Ontogenesis- and Biotic Stress-Dependent Variability of Carbohydrate Content in Snap Bean (*Phaseolus vulgaris L.*)

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Physiological examination of resistant and susceptible bean genotypes has shown that the concentration and quantitative ratios of carbohydrates measurable in leaf tissues depend on the age of leaves. During the phases of ontogenesis, the glucose and sucrose levels are the lowest in the primary leaves and highest in the youngest upper leaf. There is a continuous increase in the concentration of both carbohydrates from the oldest to the youngest leaves. The glucose/sucrose quantitative ratio decreases with ageing of the leaves until blooming. Our results indicate that the glucose concentration decreases considerably in the susceptible bean leaves after infection with the bean pathogen *Pseudomonas savastanoi* pv. *phaseolicola.* It has been proved that the glucose plays an important role in the formation of the bacterial extracellular polysaccharide (EPS) coat. Because there is a positive correlation between the age-dependent bacterial-resistance and the low sugar (especially glucose) content in older leaves of the originally susceptible bean plant, we think that in the old leaves there is not enough glucose for production of the EPS coat. Lacking EPS coat the bacterial and plant cell walls come in direct contact which permits the induction of hypersensitivity response characteristic of resistance.

Introduction

The response of bean leaves (*Phaseolus vulgaris) to Pseudomonas savastanoi* pv. *phaseolicola* is different at various developmental stages of the leaves (Velich and Szarka, 1981). According to our preliminary experiments greasy (water-soaked) spots (susceptible response) in young leaves and hypersensitive necrosis (characteristic of resistance reaction) on older leaves appear simultaneously on the same plant after bacterial inoculation. These results showed that the originally susceptible leaves become resistant with ageing.

It has been proved that surface layer or coats of bacterial exopolysaccharides (EPS) play an important role in pathogenesis (El-Banoby and Rudolph, 1979; Gross and Rudolph, 1987; Rudolph *et al.,* 1989). Further experiments indicated that the EPS encapsulation of leaf spot causing bacteria prevents immediate contact between the bacterium and the plant cell wall, so that recognition of the pathogen by plant cells is delayed (Klement, *et al.,* 1987). Without recognition bacterial cells can multiply extensively, so that water-soaking symptoms appear. When the bacterial cells are not surrounded on EPS layers the pathogen is recognized and the plant responds with rapid hypersensitive necrosis, characteristic of the resistant reaction (Klement, 1982).

Breeding experiences have proved that despite the presence of recessive genes ensuring foliage resistance, the primary leaves of certain resistant genotypes are susceptible, and hypersensitive necrosis indicating resistance is manifested only on the first trifoliate leaves (Velich *et al.,* 1994).

Different reactions of susceptible bean genotypes to the pathogen during ontogenesis may be explained with changing concentrations of certain endogenous carbohydrates in ageing leaves. In order to investigate the biochemical background of

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the contradictions observed in the bean-*Pseudomonas* host-pathogen interaction, age-dependent quantitative changes of soluble carbohydrates in bean leaves (during the disease development) were determined in correlation with bacterial multiplication.

Material and Methods

The bean plants were cultivated in commercial compost in a greenhouse. Leaf samples were collected at different phenophases, according to the stages of ontogenesis from the semi developed primary leaf stage to the appearance of young pods (Fig. 1). The dependence of carbohydrate concentrations on the age of the plant and the developmental stage of the leaves were analyzed and compared in a resistant and a susceptible genotype (the twoo genotypes were: Főnix – Hungarian variety with resistance, Cherokee – american variety with susceptibility). *Pseudomonas savastanoi* pv. *phaseolicola* (a Hungarian virulent strain, No. R 6, from the Gene Bank of the University of Horticulture and Food Industry, Budapest) pathogenic to beans was used for inoculation of leaves at different phenophases (inoculation by brush, the density of inoculum: 10^8 cells/ml), (Fig. 1). The pathogen was cultivated at 25 °C on nutrient agar medium (5450 MERCK).

Overpressured layer chromatographic separations of carbohydrates

The fresh leaves were frozen in liquid nitrogen, powdered and extracted with methanol (300 mg plant powder/600 µl of methanol: H_2O, 80:20, v/v). This suspension was centrifuged at 1500 g for 10 minutes at 4 °C. The clear supernatants were used for overpressured layer chromatographic separations (OPLC chromatograph developed by OPLC-NIT Co., Ltd., Budapest, Hungary). OPLC separations were carried out on TLC and HPTLC silica gel 60 F_{254} (Merck Co.) precoated chromatoplates using acetonitrile: H_2O (85:15, v/v), Staining was performed by aniline-diphenyl amine-phosphoric acid reagent. For densitometric determination a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), λ=540 nm was used.

