BIOCHEMICAL AND GENETIC EXAMINATIONS OF THE GENERAL DEFENSE SYSTEM OF PLANTS

Theses of PhD dissertation

Eszter Szarka

Supervisor
Dr. Éva Sárdi
senior research scientist

Associate supervisor
Dr. János Szarka

BUDAPESTI CORVINUS EGYETEM
Kertészettudományi Kar

Department of Genetics and Plant Breeding

Budapest

2008

PhD School

Name: Doctoral School of Horticultural Sciences

Field: Biological Sciences

Head of PhD School: Prof. Dr. Magdolna Tóth
Doctor of the Hungarian Academy of Sciences
Head of Department of Fruit Sciences
CORVINUS UNIVERSITY OF BUDAPEST,
Faculty of Horticultural Sciences

Supervisor: Dr. Éva Sárdi
Doctor of the Hungarian Academy of Sciences
Senior research scientist, Department of Genetics and Plant Breeding
CORVINUS UNIVERSITY OF BUDAPEST,
Faculty of Horticultural Sciences

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defense process.

……………………………...	…………………………….
Prof. Dr. Magdolna Tóth	Dr. Éva Sárdi
Head of PhD School	Supervisor

1. Introduction

Resistance breeding, which includes the field of genetics and plant pathology, is trying to make it possible by building specific resistance genes into a given plant species that the cultivation of vegetables for fresh consumptoin be free from plant-protecting agents. This workteam which has been dealing with pepper resistance breeding for three decades used to do the selection on the basis of fast tissue destruction, namely the hypersensitive response (HR), like everybody during the hystory of resistance breding. Experiences of about two decades made the research group change their strategy. One of the main steps of the change was the reconsideration of the HR, a so-called resistance response. Parallel with this they laid the resistance breeding on new bases by using the *general defense system* (Szarka and Csilléry, 1995), a plant feature recognized by them.

The genetic investigation and pathological characterization of the general defense system of pepper proved the existance of a basically new disease resistance system which blocks pathogens by preserving and strengthening the attacked cells and tissues. This was an unknown strategy til now. The general defense system protects healthy cells by an aspecific reaction which manifests itself in tissue compaction which is based on cell enlargement and cell division (Figure 1).

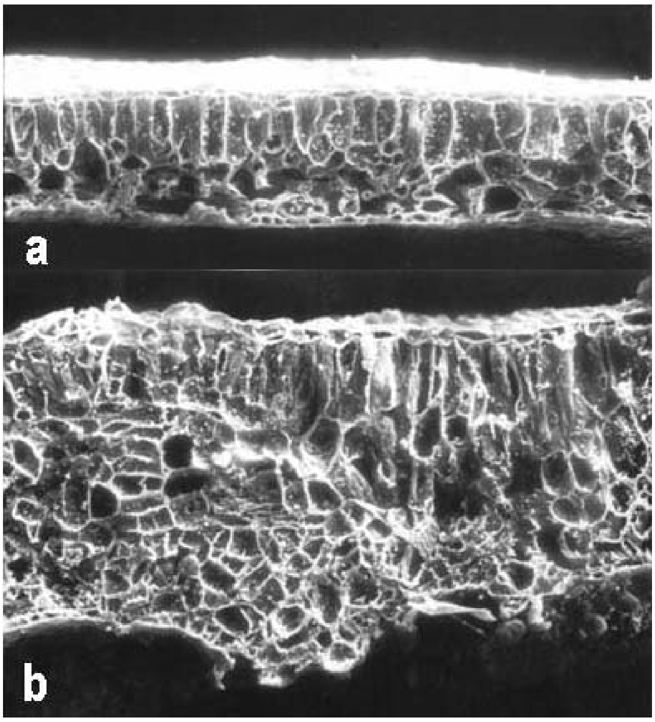

Figure 1 Cross-sections of leaves of a pepper line containing the *gds* gene. (a) not infected control leaf (b) *Xanthomonas campestris* pv. *vesicatoria* infected leaf showing cell elnargement and cell division (photo: Barbara Nagy)

In this workteam my task was to reveal the biochemical background of processes regulated by the *gds* gene encoding the *general defense system* of pepper. As a control I used the *Bs-2* specific resistance gene providing resistance against the *Xanthomonas campestris* pv. *vesicatoria* bacterium which infects pepper.

