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ORIGINAL ARTICLE

Peroxidase Activity and Isoforms in the Leaves of Apple Tree Varieties Differing in Scab Resistance

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A host of publications deals with the role of peroxidase enzyme in metabolism of damaged plant, however, peroxidase participation in the protective reactions of damaged plants still attracts the attention of many researchers, as the increase in the activity of this enzyme correlates with resistance pre-programmed by the organism itself. Therefore, it might be efficient to consider this enzyme as a marker of plant resistance marker to certain infections (Andreyeva, 1988).

One of such infections is apple tree scab – a disease causing the highest amount of damage and killing on average 30-40% of harvest. In the years with particularly epidemically hard years harvest losses may amount up to 70-80% (Sedov, 2011). Pathogenic fungus attacks leaves, at times branches and in particular fruits – both growing on the tree and stored. The infected leaves develop olive-green velutinate spots with a brim of drying skin. Small early in summer, these spots grow in size and number (Metlitsky and Metlitsky, 2008). The leaves attacked by scab sharply decrease photosynthetic activity,

which negatively affects both total tree status and fruit development and quality. Fruits damaged by scab are subject to rapid decay. Vulnerability of the same varieties differs under diverse environmental conditions. The varieties showing resistance in certain regions are easily affected by this disease in others (Kiryushchenko *et al*., 2009).

In Siberia scab attacks leaves of many apple tree varieties. In the years, which favor the disease development, some varieties in western Siberia may have the damage score up to 4.8 (Severin and Selezneva, 2010; Kalinina and Kushnareva, 2010). Unfortunately, there are no literary data on apple trees sensitivity to this pathogen in Eastern Siberia on the whole and in the Irkutsk region in particular.

The literature provides a sufficient amount of evidence on the link between plant fungicide resistance and peroxidase activity. But these data mostly refer to tobacco, potatoes, French beans and other herbaceous plants (Bestwick *et al*., 1998; Bolwell *et al*., 2002; Graskova *et al*., 2008). The available data on changes in apple tree peroxidase cover only European and American varieties and are related to temperature stress, seasonal changes and dynamics during storage (Wang and Faust, 1994; Szecsko *et al*., 2002; Patykowski *et al*., 2007).

With this in view, the present work was aimed to investigate peroxidase activity and the amount of its isoforms in the leaves of apple tree varieties grown in southern Predbaikal'ye and differing in the degree of damage caused by scab.

MATERIALS AND METHODS

Material. Apple trees of 27 varieties and species were used as a material. All the studies were carried out in 2012-13 on the basis of Siberian Institute of Plant Physiology and Biochemistry SB RAS, farms of the Irkutsk district of the Irkutsk region. The plant material for the collection lot was grown under identical agrotechnical and climatic conditions. 2-years old plantlets of berry apple tree were used as stock apples.

Variety resistance to scab was conducted based on 6 score scale (Program and methods..., 1998).

Plant extracts preparation and measurement of peroxidase activity were performed according to the standard methods (Graskova *et al*., 2008).

Native protein electrophoresis aimed at identification of peroxidase isoforms was carried out in the blocks of polyacryl-amid gel in modified system by Anderson, Borg and Mikael'son (Kolesnichenko, 2002).

RESULTS AND DISCUSSION

One of the key requirements to be met by apple tree varieties is resistance to diseases, primarily to scab. Peroxidase enzyme, as a key link of plant cell response to pathogen, undoubtedly takes part in formation of such resistance.

We split all the varieties under study into several groups based on the degree of scab-caused damage (Table 1). The following apple tree species showed no symptoms of attack by this disease: Fonarik, Altai Ruddy, Krasnoyarsk Snegirek, Krasa Buryatii, Lada, Prevoskhodnoye, Borovinka, Svetloye, Gardeners' Gift, Krasnoyarsk Seyanets, Uralsk Nalivnoye, brown

Malus baccata, Royalty. Serebryanoye Kopytse species falls under the highly resistant category (score 1). The category, which is resistant to scab (score 2), includes the following species: Ermolayev's Crab Apple, Sayan Saffron, Papirovka, Krasnaya Gorka, Purple Crab Apple, Alenushka, *Malus baccata*. Medium resistance category (score 3) was constituted by the following species: Krasnaya Grozd', Antonovka, Melba, Berkan'ka. The sensitive category (score 4) included Grushevka Moskovskaya and Orlovskoye Polosatoye.

Table 1. Peroxidase activity in leaf tissues of apple tree varieties differing in scab damage degree. The activity is expressed in conventional units per unit of raw mass.

