บทความวิจัย

Selecting Temperature for Screening Heat Tolerance in ‘Tavee 60’ Chili Seedlings

Nutkamol Masepan[1], Weerasin Sonjaroon[2, 4], Katarut Chusreeaeom[3], Srisom Suwanwong[1] and Ornusa Khamsuk[1*]

Received: 15 March 2019
Revised: 17 June 2019
Accepted: 9 July 2019

ABSTRACT

Crop yield has been affected by unfavorable growth conditions. This study aimed to find temperature for screening heat-tolerate chili pepper (*Capsicum annuum*) mutated by gamma radiation. Two-month-old seedlings were grown in the growth chamber at four temperature treatments as 27 (control), 34, 36 and 40°C for 7 days. Leaf temperature (LT), non-photochemical quenching (qN), photochemical efficiency of PSII (Fv/Fm) and electron transport rate (ETR) were determined. The results showed that leaf temperature of control plants was lower than other treatments. The qN tended to increase according to the higher temperature treatments. Fv/Fm ratio and ETR of seedlings under 40°C treatment were lower than the others. In addition, under 40°C, seedlings displayed the injury symptom after 4 days and died after 7 days. These levels of injury symptoms lead to the new qualitative parameter for future work called ‘injury index’. In conclusion, the seedlings at 40°C treatment were different from the control based on Fv/Fm. In order to get the new improved cultivar, the temperature at 40°C and Fv/Fm were selected for the future heat-tolerant screening of chili pepper seedlings mutated by gamma irradiation.

Keywords: *Capsicum annuum* L., Heat tolerance, Leaf temperature, Chlorophyll fluorescence

[1] Department of Botany, Faculty of Science, Kasetsart University, Bangkok, 10900
[2] Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, 10900
[3] Department of Applied Radiation and Isotope, Faculty of Science, Kasetsart University, Bangkok, 10900
[4] Center of Thai-French Cooperation on Higher Education and Research, Kasetsart University, Bangkok, 10900
[*]Corresponding author, email: fsciosk@ku.ac.th

Introduction

Based on a global warming study, the temperature has continuously increased by 0.8°C since 1880. Previous study also reported that the average temperature has been predicted to increase twice in the next 50 years [1]. High temperature affected both indirectly and directly to the plants. The former, indirect effect which associated water status, plants might deficit water because of the higher evaporation. High transpiration rates and low plant water potentials are results of the evaporative demand that increases with increases in day-time [2]. The latter directly effects to the plant cells. When the external temperature increases, it leads to an increase of tissue temperature inside the plant body. Plants might display withered leaves, burning and falling along with the inhibited growth of shoot and root [3]. Moreover, this high temperature affected protein denaturation and membrane plasticity causing an electrolyte leakage and some damaged mechanisms such as photosynthesis and electron transport chain which the component of PSII was located in the thylakoid membranes of chloroplast and membrane properties [4-6]. Therefore, high temperature related to every stages of plant development. For example, the germination of lettuce seeds was inhibited when received 1°C higher [7]. During the vegetative stage, high temperature reduced photosynthesis and carbon dioxide assimilation rates compared to the optimal temperature [4]. In addition, chlorophyll content and relative water content of wheat seedlings were induced under 38°C [8]. Many plants such as cowpea, tomato, cotton and rice were reported the injury from heat stress on the reproductive stage. Reproductive development of these plants was damaged by high temperature including the absence of flower and seed production [9-10].

Chili pepper is one of the important economic plants in Thailand. The value of chili product export is more than 400 million baht in 2016. However, most of yields are consumed only in the country in which some seasons the chili yield is lack due to the unfavourable growth condition. High temperature is a crucial factor that reduces yield of chili pepper because of heat stress. Furthermore, it is difficult to control the temperature under the field condition. Therefore, heat-tolerant plants may be the key for future agriculture. This study aims to select the optimum temperature and parameters for screening heat tolerance in chili pepper.

