OXIDATIVE STRESS IN *BETA VULGARIS* L. DURING THE POWDERY MILDEW ATTACK

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Abstract: The experiments, carried out in laboratory conditions, showed that during the incubation period of the fungus *Erysiphe betae* (Vanha) Welt, in the attacked sugar beet leaves, the activity of superoxide dismutase only intensifies in the susceptible variety Turbo. The activity of the oxidative enzymes increased significantly after the leaves exhibited symptoms of the disease, in all the examined varieties.

INTRODUCTION

One of the first reactions to the phytopathogenic fungi identified in the host plant is the rapid and massive release of the AOS (active oxygen species) due to the sequential reduction of molecular oxygen (O_2), i.e. the superoxide radical (O_2^-), the hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-). Subsequently to these early events, in order to minimize the destructions caused at cellular level by these free radicals, the endogenous anti-oxidative defense system made of enzymatic and non-enzymatic constituents begins to operate in the plant. Plant's protection against the free radicals released in large amount depends on the efficiency of this endogenous defense system.

At enzymatic level, there are three enzymes that function to this purpose, namely superoxide-dismutase (SOD), catalase (CAT), and peroxidase (PO). The role of peroxidase in pathogenesis is related to the oxidation of phenol compounds.

Besides these enzymes, in the infected parts of the plant attacked, acts also polyphenoloxidase, an enzyme involved in the formation of melanin compounds in the necrosed tissues. Its role is, however, controversial in what concerns resistance to diseases (Mayer, 1977).

Taking into consideration all these aspects, we considered it was necessary to study the dynamics of these enzymes' activity in the sugar beet leaves during the attack of the fungus *Erysiphe betae* (Vanha) Welt.; our aim was both theoretical and practical because we tried to identify these enzymes which may function as biochemical markers of resistance to the pathogen being examined.

To this purpose, a series of experiments were initiated in laboratory conditions, using sugar beet varieties with different levels of resistance to the attack of *E. betae*.

MATERIAL AND METHOD

Biological material, inoculation, symptom assessment

Host plant – for the experiment, sugar beet (*Beta vulgaris* L) cultivars from different varieties were used (Turbo, Ritmo, Kristall, Marian, Jamaica (Maribo Seed - Denmark) and Cremona (Hilleschog Seed - Sweden), with different levels of resistance to powdery mildew. The plants were grown under laboratory conditions, in two replicates.

Pathogen – the fungus *Erysiphe betae* (Vanha) Welt. was isolated from the experimental lots of the Station of Research and Production of Sugar Beet – Roman at the end of the summer of 1998 by transfer on plants grown in laboratory, shaking infected plants over the healthy ones. The fungus *E. betae* is strictly parasitical and does not grow on either natural or artificial culture media in laboratory, but only on live substrates.

Artificial infection

The sugar beet cultivars, 6 weeks of age, were inoculated with *Erysiphe betae* spores by direct shaking of the infected plants with 24-hour conidia (dry inoculum) onto the plants to be examined (one infected plant/20 test plants), according to the method previously described (Craita Rosu, 2001). All the selected inoculum plants

exhibited the same degree of attack and belonged to the Turbo variety. The first mycelian stains appeared on the leaves of the Turbo variety 5 days after infection.

Sample collection, enzymatic determinations/assays

Sample collection. Three leaf discs, 8 mm in diameter, were removed from sides adjacent to the infected area of each leaf and from healthy leaves (the third pair of leaves).

Peroxidase activity was determined by Moller's method (1966), using o- dianisidine as action substrate for the enzyme.

Polyphenoloxidase was assayed by spectrophotometry, using catechol as a substrate (Ermakov,A.Y., 1987).

Catalase activity was determined spectrophotometry (Beers and Sizer, 1952).

Superoxide-dismutase activity in the examined plant material was determined by spectrophotometry (Winterbourn C. and al., 1975).

RESULTS AND DISCUSSIONS

The results of the artificial infection with the fungus *Erysiphe betae* performed in laboratory conditions show a more rapid symptom evolution than in the field, favored by the established optimal conditions for spore germination and infection occurrence. The first symptoms were noted in the Turbo variety approximately 5 – 6 days after infection. In the Jamaica variety, the first whitish mycelium stains appeared after approximately 9 days as isolated colonies.

Accordingly, the events at the biochemical level follow the evolution of the symptoms (Table 1).

Table 1 - The modification of the activity ratio (infected/non-infected) of several oxidases according to variety resistance to powdery mildew and symptom evolution.

