Changing of carbohydrates by inoculation of *Pseudomonas savastanoi* pv. *phaseolicola* on bean lines with different resistance

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Summary: The *Pseudomonas savastanoi* pv. *phaseolicola* (PS) is one of the most significant stressors of bean (*Phaseolus vulgaris* L.). Chemical and agrotechnical treatments have minor importance, so breeding has great part in the protection against this pathogen. Most of the cultivars are susceptible to PS. The genetic background of resistance in the plant is a complex system. Leaf resistance is a monogenic system, but there are some modifier genes. The pathogen species can be divided into different races.

To understand the functioning of this resistance gene, experiments were carried out using bean varieties with different genotypes and near isogenic lines of bean. Eight lines were tested. Our main objective was to test bean lines with PS with high virulence.

The experiment was made in greenhouse and on field. The virulent bacterium strain has been isolated in Hungary.

The changes of carbohydrates were tested after infection. In homeostasis the level of carbohydrates (especially glucose and fructose) were higher in susceptible lines. In case of artificial and natural infection the decrease of glucose were more significant in susceptible lines than in resistant lines. In the leaf samples from systemic chlorosis the level of this carbohydrate increased.

These changes are connected with the level of resistance, but more experiments are needed to verify this assumption.

Introduction

The *Pseudomonas savastanoi* pv. *phaseolicola* (syn. *Pseudomonas syringae* pv. *phaseolicola* further on PS) is the pathogen of Halo blight one of the most important diseases of bean *(Phaseolus vulgaris).* This biogen stressor attacks the cultivars every year and causes great damages. Chemical and agrotechnical protection are inefficient, so breeding resistant hosts has great importance.

The most of conventionally bred cultivars are susceptible to PS. But there are some varieties, which have a resistance gene and some modifier genes. This gene is not expressed in juvenile stage (until first true leaf), therefore resistant varieties are also susceptible at this developmental stage. Moreover, in the pods can be found a polygenic system, which is independent of leaf resistance.

After infection with PS, systemic chlorosis and local lesions are observed on susceptible or young leaves. The systemic chlorosis is observed on young leaves grown above the infected ones. At the beginning, small water-soaked spots appear on the leaves. Later the spots enlarge and fuse together. The reddish-brown, brown necrosis is surrounded by veins. Subsequently, the necrosis is surrounded by a pale yellow halo. Under low air humidity the spots go dry. On the resistant host characteristic symptoms, called hypersensitive necrosis (HR) developed (*Velich* et. al., 1994). After re-isolation, the genome of the pathogen doesn't change.

Breeding for resistance in Hungary is based on a vertical type of resistance. The resistance is caused by a recessive gene called plr under the contribution of some modifier genes (*Velich* et al., 1988).

To understand the function of the resistance gene, we carried out experiments using bean varieties with different genotypes and isogenic bean lines. At the first step we analysed the different symptoms (local lesions, systemic chlorosis, hypersensitive necrosis) and after that some of the typical biochemical features. One of these features is the change in carbohydrates. This phenomenon can be explained by the production of extracellular polysaccharides (*EPS*, *El-Banoby - Rudolph*, 1979, *Gross*, 1987). The bacterium has a polysaccharide capsule. This capsule prevents immediate contact between the plant cell and the bacterium, the pathogen is unrecognisable for the host plant. In this case bacterial cells can multiply extensively, thus local lesions and systemic chlorosis can be detected. If the bacterium is unable to make this capsule, the plant cells recognise the pathogen and respond with hypersensitive necrosis (*Klement*, 1982). Different reactions of bean genotypes to PS can be explained by changes of the level of some endogenous carbohydrates in leaves. We tested only those leaf parts which showed the symptoms. In this case the changes are more significant, and the measurements are more exact.

Materials and methods

First, we tested two bean varieties with different genotypes: *Cherokee* (susceptible) and *Főnix* (resistant). Subsequently, we tested eight near isogenic bean lines. It means, that the lines have the same genotype, the only difference is the resistance gene and the modifier genes. Some lines have these genes, some have not, and some of them carry just modifier genes. Two different varieties were hybridised and were left in Ramsch (bulk) for 8-10 generations. The lines are the following: 1. Resistance - 90 (Al x R12 F_{10} c strain 82/1997 F_{10} - 17/1/4), 2. Susceptible with low level of resistance (moderately susceptible) - 21 (Fo x St F_{11} 971/1997) and 3. Susceptible - 2021 (*Messidor* 331, 336/1997).

