

# Differential Responses of Pak Choi and Edible Amaranth to an Elevated Temperature

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**Abstract.** Global surface temperatures are predicted to increase by 1 to 4 °C by the year 2100. To unravel the risks from rising temperature to Taiwan's summer leafy vegetable production, the phenotypical and physiological responses of two leafy crops, pak choi (*Brassica chinensis* L. cv. Quanzhou) and edible amaranth (*Amaranthus tricolor* L. cv. White leaf), were compared under an elevated temperature. A temperature increase from 28 to 32 °C resulted in lower leaf calcium, magnesium, and manganese concentrations (dry weight basis) in pak choi without significant changes in shoot dry weight, suggesting potential negative effects of the elevated temperature on pak choi leaf nutrient status. However, increased temperature promoted both root and leaf growth in edible amaranth, which may be beneficial to its yield, making edible amaranth a potential summer leafy vegetable crop for Taiwan. Furthermore, a temperature change from 28 to 32 °C resulted in a higher leaf nitrate concentration in edible amaranth, because of the lower nitrate reductase activity (NRA). Thus, suitable nitrogen fertilization rates and programs under elevated temperature conditions should be reconsidered in the future. To sum up, a future rise in summer temperatures may impose negative impacts on pak choi leaf nutrient status but positive impacts on edible amaranth production.

Daily intake of vegetables is known to be beneficial to human health, but the availability of vegetables is at risk because of the current trend of global warming. Vegetable production is greatly influenced by environmental factors, such as light intensity, temperature, and ambient CO<sub>2</sub> concentration. Furthermore, past research has revealed that different crop species may respond differently to elevated temperatures. For example, Lara and Andreo (2011) indicated that C4 plants show higher photosynthesis and growth rates under high light intensity and high temperature conditions. Although the effects of elevated temperatures on warm-season C4 cereals and weeds are widely studied (Crafts-Brandner and Salvucci, 2002; Du et al., 2009; Pompeiano et al., 2013; Wang et al., 2016), the effects of elevated temperatures on C4 vegetables such as amaranth remain largely unknown. There is an urgent need to study the physiological responses of C4 vegetables under elevated temperature conditions to fill these knowledge gaps.

Summer vegetable production in Taiwan is quite vulnerable to typhoons, pests, and diseases. After a natural disaster that causes a shortage of fresh vegetables, leafy vegetables

are normally the first to return to markets because of their short growth from seeding to harvest. Because C3 and C4 plants have been reported to respond differently to elevated temperatures, we selected one widely grown C3 summer leafy vegetable (pak choi, aka nonheading Chinese cabbage) and one important C4 summer leafy vegetable (edible amaranth) for this study. A recent study on the effects of sudden increase in temperature on photosynthesis showed that net photosynthesis of Chinese cabbage is decreased when leaf temperature is greater than around 25 °C (Oh et al., 2015). Furthermore, the effect of long-term elevated temperatures and CO<sub>2</sub> on Chinese cabbage was cultivar dependent (Choi et al., 2011). The effects of elevated temperatures on Chinese cabbage root growth and leaf nutrient status were not documented in the above-mentioned studies. However, a recent study reported that some common foods including vegetables could serve as natural sources of antioxidants if they possess a high phenolic content (Kamath et al., 2015). It is of interest to know how elevated temperatures may influence the total phenolic compounds (TPCs) in leafy vegetables. In addition, nitrate content in leafy vegetables is a major concern for vegetable consumers, especially in countries such as Taiwan where consumers prefer eating leafy vegetables. Previous reports indicated that nitrate may turn into nitrite that may then react with some amines or amides to form nitrosamines, which are known to be carcinogenic (Bruning-Fann and Kaneene, 1993; Magkos et al., 2006). Thus, it is important to

investigate how elevated temperatures may influence the levels of nutrients, TPCs, and nitrate in leafy vegetables such as pak choi and amaranth.

Leafy vegetables are cultivated extensively in central Taiwan where the climate is subtropical. Results from this study allow us to compare the root growth and the physiological responses of leaves of pak choi (a C3 vegetable) and edible amaranth (a C4 vegetable) under current (28 °C) and elevated (32 °C) temperatures and to further provide relevant information that can lead to the development of strategies to cope with the effect of elevated temperatures on leafy vegetable production in Taiwan and other subtropical regions.