Time-dependent changes of carbohydrate contents after inoculation

Bacterial multiplication and development of water-soaked spots was studied on the first trifoliates of susceptible genotypes. This phenophase was found to be the best for testing the water-soaking symptoms. Leaves were inoculated by a suspension of 10^8 cells/ml of the pathogen applied with a brush on the surface of the half developed first trifoliates. In addition quantitative changes of soluble carbohydrates in the first true leaves were

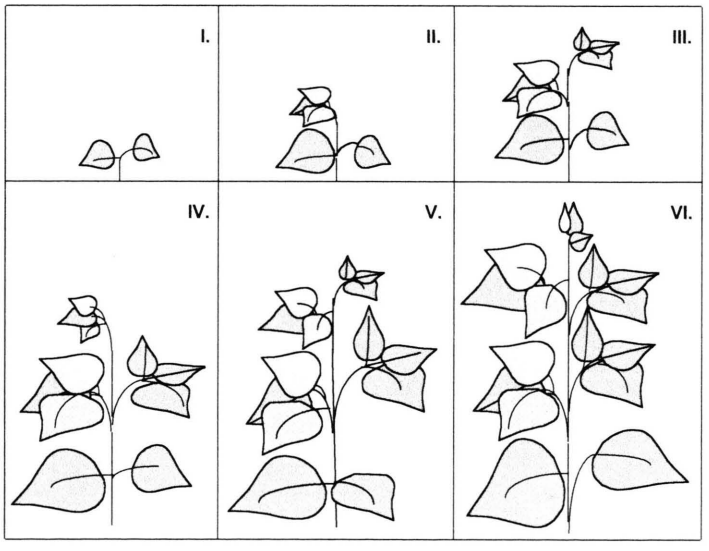

Fig. 1. Phenophases examined from the semi developed primary leaf stages (**I.**) to the appearance of young pods (**VI.**)

investigated. Samples for analysis of carbohydrates were taken 6,20,30,44,51,68 and 75 hours after inoculation. At the same time samples were taken from non-inoculated plants grown at identical conditions.

Results

During ontogenesis, from the oldest to the youngest leaves, a gradual increase of glucose and sucrose concentrations was observed (Fig. 2 and 3). Both sugars showed low concentrations in old leaves and the highest concentrations in the youngest leaves. This was true for both susceptible and resistant genotypes, but more characteristic in the susceptible genotype. With regard to the two different genotypes, the data in Tables I and II are more meaningful, displaying the glucose/sucrose quantitative ratios. Within a variety, in a plant of given age this ratio is the lowest in the oldest leaves (primary leaves) and gradually increases in the younger leaves. This correlation is not valid during blooming in any of the varieties.

Simultaneously with the determination of the carbohydrate level, the multiplication of the bacterium and the symptom development were also examined in the leaves at various ages of the susceptible genotype (Table III). The number of water-soaked spots and bacterial cells in one cm^2 area of the inoculated leaves is higher in the youngest leaves and gradually decreases in the oldest ones.

Table I. Change of the glucose / sucrose quantitative proportions during ontogenesis (resistant genotype).

Phenophases	I.	II.	III.	IV.	V.	VI.
Primary leaves	1.67	0.92	0.77	0.42	0.38	
First trifoliates		0.57	1.28	0.55	0.77	
Second trifoliates			2.21	1.95	2.79	
Third trifoliates				2.17	1.85	
Fourth trifoliate					2.23	
Pods						3.44

Table II. Change of the glucose / sucrose quantitative proportions during ontogenesis (susceptible genotype).

