Flora 242 (2018) 137–145

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

# Anatomically distinct representatives of Cactaceae Juss. family have different response to acute heat shock stress

Nataliia Nuzhyna[a,*], Kateryna Baglay[a], Anastasiia Golubenko[a], Oleh Lushchak[b]

[a] Educational and Scientific Centre "Institute of Biology and Medicine" Kyiv Taras Shevchenko National University, 1 Symona Petlyury str., Kyiv, 01032, Ukraine
[b] Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenka str., Ivano-Frankivsk, 76018, Ukraine

ARTICLE INFO

Edited by Hermann Heilmeier

*Keywords:*
*Aylostera flavistyla*
*Echinocactus grusonii*
*Mammillaria bocasana*
Hyperthermia
Antioxidant enzymes
Stem anatomy

ABSTRACT

In order to escape extreme environmental conditions plants have developed different survival strategies. The study of plant response to high temperatures is especially interesting in representatives of varied cacti families that have to resist big temperature oscillations. In the present study plants of *Aylostera*, *Echinocactus* and *Mammillaria* genera were exposed to temperatures of 40 or 50 °C for 3 h and peroxidase and superoxide dismutase activity, photosynthetic pigments and flavonoids concentration, and lipid peroxide oxidation level in the stem were determined. Plants with different anatomical structure showed distinct patterns of response to high temperature. Plants of *Echinocactus grusonii* proved the highest drought and heat resistant with strong antioxidant response and the most expressed xerophytic features of anatomical structures (epidermis and cuticle thickening, calcium oxalate accumulation, water-storage tissue development). Heat stress induced a quick increase of peroxidase activity and flavonoid concentration in this species. Relative heat resistance (40 °C) in *Mammillaria bocasana* is also facilitated by increased flavonoid concentration, chlorophyll a and carotenoid concentration and also high superoxide dismutase activity under normal conditions.

We conclude that the development of antioxidant reactions depends on anatomical protection of plants. More effective anatomical conformation to the stress conditions may be accompanied by rapid response to stress at the antioxidant biochemical level. Less effective protection at the anatomical level to hyperthermia is accompanied by the presence of a larger activity of antioxidant enzymes in conditions without short-term high-temperature stress. Plants which do not possess these qualities, *Aylostera flavistyla* in particular, suffer more from short-term high-temperature stress.

## 1. Introduction

An increase in environmental temperature is one of the common stress factors for plant organisms. The importance of this problem is growing due to planetary climate change which is predicted to come with rapid temperature changes within short periods of time (Bita and Gerats, 2013; Cao and Woodward, 1998; Jones and Moberg, 2003).

Plants of Cactaceae Juss. family show high drought and heat resistance (Nobel, 1984; Nobel et al., 1986). Representatives of different genera vary in critical temperature they can survive by using different adaptation strategies to high-temperature effects. On the other hand, during the early stages of development, plants of the Cactoideae subfamily are especially sensitive to arid conditions (Rosas et al., 2012). Many experiments on succulent species showed different adaptation strategies to arid conditions at the anatomical level (Secorun and De Souza et al., 2011; Zutta et al., 2011; Nuzhyna and Gaydarzhy, 2015; Kalashnyk et al., 2016). There are some studies devoted to changes in anatomical structure of cacti germinants in response to alterations to the environment (Ayala-Cordero et al., 2006; Nobel, 2002; Smith et al., 1984). A lot of attention is paid to biochemical response to stress adaptations. Hyperthermia, as well as other abiotic stress factors, is known to cause oxidative stress in various plants: accumulation of reactive oxygen species and intensification of lipid peroxidation (Barkasdjieva et al., 2000; Hernandez et al., 2000; Mittler et al., 2004; Zhang and Kirkham, 1994). Lipid peroxidation can be quantified by amounts of malone dialdehyde (MDA). MDA accumulation indicates an intensification of lipid oxidation or decreased degradation of damaged molecules (Lushchak, 2011). Superoxide dismutase, catalase and peroxidases establish the first chain of protection against reactive oxygen species (Grant, 2000; Sunkar, 2006). Flavonoids are known to take part in photosynthesis, lignin and suberin formation. They protect plant tissues from excessive radiation and act as antioxidants through absorption of UV light (Di Ferdinando et al., 2011; Gratão et al., 2005; Middleton et al., 2000). The pigment content of the photosynthetic

