

The effect of modified bacterial virulence to host-pathogen relationship (*Phaseolus vulgaris* L. – *Pseudomonas savastanoi* pv. *phaseolicola*)

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Summary: The *Pseudomonas savastanoi* pv. *phaseolicola* is one of the most expressive biogen stressors of the bean (*Phaseolus vulgaris* L.) in Hungary. The chemical and agrotechnological defence is inefficient, so breeding is the only workable way. The conventional cultivars are susceptible to PS while most of the new industrial varieties have genetic resistance to the pathogen. The genetic background of resistance is, however, a complex system in the bean. Leaf resistance is a monogenic system, but this gene is not expressed in juvenile stage of the host. The pathogen species can be divided into different races. After inoculation with virulent strains, typical symptoms appeared on the leaves. To understand the details of host-pathogen relationships, there were carried out experiments using bacterial strains with altered virulence. Six transposon mutants of the PS were tested. Our main objective was to test these modified bacterial strains on bean cultivars of known genetic background. First we analysed the symptoms, and then the correlation between the symptoms and the multiplication of mutant bacteria. Three cultivars (Cherokee, Inka and Főnix) were tested.

The infection by the virulent PS isolate produced typical symptoms on the three cultivars tested. Mutant bacteria (except strain 756) did not cause any significant symptoms on the hosts. The mutant 756 induced visible symptoms on the cultivars Cherokee and Inka. On Cherokee there were small water-soaked lesions, and HR (hypersensitivity reaction) was detected on Inka, but this was restricted to some cells only (mikro HR). The rate of multiplication of the wild type strain was much higher than the multiplication of the mutants. Bacteria were detected in the cotyledons and primordial leaf, but there is not any substantial number of bacteria in leaves, except for strains 757, 1212 and 1213. The rate of multiplication of strain 756 was intermediate. These, and other experiments can help to understand the genetic background of resistance and the host-pathogen relationship in the *Pseudomonas*-bean pathosystem.

Introduction

The Halo blight is one of the most important diseases of the bean (*Phaseolus vulgaris* L.). It is caused by *Pseudomonas savastanoi* pv. *phaseolicola* (syn. *Pseudomonas syringae* pv. *phaseolicola*, further on PS) and should be considered as a biogen stressor, attacking the cultivars from time to time causing detrimental damages in the leaves. The chemical and agrotechnological defence is inefficient, the only prosperous way of the defence is the construction of resistant hosts by means of breeding.

The majority of conventionally bred cultivars is highly susceptible to PS. Epidemics exerted selection pressure on

the species and varieties of reduced susceptibility survived preferably. Thus, most of the commercially used cultivated varieties are field tolerant, already.

The pathogen species can be divided into different races. Three races have been isolated in US, the race 2 has high virulence, while the other two have reduced one. The strain isolated in Hungary is comparable, but not completely identical to race 2 (Velich et al., 1994). As Taylor suggested, there are many additional races of PS (Taylor et al., 1996), and he supposed, the Hungarian race had its origin in Africa.

The genetical background of resistance is a complex system in bean. Monogenic inheritance is responsible for leaf resistance. This gene is not expressed in juvenile stage (namely until the first true leaf), which implies, that even the

resistant varieties may be susceptible at this developmental stage. Furthermore, this gene becomes inactive in the pods, while a polygenic system that is independent of leaf resistance is switched on (Szarka, 1979).

After infection with virulent PS strains, local lesions and systemic chlorosis can be observed on the susceptible leaves. The symptoms of systemic chlorosis are evident on the young leaves growing above the infected ones. First the spots are round, later they are surrounded by the veins. The originally greenish-brownish spots are fusing and eventually drying out under dry climate. Sometimes yellow halo are formed around the spots. The resistant hosts develop the characteristic symptoms of hypersensitive necrosis (HR) (Velich et al., 1994).

Breeding for resistance in Hungary is based on a vertical type of resistance. The resistance is caused by a recessive gene (*plr*) under the contribution of some other modifier genes (Velich et al., 1988). Most of the European resistant cultivars have been constructed by incorporation a single recessive gene into the genome. Szarka et al. (1979) have made extensive analysis to estimate the stability of resistance. They have reisolated bacteria in several generation of bacteria from hypersensitive necrosis, and checked the virulence of the isolates. In none of the isolate could they detect a reverted or suppressed mutant of the *plr* gene. These results proved the high stability of the pathogen. Despite the observed low frequency of mutations affecting virulence, we cannot exclude the appearance of new strains – i.e. African races (Taylor et al., 1996).