The virulent strain for the artificial inoculation isolated in Hungary as a good representation of virulent strains infecting bean cultivars under natural circumstances in Hungary. (*Szarka & Velich*, 1983). The inoculated leaves were (2 cm long) collected from the first true leaves (*Szarka & Velich*, 1983). This is the most favourable developmental stage to test resistance against PS. Samples were collected after the appearance of symptoms from resistant, susceptible and moderately susceptible varieties. In case of natural infection the bacterium strain was identical with that one which can be found in the pathogenesis under natural circumstances in Hungary. In the experiment we tried to produce a model that represents the natural infection process on the field. 1998 proved to be a good year to carry out such an experiment, because the pathogen infection level was significant. We investigated the symptoms on the first true leaves, the samples were collected just from this symptoms. As a control, samples were collected from healthy leaves. In case of most susceptible plants, samples were collected from the second true leaves to test the influence of systemic chlorosis.

The frozen (in liquid nitrogen) and powdered leaves were extracted in methanol : water = 80:20. After centrifugation, samples were put on TLC or HPTLC silica gel 60 F254 (Merck Co.) for the separation carried out by OPLC (high-pressure layer chromatographic separations, developed by OPLC-NIT Co. Ltd, Budapest). Separating fluid was acetonitrile: water = 85:15 (v/v). Development was performed by aniline - diphenyl amine - phosphoric acid reagent. Shimadzu HPTLC scanner (l = 540 nm)was used for the assessment of sacharids *(Fig.1*, Sárdi et. al., 1996).

Result and discussion

Concerning the carbohydrate concentration in the resistant genotype *(Főnix)* and the susceptible genotype *(Cherokee)* in homeostasis it can be concluded on the basis of the first true leaves that the susceptible variety has higher concentration of glucose than the resistant one. The level of fructose showed the same tendency *(Fig. 2).*

In case of artificial inoculation the concentration of glucose in the susceptible variety decreased, considerably. The

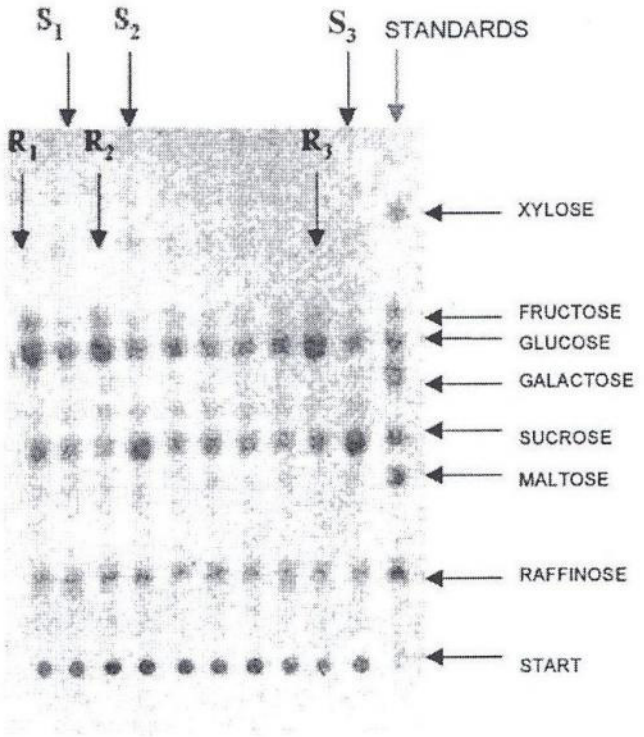
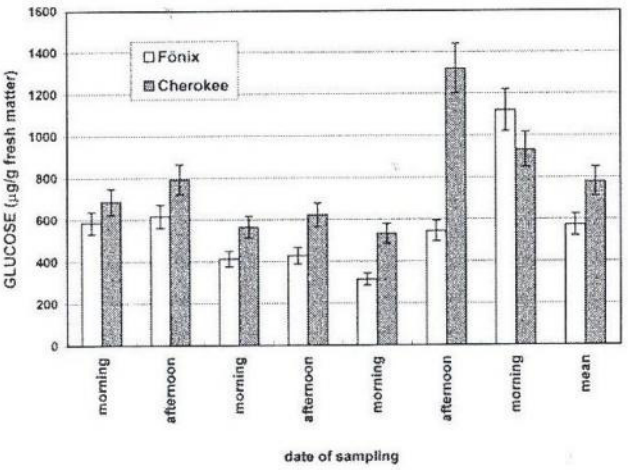