## Materials and Methods

*Plant materials and growth conditions.* Seeds of pak choi (*B. chinensis* L. 'Quanzhou') and edible amaranth (*A. tricolor* L. 'White leaf') were collected from local farmers in Yunlin County, Taiwan. Two growth chambers (Model LBG-500; Lead-Biotech Instruments Co., Ltd., Taichung City, Taiwan) were set at 28 ± 0.2 °C and 32 ± 0.2 °C to mimic the current and predicted mean summer surface air temperatures of central Taiwan, a subtropical area where leafy vegetables are widely cultivated. The tested vegetables were grown in the growth chambers from seeds. Plants were illuminated with fluorescent light [70 μmol·m<sup>-2</sup>·s<sup>-1</sup> photosynthetically active radiation (PAR)] under a day/night cycle of 13 h/11 h. The daytime average relative humidity (RH) inside the growth chambers was determined using an HOBO-U23-001 data logger (Onset Computer Corp., Pocasset, MA).

*Measurement of root growth.* Pak choi and amaranth seeds were soaked in tap water for 1 d and then planted at the bottom of a triangular trough made from a rectangular filter paper. The rectangular filter paper was then placed on the surface of a vertical plastic board with its bottom soaking in a plastic container filled with double-distilled water. For each species and each temperature tested, a set of 18 seeds were evenly planted on three vertical plastic boards (six seeds/board). This set was considered one replicate, with a total of three replicates. Root length was recorded daily for a period of 15 and 13 d. The roots of the seedlings were harvested to determine the root fresh weight (FW) and oven-dried at 70 °C for 5 d for the dry weight.

*Analysis of phenotype and leaf mineral elements, TPCs, nitrate concentrations, and NRA.* Seeds of pak choi and amaranth were planted in 9-cm × 7-cm (diameter × height) plastic pots containing Potgrond H substrates (Klasmann-Deilmann GmbH, Geeste, Germany) at a rate of three seeds per pot. The pots were set into a growth chamber at 28 or 32 °C. Plants were thinned to one plant per pot at 5 d after sowing (DAS) and fertilized with 1000-fold-diluted Hyponex solution (N:P:K = 20:20:20) (Hyponex Japan Co., Ltd., Osaka, Japan) at weekly intervals. For each species tested, a set of 15 pots with a total of

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three sets were planted in each growth chamber. From each set, six pots/plants were randomly selected for phenotypic analysis, and then, leaf samples derived from these plants were subjected to the measurement of leaf mineral elements; furthermore, leaf samples required to determine the concentration of TPCs, the nitrate concentration, and NRA were collected from the rest nine pots/plants. This set was considered one replicate, with a total of three replicates. Pak choi was harvested at 36 DAS, and amaranth was sampled at 37 DAS. Phenotypic analysis including measurement of plant height, stem diameter, leaf length (petiole plus blade length), leaf blade length, leaf width, specific leaf weight (leaf dry weight/leaf area) of the second mature leaf, leaf area, shoot FW, and shoot dry weight on a per-plant basis. The measurement of plant mineral elements was performed according to the methods previously described in Hwang et al. (2015), except that plant samples were used instead of substrate samples. The concentration of TPCs was determined by the method of Keith et al. (1958). Caffeic acid (Sigma-Aldrich, St. Louis, MO) was used to construct the calibration curve. The concentration of TPCs was then calculated and expressed as milligrams of caffeic acid equivalent per 100 g of FW (mg/100 g FW). The nitrate concentration was measured using an RQflex 10 reflection photometer (Merck, Tokyo, Japan). Nitrate reductase activity was analyzed following the method of Jaworski (1971). A KNO<sub>2</sub> solution was used as a standard solution to determine sample NRA (μmol/h/g FW).

**Statistical analysis.** For all measurements in each species, the significance of the difference between two temperature treatments was determined by calculating sample mean values and SD and analysis of *t* test using CoStat 6.2 (CoHort Software, Berkeley, CA).

