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

San-Gwang Hwang¹, Hsiao-Chien Chao, and Huey-Ling Lin

Department of Horticulture, National Chung Hsing University, 145 Xingda Road, 402 Taichung, Taiwan, Republic of China

Additional index words. total phenolic compounds, nitrate concentration, pak choi, edible amaranth, climate change

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 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 CO₂ 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; Pompeiiano et al., 2013; Wang et al., 2016), the effects of elevated temperatures on C₄ vegetables such as amaranthus 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 CO₂ 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 (Brunnering-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⁻²·s⁻¹ 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...
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$_2$ 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 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, or number of lateral roots. 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 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). 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 Bjorkman, 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$_2$ 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 stress. Pak choi grown at 32 °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). 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 Bjorkman, 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$_2$ 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 stress.
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⁻²) |
|--------------------|-----------|---------------|----------------|------------------|------------------------|----------------|---------------------------|
| 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                |
| t test             |           |               |                |                  |                        |                |                           |
| P                  | 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                |
| t test             |           |               |                |                  |                        |                |                           |
| P                  | 0.0007    | 0.0010        | 0.010          | 0.0193           | 0.0094                 | 0.19           |                           |

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.

*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* | Leaf area (cm²)/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            |
| t test             |           |           | NS                    | *                        | NS                     |
| P                  |           |           | *                     | *                        | 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            |
| t test             |           |           | 0.00003               | 0.00004                  | 0.0011                 |
| P                  | ***       | ***       | ***                   | ***                      | ***                    |

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

*Number of leaves per plant.

*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 x 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 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. 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). 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), primarily 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

HORTSCIENCE VOL. 53(2) FEBRUARY 2018 197
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 |
| t test           |           | 0.7340   | 0.4315  | 0.0046  | 0.5130  | 0.0211  |
| P                | NS        | 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 |
| t test           |           | 0.0741   | 0.0967  | 0.6632  | 0.6715  | 0.0156  |
| P                | 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.

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 |
| t test           |           | 0.2464   | 0.0404   | 0.8874   | 0.8061   |
| P                | NS        | NS      | NS     | NS     | NS      |
| Edible amaranth  | 28        | 75.43 ± 3.09 | 46.13 ± 2.92 | 55.45 ± 4.76 | 11.21 ± 1.64 |
|                  | 32        | 73.16 ± 2.82 | 57.01 ± 2.08 | 54.73 ± 1.94 | 11.21 ± 1.64 |
| t test           |           | 0.3998   | 0.0062   | 0.8201   | 0.0042   |
| P                | 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.

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) | NO3 (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          |
| t test           |           | 0.0287         | 0.0700         | 0.1204              |
| P                | NS        | NS             | 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          |
| t test           |           | 0.4062         | 0.0075         | 0.0071              |
| P                | 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.

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, as 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 Boscau 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 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.

Literature Cited

Afridi, M.M.R.K. and E.J. Hewitt. 1965. Inducible formation and stability of nitrate reductase in higher plants. II. Effects of environmental factors, antimetabolites, and amino acid on induction. J. Expt. Bot. 16:628–645.

Ben-Asher, J., A. Garcia, I. Filteff, and G. Hoogenboom. 2013. Effect of atmospheric water vapor on photosynthesis, transpiration and canopy conductance: A case study in corn. Plant Soil Environ. 59(12):549–555.

Berry, J.A. and O. Björkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31:491–543.

Boscau, M., M. Sánchez, I. Bautista, P. Donat, A. Lidón, J. Llinares, C. Liló, O. Mayoral, and O. Vicente. 2010. Phenolic compounds as stress markers in plants from gymosap habitats. Bull. Univ. Agr. Sci. Vet. Med. Cluj Napoca Hort. 67(1):44–49.

Bruning-Fann, C.S. and J.B. Kaneene. 1993. The effects of nitrate, nitrite and N-nitroso compounds on human health: A review. Vet. Hum. Toxicol. 35(6):521–538.

