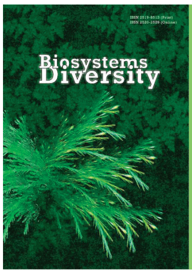

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Various antioxidant responses to hyperthermia in anatomically different species of the genus *Rosa*

N. V. Nuzhyna, O. O. Tkachuk

Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

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Taras Shevchenko
National University of Kyiv,
Simon Petliura st., 1,
Kyiv, 01032, Ukraine.
Tel.: +38-067-339-97-73.
E-mail:
nuzhynan@gmail.com,
lifeofrose@ukr.net

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The heat and drought resistance of plants depend on their anatomical and biochemical features. In the present study, the adaptive features of three species of wild rose (Rosaceae, Rosales) under the short-term impact of high-temperature stress have been characterized. Plants of the species *Rosa donetzica* Dubovik, *R. reversa* Waldst. et Kit. and *R. spinosissima* L. were exposed to a temperature of 40 degrees C for 3 hours, following which peroxidase and superoxide dismutase activity, photosynthetic pigments and flavonoids' content, and lipid peroxide oxidation level in the leaf were determined. In our investigation, the anatomical structure of leaves and drought resistance of three species of *Rosa* were studied. Xeromorphic features are the most expressed for *R. reversa* and *R. spinosissima* and almost absent for *R. donetzica*. It has been established that *R. spinosissima* is photophilous whereas *R. donetzica* is shade-tolerant. The relatively lower development of epidermic tissue in *R. donetzica* could probably contribute to more active destruction of the pigment complex under high temperature stress. The obtained data about changes in activity of peroxidase, superoxide dismutase, content of photosynthetic pigments and flavonoids, and level of lipid peroxidation indicate the low heat resistance of *R. donetzica* in comparison with the other two species. *R. reversa*, *R. spinosissima* were more tolerant to short-term hyperthermia. They showed faster antioxidant response, mainly due to the induction of peroxidase activity under stress. The species with the most expressed xerophytic features of anatomical structures have rapid antioxidant response and are more resistant to short-term hyperthermia. The induction of some activity of antioxidant enzymes "in reserve" is a less effective form of adaptation in wild roses. Such activation of enzymes is observed in plants with a more mesophytic structure. Flavonoids and superoxide dismutase were thermolabile to short-term influence of high temperature; therefore they play an insignificant role as antioxidants in the protecton against oxidative stress caused by high temperature stress in wild roses.

Keywords: leaf anatomy; drought resistance; superoxide dismutase; peroxidase; pigments.

Introduction

During the last decades there has been a steady tendency to increase in average annual air temperature (Hansen et al., 1999; Jones & Moberg, 2003; Bita & Gerats, 2013). Weather conditions are becoming more unstable – sharp temperature fluctuations and frequent thaws in winter, high temperature and moisture deficit in summer significantly influence the development of vegetation. Changes in ontogenesis of plants deserve attention from biologists and require the detailed analysis of adaptative features of plants in the current conditions of global and regional climatic dynamic (Khromykh et al., 2018).

Wild representatives of the genus *Rosa* L. have long been valuable and useful plants. Their biochemical properties enable wild roses to be used in pharmacology. Biological peculiarities and decorative qualities of wild roses are used in landscape architecture (Boskabady et al., 2011). Success of wild rose cultivation depends on many environmental factors, one of which is the temperature regime. As is known, drastic temperature fluctuations have a pronounced negative effect on plant metabolism, growth and quality of plant production. Reactive oxygen species levels and lipid peroxidation intensities tend to increase if plants are exposed to stressful conditions such as low or high temperatures, or drought (Mittler et al., 2004; Scandalios, 2005; Tuteja et al., 2013). A lot of attention is paid to biochemical response to stress adaptations. Superoxide dismutase, catalase and peroxidases establish the first chain of protection against reactive oxygen species (Grant, 2000; Sunkar, 2006; Rai et al., 2015; Harsh et al., 2016). The pigmental complex is very sensitive to environmental temperature changes (Huang, 2004; Olmos et al., 2007; Babenko et al., 2014; Gosavi et al., 2014; Rodríguez et al., 2015). Therefore, these parameters can be used as markers of changes occurring inside plants under stress. However, the existing body of studies was conducted mainly on agricultural crops (Huseynova et al., 2014; Kong et al., 2014; Talaat et al., 2014; Caverzan et al., 2016; Hasanuzzaman et al., 2018, 2019), while woody plants have received very little attention. Also the effect of drought stress on plant establishment and survival is becoming increasingly severe (Niu & Rodriguez, 2009; Asrar et al., 2012; Cai et al., 2012; Abdel-Salam et al., 2018), which makes selection of drought-tolerant plants increasingly important.

The assessment of features of leaf structure, as one of the most multifunctional vegetable organs, the exploration of biochemical changes that occur as a result of impact of unfavourable factors, allow one to define the mechanisms and the adaptation features at species as well as genus level. The anatomic features of some wild roses from different regions are described in modern scientific works (Capellades et al., 1990; Tabuchi et al., 2010; Essiett et al., 2014). But there remains insufficient research into leaf anatomic features of *Rosa* species which takes into account their heat- and drought-resistance (Tore et al., 2003).