* Corresponding author.
*E-mail addresses:* nuzhyna@univ.kiev.ua (N. Nuzhyna), dzjuba@nas.gov.ua (K. Baglay), an_hol@univ.kiev.ua (A. Golubenko), olehl@pu.if.ua (O. Lushchak).

https://doi.org/10.1016/j.flora.2018.03.014
Received 15 November 2017; Received in revised form 14 March 2018; Accepted 26 March 2018
Available online 29 March 2018
0367-2530/ 

system depends on the genotype, environmental conditions, and developmental stage. The photosynthetic apparatus of plants is one of the most sensitive to various adverse environmental factors (Chetti and Nobel, 1988). Therefore, these parameters can be used as markers of changes occurring inside plants under stress. The identification of adaptation features at the biochemical level of plants with different anatomical structure and identification of the relationship between the anatomical and biochemical indicators can help better to better understand plant adaptation to hyperthermal stress.

In the present study plants of several rare and endangered Cactaceae species from different origin were exposed to short-term high-temperature stress. The aim of the work was to determine whether the response to heat stress, such as pigment concentrations, activities of antioxidant enzymes and the intensity of lipid oxidation is different in species with different anatomical structure; also to reveal the connection between the anatomical structure and changes in the biochemical parameters. Research of stress mechanisms in various plant groups will improve the understanding of plant adaptive reactions in general.

## 2. Materials and methods

### 2.1. Plants and experimental conditions

The representatives of three species of Cactacetae family were used in this study. Plants of *Aylostera flavistyla* F. Ritt., *Mammillaria bocasana* Pos., and *Echinocactus grusonii* Hildm., were grown from seeds obtained from introduced plants of the O. Fomin Botanical Garden collection of Taras Shevchenko National University of Kyiv. The latter two species are included into the IUCN red list.

Plants were grown under identical conditions on mixed soil with content of peat, turf and sand in equal proportions (1:1:1) without additional fertilizers. Plants were regularly watered after the top layer of substrate had dried out. During the winter time plants were kept in a greenhouse on a sun-lit bench (92.5 μmol $m^{-2} s^{-1}$) at 13–15 °C /10–12 °C (day/night) and in spring at 277.5 μmol $m^{-2} s^{-1}$ and 25–26 °C/18– 20 °C (day/night). Humidity ranged from 30% day to 80% at night. The experiment itself was conducted in the first decade of May on the 1-year-old plants not adapted to high temperatures.

The species selected come from different areas; therefore their adaptability to high temperatures varies. *Aylostera flavistyla* is widespread in Bolivia (Tarija). It grows in the mountains, 2000 m above the sea level, among fragmented rocks covered by xerophytic bushes. *Echinocactus grusonii* is widespread in Central Mexico (San Luis Potosí, Hidalgo). It grows on steep slopes, in mountain gorges, on argillaceous-calcareous soils. *Mammillaria bocasana* is spread in Mexico (San Luis Potosí and Zacatecas). It grows in the mountains, 1700–2300 m above the sea level, among the rocks on volcanic rocks in the semi-desert (Anderson, 2001).

### 2.2. Anatomical and drought resistance studies

Medial stem parts of the one-year-old plants were used for anatomical studies. The height of *A. flavistyla* and *M. bocasana* plants was 2.5 cm, *E. grusonii* – 3.5 cm. The samples were fixed with FAA, then poured over with gelatin using standard procedure (Romeis, 1948). Freezing microtome OMT-28-02E (KB-Technom, Russia) was used for making cross-sections 15–20 μm thick. The cross-sections were stained with safranin, sudan, $I_2$–KI. In addition, maceration of the stems was conducted in order to study the structures of epidermis. The epidermis was described according to Zarinkamar (2007) with the microscope XSP-146TR (China). Microscopic measurements were conducted using the ocular-micrometer on the XSP-146TR microscope and Image J (Wayne Rasband, NIH). Thirty slices from 6 plants were analyzed per species.

