Saudi Journal of Biological Sciences (2015) 22, 656–663

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Morphological and physiological characterization of different genotypes of faba bean under heat stress

Manzer H. Siddiqui [a,*], Mutahhar Y. Al-Khaishany [a], Mohammed A. Al-Qutami [a], Mohamed H. Al-Whaibi [a], Anil Grover [b], Hayssam M. Ali [a], Mona Suliman Al-Wahibi [a]

[a] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[b] *Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi 110021, India*

Received 31 January 2015; revised 29 May 2015; accepted 3 June 2015
Available online 10 June 2015

KEYWORDS

Heat stress;
Faba bean;
Antioxidant system;
Proline;
Photosynthesis pigment

Abstract Heat stress (HS) is the major constraint to crop productivity worldwide. The objective of the present experiment was to select the tolerant and sensitive genotype(s) on the basis of morpho-physiological and biochemical characteristics of ten *Vicia faba* genotypes. These genotypes were as follows: Zafar 1, Zafar 2, Shebam 1, Makamora, Espan, Giza Blanka, Giza 3, C4, C5 and G853. The experimental work was undertaken to study the effects of different levels of temperature (control, mild, and modest) on plant height (PH) plant^{-1}, fresh weight (FW) and dry weight (DW) plant^{-1}, area leaf^{-1}, content of leaf relative water (RWC), proline content (Pro) and total chlorophyll (Total Chl), electrolyte leakage (EL), malondialdehyde level (MDA), hydrogen peroxide (H_2O_2), and activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzymes. HS significantly affected growth performance of all genotypes. However, the magnitude of reduction in genotypes 'C5' was relatively low, possibly due to its better antioxidant activities (CAT, POD and SOD), and accumulation of Pro and Total Chl, and leaf RWC. In the study, 'C5' was noted to be the most HS tolerant and 'Espan' most HS sensitive genotypes. It was concluded that the heat-tolerant genotypes may have better osmotic adjustment and protection from free radicals by increasing the accumulation of Pro content with increased activities of antioxidant enzyme.

1. Introduction

Heat, cold, drought and salinity are the major environmental stresses that cause severe problem in many areas of the world. The ever-increasing temperature is one of the limiting factors for plants growth and their yield, and also for geographical distributions of plants. In the coming century, plants will be affected adversely and become more vulnerable to increasing

* Corresponding author. Tel.: +966 4675872.
E-mail address: manzerhs@yahoo.co.in (M.H. Siddiqui).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.06.002
1319-562X © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University.

high ambient temperature due to anthropogenic activities that increase gases particularly carbon dioxide, methane, chlorofluorocarbons and nitrous oxides. According to the Fourth Assessment of the Intergovernmental Panel on Climatic Change (IPCC), global average temperature will be increased between 1.8 °C and 4 °C in 2100 (Sánchez et al., 2014). The increasing rate depends on the level of greenhouse gases and can even be larger if the human population and global economy keeps growing at their current rate (Sánchez et al., 2014).

Temperature plays a key role in plant growth, development, reproduction, and yield (Mittler et al., 2012). Like other organisms, plants, the sessile organisms, are constantly exposed to above the normal optimal temperatures. Globally, agriculture has suffered heavily due to the heat stress (HS) or in combination with drought and other environmental factors (Mittler, 2006). According to Lobell et al. (2011), the world wheat and maize production decreased by 3.8% and 5.5% respectively due to heat stress (HS) over the past three decades (1980–2008). Similarly, in 2010, wheat price was decreased up to 50% due to the fact that more than 20% of Russian agricultural producing areas suffered by HS (FAO, 2010; NOAA, 2011). Ortiz et al. (2008) reported that a temperature rise of 3–4 °C might cause 15–35% loss of crop yield in Africa and Asia, and 25–35% in the Middle East.

Due to global warming HS causes a series of changes in plants at morpho-anatomical, physiological, biochemical and molecular levels resulting in a drastic loss of economic yield. The effect of HS starts from the seed germination to flowering, as various stages of plant's life are temperature-dependent. Seed germination and seed vigor are adversely affected by HS that causes thermal injury or death of the seed (Khan, 1976; Grass and Burris, 1995). HS causes various physiological changes in plants such as scorching of leaves and stems, leaf abscission and senescence, shoot and root growth inhibition or reduction in number of flowers, pollen tube growth and pollen infertility, fruit damage, leading to catastrophic loss of crop yield (Bita and Gerats, 2013; Teixeira et al., 2013; Song et al., 2013; Hemantaranjan et al., 2014). HS affects the photosynthesis, respiration, water relations and membrane stability, and modulates levels of hormones, and primary and secondary metabolites (Hemantaranjan et al., 2014). HS impairs the stability of proteins, membrane integrity, RNA and activity of enzymes in chloroplast and mitochondria, resulting in an imbalance in the metabolic homeostasis (Mittler et al., 2012; Hemantaranjan et al., 2014). The disturbance in metabolic homeostasis leads to the accumulation of toxic by-products, such as reactive oxygen species (ROS) (Mittler et al., 2012). To survive or maintain steady-state balance of metabolic processes under HS, plants reprogram their transcriptome, proteome, metabolome and lipidome, thereby adjusting their composition of certain transcripts, proteins, metabolites and lipids (Mittler et al., 2012). Many heat shock proteins (HSP100s, HSP90s, HSP70s, HSP60s, HSP40s and the small HSPs (sHSPs) are accumulated in plants to mitigate the adverse effect of HS on plant metabolism (Singh and Grover, 2008; Al-Whaibi, 2011; Lavania et al., 2015a,b).