As is known,the vast majority of garden rose cultivars are budded or grafted onto rootstocks and are seldom grown on their own roots (Cai et al., 2012). The discovery of more drought-resistant and heat-resistant wild roses can help in identifying potential rootstocks for varietal roses.

Taking into account the above-mentioned facts, the aim of this work is to make a comparative analysis of adaptive features in different high-decorative species of wild rose by means of detailed study of their anatomical and biochemical features (such as pigment content, activities of antioxidant enzymes and the intensity of lipid oxidation) under the conditions of optimal temperature cultivation and after the short-term impact of high-temperature stress; also to reveal the connection between the anatomical structure and changes in the biochemical parameters. Research on stress mechanisms in various plant groups will improve the understanding of plants' adaptive reactions in general.

Materials and methods

Study species. Plants from collection of the O. V. Fomin Botanical Garden were studied. Three species of the genus *Rosa* from different natural habitats and thus with an excellent adaptation to high temperatures were selected. *R. spinosissima* L. and *R. reversa* Waldst. et Kit. are species with a very wide range. They grow in the Balkans, in West Siberia, in the Caucasus, in Asia Minor, in Central, Western and Eastern Europe, in Scandinavia, in Western China and in Mongolia. *R. donetzica* Dubovik is a species with a very limited range, growing in the basins of the Mius and Kalmius rivers, on the Donetsk and Azov Uplands and perhaps in Russia (Rostov region). This species is listed in the Red Book of Ukraine.

Anatomical methods. For anatomical study, two leaves from five plants of each species were collected from annual growth. The anatomical data were obtained from the middle part of the leaf. We fixed samples by FAA (formalin, acetic acid, alcohol), embedded in gelatin by the standard method (Romeys, 1948) and prepared leaf cross-sections (thickness of 10–15 µm) by the freezing microtome OMT-28-02E (KB-Technom, Russia), as well as skinning the epidermis from macerated leaves. The slices were stained with safranin. Microscopic measurements were performed using an Olympus System Microscope Model BX 41 (Tokyo, Japan) and Image J program (Wayne Rasband, NIH). We described the length and width of the leaf.

Drought resistance methods. To determine the drought resistance of the plants (n = 7 for each species) we measured the water content of their tissues, water deficiency and water loss after 1 hour of wilting was measured to evaluate the degree of their drought resistance according to Zhang & Tohtar' (2011) (Table 1).

$CW = (b – c) / (b – a) \times 100$, where CW – content of water in tissues (%), a – the mass of an empty sample bottle (g), b – the mass of the sample bottle with wet sample (g), c – mass of the sample bottle with dry sample (g).

$WD = (M_2 – M_1) / (M_2 – M_3) \times 100$, where WA – water deficiency (%), M_1 – the mass of stems before 24-hour water saturation (g), M_2 – the mass of stems after 24-hours saturation (g), M_3 – mass of dry sample (g).

$WL = (M_1 – M_2) / M_3 \times 100$, where WL – water loss per hour (%), M_1 – the mass of stems before wilt (g), M_2 – mass of stems after 2, 4, or 6 hours of withering (g), M_3 – the mass of a dry sample (g).

Table 1
The scale of leaf water regime parameters for assessment of relative drought resistance, by Zhang & Tohtar' (2011)

Evaluation of drought resistance	Water content of tissues, %	Water deficit, %	Average loss of water afterr 1 hour of wilting, %
Low	< 59.9	< 20.1	< 11.1
Middle	60.0–69.9	10.1 – 20.0	10.1 – 11.0
High	> 70.0	< 10.0	< 10.0

Biochemical methods. In the experiment we used leaves of three-year old wild roses from the Botanical Garden collection. The study was conducted on plants not adapted for high temperature in early June, when the day temperature was +23...+25 °C. Experimental plants in pots with soil were warmed up in a thermostat at a temperature of +40 °C for three hours. The front wall of the thermostat is made of glass thus the plants received natural daylight. We did not use any additional light sources during the thermal treatment, considering that bright light can lead to the increase of the high temperature inhibiting effect on the photosynthetic system (Foyer, 1994). The control group of plants was kept at + 25 °C. All experiments were performed four times.

Biochemical studies were performed using a spectrophotometer SF-2000 (Spectr/ Russia).

Lipid peroxidation was evaluated by malondialdehyde (MDA) content determined by colour reaction with thiobarbituric acid based on the formation in the acidic environment of a trimethyl coloured complex, which has a distinctive absorption spectrum with a maximum at $\lambda = 533$ nm (Kumar & Knowles, 1993). The amount of MDA expressed in µM/g of wet weight. Superoxide dismutase (SOD) activity was assessed by its ability to compete with nitroblue tetrazolium for superoxide radicals that originate from riboflavin photooxidation at $\lambda = 560$ nm. SOD activity was expressed in standard activity units per mg of protein (Giannopolitis & Ries, 1977). Content of protein was evaluated based on the method of Warburg & Christian (1941), at $\lambda = 280$ i $\lambda = 260$ nm and expressed in mg/g of wet weight.

Peroxidase activity was determined by the speed of benzidine oxidation to the formation of a blue product in presence of H_2O_2 and peroxidase at $\lambda = 590$ nm. Peroxydase activity was expressed in standard units per 1 g of dry weight (Sharifi & Ebrahimzadeh, 2010).