Materials and Methods

Plant materials and growth conditions

The 'Tavee 60' chili pepper seeds were grown in soil mixed with coconut shell's hair in seedling tray under open greenhouse condition (average temperatures of 33/27°C for 12 h day/12 h night, a photosynthetic photon flux density (PPFD) of 657 μmol m^{-2} s^{-1} and an average relative humidity of 70-80% for 5 days with plant data collected by a Watchdog 1450 datalogger). Seedlings were transplanted to the pot at 4 weeks after sowing. Then, 8-week-old seedlings were transferred to the growth chamber (5400R, Contherm, USA) under 4 temperature treatments as 27, 34, 38 and 40°C (70% humidity and light 180 μE for 16 hours). The experiment had 5 replications per treatments and designed with a complete randomized design (CRD).

Measurement of leaf temperature (LT)

LT is a parameter that used to indicate the ability of plant coping with high temperature [11]. The third fully expanded leaf from shoot was measured. LT was measured accompanied chlorophyll fluorescence using PAM-2500 portable fluorometer (Walz, Effeltrich, Germany).

Measurement chlorophyll fluorescence

Chlorophyll fluorescence was measured by PAM-2500 portable fluorometer (Walz, Effeltrich, Germany). The third mature leaves from shoot tip were dark adapted with a leaf clip for 30 minutes. A red light emitting diode with PAR 0.1 $\mu mol\ m^{-2}\ s^{-1}$ ($\lambda = 650$ nm) was used as first irradiated the initial fluorescence (Fo) in non-photosynthesis. A 0.8 s saturation pulse of PAR 9000 $\mu mol\ m^{-2}\ s^{-1}$ was imposed onto the leaf to determine the maximum fluorescence (Fm). Then, the variable fluorescence (Fv) was calculated as Fv = Fm-Fo and the maximal photochemical efficiency of PSII (Fv/Fm) was calculated from the formula Fv/Fm = (Fm-Fo)/Fm [12-13]. Next, the steady-state fluorescence (Fs) was recorded, the saturated pulse light was emitted to measure the maximum fluorescence of the light-adapted leaves (Fm′). The minimum fluorescence of the light-adapted leaves (Fo′) was recorded when the actinic light was turned off [14]. The actual photochemical efficiency of PSII (ΦPSII) was calculated from the formula (Fm′-Fs)/Fm′ and electron transport rate (ETR) was calculated as ΦPSII PPF × 0.5 × 0.84 [15-16]. The coeficient of non-photochemical quenching (qN) of variable fluorescence was calculated from the formula qN = (Fm - Fm′)/(Fm - Fo′) [17].

Results

Leaf temperature

LT was examined from the mature leaves. The result showed that plants under heat stress displayed high LT of seedlings grown at 27°C, was 31.96 ± 0.22°C. While the seedlings under 34, 38 and 40°C showed significantly higher LT as compared to the control (32.64 ± 0.16, 32.98 ± 0.38 and 32.78 ± 0.35°C, respectively). These 3 heat treatments were not significantly different from each other (Figure 1).

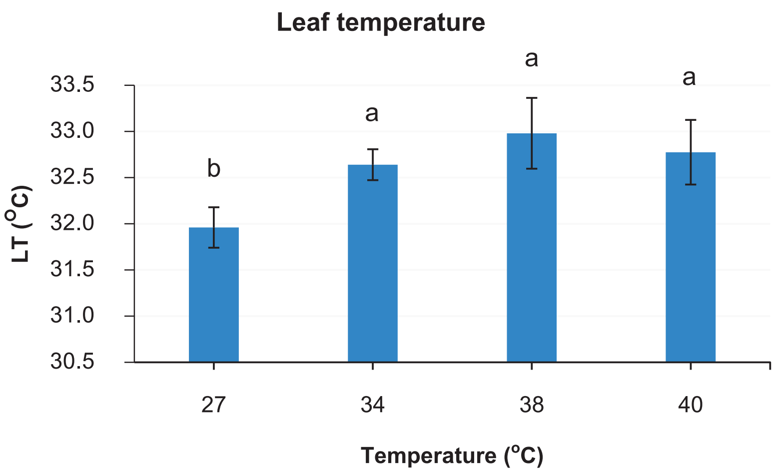

Figure 1 Effects of different temperatures on leaf temperature of 'Tavee 60' chili pepper seedlings. Data are means ± SD of five replications. The same letters indicate no significant difference at $p < 0.05$.

