

Activity and isoenzyme composition of peroxidase in the vegetative organs of Japanese quince under steppe zone conditions

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Parameters of peroxidase complex are indicators of the adaptable processes which are taking place in plants under the environment influence. In this work the general activity dynamics and peroxidase isoenzyme composition in vegetative organs of Japanese quince (*Chaenomeles* Lindl.) were studied. The four species introduced in a steppe zone of Ukraine were researched: *Ch. japonica* (Thunb.) Lindl., *Ch. speciosa* (Sweet) Nak., *Ch. cathayensis* (Hemsl.) Schneid., *Ch. × superba* (Frahm) Red.). It was determined that the enzyme activity of these species possesses specific features subject to the phases of seasonal development and in response to the action of the hydrothermal stress. The highest enzyme activity in the period of intensive growth and flowering, with a subsequent sharp drop towards the middle of summer and further smoother decrease during the phase of physiological rest were observed in the leaves of *Ch. speciosa* and *Ch. cathayensis*. A higher peroxidase activity was a characteristic of *Ch. japonica* and *Ch. × superba* in the middle of the vegetative period, which can be considered a display of adaptation processes in the conditions of an unfavourable hydrothermal regime. The dynamics of changes in the quantitative composition of isoperoxidases was similar to the dynamics of enzyme activity. As a result of the generalization of the obtained data it is possible to consider that *Ch. japonica* and *Ch. × superba* are the steadiest in the conditions of the region.

Keywords: Japanese quince, introduction, peroxidase, adaptation, stability

INTRODUCTION

Introduction of nonconventional fruit crops represents a considerable interest for the preservation of the biological variety and expansion of

the spectrum of valuable agricultural plants (Convention, 2012; Klimenko, 2007; Yermakov, 1999; Klimenko et al., 2010; Mezhsenskij, 2005; Sorokopudov et al., 2013). This group includes Japanese quince (*Chaenomeles* Lindl.). Its fruits are rich in pectins, organic acids, vitamins C, B₁, B₂, P, and macroelements such as phosphorus, potassium and calcium (Petrova, 1986; Yang et al., 2009).

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Japanese quince is a very useful product for a healthy diet: it does not contain fats, sodium, or cholesterol, and is rich in organic fibres and copper (Song et al., 2007).

Natural habitats of Japanese quince are located in the mountain areas of South-East (*Ch. cathayensis*) and East (*Ch. japonica*, *Ch. speciosa*) Asia. *Ch. × superba* is a product of natural hybridization of *Ch. japonica* and *Ch. speciosa*. Monsoonal circulation, which creates sharply expressed distinction between damp warm and dry cold seasons, is the basic feature of the climate formation in these regions. Dry seasons are winter and spring and damp seasons are summer and autumn. At the same time, climatic conditions of the Ukraine steppe zone are characterized by insufficient water delivery and high air temperatures during summer and early autumn. It is a powerful limiting factor for plant introduction.

The process of plant adaptation to unfavourable conditions is accompanied by the occurrence of specific and nonspecific reactions at all levels of the structural organization (Bobrova et al., 2010; Mitteler, 2002). Their display is controlled by simultaneous action of numerous regulatory mechanisms, one of which is dynamics of enzyme activity of the antioxidant system (Lykholat et al., 2015; Rossihina et al., 2011; Verslues et al., 2006; Kolupaev, Karpec, 2010; Kucherenko, Kapustyan, 2004). The activity and isoenzyme composition of peroxidase represents a special interest (Biryuk, 2002; Chaves, Oliveira, 2004). It is a nonspecific universal enzyme with a wide spectrum of action. It provides an optimum course of oxidation-reduction processes, takes part in breathing processes and metabolism of proteins and along with other enzymes of antioxidant protection neutralizes active forms of oxygen and provides complex protection of biopolymers (Gulen, 2004; Chirkova, 2002).

In the conditions of a droughty climate targeted researches into plant metabolism under the influence of hydrothermal stress get a special urgency (Almeselmani et al., 2006; Minnibaeva, 2003). It is known that the activity of enzymes increases in the steadiest species of plants under adverse conditions. It can occur

at the expense of the strengthening of the activity of separate components of the system or of the whole complex (Sadvakasova, Kunaeva, 1987; Haluskova et al., 2009).

Thus, ongoing studies of the peroxidase complex in the vegetative organs of Japanese quince will allow estimating the level of their stability in the conditions of introduction.