The total content of flavonoids expressed in terms of routine and absolute dry weight was assessed by the method of Trineeva et al. (2014) at $\lambda = 410$ nm and expressed as a percentage.

Pigments were extracted from plant material with 80% acetone and were determined at $\lambda = 663$, 646, 470 nm in terms of the wet weight (Lichtenthaller, 1987).

The data were analyzed in Prism Graphpad 6 (GraphPad Company, San Diego, USA, 2014). The values for different groups were compared by ANOVA followed by Tukey's multiple comparison test. A two-way analysis of variance (ANOVA) followed by a Bonferroni test was used when two factors were varied: temperatures (26 or 40 °C) and species *R. donetzica*, *R. spinosissima* and *R. reversa*. The correlation was determined by Pearson coefficient.

Results

Anatomical studies. The anatomical studies have shown that the leaves of the considered species of the genus *Rosa* are dorsoventral, hypostomatic. The surface of the lamina of *R. reversa* is more intensively covered by unicellular filiform trichomes than that of *R. donetzica*. *R. spinosissima* has no trichomes. The peculiarity of *R. reversa* is presence of glandular trichomes with multicellular pedicle and head on the abaxial side of leaf. It is noteworthy that number of trichomes on the abaxial surface is higher than on the adaxial for *R. donetzica* and *R. reversa*.

According to the results, the leaves of the researched wild roses have a similar anatomical structure: single-layer epidermis is covered with cuticle; two layers of palisade parenchyma, the spongy parenchyma consists of three layers of cells and large intercellular spaces; around the vascular bundles are many inclusions of calcium oxalate, vascular bundles are surrounded with angular collenchyma on the top and bottom.

The analysis of morphometric parameters indicates that the adaxial epidermis and its outer cell wall have larger thickness compared to the abaxial surface for all studied species (Fig. 1). However, the protective function of the lower side is offset by significantly higher trichome density. At the same time, according to the linear dimensions of the leaf, the species were ordered as follows: the smallest lamina is typical for *R. spinosissima* (length 14.0 ± 2.0 mm, width 8.0 ± 1.0 mm) → *R. donetzica* (25.7 ± 3.0 / 11.0 ± 0.5 mm) → *R. reversa* (27.2 ± 2.2 / 19.1 ± 1.9 mm).

It should be noted that the leaf thickness of *R. donetzica* is provided by development of palisade parenchyma tissue (Fig. 1). The ratio for palisade/spongy parenchyma decreases in the following order: *R. reversa* (2.25), *R. donetzica* (1.95), *R. spinosissima* (1.75). The stomata are anomocytictype. *R. spinosissima* has the lowest number of stomata (27.1 ± 2.8 pcs/mm^2), as compared to *R. reversa* with 95.2 ± 10 pcs/mm^2 and *R. donetzica* with 94.3 ± 28.7 pcs/mm^2. Indeed, *R. donetzica* possesses the largest stomata, being 49.7 ± 3.7 µm long and 35.4 ± 3.3 µm wide. The stomata of *R. spinosissima* and *R. reversa* are lower sized, 34.2 ± 3.1 / 23.6 ± 2.2 µm and 25.0 ± 1.9 / 13.7 ± 1.2 µm, respectively.

Drought resistance. The results of our studies showed that the plants of the *Rosa* genus have medium level of drought resistance in terms of water content of leaves and water deficit (except *R. reversa*) (Fig. 2). Reduction of drought resistance of these three species is caused by the relatively high loss of water per 1 hour of wilting (Fig. 2). The data on drought tolerance of the wild rose species indicate slightly lower water content of tissues in *R. donetzica* (Fig. 2), but these differences are not significant. The parameter of water-retention ability is the main indicator of plant resistance to prolonged drought and it is also similar for the abovementioned species. *R. reversa* has the lowest value of water deficiency (Fig. 2).

Biochemical studies. To establish the intensity of the stress response as a result of hyperthermia, we measured MDA as an indicator of lipid peroxidation level. MDA concentration depended on the species and the interaction between temperature and species (Table 2). The concentration of MDA in *R. donetzica* and *R. reversa* remained almost the same after stress and significantly decreased in *R. spinosissima* (Fig. 3*a*).

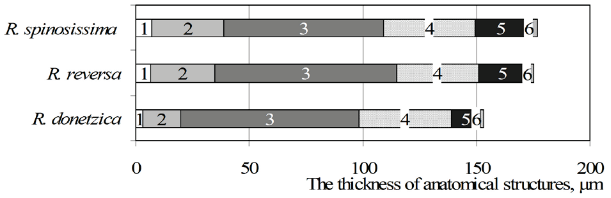

Fig. 1. Distribution of leaf anatomical structures:
1 – thickness of outer cell wall of the adaxial epidermis,
2 – thickness of adaxial epidermis, *3* – thickness of palisade mesophyll,
4 – thickness of sponge mesophyll, *5* – thickness of the abaxial
epidermis, *6* – thickness of outer cell wall of the abaxial epidermis