Both heat and drought stresses are detrimental factors for plant growth and development. A detectable inhibition in plant growth that starts at a value of daily mean temperature is called threshold temperature which varies with the plant species, and genotypes within the species (Wahid et al., 2007; Hemantaranjan et al., 2014). It is important to determine the threshold temperatures of plants that can survive under stress. It is crucial to understand the physiological processes and mechanisms involved in tolerance of plants to HS stress and possible strategies for improving crop thermotolerance. The knowledge of the physiological response of plants to stress is important for tailoring stress-tolerant crop using genetic approaches. However, we know that faba bean (*Vicia faba* L.) is an important legume crop and their cultivation particularly in arid and semi-arid regions is unsuitable because this crop is not sufficient drought and heat tolerant, as it is very susceptible to moisture and high temperature (Loss and Siddique, 1997). Keeping in view the importance of this crop for human as well as animal, the present experiment was planned to study the effect of HS on different genotypes of faba bean plants. The main objective of this experiment was to determine HS-tolerant and HS-sensitive genotypes on the basis of physio-morphological and biochemical parameters.

2. Materials and methods

To achieve the objective of the present study, ten improved genotypes of *V. faba* L. were collected from different geographical origins. Seeds of genotypes Zafar 1, Zafar 2 and Shebam1 from the General Organization for Agriculture Research, Yemen, genotypes Makamora and Espan from the local market of Riyadh, and genotypes Giza Blanka, Giza 3, C4, C5 and G853 from Agriculture Research Center, Egypt were collected. The experiments were conducted in a growth chamber (temperature 25 ± 3 °C, relative humidity 50–60%, light 90 μmol of photons m^{-2} s^{-1}; 6/8-h light/dark cycle). Seeds were grown in pots containing a mixture of sand and peat (1:1). HS treatments were subjected after 60 days of sowing. The treatment details are as follows: (i) control (UN): ambient temperature (25 °C), (ii) mild level temperature (HT1): 6 °C more than ambient temperature and (iii) modest level temperature stress (HT2): 12 °C more than the ambient temperature. Different levels of HS were imposed on plants by placing pots at the requisite temperatures for 48 h at each temperature.

The experimental pots were arranged in a simple randomized design with five replicates per treatment. Before sowing, seeds of all genotypes were surface sterilized with 1% sodium hypochlorite for 10 min, then vigorously rinsed with double distilled water (DDW) and sown in sand and peat-filled pots supplied with Raukura's nutrient solution (Smith et al., 1983). The salts used to make up the nutrient solution are as follows: Macronutrient stock solution A contained (gL^{-1}) $Mg(NO_3)_2{\cdot}6H_2O$, 4.94; $Ca(NO_3)_2{\cdot}4H_2O$, 16.78; NH_4NO_3, 8.48; KNO_3, 2.28. Macronutrient stock solution B contained (gL^{-1}) KH_2PO_4, 2.67; K_2HPO_4, 1.64; K_2SO_4, 6.62; Na_2SO_4, 0.60; NaCl, 0.33. Micronutrient supplement (mgL^{-1}) was constituted of H_3BO_3, 128.80; $CuCl_2{\cdot}2H_2O$, 4.84; $MnCl_2{\cdot}4H_2O$, 81.10; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.83; $ZnCl_2$, 23.45; ferric citrate pentahydrate, 809.84. The dilute solution applied to the plants was prepared by mixing 200 mL of each of the macronutrient stock solution with 100 mL of the micronutrient supplement and diluting to 4.5 L with DDW.

2.1. Determination of the morphological characteristics of plants

The sampling of all HS exposed plants was carried out immediately after given HS treatments for morphological analysis.

Plants were sampled after 5 d of HS treatments for measurement of growth parameters. The growth performance of faba bean plants was assessed in terms of PH plant^{-1}, FW and DW plant^{-1} and area leaf^{-1}. The leaf area was measured directly using Leaf Area Meter (Model LI-3050A, LI-COR Inc, Lincon, NE, USA). The area of three leaves (upper, middle, and lower) of each plant of the sample (consisting of five plants) was determined.

2.2. Determination of physio-biochemical characteristics of plants

Leaf RWC (%) was determined using the methods of Gulen and Eris (2003). Leaf disks were taken from the fully expanded and uniform leaves of each of the three plants (replicates) per treatment. First, the FW was recorded, and then samples were placed in a petri dish having distilled water for 4 h. Turgid weight (TW) was then recorded, and the leaf samples were placed in an incubator at 70 °C for 24 h, to determine the dry weight. Leaf RWC% was calculated by

$$\mathrm{RWC}(\%) = [(\mathrm{FW} - \mathrm{DW})/(\text{turgid weight-dry weight})] \times 100.$$

For total chlorophyll (Total Chl) determination, the youngest fully expanded leaves were extracted using 80% acetone, and the absorbance was measured using UV–vis Spectrophotometer (SPEKOL 1500; Analytik Jena AG, Jena, Germany) at 663 nm and 645 nm. Total Chl content was determined by using the established formulae (Arnon, 1949). Pro concentration was determined spectrophotometrically using the ninhydrin method of Bates et al. (1973). Fresh leaf samples were homogenized in 3% sulfosalicylic acid, followed by the addition of 2 mL each of ninhydrin and glacial acetic acid, after which the samples were heated to 100 °C. The mixture was extracted with toluene, and the free toluene was quantified at 520 nm. Malondialdehyde (MDA) content was determined according to the method of Heath and Packer (1968). Leaf samples were weighed, and homogenates containing 10% trichloroacetic acid and 0.65% 2-thiobarbituric acid were heated at 95 °C for 60 min, then cooled to room temperature, and centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was read at 532 nm and 600 nm against a reagent blank. Electrolyte leakage (EL) was used to assess membrane permeability in accordance with Lutts et al. (1995). Samples were washed 3 times with DDW to remove surface contamination, and leaf disks were cut from young leaves and placed in sealed vials containing 10 mL of DDW, followed by incubation on a rotary shaker for 24 h, after which the electrical conductivity of the solution (EC1) was determined. Then, the samples were autoclaved at 120 °C for 20 min, and the electrical conductivity was measured again (EC2) after the solution was cooled to room temperature. The electrolyte leakage was defined as $\mathrm{EC1/EC2} \times 100$ and expressed as percentage. Hydrogen peroxide (H_2O_2) levels were measured as described by Velikova et al. (2000). Fresh leaf samples were homogenized in 5 mL of 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000 rpm for 15 min and the supernatant was added to 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide. The absorbance of the supernatant was recorded at 390 nm. The content of H_2O_2 was calculated by comparison with a standard calibration curve plotted using known concentrations of H_2O_2.