Figure 1 Effect of Pseudomonas infection on the carbohydrate concentration in the leaves of resistant (R) and sensitive (S) bean genotypes



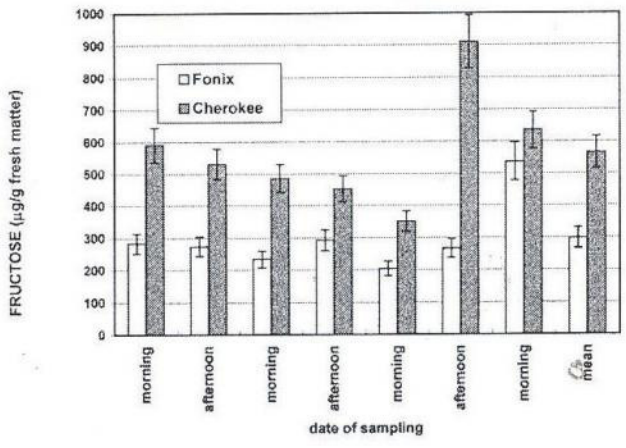


Figure 2 Glucose and fructose concentration in the resistant genotype *(Főnix)* and the susceptible genotype *(Cherokee)* in homeostasis

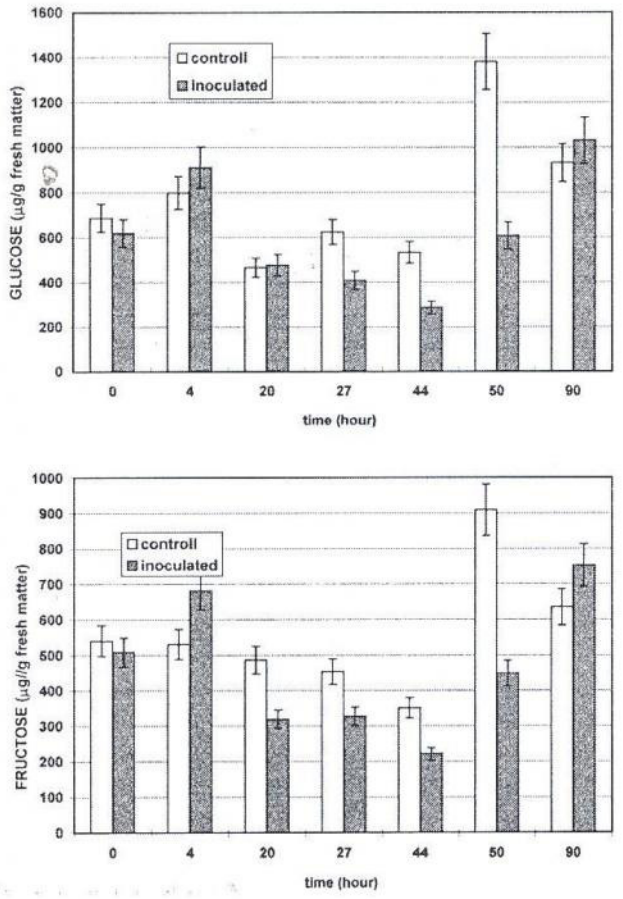


Figure 3 The quantitative change of glucose and fructose in **susceptible** *(Cherokee)* bean leaves after inoculation

