

## THE INFLUENCE OF *CERCOSPORA* LEAF SPOT ATTACK ON FREE AMINO ACIDS CONTENT IN SUGAR BEET LEAVES

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**Abstract:** In our studies, we identified a number of 19 free amino acids in the sugar beet leaves, whose content depends on the examined foliar tissue (healthy or infected), as well as on the cultivar. After infection, the necrotized tissue of the sensitive cultivar has a higher total content of free amino acids (with 22.98%) as compared to the healthy ones. The degree of these changes is lower in the resistant cultivar (3.99% against the control) in the advanced stages of the infection. Relevant, in the case of this variety is the decrease of the total number of free amino acids at the apparition of the first symptoms (with 6.91%), which indicates the enhancement of protein syntheses in the initial stages of the pathological process in the resistant plants.

### Introduction

Of the foliar diseases of sugar beet (*Beta vulgaris* var. *altissima* L.), cercospora leaf spot is the most frequent and harmful one met in all sugar beet cultivation areas in our country. Therefore, the cultivation of sugar beet resistant plants, to the attack of this phytopathogenic fungus, represents an actual trend in modern agriculture. This attempt aims both to increase sugar and root production, which – as it is well known – is strongly affected by the attack of this extremely virulent fungus, and to prevent agrobiocoenosis pollution by the implicit significant reduction of the doses of pesticides which are generally used.

In order to accomplish this aim, a number of biochemical studies need to be carried out to identify the stimulative or inhibitive nutritional factors of the growth of *Cercospora beticola* Sacc. Among these factors are the free amino acids whose content in the leaves of sugar beet cultivars, depending on the cultivar or variety, growth conditions and administer fertilisers.

There are only few studies in the literature, which monitor the influence of the content of free amino acids in the sugar beet leaves on plant resistance to cercospora leaf spot. The most noted ones are D. Brillova's [1] and Hecker's [5] but they were performed on healthy plants, in ontogenesis, and with no reference to the host-parasite relationship, *in vivo*.

Therefore, the research studies aim to elucidate a number of aspects regarding the change of the content of free amino acids in sugar beet leaves belonging to different cultivars as response to the attack of *Cercospora beticola* Sacc.

### Material and method

The sugar beet varieties selected for the trial (Turbo – resistant and Jamaica – susceptible to cercospora leaf spot) were grown in the field in three replicates, according to the method of randomised blocks. The 1.80/4.25-m parcels were former of four rows of sugar beet cultivars sown 0.45 m distance from one another. The distance between the cultivars on the row was 0.17 m. The parcel dedicated to controls was cultivated 1 km far away from the tested parcel and was protect from air currents by hemp lots. Before sowing, the field were adequate fertilized with nitrogen.

The trial was carried out in the conditions of a natural plant infection with *Cercospora beticola* Sacc., in the experimental fields of the Station of Research and Protection of Sugar Beet – Roman.

The plants emerging between 29.04.2001 and 7.05.2001, and the studies were performed at three different stages of infection: S1 (7.08.2001) at the appearance of the first symptoms of cercospora leaf spot, S2 (17.08.2001) at the moment of the spore formation, and S3 (27.08.2001), at the peak moment of the primary infection.

For analysis, a 500 mg sample (dry vegetal material - 30 minutes at 105° C) was ground in mortar and then extracted, 5 minutes by boiling, with 80% ethanol. After centrifugation, 15 minute at 10 000 g, the supernatant was refrigerated and the residue was extracted twice, using 80 % ethanol. The final supernatant was allowed to stand by night at refrigerator. Then, the content of free amino acids was determined using an automatic amino acid analyser type T-339.

The obtained values were expressed as  $\mu\text{g}/100 \text{ mg}$  dry leaf tissue.