Sample №	17.07.2012	01.08.2012	20.08.2012	Scab damage degree	
				1.08.2012	1.09.2012
1. Krasnaya Grozd'	*0.956±0.067*	*1.277±0.001*	*0.900±0.009*	2	3
2. Fonarik	**1.176±0.030**	**1.635±0.041**	**1.871±0.020**		
3. Altai Ruddy	**0.916±0.002**	**0.883±0.050**	**1.719±0.049**		
4. Antonovka	*1.568±0.102*	*1.599±0.15*	*1.276±0.052*	2	3
5. Krasnoyarsk Snegirek	0.814±0.009	0.734±0.018	0.707±0.022		
6. Krasa Buryatii	**1.155±0.034**	**1.127±0.020**	**1.600±0.138**		
7. Yermolaev's crab apple	*0.609±0.027*	*1.015±0.029*	*0.643±0.029*	1	2
8. Lada	**0.396±0.016**	**0.657±0.036**	**0.964±0.109**		
9. Melba	*1.345±0.050*	*1.664±0.047*	*0.821±0.008*	2	3
10. Prevoskhodnoye	0.789±0.023	0.504±0.010	0.553±0.014		
11. Sayan Saffron	1.090±0.028	1.808±0.023	0.883±0.037	1	2
12. Borovinka	**0.854±0.079**	**1.021±0.011**	**1.063±0.079**		
13. Svetloye	1.072±0.016	0.638±0.007	0.618±0.014		
14. Papirovka	1.043±0.011	1.171±0.064	0.422±0.013	1	2
15. Gardeners' Gift	0.895±0.023	1.067±0.009	0.678±0.022		
16. Krasnoyarsk Seyanets	0.946±0.088	0.962±0.178	0.826±0.005		
17. Berkan'ka	0.681±0.019	0.511±0.015	0.593±0.008	2	3
18. Krasnaya Gorka	0.836±0.083	0.711±0.070	0.586±0.009	2	2
19. Grushevka Moskovskaya	0.550±0.030	0.576±0.039	0.602±0.021	2	4
20. Ural'skoye Nalivnoye	0.287±0.029	0.286±0.024	0.285±0.067		
21. Crab Apple Purpurovaya	0.698±0.073	0.463±0.028	0.703±0.031	1	2
22. Alenushka	1.286±0.043	0.999±0.015	0.877±0.017	1	2
23. Serebryanoye Kopytse	*1.142±0.002*	*1.852±0.021*	*1.135±0.025*		1
24. Orlovskoye Polosatoye	*0.716±0.037*	*1.345±0.019*	*0.781±0.068*	3	4
25. Malus baccata бурая	1.299±0.095	0.827±0.020	0.969±0.024		
26. Royalty	1.037±0.004	0.611±0.012	0.808±0.022		
27. Malus baccata	0.986±0.004	0.983±0.028	0.363±0.042	1	2

The analysis of peroxidase activity data in apple tree leaf tissues demonstrated that five species classed as a group without scab-caused damage symptoms (Fonarik, Altai Ruddy, Krasa Buryatii, Lada, Borovinka) showed increase in the activity over the research period. By contrast, medium-resistant анв susceptible species and Krasnaya Grozd', Antonovka, Melba, Berkan'ka, Orlovskoye Polosatoye)

demonstrated decreasing values of the enzyme activity. Interestingly, the Ural'skoye Nalivnoye species had the lowest peroxidase activity values.

Plant response pathogen activity is often accompanied by emergence of new multiple peroxidase forms or disappearance of some other forms of this enzyme. Further tests were focused on the change in molecular forms of the enzyme under study.

The first samples were collected in July, when infection load on plants is still low and even the most sensitive apple tree varieties show no signs of disease. Iso-enzyme composition of peroxidase during this period did not show any significant differences between various groups of varieties (Table 2).

Isoform Rf 0.35 in July and August (Table 3) was not found in highly resistant varieties, but in September (Table 4) it was registered in Svetloye, Gardeners' Gift and Borovinka varieties, as well as in resistant varieties Papirovka and Sayan Saffron. In leaf tissues of Krasnaya Gorka, Grushevka Moskovskaya, Crab Apple Purpurovaya and Berkan'ka this isoform was found in July and August respectively, but was eliminated in September samples. Isoform Rf 0,43 was not identified neither in July, nor in August, but appeared in September in Krasa Buryatii, Ural'skoye Nalivnoye and Alenushka.

Though isoform Rf 0.44 in July was determined in 13 out of 27 apple tree varieties studied, the samples taken in August showed that this isoform remained only in unaffected varieties Fonarik, Prevoskhodnoye, Svetloye and Ural'skoye Nalivnoye, while in September it appeared again in Krasnoyarsk Snegirek, Krasnaya Gorka, Antonovka and Berkan'ka.

Peroxidase isoforms Rf 0.60 and Rf 0.78 were not identified in July and August, whereas in September they were found in 17 and 9 varieties respectively out of 27 apple tree varieties studied. A similar situation was observed with isoform Rf 0.70. In July it was identified in four varieties, but was absent in highly resistant genotypes. In August this isoform was identified in leaves of 9 varieties, and in September – in 15 out of 27 apple tree varieties studied.

Based on the results acquired it may be concluded that changes in peroxidase activity and a set of the enzyme molecular forms in apple tree leaf tissues is a variety-specific feature and depends on seasonal phase of plant growth and development.

Apple tree resistance to leaf scab may be supposed to be related to peroxidase activity. Further research will provide confirmation of this enzyme role in the formation of apple tree resistance to this fungic pathogen.

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