2.3. Determination of antioxidant enzymes activity

To determine the activities of antioxidant enzymes, a crude enzyme extract was prepared by homogenizing 500 mg of leaf tissue in extraction buffer (0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer, pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000*g* for 20 min at 4 °C and the supernatant was used for the enzymatic assays described below. The method of Chance and Maehly (1955) was employed to determine peroxidase (POD) (EC 1.11.1.7) activity. The activity was assayed using 5 mL of enzyme reaction solution containing phosphate buffer (pH 6.8), 50 M pyrogallol, 50 mM H_2O_2 and 1 mL of the enzyme extract diluted 20 times. The assay mixture was incubated for 5 min at 25 °C, and the reaction was terminated by the addition of 0.5 mL of 5% (v/v) H_2SO_4. Purpurogallin production was measured spectrophotometrically at 420 nm. One unit of POD activity was considered the amount of purpurogallin formed per milligram of protein per minute. The method of Aebi (1984) was used to measure catalase (CAT) (EC 1.11.1.6) activity. The decomposition of H_2O_2 was measured as the decrease in absorbance at 240 nm. In this assay, 50 mM phosphate buffer (pH 7.8) and 10 mM H_2O_2 were used in the reaction solution. Activity of superoxide dismutase (SOD) (EC 1.15.1.1) was determined based on the inhibition of nitroblue tetrazolium (NBT) photoreduction according to the method of Giannopolitis and Ries (1977). The reaction solution (3 mL) contained 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 μM ethylenediamine tetraacetic acid (EDTA), 50 mM phosphate buffer (pH 7.8), and 20–50 mL of enzyme extract. The reaction solution was irradiated under fluorescent light at 75 μM m^{-2} s^{-1} for 15 min. The absorbance at 560 nm was read against a blank (nonirradiated reaction solution). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

2.4. Statistical analysis

The data were expressed as the mean ± standard error and were analyzed statistically using IBM SPSS Ver.22 statistical software (IBM Corporation and Others, Armonk, NY, USA). The means were compared statistically using Duncan's multiple-range test at the level of $p < 0.05$.

3. Results

3.1. Heat stress experiment

In the present experiment, the performance was assessed in terms of PH plant^{-1}, shoot FW and DW plant^{-1}, area leaf^{-1}, RWC, Pro content, Total Chl EL and content of MDA and H_2O_2, and activities of CAT, POD and SOD. The effect of HS treatments on these studied parameters of genotypes was found to be significant (Tables 1–4).

The data revealed that the growth attributes of all genotypes were affected significantly depending on the temperature (Table 1). In all cultivars, PH, Shoot FW and DW, leaf area decreased gradually from the control (ambient temperature; 25 °C) to the modest temperature (37 °C). Off all the

Table 1 Effect of heat stress on growth performance of faba bean genotypes.

Treatments	Genotypes									
	Zafar 1	Zafar 2	Giza Blanka	Espan	Makamora	Shebam 1	Giza 3	C4	C5	G853
	Plant height (cm) plant^{-1}									
Control	59.00 ± 0.58^{fg}	56.00 ± 0.58^{hi}	61.00 ± 0.58^{ef}	36.67 ± 0.88^{n}	48.00 ± 0.58^{k}	48.00 ± 0.58^{k}	59.00 ± 0.58^{fg}	79.00 ± 0.58^{c}	116.33 ± 0.88^{a}	55.00 ± 0.58^{i}
HT1	56.00 ± 0.58^{hi}	43.67 ± 0.88^{l}	58.67 ± 0.88^{g}	25.67 ± 0.88^{r}	31.33 ± 0.33^{pq}	31.33 ± 0.33^{pq}	41.33 ± 0.88^{m}	70.33 ± 0.33^{d}	86.67 ± 0.88^{b}	52.33 ± 0.88^{j}
HT2	43.33 ± 0.88^{lm}	30.67 ± 0.88^{q}	33.67 ± 0.88^{o}	13.67 ± 0.33^{t}	21.67 ± 0.88^{s}	21.67 ± 0.88^{s}	33.33 ± 0.88^{op}	58.00 ± 0.58^{gh}	62.33 ± 0.88^{e}	45.00 ± 0.58^{l}
	Shoot FW (g) plant^{-1}									
Control	5.10 ± 0.12^{cde}	5.63 ± 0.48^{cd}	3.63 ± 0.18^{fg}	2.40 ± 0.06^{ij}	5.13 ± 0.03^{cde}	5.13 ± 0.03^{cde}	5.00 ± 0.06^{de}	6.00 ± 0.31^{c}	9.20 ± 0.06^{a}	4.40 ± 0.21^{ef}
HT1	3.53 ± 0.82^{fgh}	2.97 ± 0.87^{ghi}	5.17 ± 0.15^{cde}	2.20 ± 0.06^{ij}	4.73 ± 0.12^{de}	4.73 ± 0.12^{de}	4.47 ± 0.26^{ef}	4.87 ± 0.12^{de}	7.17 ± 0.15^{b}	2.53 ± 0.23^{ij}
HT2	2.43 ± 0.75^{ij}	2.63 ± 0.09^{hij}	3.53 ± 0.27^{fgh}	0.50 ± 0.06^{l}	0.73 ± 0.09^{l}	0.90 ± 0.25^{kl}	3.50 ± 0.10^{fgh}	2.70 ± 0.06^{ghij}	4.17 ± 0.12^{ef}	1.77 ± 0.09^{jk}
	Shoot DW (g) plant^{-1}									
Control	0.63 ± 0.06^{abcdef}	0.56 ± 0.04^{bcdefgh}	0.70 ± 0.23^{abc}	0.40 ± 0.01^{ghij}	0.59 ± 0.02^{abcdefg}	0.59 ± 0.02^{abcdefg}	0.57 ± 0.02^{bcdefgh}	0.76 ± 0.16^{ab}	0.81 ± 0.01^{a}	0.73 ± 0.07^{ab}
HT1	0.40 ± 0.12^{ghij}	0.37 ± 0.07^{ghij}	0.67 ± 0.06^{abcde}	0.29 ± 0.01^{ij}	0.46 ± 0.07^{defghij}	0.46 ± 0.07^{defghij}	0.45 ± 0.06^{efghij}	0.46 ± 0.02^{defghij}	0.68 ± 0.02^{ab-}	0.50 ± 0.01^{cdefgh}
HT2	0.23 ± 0.07^{j}	0.25 ± 0.01^{j}	0.41 ± 0.03^{fghij}	0.01 ± 0.00^{k}	0.24 ± 0.05^{j}	0.24 ± 0.05^{j}	0.28 ± 0.03^{ij}	0.30 ± 0.01^{ij}	0.35 ± 0.01^{hij}	0.45 ± 0.05^{efghij}
	Area (cm^{2}) leaf^{-1}									
Control	13.34 ± 0.69^{ghi}	7.63 ± 0.15^{no}	12.17 ± 0.58^{ijk}	8.53 ± 0.34^{mn}	14.79 ± 0.41^{fg}	14.79 ± 0.41^{fg}	12.59 ± 0.47^{hij}	20.00 ± 0.58^{c}	31.80 ± 0.75^{a}	15.60 ± 0.74^{ef}
HT1	11.82 ± 0.32^{ijk}	6.56 ± 0.53^{opq}	9.77 ± 0.79^{lm}	7.07 ± 0.09^{nop}	11.38 ± 0.35^{jkl}	11.38 ± 0.35^{jkl}	9.78 ± 0.53^{lm}	17.00 ± 0.58^{de}	23.37 ± 0.32^{b}	13.91 ± 0.36^{gh}
HT2	7.27 ± 0.92^{nop}	5.03 ± 0.93^{qr}	7.41 ± 0.89^{no}	4.10 ± 0.32^{r}	7.00 ± 0.47^{nop}	7.00 ± 0.47^{nop}	7.61 ± 0.26^{no}	10.63 ± 0.55^{kl}	17.50 ± 0.41^{d}	5.65 ± 0.42^{pqr}

