ORIGINAL PAPER

PHYSIOLOGICAL CHANGES IN BEAN (PHASEOLUS VULGARIS L.) LEAVES, INFECTED BY THE MOST IMPORTANT BEAN DISEA

ФИЗИОЛОГИЧНИ ИЗМЕНЕНИЯ В ЛИСТАТА ОТ ФАСУЛ (PHASEOLUS VULGARIS L.), ИНФЕКТИРАНИ С ИКОНОМИЧЕСКИ ВАЖНИ БОЛЕСТИ ПО ТАЗИ КУЛТУРА

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ABSTRACT

Gas-exchange, plastid pigments and some other physiological parameters were determined in bean (Phaseolus vulgaris L. local populations) leaves naturally infected by Xanthomonas campestris pv.phaseoli (Smith) Dye, and Pseudomonas syringae pv.phaseolicola (Bukholder) Young, Dye et Wilkie, and in healthy leaves (control).

It was established that infected leaves had lower both plastid pigments content and photosynthetic activity as well as lower yield and quality of produce.

Keywords: Phaseolus vulgaris L., Xanthomonas campestris pv.phaseoli (Smith) Dye (Xcp), Pseudomonas syringae pv.phaseolicola (Bukholder) Young, Dye et Wilkie (Psp), leaf gas exchange, chlorophyll fluorescence, plastid pigments, peroxidase activity, water content, elements of productivity

РЕЗЮМЕ

Проучено е влиянието на естественото инфектиране с Xanthomonas campestris pv.phaseoli (Smith) Dye, и Pseudomonas syringae pv.phaseolicola (Bukholder) Young, Dye et Wilkie, върху листния газообмен, пластидните пигменти и някои други физиологични показатели в листата на фасула.

Установено е, че инфектираните листа се характеризират както с понижено съдържание на пластидните пигменти и фотосинтетичната активност, така и с намален добив и качество на продукцията.

Ключови думи: Phaseolus vulgaris L., Xanthomonas campestris pv.phaseoli (Smith) Dye (Xcp), Pseudomonas syringae pv.phaseolicola (Bukholder) Young, Dye et Wilkie (Psp), листен газообмен, хлорофилна флуоресценция, пластидни пигменти, пероксидазна активност, водно съдържание, елементи на продуктивността

INTRODUCTION

Common bean (Phaseolus vulgaris L.) is a legume crop frequently and severely attacked by common (caused by Xanthomonas campestris pv. phaseoli (Smith) Dye) and halo (caused by Pseudomonas syringae pv.phaseolicola (Bulholder) Young, Dye et Wilkie) blight [1, 10, 12]. Symptoms of these diseases are very similar and it is not always possible to separate the losses caused by common blight and those due to halo blight, since they frequently occur together in the same field and on the same plants [13].

The pathogens attack all aerial plant parts, including leaf petioles, pods and seeds, but the characteristic symptoms of chlorotic borders around the necrotic lesions (Xcp) and red-brown necrotic spots (Psp) are more severe and conspicuous on leaves of susceptible cultivars.

Xcp and *Psp* could be powerful stress factors inducing significant anatomy-morphological and physiology-biochemical disorders in plant organisms [3, 5, 6, 15, 16]. The latter could have a negative effect on yields and quality of produce.

The existence of Xcp and Psp in all parts of the world indicate the necessity to identify in detail these pathogens in Bulgaria in order to help the breeding program select the right parental materials.

MATERIALS AND METHODS

The experiments were carried out in the years 2004-2005' at the field microtrials with bean plants (Phaseolus vulgaris L.) - four Bulgarian landraces from our collection (IPGR-Sadovo).

The leaf gas exchange parameters – net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) were determined with a portable photosynthetic system LCA-4 (Analytical Development Company Ltd., Hoddesdon, England). Measurements were made under a light intensity of 1400 µmol m^{-2} s^{-1} PAR, at a temperature of 25±28 ºC, an external CO_2 concentration of 450 µmol mol^{-1} and relative air humidity of 65-70%.

Parameters of chlorophyll fluorescence were determinated with a photosynthesis yield analyser MINI-PAM (H. Walz, Germany) after 30 min of dark adaptation of the plants, as described by Schreiber et al [11].

Chlorophyll (Chl) and carotenoids (Car) were extracted with 80% water acetone. The pigments were determined spectrophotometrically (Specol 11, K. Zeiss, Jena, Germany) after centrifugation of extract at 3000 rpm for 5 min [17] and calculated according Lichtenthaler and Wellburn formulae [8].