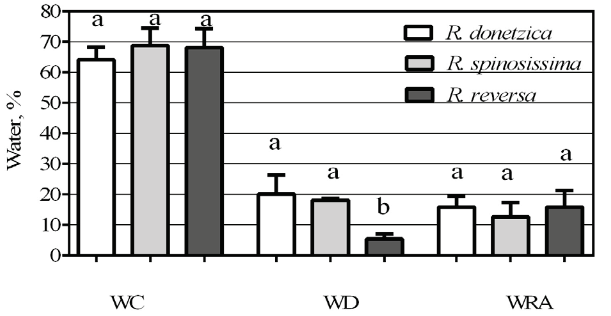

Fig. 2. Description of the drought resistance of three wild rose species: WC – water content of tissues, WD – water deficiency, WRA – water-retention ability; different letters indicate significant differences inside the parameters at P < 0.05; x ± SD, n = 7

Table 2
Two-way ANOVA of the parameters measured in plants of *R. donetzica*, *R. spinosissima* and *R. reversa* exposed to heat stress at temperatures of 40 and 26 °C in control group

Indices studied	Source	F (DFn, DFd)	P value
Malon dialdehyde	temperature	F (1, 18) = 1.40	0.2524
	species	F (2, 18) = 10.49	0.0010*
	interaction	F (2, 18) = 4.96	0.0193*
Superoxide dismutase	temperature	F (1, 18) = 14.24	0.0014*
	species	F (2, 18) = 13.28	0.0003*
	interaction	F (2, 18) = 6.62	0.0070*
Peroxidase	temperature	F (1, 18) = 4.36	0.0513
	species	F (2, 18) = 1.87	0.1832
	interaction	F (2, 18) = 8.86	0.0021*
Flavonoids	temperature	F (1, 18) = 41.80	< 0.0001*
	species	F (2, 18) = 39.60	< 0.0001*
	interaction	F (2, 18) = 3.93	0.0385*
Protein	temperature	F (1, 18) = 1.29	0.2718
	species	F (2, 18) = 998.40	< 0.0001*
	interaction	F (2, 18) = 4.07	0.0348*
Chlorophyll a	temperature	F (1, 18) = 0.53	0.4772
	species	F (2, 18) = 70.31	< 0.0001*
	interaction	F (2, 18) = 1.65	0.2201
Chlorophyll b	temperature	F (1, 18) = 47.18	< 0.0001*
	species	F (2, 18) = 15.08	0.0001*
	interaction	F (2, 18) = 31.95	< 0.0001*
Carotenoids	temperature	F (1, 18) = 2.35	0.1428
	species	F (2, 18) = 65.46	< 0.0001*
	interaction	F (2, 18) = 7.04	0.0055*

Note: * – significant differences within one of three variables – temperature, species, or their interactions.

According to our results, the effect of high temperature evokes a tendency to decrease in SOD activity for all studied species. *R. donetzica* and *R. reversa* under normal conditions form bigger storage of SOD (Fig. 3b), which enables quick response to short-term stress action, so this enzyme plays a significant role in adaptive response of these plants. It is probable that SOD action stipulates the lowest MDA level in *R. donetzica* under stress and under normal condition, compared with the other studied plants. Total SOD activity was significantly affected by temperature, species and their interaction (Table 2).

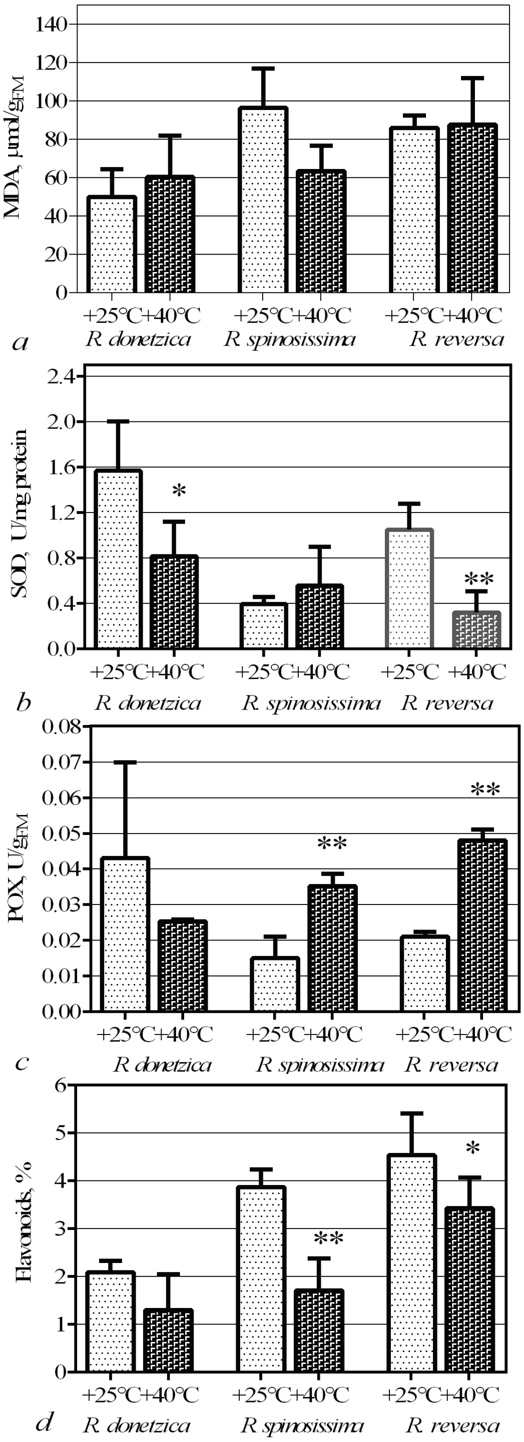

Fig. 3. The state of antioxidant system and lipid peroxidation under hyperthermia (+40 °C) and control (+25 °C): *a* – the content of malondialdehyde (MDA), *b* – superoxide dismutase activity (SOD), *c* – total peroxidase activity (POX), *d* – the content of total flavonoids; * – P < 0.05, ** – P < 0.01 compared with the control group; x ± SD, n = 5