To understand the details of host-pathogen relationships we carried experiments using transposon induced bacterial strains with altered virulence. Our main object was to test these modified bacterial strains on bean cultivars of known genetical background. At the first stage we analysed the different symptoms and the correlation between the multiplication of mutant bacteria in bean leaves and the symptoms of infection.

The mutant pathogens have been constructed by Tn5 transposon induced mutagenesis (Somlyai et al., 1986) on the principle described by Neugebauer et al., 1986. The transposon has been induced by pSUP101 plasmid, and the mutants were tested on tobacco. Three groups of bacteria could be distinguished on the basis of detectable symptoms. The first group did not cause any symptoms, the second one induced HR necrosis while the third one gave rise to local symptoms. After having tested the multiplication of mutant strains, it became clear that, they multiplied at a substantially lower rate than virulent strains, and they have longer life. In addition the experiments demonstrated that bacteria with altered virulence are spreading slower in plants.

Material and methods

Host plant cultivars:

We tested three bean cultivars : Cherokee (doesn't carry any resistance gene), Inka (carrying modifier genes) and Főnix (carrying *plr* and modifier genes). The plants were

grown in mixture of peat and perlite in 1:1 ratio in greenhouse. The temperature was 25°C during the day, and 18°C at night. The relative humidity was cc 100%.

Bacterium strains:

The virulent strain has been isolated in Hungary as a good representative strain of virulent strains occurring under natural circumstances infecting the bean varieties cultivated in Hungary (Szarka and Velich, 1983). The mutant strains were constructed by Somlyai et al., (1986). Six strains were tested in the experiments (756, 757, 1210, 1211, 1212, 1213). All of them contains a transposon incorporated into the bacterial genome (checked by DNA hybridisation) and does not require any special supplements to the growth media.

Inoculation of plants:

The inoculation was done in two ways. Either the leaves were inoculation at the first true leaf stage or the seeds were soaked in a suspension of bacteria.

In the first case the leaves were 2 cm long. Earlier experiments have shown that this was the most favourable developmental state to test the resistance against the PS. The symptoms were evaluated after one week of infection (Velich et al., 1994).

In the latter case, the experiments were carried out on Inka variety. This kind of infection made it possible to study the host-pathogen relationship at an earlier stage, i.e. before the switch on of the leaf resistance gene, which is activated at the first true leaf stage. During this early phase of development of the plant bacteria can multiply without any restriction caused by plant resistance genes. This creates a good opportunity to study the changes caused by mutant bacteria.

The seeds were soaked in 500 ml bacterial suspension (6×10^7 /ml) at room temperature for 6 hours. The seeds were planted and grown until the second true leaves stage. The multiplication of bacteria was determined in the cotyledons, in primordial leaves and in the first true leaves. Following the homogenisation and proper dilution of the samples the bacterial colonies were counted after 4 days, cultivation at 28 °C.

Results and discussion

The infection by the virulent PS isolates resulted in different symptoms on the three host cultivars tested. The susceptible Cherokee showed the typical symptoms of infection, namely largely expanded watersoaked lesions surrounded by toxic rings, toxic chlorosis in young leaves (positioned above the infected leaves). In the modifier genes carrying Inka variety the lesions were less extended, and there was no sign of toxic chlorosis. On the leaves of the resistant Főnix variety (one week after the infection) the typical HR syndromes were observed.

Mutant bacteria (except strain 756) did not cause any significant symptoms on the susceptible host (at first true

leaves stage). Though mutant 756 induced visible symptoms on Cherokee variety, but these watersoaked lesions were far smaller, than those induced by virulent strains. We could detect the HR reaction in variety Inka, but in that case the lesion was extended only to some cells surrounding (mikro HR). The strain 756 did not cause any symptoms in the resistant variety Fónix (see: Fig.1).

We can suggest two explanations for our experimental observations. 1. The transposon has been incorporated into different regions of the bacterial genome (into the virulence gene or any helper genes), which decreased or cancelled the ability of infection. 2. Mutants with impaired multiplication disposition are unable to invade the intercellular spaces, the results being in their slower spread in the plants compared to