MATERIALS AND METHODS

Plant material

Four species of Japanese quince (*Ch. japonica*, *Ch. speciosa*, *Ch. cathayensis* and *Ch. × superba*), introduced in the Botanical Garden of Oles Gonchar Dnipropetrovsk National University were the objects of research. The parameters of the peroxidase complex were determined in plant shoots and leaves. Sampling was made during the vegetative season from same-age plants within one experimental site. The activity of peroxidase in the samples was determined according to Boyarkin method (Vinnichenko et al., 2014). The definition of the isoenzyme composition of peroxidase was carried out by method of analytical isoelectric focusing (IEF) in polyacrylamide gel (PAAG) in pH 3.5–7.0 range (Rygetti, 1986; Shupranova et al., 2011).

Statistical Analysis

Classical methods of statistical processing were applied to the estimation of reliability of the obtained results by means of computer software package Excel and StatGraphics Plus. The significance value – $P < 0.05$.

RESULTS AND DISCUSSION

Only under the influence of stressful factors it is possible to reveal the display of the mechanisms of adaptation and the potential of plant stability. The impact of the Ukraine steppe zone environment can be considered one of these factors (Fig. 1).

The water deficiency caused by frequent droughts and the heat of air throughout almost entire vegetative period are capable to lead to a disbalance in the functioning of a vegetative

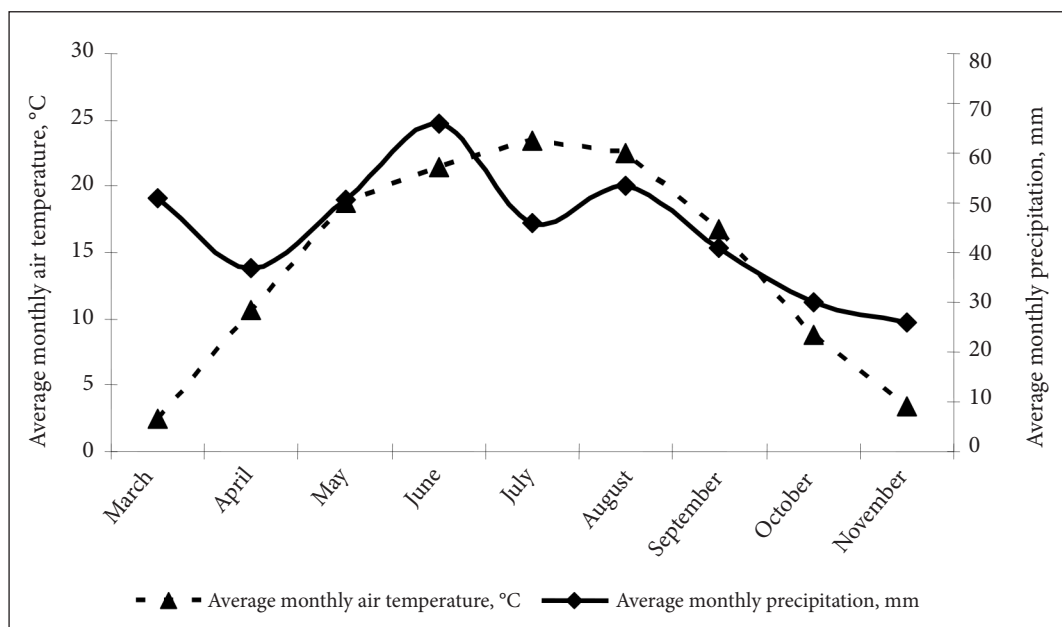


Fig. 1. Average monthly air temperature and precipitation in Dnipro city, 2011–2015

organism. The activity of peroxidase is an indicator of the plant reaction to environmental factors. The stages of ontogenesis also influence the said indicator (Andreeva, 1988).

All studied species of Japanese quince have a similar type of seasonal development. The spring expansion of buds begins in April, flowering comes at the end of April and proceeds till the end of May. The period of maturing of fruits continues from the last decade of September to the end of October. Leaf fall begins in October and only *Ch. cathayensis* continues to vegetate to the first frosts (November).

It was revealed that the highest activity of peroxidase in vegetative organs occurs in *Ch. speciosa* and *Ch. cathayensis* in May, in the phase of active growth and flowering (Fig. 2).

During this period the activity of the enzyme was average in *Ch. japonica* and *Ch. × superba*. Various levels of activity of enzyme system can be special individual indicators in various species of Japanese quince. It is necessary to notice that especially high values of the activity of enzyme are inherent in *Ch. speciosa* and *Ch. cathayensis*, which have been introduced in the conditions of the region rather recently.