Investigating the peroxidase, it worth mentioning the significant increase of its activity for *R. reversa* and *R. spinosissima*, which indicates a strong antioxidant effect under hyperthermia (Fig. 3*c*). The value of peroxidase activity in *R. donetzica* does not change significantly under stress. Peroxidase activity did not change significantly with temperature increase and species but was significantly affected by their interaction

(Table 2). According to our data, the highest number of flavonoids normally accumulates for *R. reversa* (Fig. 3*d*), which provides some antioxidant protection to these plants especially during the early stages of thermal stress. The lowest content of flavonoids in the norm was observed for *R. donetzica*. Flavonoid concentration was significantly affected by temperature, species and their interaction (Table 2).

Short-term effect of high temperature on *R. donetzica* caused the decrease in amount of chlorophylls a and b. Chlorophyll b was especially unstable under thermal stress (Table 3). The increase in amount of carotenoids due to stress and decrease of (chl *a* + chl *b*)/car index indicates the inclusion of adaptive reaction via the pigment system. The sharp increase in ratio chl *a* / chl *b* after warming is explained by the significant destruction of chlorophyll *b*. The concentration of chlorophyll *b* was significantly affected by temperature, species and their interaction (Table 2). But the concentration of chlorophyll *a* depended only on the species. Carotenoids also did not change significantly with temperature. There were no significant differences of content of chlorophyll and carotenoid after stress, for *R. spinosissima*, compared to the control group of plants (Table 3). So significant damage to light-harvesting pigments under a sharp warming to + 40 °C did not occur; there was only a trend to decrease of pigments' number. However, along with the trend to reduction in the amount of chlorophyll *a* and *b* and carotenoids, we observed a significant increase of the indicator (chl *a* + chl *b*) / car, so hyperthermia has a stronger negative effect on carotenoids than on chlorophylls of this species. After warming, the number of chlorophylls and carotenoids was the same for the control group of *R. reversa* (Table 3). However, along with regular decrease in the amount of chlorophyll *b*, we observed a tendency to increase in chlorophyll *a*. Thus, the increase of ratio chl *a*/chl *b* due to the stress was caused by multidirectional change in the number of both types of chlorophyll. Total protein concentration did not change significantly with temperature increase but was significantly affected by the species and the interaction between temperature and species (Table 2). Only in *R. spinosissima* was there an increase in the total amount of protein due to sharp warming.

Table 3

Indicators of pigment complex and protein before and after exposure to high temperature, mg/g of wet weight

Name species	Variant, °C	Protein	The indicators of pigment complex				
			chlorophyll *a*	chlorophyll *b*	carotenoid	chla/chlb	(chla+chlb)/ car
R. donetzica	+25	0.58 ± 0.0912	2.08 ± 0.037	0.78 ± 0.144	0.55 ± 0.035	2.74 ± 0.49	5.26 ± 0.54
	+40	0.50 ± 0.0059	1.94 ± 0.021***	0.17 ± 0.083*	0.69 ± 0.051**	16.80 ± 11.40*	3.07 ± 0.31**
R. spinosissima	+25	1.26 ± 0.0011	1.05 ± 0.271	0.27 ± 0.084	0.34 ± 0.086	3.92 ± 0.29	3.93 ± 0.04
	+40	1.28 ± 0.0059***	0.91 ± 0.107	0.23 ± 0.015	0.27 ± 0.024	3.93 ± 0.67	4.23 ± 0.00***
R. reversa	+25	1.26 ± 0.0085	1.35 ± 0.261	0.35 ± 0.061	0.41 ± 0.046	3.88 ± 0.42	4.13 ± 0.38
	+40	1.27 ± 0.0001	1.48 ± 0.161	0.30 ± 0.054	0.43 ± 0.069	4.94 ± 0.46*	4.14 ± 0.21

Note: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ compared with the control group, x ± SD, n = 5.

For better understanding of the relationships between anatomical and biochemical parameters, we additionally carried out the correlation analysis between different indices under the normal conditions. Under the normal conditions, if the epidermis is thinner, and therefore provides less protection from the influence of the environment, we observe a tendency to increase in the activity of antioxidant enzymes SOD and peroxidase and vice versa (Table 4). Also, when the epidermis and its outer cell wall are thinner, a lower number and larger size of stomata are observed and also an increasing number of pigments of the photosynthetic system, which is possibly connected with improved epidermal respiration and transpiration. Moreover, the accumulation of chlorophylls and carotenoids is observed at the thinner adaxial epidermis. A positive correlation was found between the thicknesses of the abaxial epidermis and its outer cell membrane. The flavonoids and proteins have high positive correlation with each other and with the thickness of epidermis. MDA has positive correlation with flavonoids and proteins and a negative one with SOD and pigments of the photosynthetic system. SOD goes up when flavonoids go down and photosynthetic pigments increase. The peroxidase activity has low correlation with most measured parameters. The thickness of the adaxial epidermis correlates negatively with the thickness of columnar parenchyma. Thickness of spongy parenchyma has no reliable correlation with any of the studied parameters.