## Results and Discussion

**Effect of elevated temperatures on root growth of pak choi and edible amaranth.** In pak choi, elevated temperatures increased root length but did not significantly affect root FW, root dry weight, or number of lateral roots. By contrast, edible amaranth roots grew more rapidly and longer as well as had greater root FW, root dry weight, and lateral root number at 32 °C relative to 28 °C (Table 1; Fig. 1). These results suggested that a temperature rise benefits young seedling root growth and development in the C4 plant, amaranth, but not in the C3 plant, pak choi—a cool-season vegetable. Koscielny and Gulden (2012) suggested that root development provides more relevant information than does shoot development in the early seedling stage, when root growth dominates shoot growth. Moreover, a recent study indicated that early seedling root growth is closely related to yield in wheat (Xie et al., 2017).

**Phenotypic analysis of pak choi and edible amaranth under elevated temperature condition.** Plants were grown to reach harvest

Table 1. Effect of elevated temperatures on root phenotypes of pak choi and edible amaranth.

Vegetable	Temp (°C)	Fresh wt (mg)	Dry wt (mg)	Length (cm)	NLR/plant <sup>z</sup>
Pak choi	28	10.85 ± 0.38	1.03 ± 0.09	5.64 ± 0.294	28.7 ± 2.11
	32	10.27 ± 0.25	0.94 ± 0.13	7.33 ± 0.905	29.6 ± 0.87
	<i>t</i> test	0.0915	0.4058	0.0376	0.5246
		NS	NS	*	NS
Edible amaranth	28	4.91 ± 0.7	0.27 ± 0.12	3.36 ± 0.112	0.14 ± 0.096
	32	9.39 ± 0.59	0.61 ± 0.06	8.42 ± 0.909	3.24 ± 0.788
	<i>t</i> test	0.0011	0.0098	0.0007	0.0025
		**	**	***	**

Values represent the mean ± SD. The measurement is taken from 15-d-old pak choi seedlings and 13-d-old edible amaranth seedlings.

<sup>z</sup>Number of lateral roots/plant.

<sup>y</sup>*P* from *t* test: NS, \*, \*\*, \*\*\* representing nonsignificant or significant at *P* < 0.05, 0.01, or 0.001, respectively.

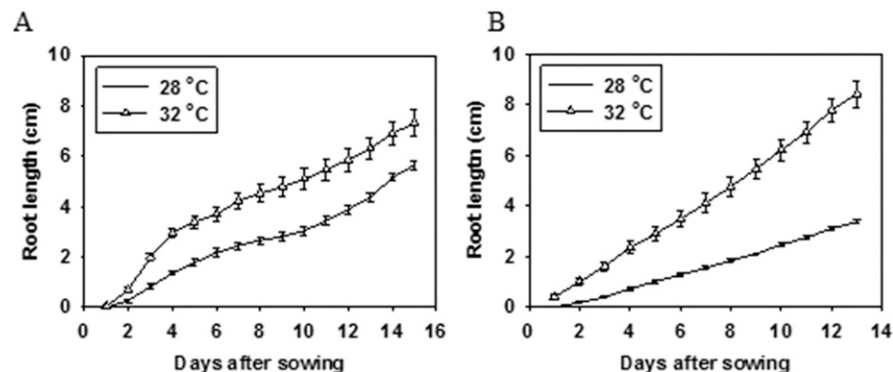


Fig. 1. Effect of elevated temperatures on the root growth of pak choi and edible amaranth. (A) Root length of pak choi grown at 28 and 32 °C air/ambient temperature. (B) Root length of edible amaranth grown at 28 and 32 °C air/ambient temperature. The error bars represent the SE of the measurements.

maturity. Pak choi grown at 28 °C showed a trend toward longer and larger leaves than that grown at 32 °C by the 36th DAS. However, there was no significant difference in plant height, stem diameter, leaf blade length, leaf width, specific leaf weight, number of leaves per plant, shoot FW per plant, and shoot dry weight per plant between both sets of plants (Tables 2 and 3). Oh et al. (2015) showed that net photosynthesis in Chinese cabbage (*Brassica campestris* subsp. *napus* var. *pekinensis*) is reduced when the temperature exceeds 25 °C, partially because of the associated high respiration rate under high temperatures. Interestingly, this study found no significant differences in most plant phenotypes (e.g., shoot FW and dry weight) between pak choi (*B. chinensis* L. ‘Quanzhou’) grown at 28 and 32 °C (Tables 2 and 3), suggesting that net photosynthesis did not decrease at 32 °C compared with 28 °C. This discrepancy may be due to a lack of difference in day/night temperatures in this study or owing to differential temperature responses between Chinese cabbage and pak choi. However, more research is needed to confirm this assumption.