- To characterize the *gds* gene, which ensures resistance against compatible and incompatible pathogens of pepper as well, it was indispensable to review and systematize the far-reaching literature in order to unambiguously place the *gds* gene in the present systematization on genetic, symptomatic and biochemical bases and to declare that we face such a form of resistance that can not be identified with the phenomena known so far.

- I tried to survey the energetic backgound of the tissue reactions of the *gds* and *Bs-2* gene by quantitative changes of glucose.

- One of the main biochemical feature of the hypersensitive reaction, which manifests itself in fast tissue destruction and which is characteristic of the specific resistance genes, is the oxidative burst. In the course of the oxidative burst hydrogen peroxide accumulates in great quantities. I investigated the changes and presumed roles of H_2O_2 concentration produced in infected tissues of plants carrying the *gds* gene.

- Salicylic acid is a general signal molecule in different host-pathogen relations which contributes to the development of resistance. High concentrations of SA, which affects the decrease of lesion number and lesion size, may result in symptomless resistance. Knowing this phenomenon, I found it neccessarry to determine the SA concentration in infected tissues of plants containing the *gds* gene which react without tissue destruction.

- In contrast with resistance responses accompanied by oxidative burst and tissue destruction the general defense reaction results in preservation of attacked tissues. It was an interesting question that what is the role of the peroxidase enzyme, which scavenges the damaging reactive oxygen species (ROS), in the general defense reaction.

- Transmethylation, which is a form of biochemical regulation based on the removal and addition of methyl groups, contributes to protection against stress effects. We studied that how does the transmethylation happen in the course of the general defense reaction, encoded by the *gds* gene, and of the specific defense reaction, encoded by the *Bs-2* gene, and what is the role of formaldehyde produced during transmethylation.

- By clearing up the genetic bases of the interaction between the *gds* and *Bs-2* gene and by studying biochemical processes behind their interaction, which is manifested in symptoms as well, my aim was to determine the role of the two types of reaction in the plant disease resistance. I investigated the patho-physiological bases of the fact that the two types of reaction regulated by the *gds* and *Bs-2* gene come into action according to an evolutionaryly determined sequence.

2. Materials and methods

Plant material

To the detailed genetic and physiological experiments we used genetically equalized doubled haploid (DH) pepper lines which were the following: the DH-99-71 which is susceptilbe to the *X. c.* pv. *vesicatoria* bacterium, the DH-99-269 carrying the *gds* gene and the DH-99-487 carrying the *Bs-2* resistance gene.

Bacteria used for inoculation

For inoculations we used a *X. c.* pv. *vesicatoria* isolate of medium agressivity originating from a Hungarian field collection. We prepared the inoculum of 10^8 cells/ml concentration from the 48 hour culture of the bacterium.

Tool and method of inoculation

The eighth or ninth almost full-grown leaves of plants in strong, vegetative stage were inoculated. We applied two kinds of infection. In the first case bacterium suspension enough to cause about 1.5 cm diameter infiltrated patches was pressed into the leaf blade. By this method we can show transport processes within a leaf from the not infected tissue parts towards the infected patches. In the other case we infiltrated the whole leaf blade with the bacterium suspension. Through this we excluded the possibility of transport within the given leaf.

The inoculum was pressed into the intercellular spaces by a syringe on the abaxial leaf surface through the stomas. We used an injection syringe without a needle and it was equipped with a piece of pierced rubber on its end to achieve suitable sealing and avoid injury of the leaf.