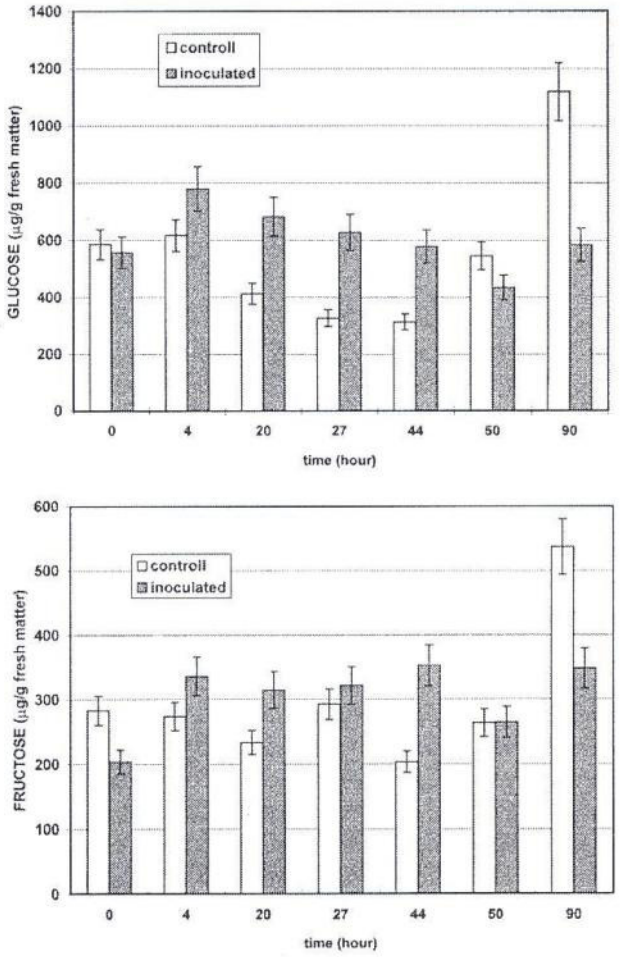


Figure 4 Change of glucose and fructose in **resistant** *(Főnix)* bean after inoculation

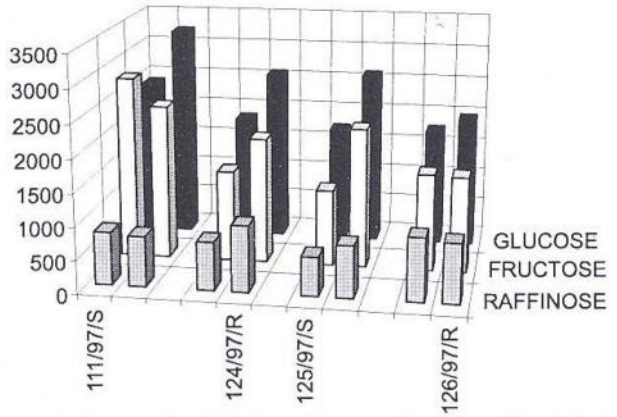


Figure 5 Change of carbohydrats (glucose, fructose, raffinose) in hear isogene lines after inoculation

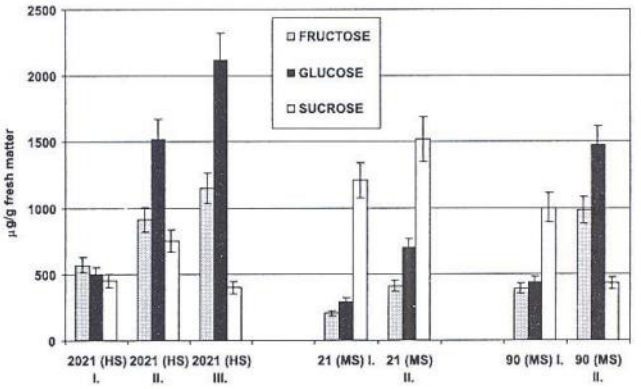


Figure 6 Change of carbohydrats in hear isogene bean lines after after natural infection

MS – moderately susceptible
HS – highly susceptible
I – inoculated first true leavs
II– controll samples
III – second true leavs with systemic chlorosis

minimum glucose level could be observed 45–52 hours after the inoculation. From this point the concentration increased. The quantitative changes of fructose content showed similar tendency as the changes of glucose content in susceptible variety *(Fig.3)*. In case of the resistant genotype *(Főnix)* we couldn't observe this connection *(Fig. 4)*.

We studied the change of carbohydrate concentration after artificial PS infection in resistant and susceptible plant of near isotonic lines. The selection and the sampling was after the assessment of pathogenicity. It could be ascertained that in the first true leaves of near isogenic lines the level of glucose was significantly lower than in first true leaves with *plr* (resistance) gene *(Fig. 5)*.

This experiment with other examinations suggest in the plant tissue without plr gene (after inoculation) lower glucose

concentration could be detected. This could be explained with higher concentration of glucose in homeostasis which is favourable to PS for making EPS capsule.

Subsequently, we tested plants after natural infection. The natural infection of PS influenced the carbohydrate concentration in different leaves *(Fig. 6)*. The carbohydrate content (especially glucose and fructose) significantly decreased as compared to healthy leaves (MSII, HSII). In various isogenic lines the level of this reduction differed from each other. This reduction was of different degree in various isogenic lines. In the samples from systemic chlorosis the level of glucose increased (HSIII).

Our results seem to prove the hypothesis of the *Pseudomonas savastanoi* pv. *phaseolicola* forming an extracellular polysaccharide coat, in connection with the role of glucose in the production of EPS.

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