However, enhanced leaf growth was observed at 37 DAS in edible amaranth cultivated at 32 °C compared with that grown at 28 °C (Tables 2 and 3). In addition to the enhanced seedling root growth (Table 1; Fig. 1B), amaranth grown at 32 °C showed greater plant height, stem diameter, leaf length, leaf blade length, leaf width, leaf number, leaf area, shoot FW, and shoot dry

weight per plant than plants grown at 28 °C (Tables 2 and 3). These results suggest that an elevated temperature exerts a positive effect on the growth of this C4 edible crop. It is generally believed that C4 plants have a higher optimum temperature for photosynthesis; however, inhibition of net photosynthesis can be observed when leaf temperature is greater than 38 °C (Berry and Björkman, 1980; Crafts-Brandner and Salvucci, 2002). It seems that the upper temperature range used in this study (32 °C) is not exceeding the optimal range for C4 photosynthesis in edible amaranth. Furthermore, previous research demonstrated that photorespiration losses in C4 plants are limited and that C4 plants have higher net photosynthetic rates at higher temperatures compared with C3 plants (Long, 1999). Farquhar et al. (1980) proposed a mathematical leaf model to describe C3 photosynthesis, and this model has been used to predict that C4 plants grown under the earth’s current CO<sub>2</sub> concentration have higher net photosynthetic rates than C3 plants when temperatures are greater than 22 °C (Collatz et al., 1998; von Fischer et al., 2008). Taken together, the results from this study suggest that a rise in the summer mean temperature from 28 to 32 °C caused by climate change has no immediate negative effect on the shoot growth of the C3 crop pak choi, but it may be beneficial to the shoot growth of the C4 plant edible amaranth. Nonetheless, it is plausible that the combined effect of higher temperatures under ambient light levels would result in greater oxidative

Table 2. Effect of elevated temperatures on shoot phenotypes of pak choi and edible amaranth.

Vegetable	Temp (°C)	Plant ht (cm)	Stem diam (mm)	Leaf length (cm)	Leaf blade length (cm)	Leaf width (cm)	Specific leaf wt (mg·cm <sup>-2</sup> )
Pak choi	28	19.9 ± 0.7	3.2 ± 0.4	19.2 ± 0.3	12.1 ± 0.4	9.6 ± 0.1	1.09 ± 0.17
	32	19.8 ± 0.5	2.7 ± 0.2	18.1 ± 0.4	11.4 ± 0.1	9.2 ± 0.3	1.04 ± 0.14
<i>t</i> test		0.94	0.13	0.02	0.06	0.11	0.75
<i>P</i> <sup>2</sup>		NS	NS	*	NS	NS	NS
Edible amaranth	28	13.9 ± 0.2	1.9 ± 0.1	8.5 ± 0.4	6.3 ± 0.3	4.1 ± 0.05	1.19 ± 0.03
	32	23.1 ± 1.7	3.2 ± 0.2	11.9 ± 1.2	8.4 ± 0.9	5.3 ± 0.3	1.27 ± 0.08
<i>t</i> test		0.0007	0.0010	0.0010	0.0193	0.0094	0.19
<i>P</i>		***	**	**	*	**	NS

Values represent the mean ± sd. The measurements were taken from the second matured leaves at 36 d after sowing (DAS) for pak choi and 37 DAS for edible amaranth.

<sup>2</sup>*P* from *t* test: NS, \*, \*\*, \*\*\* represented nonsignificant or significant at *P* < 0.05, 0.01, or 0.001, respectively.

Table 3. Effect of elevated temperatures on number of leaves (LN), leaf area, shoot fresh weight, and shoot dry weight on a per-plant basis of pak choi and edible amaranth.