Phenophases	I.	II.	III.	IV.	V.	VI.
Primary leaves	1.71	0.98	0.93	0.43	0.68	
First trifoliates		1.45	1.61	0.54	2.03	
Second trifoliates			3.91	1.04	0.68	
Third trifoliates				1.75	0.77	
Fourth trifoliate					2.51	
Pods						7.43

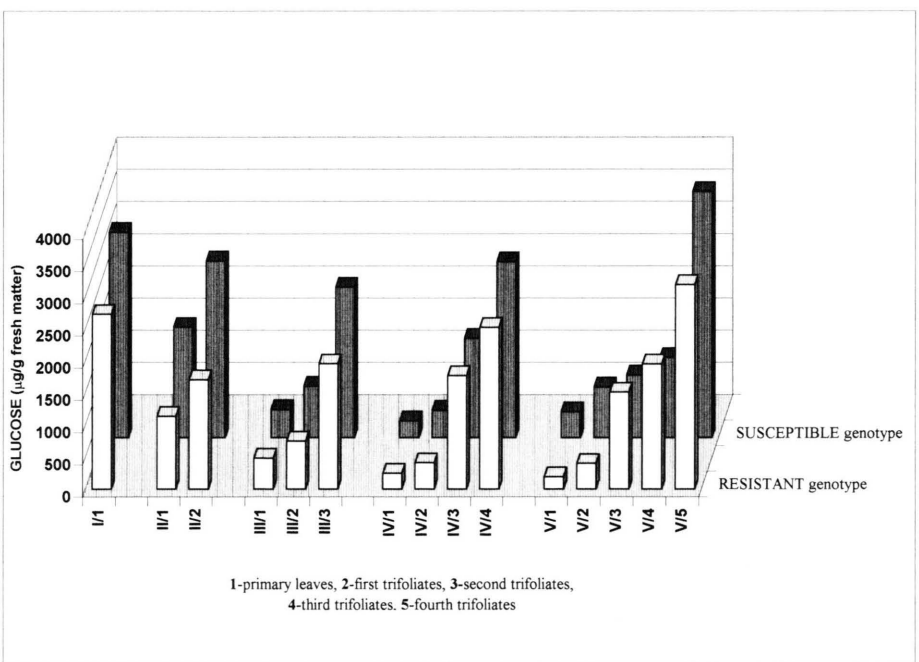

Fig. 2. Change of glucose concentration in resistant (A) and susceptible (B) bean leaves.

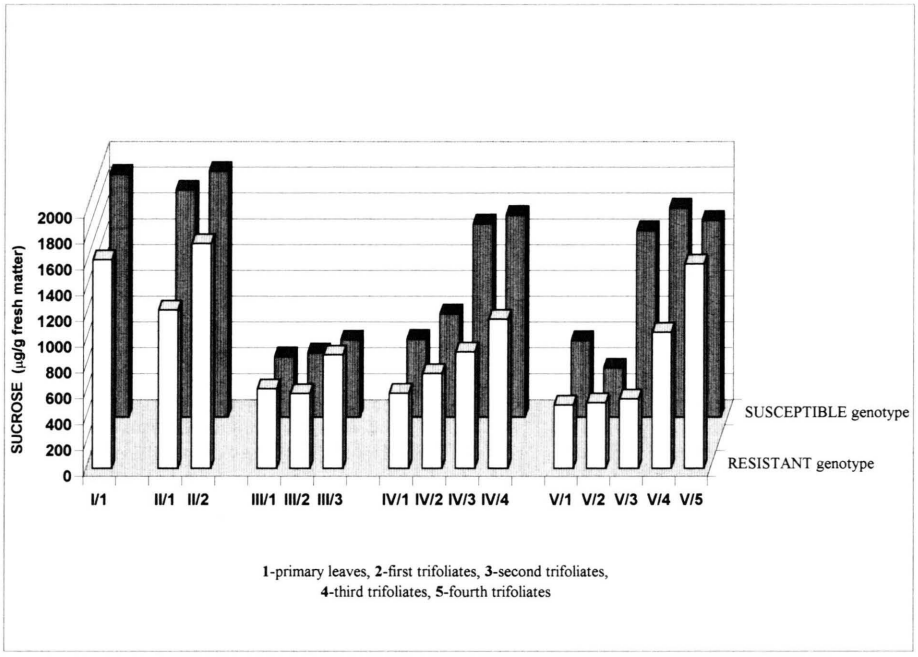

Fig. 3. Change of sucrose concentration in resistant (A) and susceptible (B) bean leaves.

Table III. Number of water-soaked spots / cm^2 leaf area of the susceptible bean genotype after inoculation with *Pseudomonas savastanoi* pv. *phaseolicola*.