Non-photochemical quenching

The qN of seedlings grown under high temperature treatment at 34, 38 and 40°C were 0.65 ± 0.03, 0.67 ± 0.11 and 0.81 ± 0.07, respectively. While qN of the control seedlings was 0.74 ± 0.07. The data revealed that qN of 34, 38 and 40°C treatments were not significantly different from the control. However, qN of heated seedlings in 40°C treatment was significantly higher than that of seedlings in 34°C treatment (Figure 2).

.

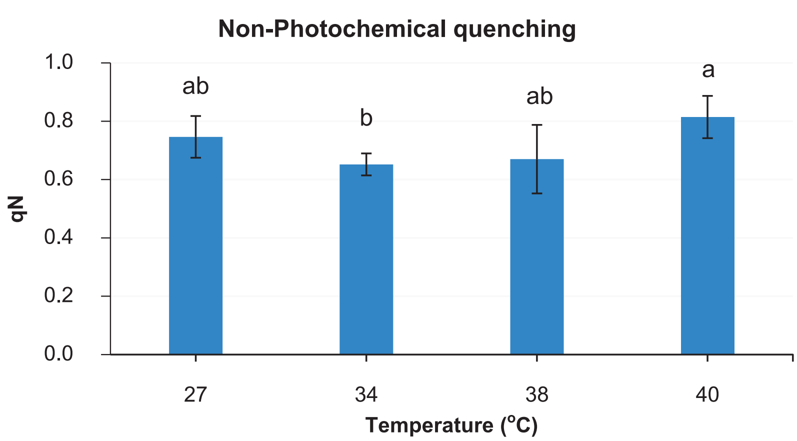

Figure 2 Effects of different temperatures on non-Photochemical quenching of 'Tavee 60' chili pepper seedlings. Data are means ± SD of five replications. The same letters indicate no significant difference at $p < 0.05$.

Photochemical efficiency

Our data showed that Fv/Fm ratio was obviously decreased in severe high temperature. Fv/Fm ratio in seedlings grown at 34 and 38°C were 0.74 ± 0.01 and 0.70 ± 0.02, respectively. Fv/Fm ratio in 27, 34 and 38°C treatments were not significantly different among the treatments. On the other hand, plants grown at 40°C showed a lower Fv/Fm ratio (0.61 ± 0.04), which was lower than other heat treatments (Figure 3).

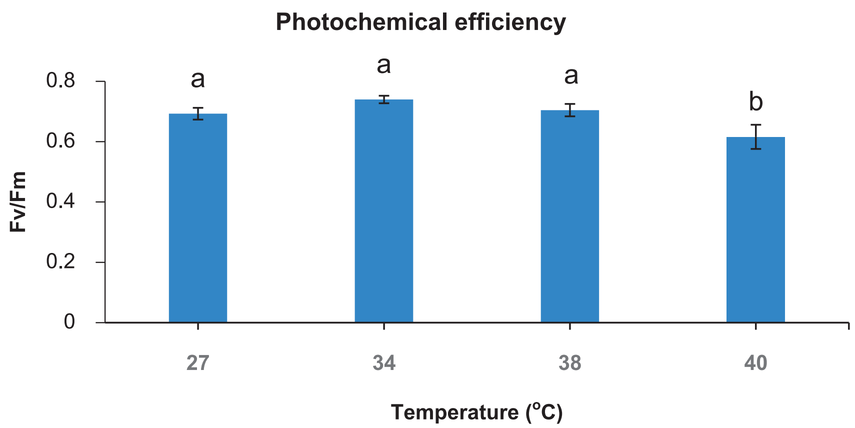

Figure 3 Effects of different temperatures on photochemical efficiency of 'Tavee 60' chili pepper seedlings. Data are means ± SD of five replications. The same letters indicate no significant difference at $p < 0.05$.

Electron transport rate

The result showed that high temperature affected the ETR. The ETR values of the heat-induced plants were 8.4 ± 2.5 and 11 ± 2.9 in 34 and 38°C treatments, respectively. In addition, the ETR of the control plants grown under normal treatment was 10 ± 2.6. The data also showed that the ETR value in 40°C treatment (1.25 ± 1.5) was greatly lower than the others (Figure 4).