Vegetable	Temp (°C)	LN/plant <sup>2</sup>	Leaf area (cm <sup>2</sup> )/plant	Shoot fresh wt (g)/plant	Shoot dry wt (g)/plant
Pak choi	28	8.2 ± 0.5	330.8 ± 13.7	14.1 ± 1.3	0.56 ± 0.11
	32	8.8 ± 0.5	277.8 ± 16.5	12.0 ± 1.0	0.44 ± 0.06
<i>t</i> test		0.19	0.01	0.09	0.17
<i>P</i> <sup>2</sup>		NS	*	NS	NS
Edible amaranth	28	6.2 ± 0.2	49.9 ± 3.1	1.4 ± 0.1	0.08 ± 0.01
	32	10.1 ± 0.3	129.8 ± 12.6	4.2 ± 0.6	0.24 ± 0.04
<i>t</i> test		0.00003	0.0004	0.0011	0.0021
<i>P</i>		***	***	**	**

Values represent the mean ± sd. The measurements were taken at 36 d after sowing (DAS) for pak choi and 37 DAS for edible amaranth.

<sup>2</sup>Number of leaves per plant.

<sup>2</sup>*P* from *t* test: NS, \*, \*\*, \*\*\* represented nonsignificant or significant at *P* < 0.05, 0.01, or 0.001, respectively.

stress levels, and possibly growth effects in the C3 species, than under the relatively lower light levels used in this study. Thus, if the higher light levels × higher temperatures do have an additive oxidative stress effect on the C3 species, a negative effect on the shoot growth of the C3 crop pak choi may occur when the summer mean temperature rises from 28 to 32 °C. By contrast, it is well established that C4 photosynthesis demands more energy relative to C3 photosynthesis; thus, the photosynthetic rate of C4 edible amaranth was more vulnerable than C3 pak choi under the low light conditions in this experiment because less adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) were likely synthesized. It is possible that the positive growth response of C4 edible amaranth to future elevated temperatures under field conditions would be even more striking than that reported in this study, where a light intensity of 70 μmol·m<sup>-2</sup>·s<sup>-1</sup> PAR was applied. Therefore, future studies should evaluate the effect of temperature rise on leafy vegetable production under ambient light levels. Furthermore, it is worth noting that summer leafy vegetable production in Taiwan normally uses plastic houses and that daily noon temperatures exceed 32 °C. Future research should include effects of extreme high temperatures and of warmer nighttime temperatures on leafy vegetable production.

Another aspect that requires further attention is that the RH inside the growth chambers was not controlled, with a daytime average value of around 46.1% ± 2.1% and 44.8% ± 1.8% at 28 and 32 °C, respectively. It seems that each growth chamber reaches its own equilibrium vapor pressure at ambient

temperatures of 28 or 32 °C with minor fluctuations during the day. Compared with the weather data provided by the Central Weather Bureau of Taiwan, the RH inside the growth chambers was lower than the ambient outdoor summer conditions in central Taiwan where the daytime RH is greater than 50%, and the daily average RH is around 80%. Tibbitts and Bottenberg (1976) indicated that leaf number, size, dry weight, and water content of butterhead lettuce all increase under 85% RH than under 50%. Similarly, Ben-Asher et al. (2013) reported that dry matter and water use efficiency in maize were higher under 85% RH than under 30%. Thus, future research should investigate the interaction between RH and photosynthetic rates in pak choi and edible amaranth. We recognize that we have conducted our experiments in a controlled environment with respect to atmospheric conditions and nutrition. In reality, however, environmental conditions outdoors in the field are considerably different where atmospheric feedback mechanisms are continually causing changes in the environment (e.g., varying temperature, humidity, and wind). Therefore, open-field studies are required to corroborate the results observed in the current study.

*Effects of elevated temperatures on pak choi and amaranth leaf nutrient status, nitrate concentration, and NRA.* The concentrations of macronutrients and micronutrients in pak choi were compared between plants grown under 28 and 32 °C. Pak choi leaves grown at 32 °C contained significantly lower concentrations of calcium (reduced by 0.51%), magnesium (reduced by 0.19%), and manganese (reduced by 17.71 ppm) than those grown at 28 °C (Tables 4 and 5). The fact that the

concentration of certain mineral elements in pak choi leaves was lower without significant changes in shoot dry weight (Table 3) when grown at 32 °C implies that 32 °C may induce some stress that affects nutrient uptake or translocation. In addition, previous research indicates that nitrate deficiency promotes increases in both primary and lateral root lengths to facilitate acquisition of meagerly accessible nutrients (Giehl et al., 2014; Linkohr et al., 2002). It is possible that pak choi seedlings developed longer roots at 32 °C (Table 1; Fig. 1) to compensate for the lower uptake efficiency of certain nutrient elements. However, more research is needed to confirm this hypothesis.