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's multiple-range test.

Table 2 Effect of heat stress on leaf relative water content (RWC), proline (Pro) content and total Chlorophyll (Total Chl) content of faba bean genotypes.

Treatments	Genotypes									
	Zafar 1	Zafar 2	Giza Blanka	Espan	Makamora	Shebam 1	Giza 3	C4	C5	G853
	RWC%									
Control	69.14 ± 0.10^{e}	66.92 ± 0.33^{f}	83.17 ± 0.66^{a}	62.71 ± 0.55^{i}	73.03 ± 0.33^{c}	73.03 ± 0.33^{c}	78.15 ± 0.28^{b}	76.89 ± 0.44^{b}	73.23 ± 0.29^{c}	71.05 ± 0.69^{d}
HT1	65.09 ± 0.38^{gh}	64.89 ± 0.50^{h}	66.26 ± 0.37^{fgh}	53.89 ± 0.05^{j}	66.14 ± 0.89^{fgh}	65.20 ± 0.50^{gh}	71.24 ± 0.58^{d}	74.03 ± 0.13^{c}	71.24 ± 0.58^{d}	66.51 ± 0.59^{fg}
HT2	44.39 ± 0.30^{m}	34.48 ± 0.46^{p}	41.46 ± 0.73^{n}	25.82 ± 0.65^{q}	45.26 ± 0.45^{m}	45.26 ± 0.45^{m}	52.09 ± 0.14^{k}	38.23 ± 0.94^{o}	49.19 ± 0.11^{l}	50.87 ± 0.26^{k}
	Pro (μg^{-1} FW)									
Control	1.19 ± 0.04^{k}	0.93 ± 0.01^{l}	0.90 ± 0.06^{l}	1.30 ± 0.03^{k}	1.34 ± 0.05^{k}	1.34 ± 0.05^{k}	1.26 ± 0.02^{k}	1.63 ± 0.30^{j}	1.25 ± 0.02^{k}	3.06 ± 0.04^{d}
HT1	1.93 ± 0.03^{i}	1.90 ± 0.03^{i}	1.17 ± 0.03^{k}	2.86 ± 0.02^{de}	2.03 ± 0.05^{hi}	2.03 ± 0.05^{hi}	2.33 ± 0.07^{g}	2.40 ± 0.04^{g}	2.77 ± 0.05^{ef}	2.27 ± 0.03^{gh}
HT2	2.54 ± 0.03^{fg}	2.93 ± 0.02^{de}	3.40 ± 0.07^{c}	1.44 ± 0.03^{jk}	2.35 ± 0.01^{g}	2.35 ± 0.01^{g}	2.68 ± 0.05^{ef}	4.73 ± 0.08^{b}	7.44 ± 0.28^{a}	1.62 ± 0.03^{j}
	Total Chl (mg g^{-1} FW)									
Control	40.07 ± 0.74^{cde}	40.47 ± 0.53^{cde}	40.87 ± 0.59^{cd}	35.00 ± 0.58^{g}	38.77 ± 0.67^{ef}	38.77 ± 0.67^{ef}	37.67 ± 0.88^{f}	41.97 ± 0.24^{bc}	48.10 ± 0.76^{a}	41.50 ± 0.42^{bcd}
HT1	38.00 ± 0.85^{f}	35.60 ± 0.60^{g}	35.40 ± 0.23^{g}	27.00 ± 0.58^{j}	38.00 ± 0.58^{f}	38.00 ± 0.58^{f}	38.00 ± 0.06^{f}	39.67 ± 0.39^{def}	42.80 ± 0.23^{b}	35.37 ± 0.35^{g}
HT2	26.83 ± 0.44^{j}	32.00 ± 0.58^{h}	27.80 ± 0.20^{j}	18.67 ± 0.88^{k}	31.03 ± 0.98^{hi}	31.03 ± 0.98^{hi}	30.70 ± 0.55^{hi}	29.73 ± 0.37^{i}	32.23 ± 0.62^{h}	29.90 ± 0.90^{i}

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's multiple-range test.