The significant decrease in peroxidase activity in *Ch. speciosa* (three times) and *Ch. cathayensis*

(four times) was observed in the middle of vegetation (the phase of secondary growth). As a rule, the given period coincides with the most adverse hydrothermal regime that probably brings about a sharp disbalance in enzyme functioning. The small increase in the activity of enzyme (by 3% and 27%, respectively) was observed at the same time in two other species of Japanese quince – *Ch. japonica* and *Ch. × superba*, which points to the progress of adaptive processes.

Peroxidase activity reaches its minimum values in the phase of physiological rest in all studied species of Japanese quince. The decrease in the activity of the enzyme by the end of vegetation is explained by the reduction of intensity of the metabolic processes in a vegetative organism and preparation for the winter rest.

The results of the study will be co-ordinated with the data of other works on the introduction of wood and shrub plants (Petrovskaya-Baranova, 1983; Zaytceva, Dolgova, 2011).

The changes in peroxidase activity can occur under the influence of adverse factors both at the expense of the already available isoenzymes and of the synthesis of new isoforms. Isoenzymes expand adaptive possibilities of an organism and raise its ecological plasticity due to their various physical and chemical properties

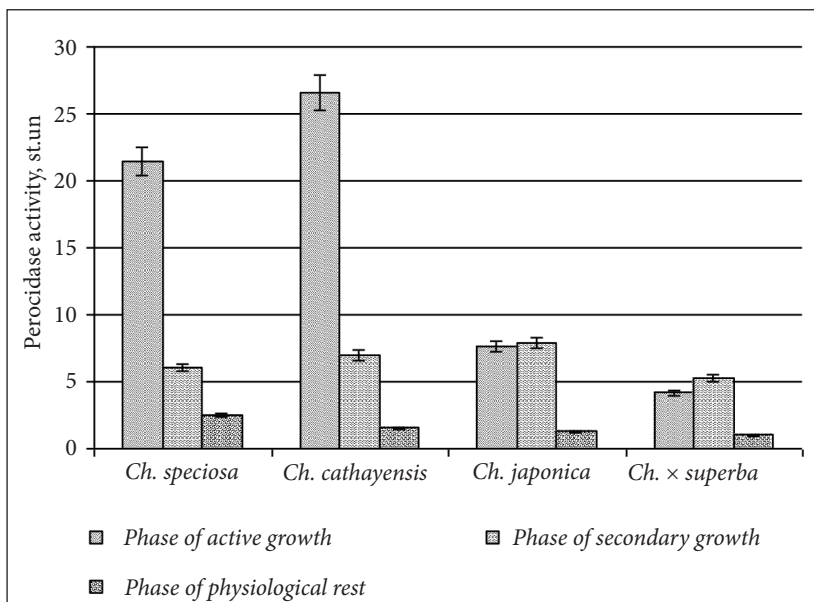


Fig. 2. Activity of peroxidase in the vegetative organs of Japanese quince throughout the vegetative period

(Lagrimini et al., 1990; Popkova, 2006). Different species of Japanese quinces have shown the specificity of the spectra of isoenzymes of peroxidase (Fig. 3).

The presence of six isoperoxidases in the pH range from 3.80 to 4.70 for the species of *Ch. japonica* in the active period of growth and flowering is registered (Table 1). The maximum activity of peroxidase is concentrated in the zone with the value of the isoelectric point (pI) being 4.30.

The presence of eight components for *Ch. speciosa* and *Ch. Cathaensis* is noted, in the range of pH from 3.80 to 5.20. It was observed that the maximum activity of peroxidase was in the zone with pI 4.30, and the minimum activity in zones with pI 5.05 and 5.20. It is necessary to note that there are only traces of components with the value pI 3.8 in the vegetative material of *Ch. cathaensis*. For *Ch. x superba*, seven isoforms with the pH range from