The concentrations of macronutrients and micronutrients in amaranth were compared between plants grown under 28 and 32 °C. The concentrations of magnesium, manganese, and copper in amaranth leaves were slightly increased by 0.08%, 10.88 ppm, and 5.88 ppm, respectively, at 32 °C compared with those at 28 °C (Tables 4 and 5), presumably because of the development of longer roots and more lateral roots at 32 °C (Table 1).

When cultivated at 32 °C, the leaves of edible amaranth contained slightly higher concentrations of nitrate than when grown at 28 °C (Table 6). Interestingly, higher NRA was observed at 28 °C compared with 32 °C in edible amaranth. This may explain why the nitrate concentration was lower in edible amaranth leaves when grown at 28 °C (Table 6). Woodin and Lee (1987) discovered that the induction rate of nitrate reductase increases as the temperature increases at a temperature range between 5 and 20 °C in *Sphagnum*. Similar results were also reported in higher plants. For example, Afridi and Hewitt (1965) subjected nitrate-deficient

Table 4. Effect of elevated temperatures on the concentration of macronutrients in pak choi and edible amaranth (dry weight basis).

Vegetable	Temp (°C)	N (%)	P (%)	Ca (%)	K (%)	Mg (%)
Pak choi	28	3.47 ± 0.20	0.49 ± 0.03	2.44 ± 0.15	5.77 ± 0.23	0.74 ± 0.09
	32	3.42 ± 0.13	0.51 ± 0.02	1.93 ± 0.04	5.60 ± 0.26	0.55 ± 0.01
<i>t</i> test		0.7340	0.4315	0.0046	0.5130	0.0211
<i>P</i> <sup>2</sup>		NS	NS	**	NS	*
Edible amaranth	28	3.98 ± 0.07	0.53 ± 0.02	1.30 ± 0.11	7.66 ± 0.25	0.87 ± 0.03
	32	3.52 ± 0.33	0.49 ± 0.02	1.26 ± 0.06	7.55 ± 0.36	0.95 ± 0.03
<i>t</i> test		0.0741	0.0967	0.6632	0.6715	0.0156
<i>P</i>		NS	NS	NS	NS	*

Values represent the mean ± SD. The measurements were taken at 36 d after sowing (DAS) for pak choi and 37 DAS for edible amaranth.

<sup>2</sup>*P* from *t* test: NS, \*, \*\* represented nonsignificant or significant at *P* < 0.05 or 0.01, respectively.

Table 5. Effect of elevated temperatures on the concentration of micronutrients in pak choi and edible amaranth (dry weight basis).

Vegetable	Temp (°C)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Pak choi	28	81.06 ± 3.37	50.41 ± 9.76	63.35 ± 2.11	10.11 ± 2.75
	32	84.93 ± 3.63	32.70 ± 3.20	63.56 ± 1.24	10.55 ± 1.00
<i>t</i> test		0.2464	0.0404	0.8874	0.8061
<i>P</i> <sup>2</sup>		NS	*	NS	NS
Edible amaranth	28	75.43 ± 3.09	46.13 ± 2.92	55.45 ± 4.76	5.33 ± 0.58
	32	73.16 ± 2.82	57.01 ± 2.08	54.73 ± 1.94	11.21 ± 1.64
<i>t</i> test		0.3998	0.0062	0.8201	0.0042
<i>P</i>		NS	**	NS	**

Values represent the mean ± SD. The measurements were taken at 36 d after sowing (DAS) for pak choi and 37 DAS for edible amaranth.

<sup>2</sup>*P* from *t* test: NS, \*, \*\* represented nonsignificant or significant at *P* < 0.05 or 0.01, respectively.

Table 6. Effect of elevated temperatures on the concentration of total phenolic compounds (TPCs), nitrate, and NRA in pak choi and edible amaranth leaves.

