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Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum*) cultivars under combined drought and heat induced oxidative stress

Askim Hediye Sekmen, Rengin Ozgur, Baris Uzilday, Ismail Turkan*

Department of Biology, Faculty of Science, Ege University, Bornova, 35100 Izmir, Turkey

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ABSTRACT

Crop losses due to combined drought and heat is predicted be greater in the future especially due to climate change. Understanding underlying mechanisms under drought and heat combination will be crucial for the selection and breeding of tolerant varieties. The objective of this study was to assess the physiological and biochemical responses of two cotton cultivars (84-S and M-503) differing in drought tolerance to the combined effects of drought and heat. The relative growth rate (RGR) of the cultivars was decreased by 62.9% in drought sensitive 84-S and reduced by 34.58% in drought tolerant M-503 due to the combined drought and heat stresses. Combined stress also enhanced lipid peroxidation (TBARS) by 170.24% and 21.9% in 84-S and M-503, respectively which suggest that drought sensitive 84-S is more sensitive to combined stress than M-503. This sensitivity to combined stress of 84-S was associated with decreased activities of catalase (CAT) and peroxidase (POX) as compared to its control, resulting in higher H_2O_2 accumulation and oxidative stress induced lipid peroxidation. On the other hand, a higher combined stress tolerance of M-503 was associated with its ability to maintain constitutive activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) and induced CAT and POX. The proline content of drought resistant M-503 was greatly enhanced under drought and the combination of drought and heat treatments as compared to 84-S. To the best of our knowledge, this is the first study conducted on the activities of antioxidant enzymes of cotton under drought and heat combination.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important textile fibres in the world, as it is cultivated on more than 32 million ha over 76 countries. Heat and drought are prevalent conditions, which can result in dramatic losses in cotton yield and fibre quality (Saranga et al., 2001; Singh et al., 2007). Sankaranarayanan et al. (2010) reported that 40–50% losses in the biomass of cotton subjected to high temperature (40/30 °C) is anticipated, as opposed to that obtained at the optimum temperatures of 30/20 °C.

Like other abiotic stresses, heat and drought are accompanied by an increased production of reactive oxygen species (ROS) such as superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\bullet$) (Miller et al., 2008; Choudhury et al., 2013). Under drought conditions, limitation of gas exchange by stomatal closure causes a loss in the balance between the light reactions and the Calvin–Benson cycle due to decreased CO_2 diffusion (Chaves et al., 2009). As a result, electron carriers can be over-reduced in chloroplasts and mitochondria resulting in the production of ROS by the transfer of electrons to molecular oxygen (Foyer and Noctor, 2012). Heat stress also causes ROS production in chloroplasts and mitochondria by disturbing membrane stability and biochemical reactions such as the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase, resulting in photorespiration and enzymes involved in cellular respiration such as complex I and III in the mitochondrial electron transport chain (Sharkey, 2005; Jaspers and Kangasjarvi, 2010). Besides this metabolic production of ROS, there are other ROS producing systems such as NADPH oxidases (NOX) in the plasma membrane, amine oxidase in the apoplast and xanthine oxidase in peroxisomes, which are all induced by environmental stimuli (Mittler, 2011; Gill and Tuteja, 2010). Overproduction of ROS under environmental stress can damage plant cells irreversibly by oxidation of cellular components such as lipids, proteins and DNA (Apel and Hirt, 2004). To scavenge damaging ROS, plants have developed detoxifying enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), glutathione reductase (GR), ascorbate peroxidase (APX), and non-enzymatic antioxidants such as ascorbate, glutathione, carotene and tocopherols (Gill and Tuteja, 2010; Suzuki et al., 2012). Apart from their destructive effects in cells,

* Corresponding author. Tel.: +90 2323112443; fax: +90 2323111036.
E-mail address: ismail.turkan@ege.edu.tr (I. Turkan).

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ROS can also act as signalling molecules in many biological processes such as stomatal closure, growth, development, and stress signalling (Demiral et al., 2011). Due to this dual role of ROS, plants are able to fine-tune their concentrations between certain thresholds by means of production and scavenging mechanisms. Since this ROS homeostasis is disrupted under stress in favour of production, constitutive and induced enzymatic antioxidant defences are considered a crucial component of plant stress tolerance (Miller et al., 2008; Suzuki et al., 2012). Accordingly, this has been an intense area of research, but the role of antioxidant enzymes in abiotic stress tolerance has still not been fully elucidated. In particular, the signalling role of ROS in plants, is still only in the very early stages of investigation (Loiacono and De Tullio, 2012; Mittler, 2011).

