase their productivity. These plants were able to maintain healthier leaves and supply photoassimilates to reproductive development and root growth. This coincides with the study that plant responses to hardening vary with the extent of temperature increase, its duration and the type of plant [14]. Similar acquired thermotolerance has been found in both woody and herbaceous plant species. It was reported that heat hardening in redbud (*Cercis canadensis* L.) seedlings showed an increase in leaf thickness and plant growth [36]. Seedling survival and recovery growth also

increased after priming in seedlings of sunflower (*Helianthus annuus*) [37].

#### IV. CONCLUSION

Temperature stress is exceptionally fluctuating in nature. Sub-lethal heat shock (priming) in the RZ had proved that it can induce thermotolerance in certain temperate crops. Therefore, effects of RZ heat hardening are beneficial for certain vegetable species such as the Canasta in this study. For Canasta, RZ heat priming resulted in higher productivity. However, the effects of RZ heat hardening on photosynthetic performance were not significant. As this is a preliminary study on the effects of RZ heat priming in the physiological changes of developing vegetables, further studies can be made by including other factors such as light.

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