Table 3 Effect of heat stress on electrolyte leakage (EL), malondialdehyde (MDA) content, and hydrogen peroxide (H_2O_2) content of faba bean genotypes.

Treatments	Genotypes									
	Zafar 1	Zafar 2	Giza Blanka	Espan	Makamora	Shebam 1	Giza 3	C4	C5	G853
	Electrolyte leakage (%)									
Control	34.33 ± 2.96^{n}	40.67 ± 2.73^{lmn}	39.67 ± 3.28^{lmn}	38.33 ± 3.53^{mn}	42.67 ± 5.36^{klmn}	47.33 ± 2.40^{jklm}	37.00 ± 1.53^{n}	40.33 ± 1.45^{lmn}	40.67 ± 4.26^{lmn}	50.67 ± 4.70^{hijk}
HT1	49.00 ± 1.73^{ijkl}	53.00 ± 4.16^{ghij}	49.33 ± 2.33^{ijkl}	57.33 ± 4.26^{fghi}	64.00 ± 3.61^{cdef}	61.67 ± 3.18^{defg}	54.33 ± 2.73^{ghij}	49.33 ± 2.96^{ijkl}	59.67 ± 2.60^{efgh}	64.33 ± 3.48^{cdef}
HT2	64.33 ± 2.91^{cdef}	67.00 ± 1.15^{bcdef}	69.00 ± 0.58^{abcde}	76.00 ± 1.53^{ab}	76.33 ± 2.19^{ab}	72.33 ± 1.20^{abc}	67.67 ± 1.33^{bcde}	70.33 ± 0.88^{abcd}	57.67 ± 5.04^{fghi}	77.67 ± 1.45^{a}
	MDA (nmol g^{-1} FW)									
Control	24.00 ± 3.06^{p}	30.67 ± 2.73^{mnop}	29.67 ± 3.28^{nop}	28.33 ± 3.53^{op}	32.67 ± 5.36^{lmno}	37.33 ± 2.40^{klmno}	27.00 ± 1.53^{p}	30.33 ± 1.45^{mnop}	30.67 ± 4.26^{mnop}	40.67 ± 4.70^{ijkl}
HT1	39.00 ± 1.73^{jklmn}	43.00 ± 4.16^{hijk}	39.33 ± 2.33^{jklm}	47.33 ± 4.26^{ghij}	54.00 ± 3.61^{defg}	51.67 ± 3.18^{efgh}	44.33 ± 2.73h^{ijk}	39.33 ± 2.96^{jklm}	49.67 ± 2.60^{fghi}	55.33 ± 3.71^{cdefg}
HT2	60.33 ± 0.88^{abcde}	55.33 ± 1.86^{cdefg}	58.33 ± 1.20^{abcdef}	64.33 ± 2.60^{abc}	67.00 ± 1.53^{ab}	62.33 ± 1.20^{abcd}	57.67 ± 1.33^{bcdef}	54.33 ± 2.91^{defg}	51.00 ± 2.08^{efgh}	67.67 ± 1.45^{a}
	H_2O_2 concentration (μmol g^{-1} leaf FW)									
Control	19.67 ± 1.76^{hijk}	21.00 ± 1.53^{hij}	17.67 ± 1.20^{ijk}	20.33 ± 1.45^{hijk}	17.00 ± 1.53^{jk}	21.33 ± 1.86^{hij}	22.00 ± 1.53^{hi}	21.00 ± 1.53^{hij}	15.67 ± 1.20^{k}	20.33 ± 1.86^{hijk}
HT1	27.67 ± 1.45^{efg}	27.00 ± 0.58^{efg}	27.33 ± 1.76^{efg}	27.67 ± 1.45^{efg}	23.00 ± 2.08^{gh}	29.67 ± 1.45^{def}	31.33 ± 1.20^{cde}	26.33 ± 0.88^{fg}	20.33 ± 0.88^{hijk}	30.33 ± 2.73^{cdef}
HT2	34.67 ± 0.88^{bc}	33.67 ± 0.88^{bcd}	35.00 ± 0.58^{bc}	37.67 ± 0.88^{b}	31.33 ± 0.88^{cde}	36.00 ± 0.58^{b}	37.00 ± 0.58^{b}	30.33 ± 2.19^{cdef}	27.00 ± 2.08^{efg}	43.67 ± 0.88^{a}

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's multiple-range test.

Table 4 Effect of heat stress on activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in faba bean genotypes.