Peroxidase activity (PA; EC 1.11.1.7) was determined according to Herzog and Fahimi [4]. The material was homogenized with 0.05M Tris-glycine buffer, pH 8.3, containing 17% sucrose (m/v). One relative PA unit (U) is equal to ΔA 470 g^{-1} min^{-1}.

Relative water content (RWC) was determined according to Morgan [9].

The analyses were made on leaves of the same physiological age in three replications.

The agroclimatic conditions for bean production in

Table 1. Leaf gas exchange parameters in healthy and naturally infected by *Xanthomonas campestris pv. phaseoli* (Smith) Dye, and *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie, Bulgarian landraces of *Phaseolus vulgaris* L.; P_N - net photosynthetic rate [µmol (CO_2) m^{-2} s^{-1}]; E - transpiration rate [mmol m^{-2} s^{-1}]; g_s - stomatal conductance [mol m^{-2} s^{-1}]; * P < 0.05; ** P < 0.01;

Таблица 1. Интензивност на листния газообмен в контролни и естествено инфектирани с *Xanthomonas campestris pv. phaseoli* (Smith) Dye, и *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie, български образци фасул *Phaseolus vulgaris* L.; P_N - скорост на фотосинтезата [µmol (CO_2) m^{-2} s^{-1}]; E - интензивност на транспирацията [mmol m^{-2} s^{-1}]; g_s - устична проводимост [mol m^{-2} s^{-1}]; * P < 0.05; ** P < 0.01;

Landraces	Variants	Parameters		
		P_N	E	g_s
2000E-01	control (healthy)	19.83±0.22	2.57±0.15	0.16±0.01
	naturally infected	12.69±0.32**	2.86±0.12*	0.18±0.02*
95E-05	control (healthy)	21.81±0.30	4.22±0.10	0.22±0.04
	naturally infected	17.88±0.36*	4.48±0.10*	0.22±0.02
99E-059	control (healthy)	20.45±0.22	3.15±0.11	0.20±0.01
	naturally infected	15.13± 0.40**	3.22±0.10	0.22±0.01*
93E-012	control (healthy)	20.98±0.16	3.46±0.09	0.18±0.03
	naturally infected	15.94±0.21**	3.67±0.12*	0.21±0.01**

Table 2. Chlorophyll *a* and *b*, and carotenoids content in healthy and naturally infected by *Xanthomonas campestris pv. phaseoli* (Smith) Dye, and *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie, bulgarian landraces of *Phaseolus vulgaris* L.; Chl. *a*, Chl. *b*, Car. [mg g^{-1} d. m.]; * P < 0.05; ** P < 0.01;
Таблица 2. Съдържание на хлорофил *a* и *b*, и каротиноиди в контролни и естествено инфектирани с *Xanthomonas campestris pv. phaseoli* (Smith) Dye, и *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie, български образци фасул *Phaseolus vulgaris* L.;
хл. *a*, хл. *b*, кар. [mg g^{-1} d. m.]; * P < 0.05; ** P < 0.01;

Landraces	Variants	Parameters		
		Chl *a*	Chl *b*	Car
2000E-01	control (healthy)	3.34±0.03	1.84±0.05	2.19±0.02
	naturally infected	1.74±0,02**	0.94±0,01**	1.74±0,01**
95E-05	control (healthy)	4.20±0.07	2.03±0.01	2.63±0.04
	naturally infected	2.73±0.06**	1.46±0.04**	2.49±0.02*
99E-059	control (healthy)	3.40±0.04	1.89±0.05	2.78±0.07
	naturally infected	2.14±0.03**	1.23±0.02**	2.08±0.04**
93E-012	control (healthy)	4.11±0.06	1.78±0.04	2.80±0.01
	naturally infected	2.79±0.05**	1.32±0.03**	1.96±0.02**

Table 3. Effects of common (*Xanthomonas campestris pv. phaseoli* (Smith) Dye) and halo (*Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie) bacterial blight on some physiological parameters in bean leaves; F_v/F_m - variable to maximum fluorescence ratio, PA - peroxidase activity [U]; RWC - relative water content [%]; * P < 0.05; ** P < 0.01;
Таблица 3. Влияние на *Xanthomonas campestris pv. phaseoli* (Smith) Dye), и *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie върху някои физиологични параметри в листата от фасул; F_v/F_m - отношение вариабилна/максимална флуоресценция, PA - пероксидазна активност [U]; RWC - относително водно съдържание [%]; * P < 0.05; ** P < 0.01;

Landraces	Variants	Parameters		
		F_v/F_m	PA	RWC
2000E-01	control (healthy)	0.796±0.03	625±12	70±1.5
	naturally infected	0.604±0.02**	900±18**	49±0.9**
95E-05	control (healthy)	0.825±0.01	720±15	74±1.4
	naturally infected	0.700±0.02*	979±14**	55±0.6**
99E-059	control (healthy)	0.781±0.05	595±18	69±1.1
	naturally infected	0.609±0.03**	892±19**	47±1.2**
93E-012	control (healthy)	0.793±0.02	686±21	72±2.0
	naturally infected	0.634±0.05**	974±16**	49±1.2**

the years of carrying out the experiment were medium favourable to favourable. In 2004 and 2005, there were strong rains in July, but temperature was higher than the mean value for the last 10 years. The rains at first helped vegetation but then complicated it as were favourable to advent of diseases.