Age of the plants (days)	**Primary leaves**	**First trifoliates**	**Second trifoliates**	**Third trifoliates**
12	9.2	–	–	–
15	1.6	3.8	–	–
22	0.7	10.0	4.3	–
25	0	1.3	4.1	6.2

Data show that the primary leaves of the bean are easily infected at the age of 12–14 days (until the first trifoliates appear). After the appearance of the trifoliates, primary leaves gradually become resistant, which is well indicated by the reduction of both the number and size of water-soaked spots per 1 cm^2 leaf area.

Fig. 4 shows the changes of the carbohydrate concentrations in the first true leaves during the bacterial infection. The carbohydrate concentrations were compared with those obtained from the non-inoculated control plants. A reduction is observed in the concentration of glucose as early as 6 hours after inoculation of leaves. The decrease reached its minimum at the 30^{th} hour. Then the glucose level increased and at the 68^{th} and 75^{th} hour sampling it was significantly higher than the starting value.

The changes in the fructose concentration with time after inoculation, display a tendency similar to that of the glucose (Fig. 4). The concentration of sucrose in the leaf tissues of the examined susceptible genotype is too low to be detectable during the very early period of infection, under the given measuring conditions even if higher amounts of samples are used.

Discussion

In the two genotypes differing significantly in susceptibility, it has already been proved that the concentrations of glucose and sucrose and their ra-

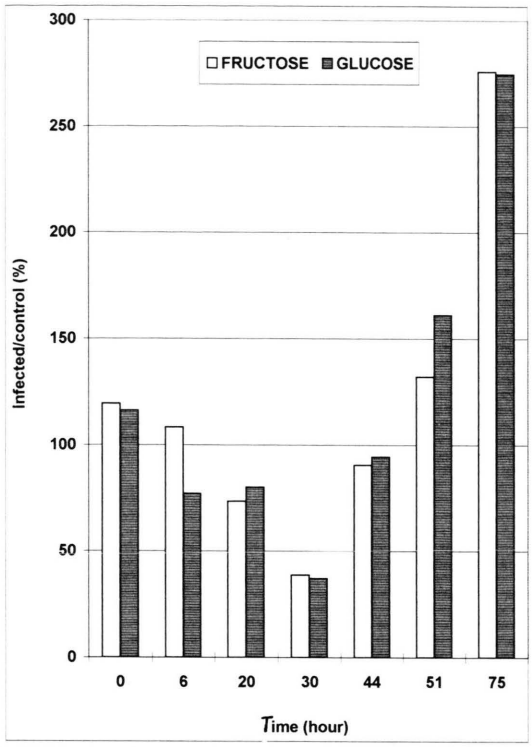

Fig. 4. The time-dependent changes of carbohydrate concentrations as percentage of control in the first trifoliates inoculated with the bacterium.

tios in the bean leaf tissues depend on the age of the plant and the developmental stage of the leaves. Thereby, it can be proved that the quantitative changes of the investigated carbohydrate components depend on age and developmental stage, irrespective of the plant genotypes. However, the water-soaking symptom develops only in the young leaves of the susceptible but not in resistant genotypes. The correlation between high sugar levels and the appearance of water-soaking symptoms in young leaves of the susceptible variety supports our former hypothesis that the high sugar contant promotes the development of EPS coat producing water-soaking symptoms. But this process is somehow inhibited in the resistant variety.

Comparing the resistant and sensitive varieties, no difference was found in the glucose/sucrose ratios of either the half grown or the full grown young primary leaves. On the other hand, the comparison of the half grown first trifoliates has proved that glucose/sucrose ratio in the resistant variety is significantly lower than in the sensitive variety. This difference, however, does not justify the lack of water-soaking symptoms in the resistant variety. We think, that in this case rather the "gene-for gene" resistant mechanism exists than the "age-dependent" resistance of leaves.

Based on changes in the endogenous carbohydrates resulting from infection, the data of the time-dependent quantitative changes of glucose can be related to the multiplication graph of *Pseudomonas savastanoi* pv. *phaseolicola* measured on the susceptible bean genotype. The low-scale reduction of glucose concentration is parallel with the initial slow multiplication of the bacterium. Parallel with the logarithmic multiplication of the pathogen, more intensive reduction of the glucose level could be observed.

Our results seem to prove our hypothesis that the *Pseudomonas savastanoi* pv. *phaseolicola* forms an extracellular polysaccharide coat in intercellular spaces which is connected to the high level of the glucose The EPS coat prevents the contact between the bacterial and plant cell thus the HR-like resistant response is not induced in the susceptible variety (Klement *et al.*, 1987).

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