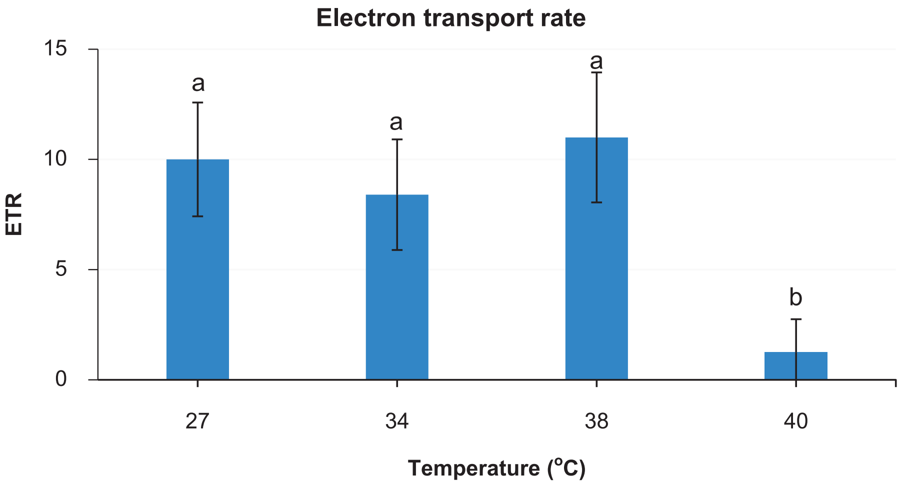

Figure 4 Effects of different temperatures on electron transport rate of 'Tavee 60' chili pepper seedlings. Data are means ± SD of five replications. The same letters indicate no significant difference at $p < 0.05$.

Conclusion and Discussion

According to the results, LT of high temperature treatments were higher than the control. It suggested that high temperature might reduce ability of heat disposal inside the leaves. For example, when plants undergo heat stress, plants normally cool their leaves by transpiration through the stomata. Evaporative cooling may help the plants survive in high temperature, but this mechanism requires sufficient water supply. High temperature condition may reduce water in soil, so plants will difficulty use this mechanism to cope with heat stress [18]. In addition, the dehydration was induced led to stomatal closure to prevent water loss, resulting in higher leaf temperature [19]. However, these LT values under high temperature conditions were not significantly different among the treatments. Because of the restriction of growth chamber size, so the seedlings were determined outside of the growth chamber. This might cause rapid cool down of leaf temperature during measurement.

The qN is non-photochemical quenching of variable fluorescence. It indicates the process of non-photochemical during the period of light which dissipated the excited energy as thermal dissipation. Furthermore, qN related to pH-gradient build-up, ATP-synthesis regulation, inactivation of reaction centers and conformational changes within thylakoid membranes [17]. According to our study, the trend of qN was increased when temperature was increased. It might be possible that heat stress damaged the photosynthetic system such as the permeability of thylakoid membranes and proteins. Therefore, the photochemical quenching was decreased leading to the increased non-photochemical quenching. In agreement with some studies such as wheat, qN was increased when the temperature increased [20]. However, the previous studies suggested that qN is a sensitive parameter adjusting rapidly during stress [21]. The lowest qN value of this study at 34°C can be described as a decreased qN because plants dissipated energy by non-photochemical at the same time as the proportion of open reaction centers was increased [11].

Photosynthesis is a sensitive process under the high temperature. It can be inhibited by heat before other symptoms are detected. The high temperature effects on PSII have been estimated by the parameters as Fo, Fm and Fv/Fm. In our study, the reduction of Fv/Fm due to the increased temperature suggested that efficiency of PSII photochemistry was reduced in the heated seedlings. The result was in agreement with some plants such as wheat [20], barley [22] and tomato [23], which the Fv/Fm values were decreased under heat stress. It might be possible that heat stress damaged membrane and proteins, including enzymes, which affected photosynthetic apparatus and photosynthetic pathway [5-6].

According to this study, the ETR values of the heat-induced plants showed the similar trend as Fv/Fm, which the values in 40°C treatment was lower than the others. ETR was decreased because the photosynthetic system was damaged by heat, especially PSII which is indicated as the primary site of heat damage to electron transport of photosynthetic process. Therefore, under high temperature, the ETR value which one of the most thermosensitive was decreased [24]. The result from this study was in agreement with Brassica plants, which ETR value was reduced under heat stress [25]. This data supported that the photosynthesis in seedlings was affected under high temperature.