Water content in tissues, water deficiency and water retention ability per hour of wilting were measured for evaluation of drought resistance using Giang method (Giang and Tokhtar', 2011) (n = 7 for each species). For measuring water content in tissues (%) the stems of the one-year-old plants were dried in individual glass bottles in a thermostat at a temperature of 105 °C to constant weight. Water deficiency (%) is defined as difference between the mass of stems before and after 24 h water saturation. Water retention ability (WRA) is an important factor to define plant resistance to long lasting drought. It characterizes the speed of water release by vegetative organs. After stems drying during 2, 4 and 6 h the average water loss per hour of wilting (%) was calculated.

### 2.3. Biochemical parameters

The plants (n = 6 in each group) were exposed to heat stress at temperatures of 40 or 50 °C for 3 h in a dry thermostat with a glass front wall to receive natural daylight. No additional light sources during the thermal treatment were used because bright light can lead to the increase of high-temperature inhibiting effect on the photosynthetic system (Foyer et al., 1994). The control groups of plants (n = 6 in each group) were held at 26 °C in an identical dry thermostat with a glass front wall to receive natural daylight (92.5 μmol $m^{-2} s^{-1}$). At the initial moment of experiment the level of air humidity in both thermostats was 40%.

Lipid peroxidation was determined by measuring malone dialdehyde (MDA) content, that forms a color adduct with thiobarbituric acid (BioChem, China) at $\lambda$ = 532 nm (Kumar and Knowles, 1993). Samples were homogenized and centrifugated (8000 × *g*, 10 min) with 1 ml of 20% trichloroacetic acid. MDA concentration is expressed in μmol per gram fresh mass (Lushchak et al., 2004).

Samples for measurement of total superoxide dismutase (SOD) activity were homogenized at 4 °C in 50 mM phosphate buffer (pH 7.8) and centrifuged at 8000 × *g* for 10 min. Total SOD activity was determined using a method which is based on SOD ability to compete with nitroblue tetrazolium (Fisher Scientific, Belgium) for superoxide radical generated by riboflavin photo-oxidation (Reanal, Hungary), absorption maximum at $\lambda$ = 560 nm. SOD activity was expressed as U/mg protein (Giannopolitis and Ries, 1977). Protein concentration was determined by Warburg and Christian method at $\lambda$ = 280 and $\lambda$ = 260 nm (Warburg and Christian, 1941) and expressed as milligram per gram of fresh mass.

For peroxidase (POX) activity measurement plant tissues were homogenized in 0.2 M acetate buffer (pH 5.0) and centrifuged at 8000 × *g* for 10 min at 4 °C and the supernatant used to determine POX activity. Peroxidase activity was determined by rate of benzidine oxidation (Khimfarminvest, Ukraine) in presence of $H_2O_2$. Changes in optical density were monitored at 590 nm (Sharifi and Ebrahimzadeh, 2010). Peroxidase activity was expressed as U/mg of protein.

Total flavonoid concentration was measured in 70% ethanol extracts spectrophotometrically at 410 nm and expressed as rutin equivalents (Trineeva et al., 2014). The pigments were measured at 470, 646 and 663 nm in plant extracts made with 80% acetone (Lichtenthaler, 1987). Pigment concentration was expressed as milligram per gram of fresh mass.

SF-2000 spectrophotometer (Spectr, Russia) was used for all biochemical measurements.

### 2.4. Statistical analysis

The data were analyzed in Prism Graphpad 6. The values for different groups were compared by ANOVA followed by Tukey's multiply comparison test. A two-way analysis of variance (ANOVA) followed by a Bonferroni test was used when two factors were varied: temperatures (26, 40 or 50 °C) and species (*Aylostera flavistyla, Echinocactus grusonii* and *Mammillaria bocasana*).