Choi, E.Y., T.C. Seo, and S.G. Lee. 2011. Growth and physiological responses of Chinese cabbage and radish to long-term exposure to elevated carbon dioxide and temperature. Hort. Environ. Biotechnol. 52(4):376–386.
Collatz, G.J., J.A. Berry, and J.S. Clark. 1998. Effects of climate and atmospheric CO₂ partial pressure on the global distribution of C4 grasses: Present, past, and future. Oecologia 114:441–454.

Crafts-Brandner, S.J. and M.E. Salvucci. 2002. Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. Plant Physiol. 129(4):1773–1780.

Du, H., Z. Wang, and B. Huang. 2009. Differential responses of warm-season and cool-season turfgrass species to heat stress associated with antioxidant enzyme activity. J. Amer. Soc. Hort. Sci. 134:417–422.

Farquhar, G.D., S. von Caemmerer, and J.A. Berry. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. Planta 149:78–90.

Giehl, R.F.H., B.D. Gruber, and N. von Wirén. 2014. It’s time to make changes: Modulation of root system architecture by nutrient signals. J. Expt. Bot. 65:769–778.

Hwang, S.G., Y.Y. Li, and H.L. Lin. 2015. The use of sawdust mixed with ground branches pruned from wax apple or Indian jujube as substrate for cultivation of king oyster mushroom (Pleurotus eryngii). HortScience 50:1220–1233.

Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissue. Biochem. Biophys. Res. Commun. 43:1274–1279.

Kamath, S.D., D. Arunkumar, N.G. Avinash, and S. Samshuddin. 2015. Determination of total phenolic content and total antioxidant activity in locally consumed food stuffs in Moodbidri, Karnataka, India. Adv. Appl. Sci. Res. 6(6):99–102.

Keith, R.W., D.L. Tourneau, and D. Mahlum. 1958. Quantitative paper-chromatographic determination of phenols. J. Chrom. A 1:534–536.

Koscielny, C.B. and R.H. Gulden. 2012. Seedling root length in Brassica napus L. is indicative of seed yield. Can. J. Plant Sci. 92:1229–1237.

Lara, M.V. and C.S. Andreo. 2011. C₄ Plants adaptation to high levels of CO₂ and to drought environments. In: A. Shanker (ed.). Abiotic stress in plants—Mechanisms and adaptations. InTech, Rijeka, Croatia.

Linkohr, B.I., L.C. Williamson, A.H. Fitter, and H.M.O. Leyser. 2002. Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. Plant J. 29(6):751–760.

Long, S.P. 1999. Environmental responses, p. 215–249. In: R.F. Sage and R.K. Monson (eds.). C₄ plant biology. Academic Press, San Diego, CA.

Magkos, F., F. Arvaniti, and A. Zampelas. 2006. Organic food: Buying more safety or just peace of mind? A critical review of the literature. Crit. Rev. Food Sci. Nutr. 46:23–56.

Oh, S., K.H. Moon, E.Y. Song, I.C. Son, and S.C. Koh. 2015. Photosynthesis of Chinese cabbage and radish in response to rising leaf temperature during spring. Hort. Environ. Biotechnol. 56(2):159–166.

Pompeiano, A., M. Volterrani, and L. Guglielminetti. 2013. Physiological responses of C₄ grasses to prolonged heat stress. Adv. Hort. Sci. 27(3):127–132.

Tibbitts, T.W. and G. Bottenberg. 1976. Growth of lettuce under controlled humidity levels. J. Amer. Soc. Hort. Sci. 101:70–73.

von Fischer, J.C., L.L. Tieszen, and D.S. Schimel. 2008. Climate controls on C₃ vs. C₄ productivity in North American grasslands from carbon isotope composition of soil organic matter. Glob. Change Biol. 14:1141–1155.

Xie, Q., K.M.C. Fernando, S. Mayes, and D.L. Sparkes. 2017. Identifying seedling root architectural traits associated with yield and yield components in wheat. Ann. Bot. 119:1115–1129.

Wang, D., S.A. Heckathorn, K. Mainali, and R. Tripathee. 2016. Timing effects of heat-stress on plant ecophysiological characteristics and growth. Front. Plant Sci. 7:1629.

Woodin, S.J. and J.A. Lee. 1987. The effects of nitrate, ammonium and temperature on nitrate reductase activity in Sphagnum species. New Phytol. 105:103–115.