Vegetable	Temp (°C)	TPC (mg/100 g)	NO <sub>3</sub> (µg/g FW)	NRA (µmol/h/g FW)
Pak choi	28	65.56 ± 1.28	6,454.04 ± 363.60	0.52 ± 0.06
	32	71.43 ± 2.75	6,981.77 ± 79.59	0.40 ± 0.09
<i>t</i> test		0.0287	0.0700	0.1204
<i>P</i> <sup>2</sup>		*	NS	NS
Edible amaranth	28	61.17 ± 3.69	3,254.65 ± 240.45	0.85 ± 0.02
	32	58.95 ± 1.92	4,160.71 ± 202.17	0.70 ± 0.05
<i>t</i> test		0.4062	0.0075	0.0071
<i>P</i>		NS	**	**

FW = fresh weight; NRA = nitrate reductase activity.

Values represent the mean ± SD. The measurements were taken at 36 d after sowing (DAS) for pak choi and 37 DAS for edible amaranth.

<sup>2</sup>*P* from *t* test: NS, \*, \*\* represented nonsignificant or significant at *P* < 0.05 or 0.01, respectively.

cauliflower leaf tissue to various temperatures within the range of 22 to 32 °C for 3 h with the treatment of nitrate and found that the induction rate of nitrate reductase decreased as temperature decreased. Intriguingly, when the temperature treatment extended to 6 or 9 h, the induction rate of nitrate reductase in the cauliflower leaf was greater at 22 °C than at 32 °C, which may be because of the increased inactivation rate of nitrate reductase under long-term high temperature conditions. Consistently, a higher turnover rate of NRA at high temperatures, possibly due to the induction of a certain inactivation enzyme, was suggested by Woodin and Lee (1987). In line with these previous reports, results from this study indicate that long-term exposure to 32 °C inhibits NRA in C4 amaranth (Table 6).

*Effect of elevated temperatures on the concentration of TPCs in pak choi and*

*amaranth leaves.* Pak choi leaves grown at 32 °C had a higher concentration of TPCs than those grown at 28 °C, whereas no significant difference was noticed in amaranth leaves between both temperatures (Table 6). According to Boscaiu et al. (2010), one of the common plant responses to stress is to generate more plant antioxidants, such as TPCs and total flavonoids. Consistent with the longer seedling root length and the reduced concentration of certain nutrient elements in leaves, the significantly higher amount of TPCs in pak choi leaves at 32 °C further suggested that pak choi suffered from some stress at this temperature. This higher level of TPCs in pak choi leaves may function to scavenge excessively produced reactive oxygen species (ROS) so that the net photosynthesis remained unaffected, as indicated by the similar shoot dry weight observed in plants grown at 28 and

32 °C (Table 3). Nevertheless, additional research is required to determine whether pak choi leaves have different rates of ROS formation, ROS scavenging, and/or net photosynthesis at 32 °C relative to 28 °C.

## Conclusion

Results from this study suggest that an increase in the mean summer temperatures from 28 to 32 °C may induce longer seedling root length in the C3 crop pak choi. However, this temperature increase did not significantly alter root FW, root dry weight, or number of lateral roots. In addition, leaf calcium, magnesium, and manganese concentrations decreased, however, without a significant change in shoot dry weight, representing a possible decline in the uptake or translocation of certain nutrients under elevated temperature conditions. By contrast, an increase in the mean summer temperature had positive growth effects on the C4 crop edible amaranth. These results are consistent with the general idea that C4 plants are more resistant to high temperatures. Furthermore, leaf nitrate concentration in edible amaranth was slightly higher at 32 °C than that at 28 °C, suggesting that nitrate could accumulate to a larger extent in edible amaranth leaves under elevated temperature conditions. Thus, nitrogen application rates and programs should be reevaluated to reduce nitrate accumulation in edible amaranth under elevated temperature conditions. Overall, a future rise in summer temperatures may impose negative impacts on pak choi leaf nutrient status but positive impacts on edible amaranth production. Thus, edible amaranth seems to be a favorable choice as a summer leafy vegetable crop in Taiwan, as well as in other countries, should the surface air temperature continue to climb.

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