Treatments	Genotypes									
	Zafar 1	Zafar 2	Giza Blanka	Espan	Makamora	Shebam 1	Giza 3	C4	C5	G853
	CAT activity (units mg^{-1} protein min^{-1})									
Control	156.33 ± 2.19i	142.67 ± 1.45jk	143.00 ± 1.15jk	146.67 ± 1.86j	165.00 ± 1.73hi	136.00 ± 2.31 k	139.33 ± 0.88jk	171.67 ± 3.71gh	180.00 ± 3.61 fg	191.00 ± 2.08e
T1 at 32 °C	185.00 ± 2.31ef	186.33 ± 2.73ef	158.67 ± 0.88i	158.67 ± 2.03i	177.33 ± 2.19 fg	159.00 ± 4.36i	183.67 ± 2.60ef	206.33 ± 2.73 cd	224.00 ± 2.65b	209.00 ± 3.21 cd
T2 at 38 °C	223.67 ± 2.73b	201.67 ± 4.91d	185.33 ± 2.03ef	179.67 ± 0.33 fg	191.00 ± 3.79e	172.00 ± 1.73gh	213.67 ± 6.44c	237.33 ± 6.77a	244.33 ± 2.33a	236.33 ± 2.40a
	POD activity (unit min^{-1} g^{-1} FW)									
Control	10.20 ± 0.80 m	20.33 ± 0.32 h	13.63 ± 0.18kl	16.27 ± 0.27jk	19.83 ± 0.15hi	11.27 ± 0.27 lm	16.43 ± 0.24j	17.30 ± 0.76ij	22.50 ± 1.22fgh	13.70 ± 0.12kl
T1 at 32 °C	13.77 ± 1.29kl	24.37 ± 0.77cdefg	17.23 ± 1.29ij	20.10 ± 0.62 h	24.77 ± 1.07cdef	15.70 ± 1.60jk	21.87 ± 1.17gh	20.50 ± 0.23 h	26.17 ± 0.84bcde	17.50 ± 1.22ij
T2 at 38 °C	21.70 ± 0.61gh	26.27 ± 0.44bcd	23.90 ± 0.64defg	23.50 ± 0.91efg	27.57 ± 0.64ab	19.83 ± 0.71hi	25.93 ± 0.61 bcde	26.63 ± 0.68abc	29.00 ± 0.67a	23.67 ± 1.61defg
	SOD activity (units mg^{-1} protein min^{-1})									
Control	11.33 ± 1.86lmn	13.00 ± 1.53klm	12.33 ± 0.88klmn	8.67 ± 2.19n	9.33 ± 1.45mn	8.33 ± 0.88n	9.67 ± 0.67mn	9.33 ± 1.86mn	11.33 ± 0.88lmn	15.00 ± 1.53hijkl
T1 at 32 °C	17.33 ± 1.45fghij	18.67 ± 1.45defgh	18.33 ± 1.20efghi	16.33 ± 1.20ghijk	15.00 ± 2.08hijkl	12.00 ± 1.53lmn	14.00 ± 1.15jkl	14.33 ± 0.88ijkl	17.33 ± 1.20fghij	20.33 ± 1.76cdefg
T2 at 38 °C	24.00 ± 1.53bc	24.33 ± 0.33abc	25.00 ± 0.58ab	22.67 ± 0.88bcd	21.67 ± 0.88bcde	18.67 ± 0.33defgh	21.00 ± 0.58bcdef	18.67 ± 0.88defgh	24.00 ± 0.58bc	28.00 ± 0.58a

Means followed by a similar letter within a column for each parameter are not significantly different at the 0.05 level of probability by Duncan's multiple-range test.

genotypes, 'C5' and 'C4' had the highest PH, Shoot FW and DW while genotype 'Espan' had the lowest at HS treatments. However, among all studied genotypes, 'C5' gave the maximum values for almost all parameters. Similarly, RWC, Pro accumulation, Total Chl content, EL, and MDA content were measured in all genotypes at different HS treatments (Tables 2 and 3). The effect of treatments on these parameters was found to be significant for all genotypes. Leaf RWC decreased with increasing temperature. A similar trend was observed in all cultivars. However, among the genotypes 'C5' and 'Giza 3' gave maximum values for RWC under HS. The accumulation of Pro increased with increasing levels of HS. The highest accumulation of Pro was observed in genotype 'C5' as compared to other genotypes at modest HS while content of Pro was higher in 'Giza Blanka' than all cultivars at ambient temperature (control). Total Chl content was found to be temperature dependent in all genotypes. Under HS, the lowest reduction of Total Chl was observed in genotypes 'C5' and 'Zafar-2' while the highest inhibition was found in genotype 'Espan'. EL, and MDA and H_2O_2 content were increased with increasing levels of temperature (Table 3). Also, genotypes 'C5' showed the lowest EL, and MDA and H_2O_2 content in comparison to the remaining genotypes under HS. However, cultivar 'Espan' exhibited the highest EL, and MDA and H_2O_2 content.

Activities of antioxidant enzymes (CAT, POD, and SOD) were significantly increased with increasing levels of HS in plants of all genotypes (Table 4). Under severe HS conditions, genotypes 'C5' and 'C4' showed similar CAT activity. Also, under HS, the highest enzyme activity was noted in genotype 'C5' and 'Zafar 1' for SOD. However, genotype 'C5' gave the maximum value for POD enzyme activity. Moreover, the magnitude of increase in these enzymes activities in genotype 'C5' was higher than other genotypes of faba bean under HS. Genotype 'Espan' exhibited the lowest enzymes activities under HS.

4. Discussion

4.1. Heat stress treatments

V. faba genotypes responded comprehensively in terms of growth and physiological characteristics and differential resistance to HS (Table 1). The HS exhibited a negative effect on all studied faba bean genotypes. The observed decrease in growth parameters (PH, Shoot FW and DW, leaf area) with increasing levels of temperature over the control may be due to the loss of turgidity and RWC (Table 2) (Kesici et al., 2013). The high level of temperature stress might be altered cell division and cell elongation resulted in reduced leaf area in all genotypes (Table 1). Rahman (2004) and Ahamed et al. (2010) in wheat, Al-busaidi et al. (2012) in *Jatropha curcas* reported that high temperature causes a significant negative influence on the plant growth. Among the studied genotypes, 'C5' responded better by giving maximum values for these characteristics, and found to be tolerant to HS, whereas 'Espan' found to be sensitive to HS. Leaf RWC has been established as an indicator of state of water balance of plants essentially in terms of the physiological consequences of cellular water deficit. In the present experiment, leaf RWC of all genotypes decreased significantly depending on temperature (Table 2). In all genotypes, RWC declined linearly from ambient to modest temperature. Among the cultivars, 'Giza-3' and 'C5' exhibited the highest RWC while 'Espan' had the lowest RWC under HS. This finding coincides with earlier studies of Kesici et al. (2013). Therefore, it was revealed that among the genotypes differed leaf RWC could indicate a corresponding difference in leaf hydration, leaf water deficit and physiological water status in plants. Pro accumulation is an important metabolic parameter that has a significant effect on cellular redox potential thereby lowering the generation of free radicals that is associated with the tolerance of plants to stress (Alia et al., 1993; Hare and Cress, 1997; Siddiqui et al., 2009). In this study, Pro accumulation was recorded to be the highest in stressed plants as compared to the control. The maximum accumulation of Pro was found in genotypes 'C5' and minimum Pro accumulation was found in genotype 'Espan' (Table 2). Bohnert and Jensen (1996) reported that organic solutes at higher concentrations improved enzyme activity and maintained the intercellular biochemistry. We infer that accumulation of Pro might have a role in tolerance of genotype 'C5' to HS.