For better understanding of the relationships between biochemical indicators during temperature stress we carried out a correlation analysis. For each parameter (e.g. MDA), data for each species at control conditions (+25 °C) and +40 °C were inputted (Table 5). With an increase of stress and, thus, the increase of the lipid peroxidation, SOD activity significantly decreases and vice versa. Also, the amount of photosynthetic pigments reduces when MDA concentration goes up (Table 5). Thus we observed a positive correlation of MDA with the amount of flavonoids and proteins under stress, which is smaller compared with the control conditions. Positive correlation was determined between the activity of SOD and the amount of chlorophylls, as a result of the action of antioxidant defense mechanisms. The peroxidase activity has low correlation with every measured parameter.

Discussion

The anatomical studies have shown that *R. spinosissima* has a thick epidermis and its outer cover on both sides, which is responsible for its having the thickest lamina, while *R. donetzica* has the thinnest leaf covered with a thin epidermis (Fig. 1). As is known, thickening of the epidermis and its outer cell wall due to the cuticle and the waxy bloom indicates resistance to water deficit (Levitt, 1972). Thus, according to the given parameters, the more drought resistant species are *R. spinosissima* and *R. reversa*, and the least drought-tolerant species is *R. donetzica*. The thickest lamina is typical for *R. spinosissima*. At the same time, a xeromorphic feature is also reduction of the linear dimensions of the leaf. The smallest lamina is typical for *R. spinosissima* too. As is known from the literature, higher development of palisade parenchyma compared to spongy parenchyma is a xeromorphic feature of leaves (Ezau, 1977). The highest ratio for palisade/spongy parenchyma is typical for *R. reversa*.

The largest number of stomata, along with their small size is observed in *R. reversa*. It shows an increased ability of water evaporation, which reduces overheating compared to other species. Therefore, increase of stomata density is a positive feature of the epidermis of plants under hyperthermia conditions but decreases drought tolerance of plants (Torre et al., 2003; Zandalinas et al., 2016). The lowest number of stomata is typical for *R. spinosissima*. On the other hand, a relatively large area of epidermal cells is an indicator of the relatively lower drought resistance of *R. spinosissima* and *R. donetzica*.

Increasing density of stomata indicates out an enhanced ability to evaporate water, which helps to reduce the overheating of plants, facilitating their adaptation to high environmental temperatures, which is often accompanied by drought. But it has a negative effect when drought is not accompanied by high temperature. This is confirmed by other scientists: the more drought-resistant species of the genus *Rosa* are characterized with a significant lower number of stomata per area unit (Torre et al., 2003). Drought resistance of *R. reversa*, with a relatively large number of stomata, is probably balanced by stress avoidance mechanisms: leaf conductance and the duration of maximum stomatal opening decreased in order to control water loss via transpiration, contributing to maintenance of leaf turgor in plants under water stress (Cruz et al., 2012), and also the small size of stomata.

In our study, the lowest loss of water per 1 hour was observed for *R. spinosissima* (Fig. 2), which is partly connected with the lowest number and size of stomata, and thus with decrease of water loss for transpiration under temperature increase. *R. reversa* has the lowest value of water deficit (Fig. 2), which confirms the resilience of these plants under water scarcity. Reduction of the leaf thickness in *R. donetzica* indicates low resistance to water deficit (Hickey & King, 2001).

The results of our multi-year phenological observations on plants of the genus *Rosa* in the Botanical Garden have shown that the most drought-resistant species of the studied wild roses is *R. reversa* – a species with a very wide range. The representatives of this species withstand prolonged summer drought extremely well, and annually grow and develop well and fruit abundantly. *R. spinosissima* appeared quite drought resistant under the summer drought conditions, and despite the leaf turgor loss in the afternoon summer heat, fruits abundantly every year. *R. donetzica* is vulnerable to the summer drought. This species requires additional watering during the summer drought, without which it gradually loses some leaves and fruits by the end of summer. The absence of significant changes in the concentration of MDA in *R. donetzica* and *R. reversa* indicates their relative resistance to the sharp influence of high temperature. At the same time, the amount of MDA in *R. spinosissima* significantly decreased, which probably can be explained by the fast effective working of the antioxidant system of these plants. Decrease of MDA content in the leaf was also observed immediately after the experiment by other researchers (Savicka & Škute, 2010; Zandalinas et al., 2016).

