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ORIGINAL ARTICLE

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

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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

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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.