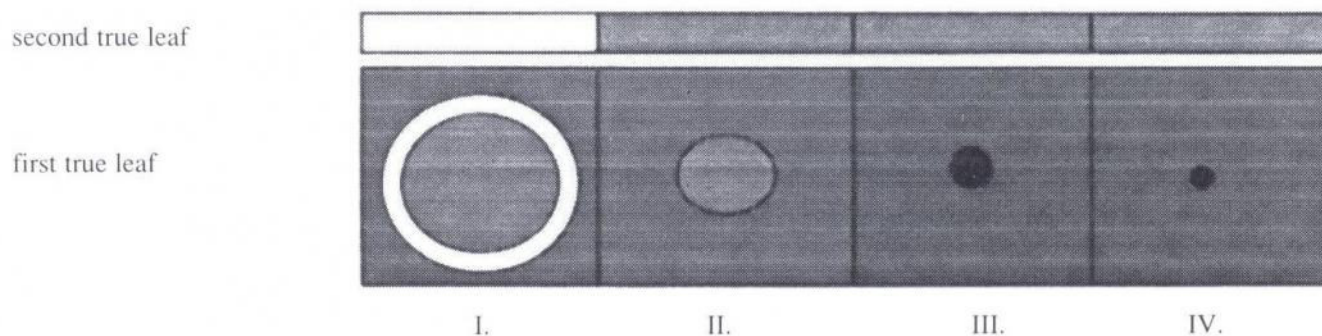


Fig.1 The symptoms of the different *Pseudomonas* strains (wild type and mutants) on the bean varieties of different resistance (magnified 20 x).

- I. wild type of PS – susceptible variety (Cherokee): watersoaked lesions with yellow halo on the first true leaf, systemic chlorosis on the young leaves above the infected leaf.
- II. strain 756 of PS – susceptible variety (Cherokee): small watersoaked lesions on the first true leaf, and there is no systemic chlorosis on the young leaves.
- III. wild type of PS – resistant variety (Fónix) : hypersensitive necrosis on the first true leaf, there is no systemic chlorosis on the second true leaf.
- IV. mutant 756 of PS – variety with modifier genes (Inka) : mini hypersensitive lesions and there is no systemic chlorosis on the young leaf.

The comparison of the symptoms observed in a compatible host and the multiplication of the mutant bacteria in the tested host revealed quite striking correlation. The rate of wild type strains was higher a magnitude than those of the mutants studied (see: table 1).

After seed infection in the cotyledons of the variety Inka, the multiplication of strain 1210 was the highest, while that of strain 757 was lowest. Strain 756 showed a medium rate of multiplication.

Strange enough, in the primordial leaves (after seed infection) the strain 757 multiplied at the highest rate, while strain 1211 showed the lowest multiplication rate.

Next we have investigated the number of bacteria (after seed infection) at the first true leaf stage. We could not detect any substantial number of bacteria in leaves, except for 757, 1212 and 1213. This can be easily explained by the well-known characteristic of variety Inka being less susceptible to PS. In the case of susceptible varieties the pathogen bacterium can be transmitted into the first true leaves after seed infection, while this transmission is hampered in the resistant varieties. The expression of a resistance gene (*plr*) prevents the multiplication of the infecting bacteria (this is the case in the resistant variety Fónix). There is another reason, which explains the lower replication rate of mutant bacteria. There are multiplying at substantially lower rate than their virulent counterparts occurring in the nature and these strains are moving slower in the plant.

Table 1 The multiplication of bacterium strains on the different developmental stages of the host plant after seed inoculation

strains	cotyledon	primordial leaf	first true leaf
control*	$1.15 \cdot 10^5$	$4.46 \cdot 10^4$	–
756	$1.9 \cdot 10^7$	$1.09 \cdot 10^5$	–
757	$6.82 \cdot 10^6$	$9.2 \cdot 10^5$	$2.2 \cdot 10^5$
1210	$4.59 \cdot 10^7$	$8.28 \cdot 10^5$	–
1211	$1.59 \cdot 10^7$	$7.03 \cdot 10^4$	–
1212	$7.32 \cdot 10^6$	$1.9 \cdot 10^5$	$5 \cdot 10^5$
1213	$1.86 \cdot 10^7$	$2.53 \cdot 10^5$	$1.25 \cdot 10^5$

These data are means of six experiments.

*We tested the degree of infection on the control sample, because, if the seeds are infected, we cannot disinfect that. On the control the number of the bacteria was just 1,6–3,7% of the minimum number of mutant bacteria.

the wild type strain. This is why a limited population of these bacteria was present in the leaves. The reaction of the plants was more radical, and they blocked the multiplication of bacteria in a more intense way.

The multiplication of strain 756 showed a intermediate tendency, which makes it more plausible that the mutation of virulence gene is not the only factor causing the symptoms observed after bacterial infection. Further investigations with this strain could help to explain the biochemical and genetical background of host-pathogen relationship in the *Pseudomonas* – bean system.

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