Table 4
The correlation between anatomical and biochemical parameters under normal conditions (n = 9)

Characteristics	Superoxide dismutase	Peroxidase	Flavonoid	Chlorophyll a	Chlorophyll b	Carotenoid	Protein	Palisade mesophyll	Sponge mesophyll	Adaxial epidermis	CWD	Abaxial epidermis	CWB	Length of stomata	Width of stomata	Number of stomata
Malon dialdehyde	–0.69*	–0.46	0.69*	–0.80**	–0.88**	–0.69*	0.86**	–0.66	0.03	0.74*	0.50	0.63	0.83**	–0.75*	–0.52	0.91***
Superoxide dismutase	–	–0.12	–0.68*	0.50	0.76*	0.38	–0.67	0.44	0.05	–0.57	–0.38	–0.51	–0.33	0.77*	0.59	–0.64
Peroxidase	–	–	–0.42	0.51	0.43	0.44	–0.63	0.25	–0.03	–0.47	–0.17	–0.35	–0.57	0.43	0.43	–0.60
Flavonoid	–	–	–	–0.63	–0.77*	–0.58	0.87**	–0.35	0.29	0.73*	0.32	0.56	0.59	–0.87**	–0.86**	0.86**
Chlorophyll a	–	–	–	–	0.89**	0.97***	–0.84**	0.69	0.08	–0.81**	–0.82**	–0.40	–0.63	0.75*	0.53	–0.88**
Chlorophyll b	–	–	–	–	–	0.80**	–0.93***	0.57	0.06	–0.84**	–0.60	–0.47	–0.60	0.89**	0.63	–0.92***
Carotenoid	–	–	–	–	–	–	–0.74*	0.68	0.07	–0.81**	–0.82**	–0.24	–0.58	0.67*	0.47	–0.81**
Protein	–	–	–	–	–	–	–	–0.57	–0.00	0.85**	0.52	0.63	0.67*	–0.93***	–0.80**	0.98***
Palisade mesophyll	–	–	–	–	–	–	–	–	0.56	–0.75*	–0.79*	–0.32	–0.45	0.64	0.53	–0.62
Sponge mesophyll	–	–	–	–	–	–	–	–	–	–0.28	–0.39	0.24	0.35	0.19	0.13	0.09
Adaxial epidermis	–	–	–	–	–	–	–	–	–	–	0.59	0.34	0.56	–0.87**	–0.72*	0.86**
CWD	–	–	–	–	–	–	–	–	–	–	–	0.18	0.24	–0.58	–0.45	0.55
Abaxial epidermis	–	–	–	–	–	–	–	–	–	–	–	–	0.72*	–0.49	–0.47	0.65
CWB	–	–	–	–	–	–	–	–	–	–	–	–	–	–0.45	–0.32	0.80**
Length of stomata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.91***	–0.88**
Width of stomata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–0.73*

Note: CWD – thickness of outer cell wall of the adaxial epidermis, CWB – thickness of outer cell wall of the abaxial epidermis; * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$.

Table 5
The correlation between biochemical parameters under hyperthermia

	Superoxide dismutase	Peroxidase	Flavonoid	Chlorophyll a	Chlorophyll b	Carotenoid	Protein
Malon dialdehyde	–0.64**	0.13	0.54*	–0.38	–0.38	–0.24	0.60**
Superoxide dismutase	–	–0.28	–0.47*	0.53*	0.51*	0.45	–0.71**
Peroxidase	–	–	0.09	0.04	–0.01	–0.05	0.18
Flavonoid	–	–	–	–0.31	–0.06	–0.37	0.61**
Chlorophyll *a*	–	–	–	–	0.52*	0.91***	–0.82***
Chlorophyll *b*	–	–	–	–	–	0.17	–0.37
Carotenoid	–	–	–	–	–	–	–0.82***

Note: * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$, n = 18.

Biochemical studies have shown that the effect of hyperthermia evokes a decrease in SOD activity for *R. donetzica* and *R. reversa*, which is confirmed by literature data (Zhang & Kirkham, 1994; Nuzhyna et al., 2018). According to Barkasdjieva et al. (2000) and Rizhsky et al. (2002), temperature increase causes denaturation of this enzyme in plants. Along with this, it is necessary to note a significantly higher level of SOD activity under normal conditions in *R. donetzica* and *R. reversa* compared to *R. spinosissima*. However, for *R. spinosissima* there is a tendency to increase the activity of SOD under temperature stress.

The increase of peroxidase activity (as for *R. reversa* and *R. spinosissima*) in response to high temperature treatment have been noted by other researchers in other plants (He & Huang, 2010; Ardelean et al., 2014; Nuzhyna et al., 2018). *R. donetzica* has the highest peroxidase activity and SOD activity in normal conditions. It allows it to use these antioxidant enzymes rapidly under stress. In our opinion, the creation of a pool of active antioxidant enzymes is less effective compared to the rapid inclusion of protective antioxidant systems in a stressful situation for these plants.

It is known that one of the many functions of flavonoids is the antioxidant one (Di Ferdinando et al., 2011; Wang et al., 2015). According to the literature, hyperthermia has different effects on flavonoids, depending on their type and location in the plant (Raghuwanshi et al., 1994). In addition, we can say that we observed only reduction of the amount of flavonoids in the leaves of all studied species under warming up to +40 °C. It is thought that decrease in flavonoid accumulation, under a high temperature, could result from different causes, such as accelerated degradation of these compounds and inhibition of mRNA transcription of the flavonoid biosynthetic genes (Mori et al., 2007).