The data were statistically analysed. The differences between control and other variants were evaluated according to Student's t-criterion [18].

RESULTS AND DISCUSSION

The effects of common and halo bacterial blight on the photosynthetic rates of healthy and diseased bean leaves were determined by gas exchange analysis (Table 1).

The results showed that the leaf gas exchange rate in the infected leaves of all investigated forms was changes. The net photosynthetic rate was strongly suppressed - by 18% (95E-01) to 36% (2000E-01).

There is a consensus that a decrease of photosynthesis is

Table 4. Elements of productivity of healthy and naturally infected by *Xanthomonas campestris pv. phaseoli* (Smith) Dye, and *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie, Bulgarian landraces of *Phaseolus vulgaris* L.; * P < 0.05; ** P < 0.01;

Таблица 4. Елементи на продуктивността в контролни и естествено инфектирани с *Xanthomonas campestris pv. phaseoli* (Smith) Dye, и *Pseudomonas syringae pv. phaseolicola* (Bukholder) Young, Dye et Wilkie, български образци фасул *Phaseolus vulgaris* L.; * P < 0.05; ** P < 0.01;

Landraces	Variants	Parameters		
		Number of pods per plant	Number of seeds per plant	100 seeds weight [g]
2000E-01	control (healthy)	5,3±0.25	11.7±1.0	32,4±1.2
	naturally infected	2,0±0.08**	4.0±0.05**	23,9±2.3**
95E-05	control (healthy)	15,1±1.2	37.7±2.8	32,9±1.6
	naturally infected	8,5±0.61**	21.5±2.2**	28,7±2.5*
99E-059	control (healthy)	11,5±0.79	22.3±4.1	38,2±1.9
	naturally infected	6,2±0.52**	12.3±1.9**	31,3±2.2*
93E-012	control (healthy)	13,9±0.62	29.4±2.6	42,4±2.8
	naturally infected	6,1±0.40**	15.4±1.3**	37,3±2.1*

attributed to both stomatal and non-stomatal limitations [14]. As it was showed in Table 1 transpiration rate and stomatal conductance in the infected leaves were higher than controls. That's why it can be assumed that mainly non-stomatal limitation of photosynthesis take place. These have been attributed to damages in the primary photochemical (inhibited functional activity of PSII and reduced amount of photosynthetic pigments) and biochemical processes [7].

Xcp and Psp induced changes in content of photosynthetic pigment (Table 2). A statistically significant decrease by 24% (95E-05) to 48% (2000E-01) in chlorophyll a content was detected. There was a similar effect on the content of chlorophyll b. We suggest that the reduced total chlorophyll content in infected plants was due on the activation of its enzymatic degradation.

The stronger decrease of chlorophyll content as compared to that of carotenoids provoked changes in their interrelations.

The F_v/F_m ratio is a parameter for the potential PSII efficiency in the photochemical reactions. It is know that in healthy leaves this ratio is in the range of 0.75-0.85 [2]. The significant F_v/F_m decrease in the infected plants (by about 20%) was indicative of serious PSII disturbances (Table 3).

Under stress plants possess several tissue antioxidant enzymes for protection against the potentially cytotoxic forms of activated oxygen species, among them peroxidase (PA) being most important. The results showed, that the PA activity in infected leaves was increased by 36 to 50% above the control.

The changes in the relative water content (RWC) of all investigated forms were significant. RWC in infected leaves was by 26% to 32% lower comparison to healthy such.

Xcp and *Psp* gave the negative effect on productivity of plants (Table 4). All studies forms had lower number of pods and seeds per plant. The weight of 100 seeds was strongly reduced as a result of slight growth of them.

The best, in terms of having the least difference in comparison to the control, was 95E-05 population.

On the basis of the physiological parameters studied the following conclusion can be made: The studies forms had differ in their resistance to common and halo blight. Some of them had better and some worse tolerance to diseases. On the basis of the data obtained we could specify 95E-05 as population with a higher level of resistance to common and halo blight comparison to the other three.

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