Based on the data, the increase of LT and qN, as well as the decrease of Fv/Fm and ETR were due to high temperature. It suggested that high temperature affected photosynthesis of the seedlings. Our data was in agreement with many plants such as wheat [20], rice [26] and tomatoes [22] under heat stress condition. The increasing of temperature caused increased LT and cell components leading to thermal stability of membrane and proteins including thylakoid membrane and photosynthetic enzymes. Therefore, photosynthesis was interrupted and affected to qN, Fv/Fm and ETR values. In addition, high temperature affected to morphology of seedlings as well. From the results, under 27, 34, 38 and 40°C the seedlings could survive more than 7 days. But only at 40°C, the seedlings displayed the injury symptoms after 4 days as wilted young leaves, leaf necrosis, fall leaf and died in later within 7 days (Figure 5).

Figure 5 Effects of different temperatures on injury symptoms of 'Tavee 60' chili pepper seedlings in 7 days.

In our study, the chlorophyll fluorescence parameters of the seedlings at 40°C treatment are quite different from the controls. Therefore, the temperature at 40°C was selected for the future screening of heat tolerance in chili pepper seedlings because this temperature level was the lowest temperature causing the heat stress symptoms. Moreover, these injury symptoms were used to generate an injury index. The injury levels were scored as 0 = not showed injury, 1 = young leaves wilted and/or some leaf necrosis, 2 = many leaves wilted and 3 = all leaves wilted (Figure 6).

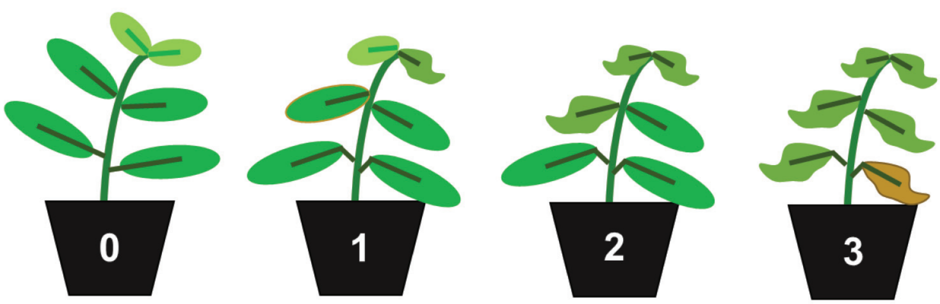

Figure 6 Injury index levels (relates to revealed injury symptoms).

The results of Fv/Fm and ETR were the most obvious parameters showing the difference between the control and the heated seedlings. Previous study suggested that ETR must be used carefully, especially under the stress conditions because this value is sensitive. It might be changed if the chlorophyll content was decreased [27]. While Fv/Fm ratio was reported that it could be used as an early indicator of heat tolerance in tomato [22]. Therefore, Fv/Fm was selected to determine heat tolerance in chili pepper seedlings.

In conclusions, high temperature can affected the chili seedlings indicated by the increased LT and qN and the decreased Fv/Fm and ETR. For our future work, the chili pepper seedlings mutated by gamma ray irradiation will be screened for heat tolerance at 40°C, and Fv/Fm and injury index will be used as primary indicators.