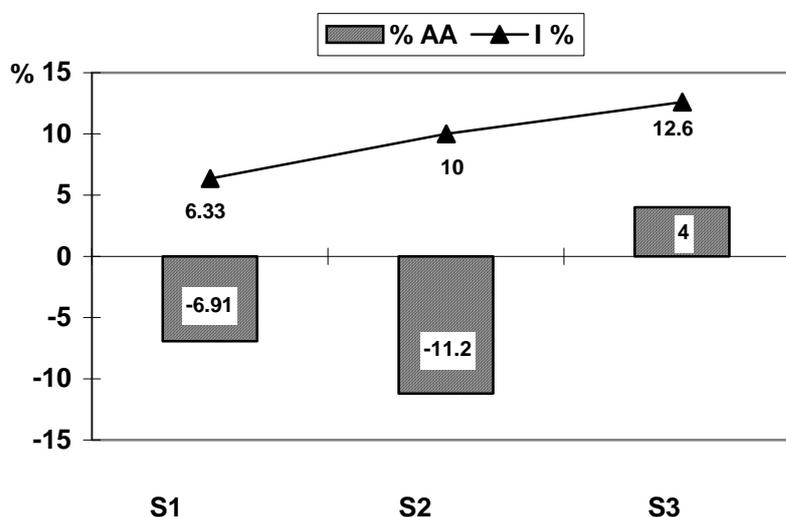
In order to assess the intensity of the attack, a 10-class marking system was employer [9].

### Results and discussions

The research studies performed on a number of 19 free amino acids (i.e. aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine,  $\delta$  - aminobutyric acid, ornithine, lysine, histidine, and arginine) showed that throughout the experimental period (7.08.2001 – 27.08.2001), the total content of free amino acids decreases quantitatively in the healthy sugar beet cultivars. Fluctuations were noticed however, in the content of each amino acid, some decreasing, others increasing quantitatively, with particularities for each cultivar.

The most important feature, from the point of view of the free amino acids content change in the sugar beet leaves attacked by *Cercospora beticola* Sacc., is their quantitative increase in the necrotic tissues in the advanced stages of infection. The higher the increase, the higher the intensity of the attack (in the susceptible cultivar as compared to the resistant one and as the pathogen evolves within the tissues).

The dynamics of the evolution of the free amino acids content depends on the resistance or the susceptibility of plants.



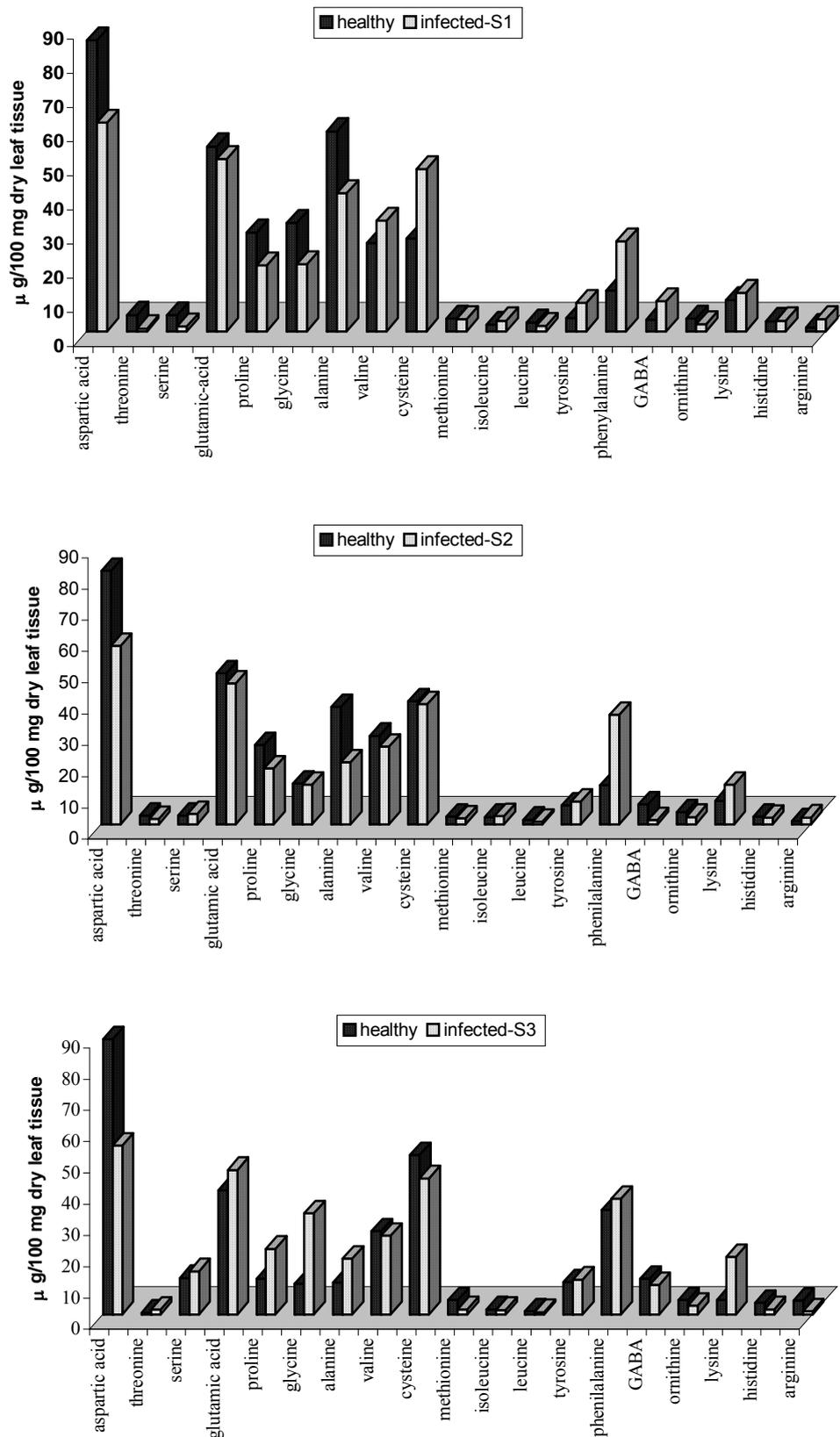
**Fig. 1: Percentual variation (%) of the total number of free amino acids (AA) against the control in sugar beet leaves (Turbo cultivar), depending on the intensity of the attack (I%) of *Cercospora beticola* Sacc. ( S1, S2, S3 – disease progress stages)**

