



Interspecific differences of antioxidant ability of introduced *Chaenomeles* species with respect to adaptation to the steppe zone conditions

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Plants of the genus *Chaenomeles* are traditionally used in the countries of South-East Asia, due to their high nutritional and health-promoting properties. However, the successful introduction of species promising for gardening from geographically remote areas requires the study of plant ontogeny under the conditions of new habitat. This is a very substantial problem for the steppe zone, where the continental climate has features of aridity and complicates the process of increasing the diversity of fruit crops by introducing the desired species. The present study aims to assess the effectiveness of the protective enzymatic system of different *Chaenomeles* genotypes subject to a steppe climate as well as the accumulation of the biologically active compounds with high antioxidant capacity. The study was performed on the basis of the introduced horticultural plants collection in the Botanical Garden of the DNU, and the *Chaenomeles* fruits, leaves, and the seeds were examined. The highest activity of catalase, benzidine-peroxidase and guaiacol-peroxidase, and the greatest enzymes activation during vegetation were found in leaves of *Ch. cathayensis* and *Ch. speciosa*, while the lowest activity was in leaves of both Japanese species. The biggest total phenolic content in the isopropanolic plant extracts, determined by Folin–Ciocalteu assay, was found in leaves of *Ch. × superba*, *Ch. × californica* and *Ch. cathayensis* (44.8, 52.8, and 43.6 mg GAE/g WW); a less high level was found in leaves of *Ch. japonica* and *Ch. japonica* var. *maulei* (43.1 and 40.2 mg GAE/g), while the lowest was in leaves of *Ch. speciosa* (29.3 mg GAE/g). The total flavonoids content determined using the aluminum chloride method, did not differ by variety or species in the plant leaves, being in the range of 2.6–2.9 mg of RE per g WW (accordingly, in leaves of *Ch. japonica* var. *maulei* and *Ch. × californica*). The high total reducing power determined by potassium ferricyanide assay was found in leaves of both hybridogenic species and *Ch. cathayensis* (respectively, 11.6, 14.1, and 11.4 AAE/g WW); leaves of both Japanese species had slightly lower values and the lowest was in leaves of *Ch. speciosa* (7.7 AAE/g). In the *Chaenomeles* fruits, the total phenolic content was the lowest in *Ch. speciosa* (17.8 mg GAE/g), average in both Japanese species (28.7 and 27.8 mg GAE/g), and the highest (33.3 mg GAE/g) was in *Ch. cathayensis*. The flavonoid accumulation was highest in the fruits of *Ch. cathayensis* and *Ch. japonica* var. *maulei* (0.67 and 0.63 mg RE/g), intermediate in both hybridogenic species and *Ch. japonica* (accordingly, 0.57, 0.42 and 0.38 mg RE/g), and the lowest in *Ch. speciosa* (0.30 mg RE/g). The total reducing power of *Chaenomeles* fruit was lower as compared to leaves, and decreased from 11.2 to 5.7 mg AAE/g in the series *Ch. cathayensis* > *Ch. × californica* > *Ch. japonica* > *Ch. japonica* var. *maulei* > *Ch. × superba* > *Ch. speciosa*. High correlation coefficients between total reducing power and total phenols content in the *Chaenomeles* leaves and fruits (respectively, $r = 0.96$ and $r = 0.95$, $P < 0.05$) confirm the significant contribution of phenolic compounds to the antioxidant capacity. The study results indicate a high antioxidant capacity of the *Chaenomeles* species in the conditions of the steppe climate due to the antioxidant enzymes activity and the accumulation of a significant amount of phenolic metabolites in leaves and fruits.

Keywords: *Chaenomeles*; fruits; leaves; antioxidant enzymes; phenols; flavonoids; total reducing power

Introduction

Horticultural plants are an inexhaustible resource for human food (Ros-García et al., 2004), but their role as a source of the biologically active compounds for health is not less important (Beecher, 2003; Vrhovsek et al., 2004; Górnaś et al., 2014). The antioxidant properties of plant second metabolites, which have a different chemical structure, contribute to the neutralization of oxidative stress, thereby facilitating or eliminating the diseases caused by it (Costa et al., 2009; Sahan et al., 2012). Many of these compounds are able to provide preventive and therapeutic effects, supplementing or even replacing traditional medicines, and can also serve as plant raw materials for the production of medicaments (Cheynier, 2005; Mojzer et al., 2016).

The introduction of alien fruit plants into cultivation in different regions contributes to the expansion of the local spectrum of species possessing health promoting properties for people. However, it is necessary to take into account the fact that the accumulation of biologi-

cally active compounds in plants depends to a large extent on the region of origin of species and varieties, and the new environmental conditions (Alexeyeva et al., 2016), including the effect of climatic factors (McGhie et al., 2005; Hafez-Taghva et al., 2016). This is particularly relevant for the Steppe Dnieper of Ukraine, where the climate has continental features and is not equally favourable for all introduced plant species, even within the same genus. Therefore, an assessment of the stability and metabolic activity of introduced fruit plants is necessary, since it enables the optimal selection of the most promising species for industrial cultivation. Adaptation of desired fruit plants to unfavourable environmental conditions can be improved with the help of modern methods, such as the treatment with the synthetic plant growth regulators (Shcherbyna et al., 2017).