References

1. Field, C. B., Barros, V., Stocker, T. F., Qin, D., Dokken, D. J., Ebi, K. L., Mastrandrea, M. D., Mach, K. J., Plattner, G. K., Allen, S. K., Tignor, M., & Midgley, P.M. (2007). *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation.* Cambridge. Cambridge University Press. p. 1-19.
2. Hall, A. E. (2001). Crop Responses to Environment. Florida. CRC Press LLC.
3. Omae, H., Kumar, A. & Shono, M. (2012). Adaptation to high temperature and water deficit in the common bean (*Phaseolus vulgaris* L.) during the reproduction period. *Journal of Botany,* 1-6.
4. Al-Khatib, K. & Paulsen, G. M. (1999). High-temperature effects on photosynthetic processes in temperate and tropical cereals. *Crop Science, 39,* 119-125.
5. Taiz, L., & Zeiger, E. (2006). Plant Physiology. 4th edition. Massachusetts. Sinauer Associates Inc.
6. Feng, B., Liu, P., Li, G., Dong, S. T., Wang, F. H., Kong, L. A., & Zhang, J. W. (2014). Effect of heat stress on the photosynthetic characteristics in flag leaves at the grain-filling stage of different heat-resistant winter wheat varieties. *Journal of Agronomy and Crop Science, 200,* 143-155.
7. Argyris, J., Truco, M. J., Ochoa, O., Knapp, S. J., Still, D. W., Lenssen, G. M., Schut, J. W., Michelmore, R. W., & Bradford, K. J. (2005). Quantitative trait loci associated with seed and seedling trait in *Latuca. Theoretical and Applied Genetics, 111,* 1365-1376.
8. Hasanuzzaman, M., Nahar, K., Alam, M. M., & Fujita, M. (2012). Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum*) seedlings by modulation the antioxidant defense and glyoxalase system. *Australian Journal of Crop Science, 6*(8), 1314-1323.
9. Hall, A. E. (1992). Breeding for heat tolerance. *Plant Breeding Reviews, 10,* 129-168.
10. Kuo, C. G. (1993). *Adaptation of food crops to temperature and water stress, Taiwan.* Asian Vegetable Research and Development Center. p. 271-284.
11. Pastenes, C., & Horton, P. (1996). Effect of high temperature on photosynthesis in beans. *Plant Physiology, 112,* 1245-1251.

12. Van Kooten O., Snel J. F. H. (1990). The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research, 25,* 147-150.
13. Demmig-Adams, B., & Adams, W. W. III. (1996). Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. *Planta, 198,* 460-470.
14. Lichtenthaler, H. K., Buschmann, C., & Knapp, M. (2005). How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. *Photosynthetica, 43,* 379-393.
15. Bilger, W., & Björkman, O. B. (1990). Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in *Hedera canariensis. Photosynthesis Research, 25,* 173-185.
16. Krall, J. P., & Edwards, G. E. (1992). Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiologia Plantarum, 86,* 180-187.
17. Brestic, M., & Zivcak, M. (2013). PSII fluorescence technique for measurement of drought and high temperature stress signal in crop plants: Protocols and applications. *Molecular Stress Physiology of Plants,* 87-131.
18. Urban, J., Ingwers, M. W., McGuire, M. A., & Teskey, R. O. (2017). Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides x nigra. Journal of Experimental Botany,* 68, 1757-1767.
19. Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Plant Science, 11*(1), 15-19.
20. Lu, C. M., & Zhang, J. H. (2000). Heat-induce multiple effects on PSII in wheat plants. *Journal of Plant Physiology, 156,* 259-265.
21. Lichtenthaler, H. K. (1988). *Applications of chlorophyll fluorescence.* Dordrecht. Kluwer Academic. p. 143-149.
22. Kalaji, H. M., & Guo, P. (2008). Chlorophyll fluorescence: A useful tool in Barley plant breeding programs. *Photochemistry Research Progress,* 448-471.
23. Zhou, R., Yu, X., Kjaer, K. H., Rosenqvist, E., Ottosen, C., & Wu, Z. (2015). Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. *Environment and Experiment Botany, 118,* 1-11.
24. Heckathorn, S. A., Downs, C. A., Sharkey, T. D., & Coleman, J. S. (1998). The Small, Methionine-Rich Chloroplast Heat-Shock Protein Protects Photosystem II Electron Transport during Heat Stress. *Plant Physiology, 116,* 439-444.

25. Diaz, M., De Haro, V., Munoz, R., & Quiles, M. J. (2007). Chlororespiration is involved in the adaptation of *Brassica* plants to heat and high light intensity. *Plant, Cell and Environment, 30,* 1578-1585.
26. Han, F., Chen, H., Li, X. J., Yang, M. F., Liu, G. S., & Shen, S. H. (2009). A comparative proteomic analysis of rice seedlings under various high-temperature stresses. *Biochemica et Biophysica Acta - Proteins and Proteomics, 1794,* 1625-1634.
27. Tozzi, E. S., Easlon, H. M., & Richards, J. H. (2013). Interactive effect of water, light and heat stress on photosynthesis in Fremont cottonwood. *Plant, Cell and Environment, 36,* 1423-1434.