Thus, in the Turbo cultivar (resistant) a quantitative decrease of free amino acids content was noticed, as compared to the controls (healthy plants): with 6.91 % at the apparition of the symptoms and with 11.2 % after 10 days (Fig. 1 and 2). This may be explained by the quantitative decrease of some free amino acids, either as a result of their use as nutrients by the fungus, or – especially – due to the enhancement of protein syntheses resulting in the formation of antifungal protids and proteins. Such proteins were identify in the sugar beet leaves attacked by cercospora leaf spot, in the apoplast [6, 7]. Both types of protein contain cystein residues.

Although the content of free amino acids decreases on the whole, the trials revealed a quantitative increase in the content of some amino acids which play an important part in the development of the new metabolic routes of fungicides compounds synthesis, such as: cystein, tyrosine, and phenylalanine.

In stage S3 was noticed the same behaviour as in the case of the susceptible plants, the degenerative processes in the necrotic symptoms areas being the same (a 4 % increase against the control). However, the level of these processes in the case of entire leaves is much lower because the attacked area is significantly smaller than in the sensitive leaves.

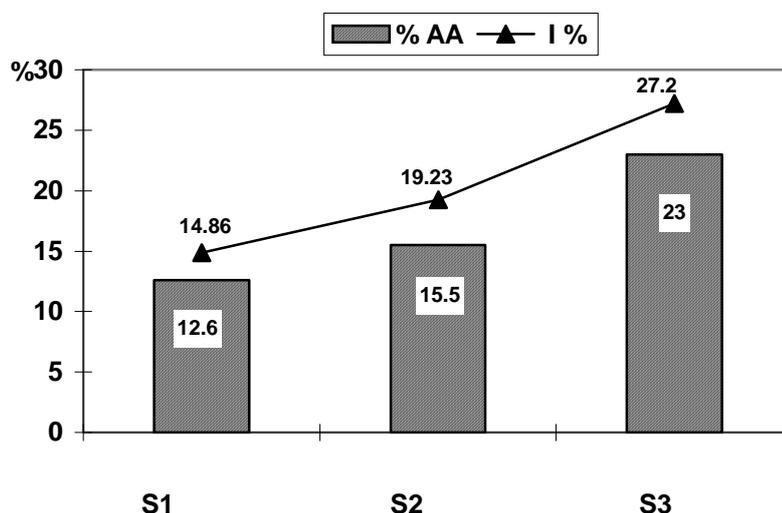
In the susceptible cultivar (Jamaica), the increase of the content of amino acids was progressive, as compared to the control: from 12.6 % in stage S1 to 23 % in stage S3 (Fig. 3 and 4). This value significantly stresses the unbalanced nitrogen metabolism in the compatible relationship.