Analyzing the correlations under the normal conditions, we found that MDA slowly accumulates as a result of the influence of the external environment and the vital activity of the organism, therefore, the less the SOD, the greater the MDA and vice versa (Table 4). The increase in the amount of MDA is accompanied by a decrease in the content of chlorophylls and carotenoids, probably due to the destruction of pigments. The greater activity of SOD may help to improve the work of the photosynthesis apparatus due to the neutralization of the active forms of oxygen formed during the functioning of the organism. Also, the higher activity of SOD reduces the need for the synthesis of additional antioxidants, such as flavonoids. The positive correlation of flavonoids, proteins and the thickness of the epidermis may be connected with the localization of flavonoids mainly in the epidermis (Hernández et al., 2009). Data about autonomy of peroxidase were also obtained by Rai et al. (2015). It is possible that under abrupt warming, the number of heat shock proteins increases as a protective reaction to temperature stress and to increased MDA concentration. It is important to note that the correlations between the biochemical parameters under the stress conditions are the same as under the normal conditions (Table 4, 5). The total amount of protein of *R. donetzica* decreased after warming, perhaps because of degradation (Table 2). In *R. spinosissima* protein increased after stress, possibly due to the formation of heat shock proteins.

In the control group, the concentration of chlorophyll *a* and *b* and carotenoids was different among the three species. It is known that the content of chlorophylls in the leaf reflects the adaptability of plants to a particular light intensity. The relatively lower rates of chlorophyll content for *R. spinosissima* show that it is the most photophilous among the studied species. Whereas, the highest chlorophyll content for *R. donetzica* shows the highest shade-tolerance among the studied wild roses. We can say that thermal stability of the photosynthetic apparatus is higher at lower content of light-harvesting pigment. Thus, the content of pigments in *R. donetzica* is twice as high as in the other two species, and the photosynthetic system of these plants was the most unstable under temperature stress. Other researchers also note the inhibition of photosynthetic activity (primarily due to chlorophylls) as a result of stress, particularly under hyperthermia and hypothermia (Zhang et al., 2010; Ashraf & Harris, 2013; Babenko et al., 2014). Among the effects of high temperature, there are destructive changes to the photosynthetic apparatus, which cause reduction of photochemical efficiency of photosystem II as a sensitive component of photosynthesis (Barnabas et al., 2008; Chen et al., 2012). The significant changes in quantitative parameters of the pigment system were not found in *R. spinosissima* and *R. reversa*. Also, the ratio chl *a* / chl *b* for *R. spinosissima* and (chl *a* + chl *b*) / car for *R. reversa* remained stable after the stress.

The research has shown that plants of *R. spinosissima* are the most photophilous among the studied species. By contrast, *R. donetzica* is the most shade-tolerant. Short-term influence of high temperature (40 °C) has negatively affected the photosynthetic system of *R. donetzica* and had almost no damaging effect on *R. reversa* and *R. spinosissima*. The flavonoids are less involved in the antioxidant reactions under hyperthermia in plants of all studied species. Thus, the plants of *R. donetzica* are the least heat-resistant.

Thus, the results of the research on anatomical features of the leaf structure of three *Rosa* species have shown that *R. donetzica* has the lowest drought and heat resistance. This is confirmed by our long-term phenological observations of the wild rose in the Botanic Garden. Low resistance to the considered damaging factors is caused by the relatively weak development of epidermal tissue which, in turn, leads to the greater destruction of the pigment complex. Also, the antioxidant system is not activated under short-term stress, and the protection mainly occurs due to the accumulated enzymes in normal conditions.

The plants of *R. reversa* were the most drought-resistant and heat-resistant. In our opinion, representatives of this species can successfully withstand the conditions of water scarcity and high temperature, due to the presence of the following anatomical features: a large number of unicellular covering and glandular trichomes (which reduce the influence of insolation and therefore overheating of the lamina and reduce turbulent airflow near epidermis, reducing transpiration), thicker outer epidermal cell wall; maximal ratio of palisade to spongy parenchyma (compared to other studied species). The highest number of stomata, tolerance of the photosynthetic complex to hyperthermia, the presence of a flavonoid reserve in normal conditions and intense peroxidase activation after exposure to stress factors also contributes to the heat-resistance of these plants.

The *R. spinosissima* plants were less drought resistant by the anatomical features. The absence of trichomes on the surface of this species is compensated by the considerable thickness of epidermis and its outer cell wall, by the minimal number of stomata (relative to the considered species), by the highest water storage ability. These plants can be considered as heat resistant. The anatomical and biochemical parameters confirm this. The antioxidant system of those wild roses functions similarly to that of *R. reversa*, but less intensively.

Conclusions

R. reversa and *R. spinosissima* plants can be considered as drought resistant and heat resistant. These species with fast antioxidant response are more resistant to short-term hyperthermia. The synthesis of antioxidant enzymes "in reserve" is a less effective form of adaptation. Low development of the epidermal tissue of *R. donetzica* probably facilitated higher destruction of the pigment complex. The detected anatomical and biochemical parameters of *R. donetzica* support lower adaptive capacity to sharp changes in environmental conditions. These plants are the least heat resistant and drought resistant. This may determine the significant limitation of the natural range of this species. These data should be considered for successful cultivation of these species of wild roses.

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