In the steppe zone of Ukraine, the introduction of horticultural plants from remote geographical areas has been carried out in the Botanical Garden of Dnipro National University for many years, including several natural and hybridogenic species of the genus *Chaenomeles*. The adap-

tation of these introduced plants to the new habitat was assessed from a phenological position, but the plants' capacity for biosynthesis and the accumulation of secondary metabolites has not been investigated. Meanwhile, the fruits and other plant organs of the *Chaenomeles* species are a rich source of polyphenols (Miao et al., 2016), organic acids, alcohols, ketones, aldehydes, vitamin C (Watychowicz et al., 2017), triterpenes and procyanidins (Du et al., 2016), as well as the macro- and microelements (Baranowska-Bosiacka et al., 2017). It is natural that the fruits of various species of the *Chaenomeles* genus have been used for centuries in China and other countries of Southeast Asia for the treatment of cardiovascular diseases, anemia, rheumatism and gout (Du et al., 2013; Watychowicz et al., 2017).

Nowadays, the numerous studies confirm the high prophylactic and therapeutic effects of preparations obtained from the fruits, leaves and twigs of various *Chaenomeles* plants, including their anti-inflammatory (Strugała et al., 2016; Han et al., 2016; Suh et al., 2017), anti-influenza (Zhang et al., 2014), and hepatoprotective activity (Ma et al., 2016; Baranowska-Bosiacka et al., 2017; Zakłos-Szyda & Pawlik, 2018), as well as their effectiveness in the prevention and treatment of diabetes (Watychowicz et al., 2017). In addition, the significant anti-carcinogenic properties of the extracts from *Ch. japonica* fruits, particularly against breast cancer (Lewandowska et al., 2013) and colon cancer (Gorlach et al., 2011; Owczarek et al., 2017) has been established. Recent studies have revealed immunoregulatory and antiparkinsonian properties of the extracts from *Ch. speciosa* fruits (Zhang et al., 2014). The vast majority of study results indicate that biological activity of the preparations are to the greatest extent associated with the presence of the polyphenols (Hamauzu et al., 2006; Brglez et al., 2016; Zakłos-Szyda & Pawlik, 2018). The aim of the present work was to assess the adaptive capabilities of different *Chaenomeles* genotypes to the arid climate of the Steppe Dnieper, taking into account the activity of antioxidant enzymes and the content of secondary metabolites in leaves and fruits of plants.

Materials and methods

Study area. The research was performed in 2016–2017 in the Botanical Garden of Oles Honchar Dnipro National University (48°26'14" N, 35°02'35" E). The original soil cover is usual low-humus medium loamy chernozems; the humus content in the plow layer is 4–5%. The regional climate has distinct continental features, such as seasonal droughts with high temperatures, unstable moisture and dry hot winds. During the vegetation period of the *Chaenomeles* plants, several periods with low relative humidity (in the range 31–52%) were noted, in particular in April and June, and especially long in July and August. Drought periods were accompanied by a high air temperature, which reached 25–30 °C in the second decade of June, 32 °C in July, and 35 °C in August.

Data collection. The study objects were the leaves, ripe fruits, and seeds of some natural and hybridogenous plant species of the genus *Chaenomeles* Lindley (family of Rosaceae, subfamily Maloideae).

Table 1
Phenological differences of genus *Chaenomeles* species in 2017

Introduced species	Introduction time	Source of the introduction	Phenological phases of plant growth		
			leaves appearance	florescence	ripe fruit appearance
<i>Ch. speciosa</i>	1996	Moldova, Kishinev	18.03	25.03–19.04	27.09–05.10
<i>Ch. cathayensis</i>	1996	Moldova, Kishinev	21.03	29.03–13.04	10.10.–25.10
<i>Ch. japonica</i>	1996	Moldova, Kishinev	20.03	27.03–10.04	21.09–28.09
<i>Ch. japonica</i> var. <i>maulei</i>	1955	Tajikistan, Khorog	19.03	26.03–13.04	14.09–01.10
<i>Ch. × superba</i>	1996	Moldova, Kishinev	19.03	29.03–14.04	25.09–10.10
<i>Ch. × californica</i>	2006	Crimea, Yalta, State Nykytsky botanical garden	20.03	28.03–12.04	25.09–10.10

Extracts containing the phenolic compounds were obtained by holding the raw crushed plant material (200 mg of fruit flesh, and 500 mg of leaves) in 5 ml of 80% isopropanol for 24 hours at room temperature. After this, the crude extracts were filtered and stored at 4 °C until analysis. The Folin – Ciocalteu method (Singleton et al., 1999) in modification (Nwanna et al., 2013) was used for the total phenolic content measurement. In brief, 0.2 ml of plant extract diluted with 0.2 ml of distilled water was oxidized with 1 ml of 10% Folin –

Systematic and taxonomic characterization of the species was made in accordance with Rumpunen (2002) and Watychowicz et al. (2017).

Ch. japonica (Thunb.) Lindl. (Japanese quince) is common in central and south Japan at an altitude 100–2,100 m on hillsides, and on riverbanks and lakeshores. It is a dwarf shrub 0.6–1.2 m in height with ovoid leaves. The flowers are small, most often the colour of a salmon or an orange. Small fruits are similar in shape to apples that mature early and have an extraordinary fragrance. This is the most enduring species in the genus *Chaenomeles*.