Cultivars	I	Infected/non infected ratio									
		Days after infection									
		5 days					*10 days*				
		SOD	CAT	POs	POi	PPO	SOD	CAT	POs	POi	PPO
Turbo	6.8	1,49	1,10	1,55	1,10	1,41	1,02	1,44	2,90	1,16	2,46
Jamaica	3.1	0,97	0,96	1,00	1,22	1,09	0,77	1,23	2,78	1,21	1,26

(I= intensity of attack, on a scale from 0 to 9)

In addition, we tried to establish a correlation between sugar beet resistance to powdery mildew and peroxidase activity. For the experiment, a number of 8 cultivars was assayed for the peroxidase activity, both fractions, 10 days after the artificial infection with *Erysiphe betae*.

Based on the results of the experiments, we may assert that there is a negative correlation between the resistance of sugar beet varieties to powdery mildew and peroxidase activity, this biochemical parameter not being representative for detecting sugar beet cultivars' resistance to the fungus attack.

The most common effect met in almost all host-parasite relationships is the respiration enhancement in the infected tissues and the adjoining ones. In the case of powdery mildew – producing fungi, this enhancement is also due to the respiration of the fungus itself, which is more intense than that of the plant.

Table 2 - Peroxidase activity in sugar beet leaves 10 days after the infection with *Erysiphe betae*

Cultivar	I%		*Soluble peroxidase activity*			*Ionicaly bound peroxidase activity*		
			U PO/g/min					
	Mean	*Limits value*	*Heathy*	*Infected*	*I/N ratio*	*Healthy*	*Infected*	*I/N ratio*
Cremona	8,0	7–9	*16,03± 1,06	51,24 ± 1,98	3,19	3,22 ± 0,18	3,92 ± 0,41	1,21
Turbo	7,2	6–8	15,81 ± 1,02	49,33 ± 1,95	3,12	2,68 ± 0,26	3,64 ± 0,21	1,35
Ritmo	6,7	6–7	14,15 ± 1,12	48,36 ± 1,42	3,41	1,98 ± 0,22	2,56 ± 0,25	1,29
Kristall	6,0	5–7	14,26 ± 0,98	41,78 ± 1,87	2,92	1,73 ± 0,23	2,24 ± 0,18	1,29
Marian	6,0	5–7	15,51 ± 1,02	40,66 ± 1,69	2,62	2,18 ± 0,31	2,58 ± 0,22	1,18
Jamaica	3,5	1–4	15,48 ± 0,99	42,31 ± 1,88	2,73	1,41 ± 0,18	1,72 ± 0,16	1,21

(I= grade of the attack intensity;
* = arithmetic mean of the values and the standard error; (n=8)

However, while the increase in the level of catalase, soluble peroxidase and polyphenoloxidase activities is in direct correlation with the degree in which the plant is affected, in the case of the ionic fraction of peroxidase the activity varies irrespective of the resistance of the varieties to powdery mildew, increasing gradually as the attack progresses, but approximately with the same magnitude in all the varieties being tested.

The more powerful enhancement of the soluble peroxidase activity in the Turbo and Cremona varieties, as shown, proves that in these varieties the non-specific resistance mechanisms set into action early. In the powdery mildew attack, however, such a reaction is not efficient, the subsequent evolution of the fungus not being hindered; on the contrary, the epiphyte development of the mycelium stimulates the respiration process and, implicitly, the activity of the enzyme.

Since the specialized literature (Eugenia Eliade,1990) indicates for the fungus *E.betae* the formation of lignin and suberin papillae as a resistance mechanism against the penetration of epidermal cell wall by specialized hyphae differentiated into appressoria, the activity of the peroxidase fraction ionically bound is expected to be higher in the variety more resistant to powdery mildew such as the Jamaica variety; this assumption can be made since the peroxidase bound to the cell wall is involved in the polymerization of the phenol monomers of lignin and suberin, acting in a first line of defense against pathogen invasion. However, this fact has not been noted, the level of the enzymatic activity being constitutively higher in the susceptible varieties, and the enhancement of activity after the infection having similar magnitudes in the varieties.

CONCLUSIONS

The activity of the oxidative enzymes increases progressively after the apparition of the *E.betae* mycelium on the foliar surface, thus proving their role in the general defense mechanism of the plant against the oxidative stress.

During the incubation period no powerful stimulation of the examined enzymes was noted, except for the superoxide-dismutase.

None of the determined markers can be used for screening the sugar beet varieties resistant to powdery mildew attack.

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