**Fig. 2: Influence of *Cercospora beticola* Sacc. attack on the content of free amino acids in the sugar beet leaves (Turbo cultivar) in various stages of the infection (S1,S2 and S3)**

In the most advanced stage of infection, S3, although most of amino acids can be found in greater amounts than in the control (aspartic acid, glutamic acid, glycine, alanine, valine, cysteine, methionine, lysine and histidine in particular), there are a number of amino acids whose content decreases as compared to the healthy cultivars, such as: tyrosine, phenylalanine,  $\delta$  - aminobutyric acid and arginine.

The performed trials showed that the disintegration of the various cell structures is accompanied by protein degradation. Such effect is produced because of the activation of proteolytic enzymes in the attacked host tissues, a phenomenon common with many host-pathogen relationships [3, 4]. Extra cellular proteolytic enzymes synthesized by the fungus also take part in proteolysis, to provide directly assimilable nutrients (free amino acids) for it [2].

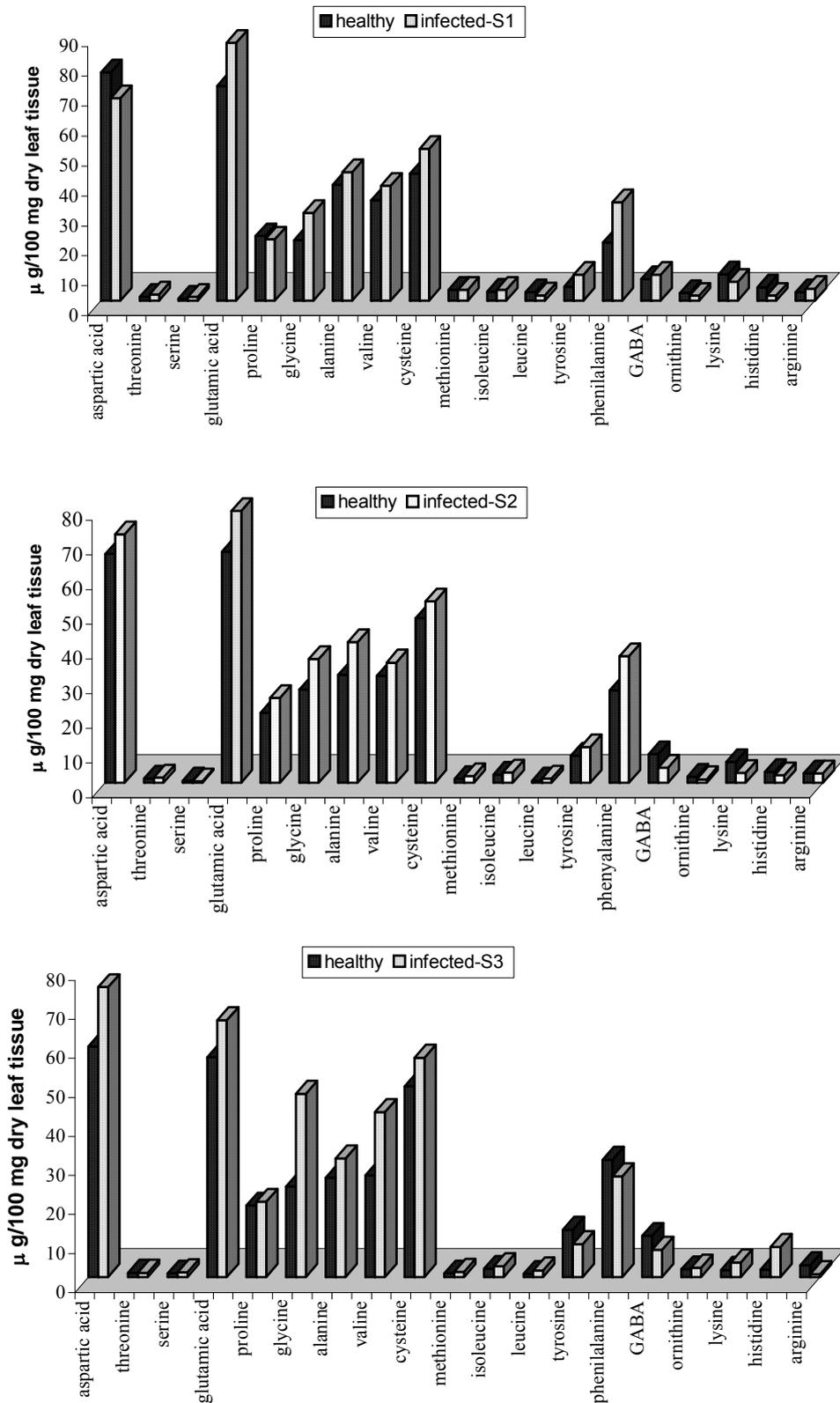


**Fig. 3: Percentual variation (%) of the total number of free amino acids (AA) against the control in sugar beet leaves (Jamaica cultivar), depending on the intensity of the attack (I%) of *Cercospora beticola* Sacc. ( S1, S2, S3 – disease stages)**

Indeed, a higher content of amino acids which in general enhance susceptibility to fungal diseases was identified in the infected tissues (in both varieties), such as: aspartic acid, glutamic acid, and glycine. In this respect, the trials performed *in vivo* confirm the results of those *in vitro* regarding the influence of free amino acids on the nutrition of the fungus *Cercospora beticola* Sacc. [8], the above mentioned amino acids being the most propitious to the vegetative growth of the fungus. Moreover, Jamaica cultivar contains a higher quantity of glutamic-acid, which was considered the only amino acid that can be used in identifying susceptible varieties [5].

The quantitative increase of certain amino acids in the infected tissues may be due both to their synthesis *de novo* in the host cells and to the contribution of the

pathogen, the hyphae of the fungus absorbing and retaining a part of the amino acids for the synthesis of its own proteins.



**Fig. 4: Influence of *Cercospora beticola* Sacc. attack on the content of free amino acids in the sugar beet leaves (Jamaica cultivar) in various stages of the infection (S1, S2 and S3)**

The comparing of the results obtained in this stage (S3) leads to the conclusion that of all the amino acids present in greater amounts in the infected tissues, aspartic acid, glutamic acid, and glycine are those defining the necrotized tissue (their content is the highest as compared to the control).