Ch. japonica var. *maulei* was introduced from Japan in 1869 by Maule, the owners of nurseries at Bristol, England. This plant is taller, has single flowers, painted in colors from pink to orange.

Ch. speciosa (Sweet) Nakai (flowering quince) is found in China in mountainous terrain at different altitudes. The species is winter hardy and drought-resistant. It is a deciduous or semi-evergreen shrub 2–3 m in height. The leaves are ovate with jagged edges, the flowers are often red, but can be white and pink. The fruits are very diverse in shape, size, and time of ripening.

Ch. cathayensis (Hemsl.) Schneider (Chinese quince) is found in the wild in China and southern Tibet at an altitude of over 3,000 m, but its winter hardiness is average, as well as drought resistance. Usually it is a shrub up to 3 m high, but can be formed as a tree. The leaves of this plant are elliptical with notches, flowers are white or pink. The fertility is plentiful, but the fruits ripen late; the shape of the fruit is ovoid, the length reaches 15–20 cm.

Ch. × superba (Frahm) Rehder (*Ch. japonica* × *Ch. speciosa*, *Superba* group) is a shrub 2 m in height, the leaves are similar to *Ch. japonica* leaves. Flowers are of medium size and may be white, pink, orange or red. Fruits are more often in the form of apples, larger than in *Ch. japonica* and they mature later.

Ch. × californica Clarke ex Weber [*Ch. cathayensis* × (*Ch. × superba*), *Californica* group] is a shrub more than 2 m in height with lanceolate leaves. Flowers are large, usually pink or red, or mixed colours. It produces a variety of fruits ranging from medium to large, the shape is ovoid or apple. This hybrid has an average frost resistance and drought resistance.

All plants of *Chaenomeles* genus are alien species in the Steppe Dnieper, and were introduced in the region several decades ago. Plant leaves were selected during the period of flowering, and the fruits were selected as they mature, according to phenological phases (Table 1).

Data analysis. Determination of the antioxidant enzymes activity in plant leaves was performed by spectrophotometric method in the supernatants after centrifugation of the crude extracts (100 mg of plant fresh mass homogenized in Tris-HCl buffer, pH 7.0). Catalase activity (CAT) was detected at 410 nm by ammonium molybdate method in accordance with Göth (1991), and calculated using a calibration graph prepared on H₂O₂ solutions. Activity of benzidine-peroxidase (BPO) was measured at 490 nm by the method of Gregory (1966), and activity of guaiacol-peroxidase (GPO) was detected at 470 nm according to the method of Ranieri et al. (1997).

Ciocalteu reagent, and neutralized by 7.5% Sodium carbonate solution. The reaction mixture was incubated for 40 minutes at 45 °C and cooled. Optical density of the samples was measured at a wavelength of 726 nm. The total phenolic content was calculated using a calibration graph prepared on the solutions of Gallic acid (GA), and expressed as mg GA equivalents per 100 g of wet weight (mg GAE/100 g WW).

The aluminum chloride spectrophotometric method (Pełal & Pyrzyńska, 2014) was used for the measurement of total flavonoids content

in the *Chaenomeles* plants. Briefly, 1 ml of aluminum chloride (AlCl₃) solution, and 1 ml of sodium acetate were added to 2 ml of isopropanol extract. The reaction mixture was maintained for 10 minutes at room temperature, and the optical density measured at 425 nm. The content of flavonoids was calculated using a calibration graph prepared on rutin solutions, and the result was expressed as mg rutin equivalents per 100 g of plant wet weight (mg RE/100 g WW).

The total reducing power (TRP) of the *Chaenomeles* plants was determined in accordance with Pulido et al. (2000). Briefly, 1 ml of extract was mixed with 1 ml of sodium phosphate buffer (pH 6.6) and 1 ml of potassium ferricyanide. Reactive mixture was incubated at 50 °C for 20 min. Then, 1 ml of 10% trichloroacetic acid was added, and the mixture centrifuged. Then, 1 ml distilled water, and 0.2 ml of 0.1% ferric chloride solution was added to 1 ml of the supernatant, and the absorbance was measured at 700 nm. The calibration graph was constructed by solutions of ascorbic acid (AA). The total reduction power of plant extracts was expressed as AA equivalents per g of plant wet weight (mg AAE/g WW) in accordance with the method of Augustus et al. (2015).

The electrophoretic analysis was performed in accordance with the method of Laemmly (1970) in 13.5% acrylamide gel. Protein samples (50 µg/ml) were boiled for 5 min at 95 °C and applied to the respective wells in the gel. Analysis was run at 20 mA in the electrophoresis unit VE-20 (Russia). Protein standards were run in parallel with the samples. Bands were stained with 0.3% Coomassie Brilliant Blue G-250.

Data obtained were expressed as mean ± standard deviation (SD) of three measurements. Statistical analyses were performed with ANOVA. Correlation analysis examined the interrelations between the study indexes of the metabolite's content and the reducing power ($P < 0.05$). Tukey's criterion of an honestly significant difference (HSD) in group mean was used to distinguish between samples of mean values (Mcdonald, 2014). This criterion makes it possible to conduct multiple pair comparisons of mean values. Differences were considered statistically significant at $P < 0.05$.