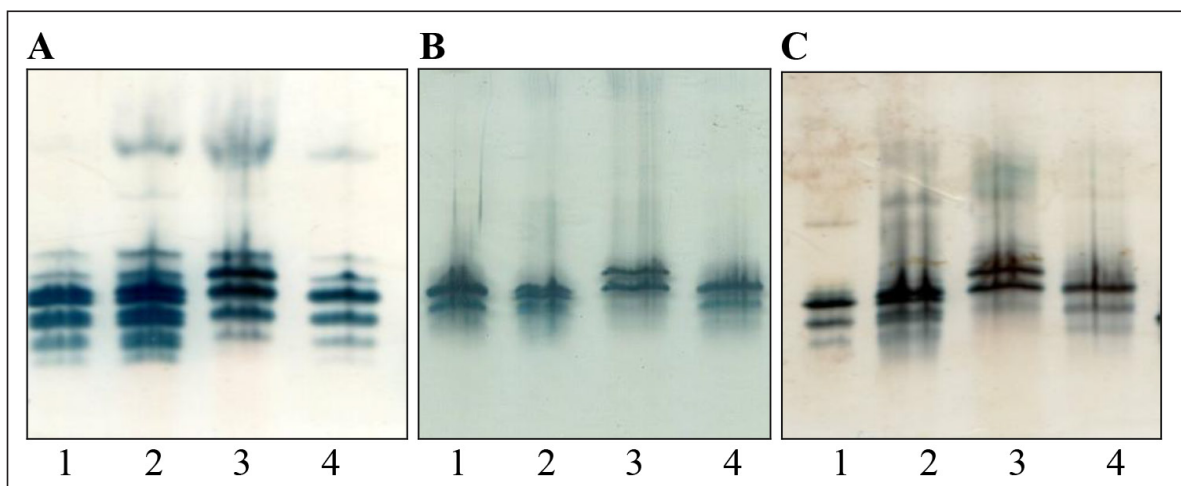


Fig. 3. IEF-PAGE analysis of the peroxidase system of the vegetative organs of Japanese quince during different periods of vegetation (A – the phase of active growth and flowering, B – the phase of secondary growth and ripening of sprouts, C – the phase of physiological rest): 1 – *Chaenomeles japonica*; 2 – *Chaenomeles speciosa*; 3 – *Chaenomeles cathayensis*; 4 – *Chaenomeles x superba*

Table 1. Values of isoelectric points of peroxidase isoenzymes in the vegetative organs of various species of *Chaenomeles* genus (the phase of active growth and flowering)

Values of pI	Species of <i>Chaenomeles</i> Lindl.			
	<i>Ch. japonica</i>	<i>Ch. speciosa</i>	<i>Ch. cathaensis</i>	<i>Ch. × superba</i>
3.80	+	+	tr.	tr.
3.90	++	++	+	+
4.05	+++	+++	-	-
4.10	-	-	++	++
4.30	++++	++++	++++	+++
4.48	+	+	+++	-
4.50	-	-	-	+
4.70	+	+	++	-
4.75	-	-	-	tr.
5.05	-	tr.	tr.	tr.
5.20	-	tr.	tr.	-

Note: intensity of isoperoxidases colouring: tr. – traces; + – very poor; ++ – poor; +++ – strong; ++++ – very strong.

3.80 to 5.05 were determined, one of which is also a characteristic of *Ch. speciosa*. Absence of components with values of pI 4.05, 4.48, and 4.70 and an occurrence of the isoform with the value of pI 4.50 were established.

It was revealed that the reduction in the quantity of isoforms for all investigated species was in the middle of vegetation. Absence of components with value of pI 5.10 in the vegetative material of *Ch. cathaensis* and *Ch. × superba*, and with the value of pI 5.15 in *Ch. japonica* and *Ch. speciosa*, as well as an occurrence of the isoform with the value of pI 5.21 at *Ch. cathaensis* were determined (Table 2). The maximum activity of peroxidase was established in the zone with the value

of pI 5.20 for all investigated species. It is necessary to point out that pH is displaced to less sour isoperoxidases in an unfavourable hydrothermal regime. Changes in the peroxidase isoenzyme composition were also noticed in September.

The majority of the components remain as traces. It corresponds to the data on the general decrease in the peroxidase activity during this period. Thus the maximum enzyme activity is observed in the zone with the value of pI 4.80 for all investigated species, except *Ch. cathaensis* in which a high activity of peroxidase in zones with pI 4.90 and 4.95 was shown (Table 3).

Isoforms with the value of pI 4.90 and 5.23 were inherent only in this species. Isoenzymes

Table 2. Values of the isoelectric points of peroxidase isoenzymes in the vegetative organs of various species of genus *Chaenomeles* (the phase of secondary growth)

Values of pI	Species of <i>Chaenomeles</i> Lindl.			
	<i>Ch. japonica</i>	<i>Ch. speciosa</i>	<i>Ch. cathaensis</i>	<i>Ch. × superba</i>
5.05	-	tr.	-	-
5.10	++	+	-	-
5.15	-	-	tr.	++
5.20	++++	++++	+++	+++
5.21	-	-	+++	-

For the intensity of isoperoxidases colouring, see Table 1.