From a practical point of view, the increase of the content of free amino acids in the attacked plants has a negative impact on the sugar extraction from roots because they cause the retaining of sugar in molasses and may lead to Maillard reactions.

### Conclusions

The content of glutamic acid in healthy sugar beet leaves is higher in the susceptible cultivar than in the resistant one, which may influence plant susceptibility to the attack of *Cercospora beticola* Sacc.

After infection, in the first two stages of the disease, the content of free amino acids in the tissue of resistant leaves was lower than in the healthy leaves, which shows that an enhancement of the protein synthesis takes place in these tissues, in order to produce antimicrobial peptides and proteins. At an individual level, in the Turbo cultivar, a quantitative increase of tyrosine and phenylalanine was notice, both the compounds being involved in the hypersensitivity reaction.

In the tissues of the sensitive cultivars (Jamaica cultivar) the amount of free amino acids increases progressively, which shows a strong unbalance in nitrogen metabolism in the susceptible plants.

Of the amino acids identified in greater quantities in the necrotized tissues, we may enumerate the following: aspartic acid, glutamic acid and glycine. Also, were present amino acids like alanine, valine, cysteine, methionine, lysine, and histidine.

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### **INFLUENȚA ATACULUI DE CERCOSPORIOZĂ ASUPRA CONȚINUTULUI ÎN AMINOACIZI LIBERI DIN FRUNZELE DE SFECLĂ DE ZAHĂR**

#### **(Rezumat)**

În experimentele noastre, am identificat, în frunzele de sfeclă de zahăr, un număr de 19 aminoacizi liberi, al căror conținut variază în funcție de starea țesutului foliar (sănătos sau infectat) analizat și de soi.

Postinfecțional, țesuturile necrozate ale soiului sensibil, au un conținut mai ridicat de aminoacizi liberi totali (cu 23%) față de cele sănătoase (plante martor). Nivelul acestor modificări este mai scăzut în cazul soiului rezistent (4% față de martor), în stadiile avansate ale infecției. Relevantă este, însă, în cazul soiului rezistent, scăderea numărului de aminoacizi liberi totali (cu 6,91% față de martor), în momentul apariției simptomelor, ceea ce semnifică intensificarea sintezelor proteice în stadiile inițiale ale procesului patologic, la plantele rezistente.