Results

The highest catalase (CAT) activity in the leaves of all *Chaenomeles* species was found in April, then declining until June in leaves of *Ch. japonica* var. *maulei* and hybridogenic species *Ch. × superba* and *Ch. × californica* ($P < 0.01$), or until July in all other species (Table 2). In the leaves of all species, catalase activity increased sharply in August relative to July. In August, the activity of catalase in the leaves of all species reached 39–74% of the level in April ($P <$

0.01), excluding the species *Ch. japonica* var. *maulei*, in the leaves of which the enzyme activity in August exceeded the April index by 2.3 times ($P < 0.01$).

Dynamics of the peroxidase activity in the leaves of *Chaenomeles* plants had species-specific features. The activity of plant benzidine peroxidase (BPO) increased in May in comparison with April only in the leaves of species *Ch. cathayensis* (by 62%, $P < 0.05$), whereas in the leaves of all other species the enzyme activity decreased reaching only 33–46% ($P < 0.05$) of the April level (Table 3). In June, a noticeable activation of benzidine peroxidase was revealed in leaves of all *Chaenomeles* plants, especially significant in the species *Ch. japonica*, *Ch. japonica* var. *maulei*, and *Ch. × californica* (in 2.0, 2.5 and 3.2 times respectively, $P < 0.05$). In July, the increase in enzymatic activity continued only in the leaves of *Ch. cathayensis*, whereas in the leaves of other species the BPO activity decreased in comparison with June, especially in both Japanese species (by 37% and 25%, respectively, $P < 0.05$).

The activity of guaiacol peroxidase (GPO) in plant leaves in May exceeded the April level in all *Chaenomeles* species, except for *Ch. japonica* var. *maulei* and *Ch. × superba*, where the activity decreased slightly (Table 4). In the leaves of *Ch. cathayensis* and *Ch. japonica* the highest GPO activity was found in May, exceeding the April level by 3.2 and 1.3 times, respectively ($P < 0.01$), whereas in leaves of all other species the highest enzyme activation was revealed in June. In August, the leaves of all species, excluding *Ch. cathayensis*, had high activity of guaiacol peroxidase, exceeding the April level by 11–12%, or even more than twice in the leaves of species *Ch. japonica* var. *maulei* and *Ch. × californica* ($P < 0.01$). The total phenolic compounds in the leaves of *Chaenomeles* species as determined by Folin – Ciocalteu assay, was expressed as gallic acid equivalents (GAE) by reference to a standard curve ($y = 304.15x$, and $R^2 = 0.99$). The total flavonoids content in leaves of various species of *Chaenomeles* was determined using aluminum chloride method, and is expressed as rutin equivalent (RE) (equation of regression $y = 0.0139x$, $R^2 = 0.999$). The total reducing power (RP) of the leaves of *Chaenomeles* different species was expressed as the equivalent of ascorbic acid (AAE) (equation of linear regression $y = 252.05x - 32.316$, $R^2 = 0.993$). In the leaves of studied plants, the total content of phenols varied notably, while flavonoids content did not differ in variety (Table 5). The content of the phenolic compounds as well as the flavonoids content in the ripe fruits of all *Chaenomeles* species (Table 3) was lower than in the leaves of studied plants (Table 6). Electrophoretic analysis of the proteins from plant seeds of the genus *Chaenomeles* revealed a wide spectrum of the polypeptides in the molecular weight range 9.6–83.2 kD (Fig. 1).

Table 2

Changes of catalase activity in leaves of the *Chaenomeles* plants during vegetation ($\bar{x} \pm SD$, $n = 3$, $P < 0.01$)

Species	Catalase (CAT) activity, $\mu\text{M H}_2\text{O}_2/\text{s g WW}$				
	April	May	June	July	August
<i>Ch. speciosa</i>	25.34 ± 0.33 ^a	19.23 ± 0.09 ^b	1.06 ± 0.09 ^{cd}	0.48 ± 0.01 ^{cd}	14.71 ± 0.56 ^c
<i>Ch. cathayensis</i>	32.41 ± 0.40 ^a	21.75 ± 0.09 ^{bc}	0.80 ± 0.16 ^{cd}	0.65 ± 0.01 ^{cd}	20.70 ± 1.87 ^{bc}
<i>Ch. japonica</i>	35.00 ± 1.05 ^a	9.23 ± 0.12 ^b	0.89 ± 0.08 ^{cd}	0.48 ± 0.01 ^{cd}	26.30 ± 0.98 ^c
<i>Ch. japonica</i> var. <i>maulei</i>	16.88 ± 0.66 ^a	9.71 ± 0.08 ^b	0.89 ± 0.08 ^c	3.14 ± 0.24 ^d	38.20 ± 0.96 ^e
<i>Ch. × superba</i>	31.38 ± 0.58 ^a	11.32 ± 0.16 ^{bc}	1.44 ± 0.40 ^c	3.15 ± 0.05 ^d	12.08 ± 0.10 ^{bc}
<i>Ch. × californica</i>	24.22 ± 0.32 ^a	5.01 ± 0.10 ^b	0.18 ± 0.01 ^{cd}	0.92 ± 0.09 ^{cd}	13.35 ± 0.81 ^e

Note: the same letters indicate statistically insignificant differences in the means of the compared pair according to Tukey's criterion (HSD).