Table 3. Values of the isoelectric points of peroxidase isoenzymes in the vegetative organs of various species of genus *Chaenomeles* (the phase of physiological rest)

Values of pI	Species of <i>Chaenomeles</i> Lindl.			
	<i>Ch. japonica</i>	<i>Ch. speciosa</i>	<i>Ch. cathaensis</i>	<i>Ch. × superba</i>
4.27	tr.	tr.	–	–
4.50	tr.	tr.	–	tr.
4.60	+	+	–	–
4.75	–	–	–	+
4.80	++	+++	tr.	++
4.90	–	–	+++	–
4.95	–	–	+++	tr.
5.00	–	tr.	–	–
5.10	tr.	–	–	–
5.18	–	tr.	tr.	–
5.23	–	–	tr.	–

For the intensity of isoperoxidases colouring, see Table 1.

with value of pI 4.95 were found in small quantities in the leaves of *Ch. × superba*. In the vegetative material of *Ch. japonica* and *Ch. speciosa* components in the pH range of 4.27 to 4.60 were stable. In the enzyme spectrum of the vegetative material of *Ch. speciosa* the isoform with the value of pI 5.00 which is absent in other species of Japanese quince, was determined.

CONCLUSIONS

The conducted research revealed that the presence of a considerable quantity of peroxidase isoforms in the phase of active growth and flowering points to the progress in this period of intensive metabolic processes in the vegetative organs of Japanese quince. However, high activity of the enzyme in *Ch. speciosa* and *Ch. cathaensis* can indicate not only a specific difference, but also too big expenses of energy which is spent by plants for homeostasis maintenance.

An unfavourable hydrothermal regime leads to a sharp disbalance in the functioning of peroxidase of these species. At the same time, despite the reduction of isoenzyme spectrum in *Ch. japonica* and *Ch. × superba*, the general activity of peroxidase rises a little, that points to the adaptation of these plants to stressful factors.

Thus, in the conditions of the steppe zone of Ukraine it is possible to consider that *Ch. japonica* and *Ch. × superba* are the steadiest species and *Ch. speciosa* and *Ch. cathaensis* are the species of average stability. It is necessary to apply additional agrotechnical methods for their successful cultivation in this region.

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JAPONINIO SVARAINIO VEGETACINIŲ ORGANŲ AKTYVUMAS IR PEROKSIDAZĖS IZOFERMENTŲ SUDĖTIS STEPIŲ ZONOS SĄLYGOMIS

Santrauka

Peroksidazės kompleksas atspindi prisitaikymo procesus, vykstančius augaluose veikiant aplinkai. Šiame darbe buvo tiriama japoninio svarainio (*Chaenomeles* Lindl.) vegetacinių organų bendra veiklos dinamika ir peroksidazės izofermento sudėtis. Iširtos keturios svarainių rūšys, introdukuotos į Ukrainos stepių zoną: *Ch. japonica* (Thunb.) Lindl., *Ch. speciosa* (Sweet) Nak., *Ch. cathayensis* (Hemsl.) Schneid., *Ch. × superba* (Frahm) Red. Nustatyta, kad šių rūšių fermentinis aktyvumas priklauso nuo sezoninio vystymosi fazių ir atsako į hidroterminį stresą. Didžiausias fermento aktyvumas užfiksuotas *Ch. speciosa* ir *Ch. cathayensis* lapuose intensyvaus augimo ir žydėjimo laikotarpiu, vėliau jis staigiai mažėjo iki vasaros vidurio ir palaipsniui mažėjo fiziologinio poilsio fazėje. Aukštesnis peroksidazės aktyvumas buvo būdingas *Ch. japonica* ir *Ch. × superba* rūšims vegetacinio laikotarpio viduryje, ir jį galima laikyti prisitaikymo procesų rodikliu nepalankaus hidroterminio režimo sąlygomis. Peroksidazės kiekybinės sudėties pokyčio dinamika, tiriant izofermentų elektroforetinį spektrą, buvo panaši į bendrą jų aktyvumą. Tyrimo duomenimis, *Ch. japonica* ir *Ch. × superba* yra patvariausios regiono sąlygomis.

Raktažodžiai: japoninis svarainis, peroksidazė, prisitaikymas, stabilumas