Total Chl is an important biomolecule in plants for photosynthesis. In all genotypes, Total Chl synthesis was impaired with increasing temperature (Table 2). The highest content of Total Chl was recorded in genotype 'C5' and 'Zafar 2'. The change in Total Chl content in plant is a typical response of stress. Under stress, a decrease in Total Chl biosynthesis in faba bean cultivars may be due to inhibition of photosynthetic electron transport chain (Mohanty et al., 1989) and the enzymes of Chl biosynthesis, such as δ-aminolevulinic acid (ALA) synthase, ALA dehydratase, and porphobilinogenase (Tewari and Tripathy, 1998; Shalygo et al., 1999). Also, high temperature alters the anatomical structure in leaves, such as shape of chloroplasts, swelling of stromal lamellae, clumpy vacuoles that change the structural organization of thylakoids and form antenna-depleted PS II, and resulted in reduced photosynthetic and respiratory activities (Zhang et al., 2005; Lipiec et al., 2013). Therefore, the amount of Chl content strongly depends on the species' physiological responses and their ability to tolerate stress. Under different environmental stresses, EL, and content of MDA and H_2O_2 in plants play a key role as an indicator for oxidative damage (Siddiqui et al., 2013, 2014). In all plants of genotypes, EL, and content of MDA and H_2O_2 increased with increasing levels of temperature (Table 3). However, the lowest EL, and content of MDA and H_2O_2 was reported in genotype 'C5'. This indicates that cultivar 'C5' was more tolerant to HS than these nine cultivars. The lowest level of EL, and content of MDA and H_2O_2 in genotype 'C5' might be due to the highest accumulation of Pro (Table 2) that acts as an antioxidant and reduces the generation of free radicals and lipid peroxidation (Siddiqui et al., 2009; Alia et al., 1993). It is well established that antioxidant enzymes are extremely important as a part of defense mechanisms in plants, through which plants can endure under unfavourable environmental conditions by detoxifying reactive oxygen species (ROS), as we know that ROS causes harmful effect on plants by reacting with a large variety of biomolecules, such as DNA, protein and carbohydrates (Siddiqui et al., 2013). In the present experiment, activities of antioxidant enzymes (POD, CAT and SOD) in plants of all genotypes were substantially increased with increasing temperature (Table 4). However, under HS, the highest enzyme activities were noted in genotypes 'C5' and 'C4' for CAT, only 'C5'

for POD, and 'C5' and Zafar-1 for SOD. Overall, genotypes 'C5' and 'Espan' exhibited the highest and lowest enzyme activities under HS, respectively. The highest activity of these enzymes might help genotype 'C5' to be more tolerant than the others.

5. Conclusion

From the findings of the present studies, we conclude that genotype 'C5' has a better ability to tolerate high temperature, while genotype 'Espan' is relatively more sensitive to HS than 9 cultivars of *V. faba*. Under HS, genotypes of faba bean exhibited genotypic variations due their differential responses for morpho-physiological and biochemical characteristics. All morphological, physiological and biochemical characteristics of 10 genotypes of faba bean were reduced under HS. We observed that genotype 'C5' was most resistant to HS, and it has a better ability to retain more RWC and accumulation of Pro. Also, genotype 'C5' was relatively more efficient to reduce oxidative damage by increasing activities of CAT, POD and SOD. For further study, theses genotypes can be used to find the molecular mechanism(s) involved in tolerance of faba bean plants to HS. These genotypes can be used for further studies to uncover relation of pollen heat shock proteins with pollen viability and to show whether lack/inadequate expression of specific heat shock proteins has any role in oxidative damage to faba bean plants as well as pollens.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