Table 3

Changes of benzidine-peroxidase activity in the leaves of *Chaenomeles* plants during vegetation (Mean ± SD, $n = 3$, $P < 0.05$)

Species	Benzidine-peroxidase (BPO) activity, mM benzidine/min g WW				
	April	May	June	July	August
<i>Ch. speciosa</i>	181.59 ± 0.29 ^a	83.99 ± 1.38 ^b	147.94 ± 2.32 ^c	121.81 ± 3.38 ^d	114.90 ± 4.75 ^{bc}
<i>Ch. cathayensis</i>	117.20 ± 0.99 ^a	190.34 ± 1.24 ^b	195.28 ± 2.20 ^{bc}	214.45 ± 3.46 ^d	89.28 ± 5.65 ^e
<i>Ch. japonica</i>	159.43 ± 6.52 ^a	65.93 ± 0.32 ^b	130.33 ± 3.40 ^c	82.26 ± 3.44 ^d	157.46 ± 5.95 ^{ac}
<i>Ch. japonica</i> var. <i>maulei</i>	104.24 ± 1.39 ^a	38.75 ± 1.58 ^b	94.08 ± 2.77 ^c	73.37 ± 2.67 ^d	129.92 ± 6.39 ^e
<i>Ch. × superba</i>	188.69 ± 3.36 ^a	63.17 ± 1.01 ^b	119.88 ± 0.79 ^c	108.52 ± 3.48 ^d	145.21 ± 7.71 ^e
<i>Ch. × californica</i>	149.22 ± 1.20 ^a	55.00 ± 1.19 ^b	177.53 ± 1.58 ^c	151.22 ± 2.93 ^{ad}	153.11 ± 5.55 ^{ade}

Note: see Table 2.

Table 4Changes of guaiacol-peroxidase activity in the leaves of *Chaenomeles* plants during vegetation ($\bar{x} \pm \text{SD}$, $n = 3$, $P < 0.01$)

Species	Guaiacol-peroxidase (GPO) activity, mM guaiacol/min g WW				
	April	May	June	July	August
<i>Ch. speciosa</i>	16.58 \pm 0.10 ^a	25.80 \pm 1.19 ^b	26.81 \pm 0.96 ^{bc}	16.56 \pm 1.30 ^{ad}	19.40 \pm 0.93 ^c
<i>Ch. cathayensis</i>	16.34 \pm 0.02 ^a	51.53 \pm 2.19 ^b	42.86 \pm 0.60 ^{bc}	30.03 \pm 1.67 ^d	10.27 \pm 0.41 ^e
<i>Ch. japonica</i>	15.54 \pm 0.23 ^a	20.48 \pm 1.53 ^b	16.05 \pm 0.35 ^{ac}	15.37 \pm 0.62 ^{acd}	16.27 \pm 0.72 ^{ade}
<i>Ch. japonica</i> var. <i>maulei</i>	13.86 \pm 0.93 ^a	12.84 \pm 1.10 ^{ab}	27.89 \pm 0.39 ^c	14.87 \pm 0.94 ^{abd}	31.55 \pm 1.48 ^c
<i>Ch. \times superba</i>	20.88 \pm 0.23 ^a	17.71 \pm 1.17 ^b	20.75 \pm 0.72 ^{ac}	17.34 \pm 1.03 ^{bd}	24.00 \pm 0.73 ^c
<i>Ch. \times californica</i>	12.46 \pm 0.15 ^a	14.41 \pm 0.70 ^{ab}	54.91 \pm 0.17 ^c	21.78 \pm 1.07 ^d	32.71 \pm 2.40 ^c

Note: see Table 2.

Table 5Total phenolic, total flavonoids content and the reducing power in the leaves of *Chaenomeles* plants

Species	Total phenolic content, mg	Total flavonoids content, mg	Total reducing power, mg
	GAE/100 g WW	RE/100 g WW	AAE/100 g WW
<i>Ch. speciosa</i>	2928.0 \pm 142.2	274.2 \pm 9.8	772.0 \pm 10.5
<i>Ch. cathayensis</i>	4355.2 \pm 119.3	275.9 \pm 8.9	1137.9 \pm 47.5
<i>Ch. japonica</i>	4307.1 \pm 111.3	273.5 \pm 11.4	1054.7 \pm 30.5
<i>Ch. japonica</i> var. <i>maulei</i>	4017.3 \pm 105.6	263.5 \pm 10.2	1057.3 \pm 37.2
<i>Ch. \times superba</i>	4476.1 \pm 122.2	274.3 \pm 9.0	1157.2 \pm 37.8
<i>Ch. \times californica</i>	5277.0 \pm 132.3	285.1 \pm 9.3	1413.6 \pm 33.8

Table 6Total phenolic, total flavonoids content and the reducing power in *Chaenomeles* fruits