Current research on climate change predicts that plants will be subjected to a combination of drought and heat more often in forthcoming years, than under the present conditions (IPCC, 2007). The response of plants to a combination of these stresses is more complex than the response to a single stress such as drought or heat, which is discussed in detail by Mittler (2006). Hence, understanding the biochemical mechanisms underlying the tolerance of economically important crop plants such as cotton to combination of heat and drought, occurring together, is essential for the selection of suitable genotypes for improved yield. However, previous studies investigating the ROS scavenging capacity of cultivated plants have focused mainly on the effects of these two stresses applied separately. For instance, the antioxidant responses of wheat (Zhang and Kirkham, 1994; Selote and Khanna-Chopra, 2010; Hameed et al., 2011), bean (Turkan et al., 2005), rice (Sharma and Dubey, 2005; Basu et al., 2010), maize (Bai et al., 2006), and sugarcane (Cia et al., 2012) have been evaluated following drought stress alone. On the other hand, the antioxidant defence capacities of wheat (Sairam et al., 2000; Almeselmani et al., 2006), rice (Kumar et al., 2012), maize (Gong et al., 1997; Kumar et al., 2012) were investigated in response to heat stress alone.

Similarly, the antioxidant responses of cotton have been studied only under drought stress (Ratnayaka et al., 2003; Deeba et al., 2012) and salinity (Gossett et al., 1994; Meloni et al., 2003). The response of cotton to heat stress in terms of antioxidant defence has also evaluated in different studies (Mahan and Mauget, 2005; Gür et al., 2010). However, there have been no studies conducted on ROS formation and ROS detoxification by cotton grown under combination of drought and heat stresses.

Therefore, in the present study, we have attempted to elucidate ROS formation and the ROS detoxification capacity of the antioxidant defence systems of two cotton cultivars differing in drought tolerance, which have been grown under the combined effects of drought and heat stress. In this investigation, the physiological and biochemical characteristics of two cotton cultivars, drought-sensitive Nazilli 84-S (Yildiz-Aktas et al., 2009) and drought-tolerant Nazilli M-503 (Sahin et al., 2000) were evaluated under drought and heat stress and their combination. The enzyme activities analyzed included SOD (isoforms), CAT, POX, APX, GR and NOX. In addition, the relative growth rate, photosynthesis stress indicator F_v/F_m, H_2O_2 content and lipid peroxidation level were also determined.

2. Materials and methods

2.1. Plant material and stress applications

Drought sensitive Nazilli 84-S (Yildiz-Aktas et al., 2009) and drought tolerant Nazilli M-503 (Sahin et al., 2000) cotton cultivars were used in this study, which were obtained from Nazilli Cotton Research Institute (NCRI) (Nazilli, Aydın, Turkey). Seeds were sterilized in 70% ethanol solution (2 min) and then rinsed five times in distilled sterile water. Seeds were germinated at 25 °C for 3 days under a 16 h light/8 h dark cycle in pots filled with perlite. Seedlings were grown for three weeks under controlled conditions (light/dark regime of 16/8 h at 25 °C, relative humidity of 70%, photosynthetic photon flux density of (PAR) 350 μmol m^{-2} s^{-1}) and were sub-irrigated every other day with a half-strength Hoagland's solution (Hoagland and Arnon, 1950). After three weeks, seedlings that had the same growth were randomly separated into four groups (control, drought, heat, drought + heat). For drought stress, cotton seedlings were treated with 40% PEG 6000 for 10 days. Heat stress was gradually applied to 31 day old seedlings from 30 °C to 45 °C (1 °C/15 min) and plants were incubated for an additional 2 h at 45 °C. For combination of these stresses, drought treated plants were subjected to heat treatment at the end of the 10th day of drought treatment. Non-stressed plant groups were used as controls (experimental design is shown in Fig. 1). All groups were harvested on the 31st day. Fully expanded 3rd leaves of seedlings were harvested and then stored at −80 °C until further analysis. Leaves were counted from bottom to top. All the experiments were repeated 3 times.

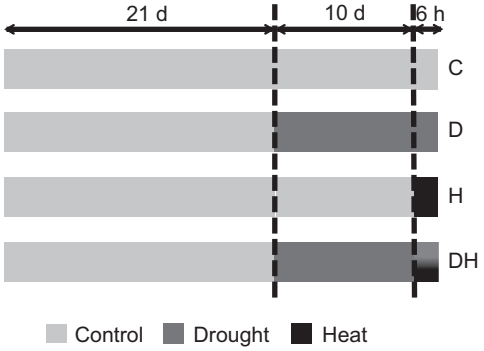

Fig. 1. Scheme summarizing experimental design.

2.2. Growth analysis

Three-week-old seedlings were harvested for RGR calculation before treatments (0 day). After the treatments, random plants for control, drought, heat and drought + heat groups ($n = 6$) were used for the growth analyses and were separated to shoot and root fractions. Shoots were dried at 70 °C for 72 h and dry weights were used to calculate the relative growth rate (RGR) of shoots according to the spreadsheet provided by Hunt et al. (2002). 10.25 d was used duration of treatment in the spreadsheet for 10 d + 6 h of treatment time.

2.3. Leaf relative water content (RWC)

3rd leaves ($n = 6$) were obtained from each treatment group and their FW was determined. The leaves were floated on deionised water for 6 h under low irradiance and then the turgid tissue was quickly blotted to remove excess water and their turgid weights (TW) were determined. DW was determined after the leaves were dried in the oven. The relative water content (RWC) was calculated by the following formula:

$$\mathrm{RWC}(\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

2.4. Chlorophyll fluorescence

The maximum quantum yield of PSII (F_v/F_m) of leaves ($n = 6$) for each treatment group was measured using a Plant Efficiency