This project was supported by NSTIP strategic technologies program number (11-BIO1922-02) in the Kingdom of Saudi Arabia.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Ahamed, K.U., Nahar, K., Fujita, M., Hasanuzzaman, M., 2010. Variation in plant growth, tiller dynamics and yield components of wheat (*Triticum aestivum* L.) due to high temperature stress. Adv. Agric. Bot. 2, 213–224.
Al-busaidi, A., Ahmed, M., Chikara, J., 2012. The impact of heat and water stress conditions on the growth of the biofuel plant *Jatropha curcas*. Int. J. Environ. Stud. 69, 273–288.
Alia, Saradhi, P.P., Mohanty, P., 1993. Proline in relation to free radical production in seedlings of *Brassica juncea* raised under sodium chloride stress. Plant Soil 155 (1), 497–500.
Al-Whaibi, M.H., 2011. Plant heat-shock proteins: a mini review. J. King Saud Univ. Sci. 23, 139–150.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24, 1–15.
Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 9, 205–207.
Bita, C.E., Gerats, T., 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front. Plant Sci. 4, 1–18.
Bohnert, H.J., Jensen, R.G., 1996. Strategies for engineering water-stress tolerance in plants. Trends Biotechnol. 14 (3), 89–97.
Chance, B., Maehly, A.C., 1955. Assay of catalase and peroxidases. Methods Enzymol. 2, 764–775.
FAO, 2010. FAO Cuts Wheat Production Forecast but Considers Supplies Adequate, <http://www.fao.org/news/story/tr/item/44570/icode/en/>.
Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol. 59, 309–314.
Grass, L., Burris, J.S., 1995. Effect of heat stress during seed development and maturation on wheat (*Triticum durum*) seed quality. I. Seed germination and seedling vigor. Can. J. Plant Sci. 75 (4), 821–829.
Gulen, H., Eris, A., 2003. Some physiological changes in strawberry (*Fragaria* × *Ananassa* cv. 'Camarosa') plants under heat stress. J. Hortic. Sci. Biotechnol. 78, 894–898.
Hare, P.D., Cress, W.A., 1997. Metabolic implications of stress induced proline accumulation in plants. Plant Growth Regul. 21, 79–102.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.
Hemantaranjan, A., Bhanu, A.N., Singh, M.N., Yadav, D.K., Patel, P.K., Singh, R., Katiyar, D., 2014. Heat stress responses and thermotolerance. Adv. Plants Agri. Res. 3, 1–10.
Kesici, M., Gulen, H., Ergin, S., Turhan, E., Ahmet, I.P.E.K., Koksal, N., 2013. Heat-stress tolerance of some strawberry (*Fragaria* × *ananassa*) cultivars. Notulae Bot. Hortic. Agrobot. Cluj-Napoca 41 (1), 244–249.
Khan, R.A., 1976. Effect of high-temperature stress on the growth and seed characteristics of barley and cotton. Basic Life Sci. 8, 319–324.
Lavania, D., Dhingra, A., Siddiqui, M.H., Al-Whaibi, M.H., Grover, A., 2015a. Current status of the production of high temperature tolerant transgenic crops for cultivation in warmer climates. Plant Physiol. Biochem. 86, 100–108.
Lavania, D., Siddiqui, M.H., Al-Whaibi, M.H., Singh, A.K., Kumar, R., Grover, A., 2015b. Genetic approaches for breeding heat stress tolerance in faba bean (*Vicia faba* L.). Acta Physiol. Plant 37, 1737. http://dx.doi.org/10.1007/s11738-014-1737-z.
Lipiec, J., Doussan, C., Nosalewicz, A., Kondracka, K., 2013. Effect of drought and heat stresses on plant growth and yield: a review. Int. Agrophys. 27 (4), 463–477.
Lobell, D.B., Schlenker, W., Costa-Roberts, J., 2011. Climate trends and global crop production since 1980. Science 333, 616–620.
Loss, S.P., Siddique, K.H.M., 1997. Adaptation of faba bean (*Vicia faba* L.) to dryland mediterranean-type environments I. Seed yield and yield components. Field Crop Res. 52 (1), 17–28.
Lutts, S., Kinet, J.M., Bouharmont, J., 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J. Exp. Bot. 46, 1843–1852.
Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11, 15–19.
Mittler, R., Finka, A., Goloubinoff, P., 2012. How do plants feel the heat? Trends Biochem. Sci. 37 (3), 118–125.
Mohanty, N., Vass, I., Demeter, S., 1989. Impairment of photosystem 2 activity at the level of secondary quinone acceptor in chloroplasts treated with cobalt, nickel and zinc ions. Physiol. Plant 76, 386–390.
NOAA, 2011. NOAA National Climatic Data Center, State of the Climate: Global Hazards for July 2010. Retrieved from: <http://www.ncdc.noaa.gov/sotc/hazards/2010/7>.
Ortiz, R., Sayre, K.D., Govaerts, B., Gupta, R., Subbarao, G.V., Ban, T., Hodson, D., Dixon, J.M., Ortiz-Monasterio, J.I., Reynolds, M., 2008. Climate change: can wheat beat the heat? Agric. Ecosyst. Environ. 126 (1), 46–58.
Rahman, M.M., 2004. Response of wheat genotypes to late seedling heat stress (MS thesis). Department of Crop Botany, Bangabandhu

Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

Sánchez, B., Rasmussen, A., Porter, J.R., 2014. Temperatures and the growth and development of maize and rice: a review. Global Change Biol. 20, 408–417.

Shalygo, N.V., Kolesnikova, N.V., Voronetskaya, V.V., Averina, N.G., 1999. Effect of Mn^{2+}, Fe^{2+}, Co^{2+} and Ni^{2+} on chlorophyll accumulation and early stages of chlorophyll formation in greening barley seedlings. Russ. J. Plant Physiol. 46, 496–501.

Siddiqui, M.H., Mohammad, F., Khan, M.N., 2009. Morphological and physio-biochemical characterization of *Brassica juncea* L. Czern. & Coss. genotypes under salt stress. J. Plant Int. 4, 67–80.

Siddiqui, M.H., Al-Whaibi, M.H., Sakran, A.M., Ali, H.M., Basalah, M.O., Faisal, M., Alatar, A., Al-Amri, A.A., 2013. Calcium-induced amelioration of boron toxicity in radish. J. Plant Growth Regul. 32, 61–71.

Siddiqui, M.H., Al-Whaibi, M.H., Faisal, M., Al Sahli, A.A., 2014. Nano-silicon dioxide mitigates the adverse effects of salt stress on *Cucurbita pepo* L. Environ. Toxicol. Chem. 33, 2429–2437.

Singh, A., Grover, A., 2008. Genetic engineering for heat tolerance in plants. Physiol. Mol. Biol. Plants 14, 155–166.

Smith, G.S., Johnston, C.M., Cornforth, I.S., 1983. Comparison of nutrient solutions for growth of plants in sand culture. New Phytol. 94, 537–548.

Song, Y.H., Ito, S., Imaizumi, T., 2013. Flowering time regulation: photoperiod-and temperature-sensing in leaves. Trends Plant Sci. 18 (10), 575–583.

Teixeira, E.I., Fischer, G., van Velthuizen, H., Walter, C., Ewert, F., 2013. Global hot-spots of heat stress on agricultural crops due to climate change. Agric. Forest Meteorol. 170, 206–215.

Tewari, A.K., Tripathy, B.C., 1998. Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. Plant Physiol. 117, 851–858.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59–66.

Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R., 2007. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61, 199–223.

Zhang, J.H., Huang, W.D., Liu, Y.P., Pan, Q.H., 2005. Effects of temperature acclimation pretreatment on the ultrastructure of mesophylls in young grape plants (*Vitis vinifera* L.cv Jingxiu) under cross temperature stresses. J. Integr. Plant Biol. 47, 959–970.