Species	Total phenolic content, mg	Total flavonoids content, mg	Total reducing power, mg
	GAE/100 g WW	RE/100 g WW	AAE/100 g WW
<i>Ch. speciosa</i>	1784.4 \pm 98.1	29.8 \pm 2.1	565.8 \pm 15.7
<i>Ch. cathayensis</i>	3331.7 \pm 127.3	66.5 \pm 4.6	1121.7 \pm 27.5
<i>Ch. japonica</i>	2869.2 \pm 81.8	38.4 \pm 3.3	885.6 \pm 19.5
<i>Ch. japonica</i> var. <i>maulei</i>	2783.0 \pm 75.2	62.6 \pm 5.1	872.3 \pm 21.9
<i>Ch. \times superba</i>	2676.5 \pm 118.8	56.8 \pm 3.6	784.8 \pm 16.8
<i>Ch. \times californica</i>	2636.0 \pm 89.1	41.9 \pm 2.9	937.7 \pm 22.3

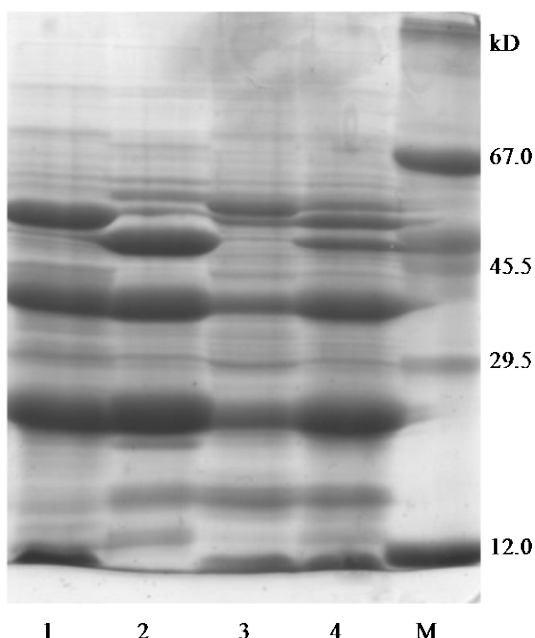


Fig. 1. Electrophoretic spectra (SDS-PAGE) of the seed proteins of the *Chaenomeles* plants: M – protein markers (cytochrome C, 12.0 kD; carbonic anhydrase, 29.5 kD; ovalbumin, 45.5 kD; bovine serum albumin, 67.0 kD); 1 – *Ch. japonica*; 2 – *Ch. cathayensis*; 3 – *Ch. \times superba*; 4 – *Ch. speciosa*

The number of protein bands in the spectra varied from 16 in the seeds of species *Ch. japonica* to 19 bands in the seeds of *Ch. speciosa* and *Ch. cathayensis*, and to 22 in the seeds of the hybrid *Ch. \times superba*.

Dominant components in the protein complex of seeds were also different in the species from different regions of origin. The polypeptides with a molecular weight of 19.7 and 29.3 kD predominated in the seeds of *Ch. speciosa*, and the components of 18.1, 29.3 and 38.7 kD dominated in the composition of the seed proteins of *Ch. cathayensis*. In the seeds of *Ch. japonica* fruits, the components with a molecular weight of 18.1, 31.6 and 46.0 kD were most expressed.

Discussion

The functioning of catalase is the main metabolic pathway to protect the photosynthetic process, eliminating excess hydrogen peroxide, which is formed in plant cells under the influence of unfavourable environmental conditions (Huseynova et al., 2015). The effect of drought, high temperature and intense sunlight on plants reduces the effectiveness of photosynthesis, instead reinforcing the process of photorespiration and causing excessive accumulation of reactive oxygen species, including hydrogen peroxide (Luna et al., 2005). In our study, high catalase activity over a long period (from April to June) was noted in the leaves of species *Ch. cathayensis* and *Ch. speciosa* that originate from the mountainous regions of China and are more tolerant of drought (Table 2). In the hybridogenic species *Ch. \times californica* and *Ch. \times superba*, as well as in *Ch. japonica* var. *maulei*, the catalase activity in leaves declined gradually in the interval from April to June, but increased sharply in July, indicating a moderate tolerance of these species to the arid character of the regional climate. In the leaves of *Ch. japonica* catalase activity was maximal in April, quite high in May, but significantly decreased in June and July, against the background of the effect of high temperature and a decrease in air humidity.

The functions of peroxidases in the plant cells are diverse and numerous, including participation in eliminating excess hydrogen peroxide (Halliwell, 2006) and in the phenols metabolism (Allison & Schultz, 2004). At the same time, the activation of benzidine peroxidase and guaiacol peroxidase under the action of water deficiency can be more likely associated with the involvement of enzymes in the lignification process than with the protection of plants from oxidative stress (Lee et al., 2007). Among the *Chaenomeles* plants, the highest activity of benzidine peroxidase was found in both species of Chinese origin and hybridogenic species *Ch. \times californica* and *Ch. \times superba* (Table 3). Whereas, in leaves of *Ch. cathayensis* the enzyme activity increased significantly until August, in other species BPO activity declined sharply in May, but then increased significantly, remaining high during the period of the greatest increase in drought and heat. In both Japanese species, the activity of benzidine peroxidase in the leaves was reduced during the vegetation, reaching a high level only in August, indicating a lower tolerance of these species. Higher activity of guaiacol peroxidase at the beginning of vegetation and the most significant activation of the enzyme during the growing season were found in the leaves of *Ch. cathayensis*, *Ch. speciosa* and *Ch. \times californica* (Table 4). The activity of GPO in the leaves of both Japanese species was less significant during the vegetation, remaining at the same level in August in *Ch. japonica* leaves, while increasing more than twice in the leaves of *Ch. japonica* var. *maulei*.

The health promoting abilities of fruit plants are largely determined by the high antioxidant capacity of plant metabolites, which can be realized as free radical scavenging activity, and by reduction or

chelation of metals as well (Costa et al., 2009; Du et al., 2013). In the present paper, the antioxidant level of the *Chaenomeles* leaves and fruits was estimated as the total reducing power (TRP) and expressed as the ascorbic acid equivalents. Leaves of *Ch. × californica* had the highest TRP (14.1 mg AAE/g WW), while reducing power in leaves of *Ch. speciosa* was almost two times less (Table 5). Leaves of hybridogenic species *Ch. × superba* and *Ch. cathayensis* as well showed high reduction ability; whereas it was slightly lower in leaves of both Japanese species. However, in general, the total reducing power of *Chaenomeles* leaves can be estimated as substantial, given that reducing power of *Thymus vulgaris* leaves reached 0.94 mg AAE/g WW only (Eghdami et al., 2013), and the total reducing capacity of *Cyperus erectus* plants (20.0 mg AAE/g DW) was estimated as high (Augustus et al., 2015). The study results indicate that leaves of the *Chaenomeles* species can be considered as plant raw materials with high antioxidant activity. The output coincides with the data of Han et al. (2016) on the radical scavenging activities and anti-inflammatory effects of different *Ch. sinensis* leaf extracts.

The total reducing power in the fruit flesh of *Chaenomeles* species was lower as compared to leaves, and *Ch. cathayensis* fruits showed the maximal level while *Ch. speciosa* the minimal, respectively, 11.2 and 5.7 mg AAE/g WW (Table 6). The antioxidant capacity of *Ch. japonica* and *Ch. japonica* var. *maulei* fruits occupied the middle position between the TRP of the hybridogenic species *Ch. × superba* and *Ch. × californica* (7.9 and 9.4 mg AAE/g respectively). In total, the antioxidant potential of *Chaenomeles* fruits is comparable to the level shown by Mitic et al. (2016) for other fruit crops, including nectarine (14.36–17.58 mg AAE/g), plum (32.43–58.30 mg AAE/g), and peach (12.31–24.55 mg AAE/g) as well as to the reducing power of *Berberis* fruits defined in the range 5.0–9.6 mg AAE/100 g DW (Khromykh et al., 2018).

Polyphenols are abundantly present in a majority of fruits, herbs and vegetables, and can play the protective role against reactive oxygen and nitrogen species, UV light, plant pathogens, parasites and predators (Brglez et al., 2016). These secondary plant metabolites represent a large and diverse group of substances including simple polyphenols, catechins, anthocyanins, glycosides of flavones, flavonols, isoflavones and flavanones and other compounds (Sakakibara et al., 2003; Jafari et al., 2014). In our study, the total phenolic content in the *Chaenomeles* fruits isopropanol extracts was the lowest in *Ch. speciosa* (17.84 mg GAE/g WW), average in *Ch. japonica* and *Ch. japonica* var. *maulei* (28.69 and 27.83 mg GAE/g), and the highest (33.32 mg GAE/g) in *Ch. cathayensis* fruits (Table 6). As for the hybridogenous species *Ch. × californica* and *Ch. × superba*, the content of phenols in their fruits reached an average level relative to the parent plants. The results obtained differ from the data on the phenol content in the acetone extracts from the fruits of *Ch. speciosa* (46.92 mg GAE/g) and *Ch. japonica* (19.35 mg GAE/g) collected in the Botanical Garden of Beijing in China (Du et al., 2013). It is obvious that the differences in the accumulation of antioxidant metabolites in the fruits of the same plant species are due to the influence of environmental conditions, which vary widely in these two regions. The study results suggest also that the adaptation of Japanese and Chinese species to the soil and semi-arid climatic conditions of the Steppe Dnieper was accompanied by more significant accumulation of the antioxidant compounds in the fruits of *Ch. japonica* plants than *Ch. speciosa*. This assumption agrees with the data of Nichols et al. (2015) that the high level of phenolic compounds accumulation positively correlated with the improvement of the stability of clover plants to drought. In general, the content of phenolic compounds in the *Chaenomeles* fruits significantly exceeded those for some other fruit crops, including peach (0.69–4.00 mg GAE/g), nectarine (0.93–1.83 mg GAE/g), and plum (3.91–13.53 mg GAE/g), reported by Mitic et al. (2016). In the fruits of different apple varieties cultivated in Italy the mean content of total polyphenols lay between 66.2 and 211.9 mg/100 g of FW (Vrhovsek et al., 2004), while other data (Wojdyło et al., 2008) showed the variation in the range from 523 to 2,724 mg GAE/100 g DW in a group of 67 different apples varieties.

The total phenolic content in leaves of *Chaenomeles* plants measured during the flowering exceeded that in the fruits of each species,

and varied notably (Table 5). The most polyphenols accumulation was revealed in leaves of hybridogenic species *Ch. × californica* (52.8 mg GAE/g WW), while the lowest was in *Ch. speciosa* leaves (29.3 mg GAE/g). Variability in the accumulation of phenols (40.7–160.0 mg GAE/g) was established in leaves of *Marrubium vulgare* plants from different geographical origins (Bouterfas et al., 2016). A similarly wide range of the polyphenols accumulation (11.19–51.49 mg GAE/g) in *Curcuma longa* plants from different ecological niches of Western Himalayas was reported by Arya et al. (2015). Thus, the variability of the secondary metabolites accumulation in *Chaenomeles* leaves and fruits is an indicator of the adaptation success of introduced species from the different geographical origin to climatic conditions of the Steppe Dnieper.

Flavonoids are a subclass of the polyphenols, and they form a large group of food constituents, many of which alter metabolic processes and have a positive impact on health (Beecher, 2003), possess preventive and therapeutic potential (Owczarek et al., 2017). In the leaves of *Chaenomeles* plants, the content of flavonoids varied very slightly, ranging from 2.64 mg RE/g WW in the species *Ch. japonica* var. *maulei* to 2.85 mg RE/g in *Ch. × californica* (Table 5). As for the plant fruits, the highest flavonoids accumulation was revealed in *Ch. cathayensis* and *Ch. japonica* var. *maulei* (0.67 and 0.63 mg RE/g WW respectively), while the lowest level (0.30 mg RE/g WW) was in the fruits of *Ch. speciosa* (Table 6). The fruits of Japanese quince and the hybrid species *Ch. × superba* and *Ch. × californica* had an intermediate flavonoid content, respectively, 0.38, 0.57, and 0.42 mg RE/g WW. The results obtained are consistent with the data (Hafez-Taghva et al., 2016) on the accumulation of flavonoids in the range 0.091–0.618 mg RE/g in *Ch. japonica* fruits. Dissimilar levels of the flavonoids accumulation in fruits of different species can be attributed to the general diversity of the phenolic compounds, which was found within the genus *Chaenomeles* (Watychowicz et al., 2017; Miao et al., 2016; Lewandowska et al., 2013).

Correlation analysis revealed the positive relationship between the total reducing power and the total phenols content, and total flavonoids content in leaves of the *Chaenomeles* plants (respectively, $r = 0.96$ and $r = 0.88$, $P < 0.05$). Consequently, phenols in general, and flavonoids in particular, make an equally significant contribution to the overall high antioxidant activity of the leaves. As for the fruits of the *Chaenomeles* species, strong correlation was established between the total reducing power and the total phenols content ($r = 0.95$, $P < 0.05$), whereas a lower correlation coefficient ($r = 0.66$) was found between the total reducing power and total flavonoids content. The explanation for the low level of correlation may be due to the fact that flavonoids are widespread in nature, but not evenly (Beecher, 2003), most likely even within the same genus of plants.

Storage proteins make up the bulk of the seed protein and play an important role in their quality, as the success of germination and further growth of the seedlings in extreme environmental conditions largely depends on their content, rate of transformation and use in the metabolic processes (Radhika & Rao, 2015). The polypeptide composition of the storage proteins of *Ch. japonica* seeds had fewer components than the seeds of species from the Chinese region, *Ch. speciosa* and *Ch. cathayensis* (Fig. 1). Consequently, the distinction of seeds polypeptide composition of the *Chaenomeles* species from different regions can make a significant contribution to the growth and resistance of these species in their natural areas and in new habitats as well. This assumption is consistent with the data (Bartish et al., 2000; Rumpunen, 2002) that the species *Ch. japonica* differs clearly from *Ch. speciosa* and *Ch. cathayensis* in the aspects related to spontaneous hybridization. The differences in the polypeptide composition of seed storage proteins serve as yet further evidence of the wider range of adaptive capabilities of the *Chaenomeles* species of Chinese origin to the conditions of the steppe climate.

Conclusion

A comparative study of the antioxidant enzymes activity and the second metabolites accumulation in leaves and fruits of several natural and hybridogenic genotypes of the genus *Chaenomeles* were car-

ried out. Differences in the antioxidant capacity of plants due to the peculiarities of Chinese and Japanese species adaptation to the conditions of steppe climate have been established. In leaves, the highest activity of antioxidant enzymes and their greatest activation under the influence of heat and drought during vegetation were found in *Ch. cathayensis* and *Ch. speciosa*, and the lowest in leaves of both Japanese species. The total antioxidant capacity and phenolic content reached the highest level in the leaves of hybridogenic species *Ch. × superba* and *Ch. × californica*, as well as *Ch. cathayensis*. In general, the total antioxidant capacity and metabolite content in the fruits of all *Chaenomeles* species was found to be lower compared to leaves. The total reducing power and the accumulation of metabolites were the greatest in fruits of *Ch. cathayensis*, while *Ch. speciosa* fruits had unexpectedly low levels. The significant contribution of phenolic compounds to the antioxidant capacity of leaves and fruits was confirmed by high correlation coefficients. The study results showed that leaves and fruits of all *Chaenomeles* species are sufficiently rich sources of natural antioxidants. Nevertheless, the specific differences established in the work led to the conclusion that species *Ch. cathayensis* and *Ch. × californica* are the most promising species for cultivation in the steppe zone in order to obtain fruits with a rich content of antioxidant compounds.

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