In order to compare the operation of the *gds* and *Bs-2* gene in uninjured and injured plant cells we put such a mechanical pressure on the leaf in a small point that it caused just infiltration of the tissues. Following this we performed the infection on such a way that the injured, infiltrated tissue point was/got in the middle of the infected tissue part of 2 cm in diameter.

Systematization of symptoms for genetic analyses

7-8 days after the inoculation with the *X. c.* pv. *vesicatoria* bacterium, the inoculated tissue parts of leaves of susceptible plants became water-soaked. 3-4 days after the inoculation the plant response encoded by the dominant *Bs-2* specific resistance gene manifested itself in slight purple colouration of infected tissues sometimes accompanied by tissue destruction. The phenotype referring to the *gds* gene, which regulates the general defense reaction characterzied by high reaction speed, develped only on the 8th-10th day. The explanation for this seeming contradiction is that owing to the low stimulus threshold the general defense reaction blocked the destruction of the bacterium so fast that it became visible only after several days of growth of the leaves in the form of tissue thickening and a slight chlorosis.

Sampling

Considering the rapidity of plant defense reactions, we collected samples immediately after inoculation and during the following 24 hours.

For biochemical studies leaf discs of 1.5 cm diameter were cut with a punch from the infiltrated tissue part and within the same leaf from such parts that lie towards the base of a leaf which were not touched by the inoculum. In the case of the whole leaf inoculation we homogenized the entire leaf as a sample.

Methods of biochemical and physiological experiments

We used chromatographic methods for studying carbohydrate compounds (Sárdi *et al.* 1996), different methylated compounds and formaldehyde (Gersbeck *et al.* 1989) and salicylic acid (after Raskin *et al.* 1989). We determined peroxidase enzyme activity (Shannon *et al.* 1966) and hydrogen peroxide concentrations (Toldi *et al.* 2008) by spectrophotometric methods.

3. Results and Discusion

The *gds* gene, which encodes a so far unknown defense process and encodes the general defense system in pepper, being effective in non-host and host-pathogen interactions as well and ensuring a broad spectrum resistance fulfills the role of the plant immune system. Beside symptoms we also tried to characterize the general defense system by fundamental biochemical processes and to reveal its role in disease resistance in accordance with the *Bs-2* specific resistance gene.

3.1. The economical operation of the general defense system

One of the most suitable compounds to show physiological changes as a consequence of an infection is glucose which constitutes the energetical basis of plants. We studied changes of the quantity of glucose in the infected and the neighbouring, not infected tissue parts as well.
The changes in the glucose content of the inoculated tissues of plants giving susceptible, specific and general defense reaction respectively, were different both in time and quantity and we could compare them well with the symptoms and characteristic tissue alterations of the given host plants.
In the susceptible host-pathogen relationship, where the pathogen has an advantage over the plant, the disease is developing slowly which is accompanied by intense glucose comsumption. This leads to the exhaustion of reserves of the plant and occasionally to the destruction of the plant.
The quantity of glucose in the inoculated tissues of pepper plants with the *Bs-2* gene suddenly decreased following the infection but after one hour the glucose content was restored. This may be explained by the glucose transport coming from the neighbouring tissues. On the basis of these the specific defense reaction is one-off and energy-saving and it is able to ensure effective local responses.
In contrast with the specific defense reaction the general defense system ensured by the *gds* gene is operating continuously thus its glucose consumption is greater. This was also proved by the continuous glucose transport coming from the neighbouring not infected tissues. However, the greater glucose consumption, neccessarry to the reinforcement of the cell walls and the process of cell enlargement, is well compensated by the fact that the attacked tissues will not die.
As we studied artificially infected leaf parts of identical size we can survey just the energy demand of uniform infected tissue parts but not the absolute quantites of carbohydrates. In this case the carbohydrate consumption of the general defense reaction is greater than that of the specific defense reaction but in the case of a natural infection the reaction of the *gds* gene affects only 10-15 cells around the point of infection. Thus this is far more economical than the hypersensitive reaction which is accompanied by a lesion of several mm in diameter.

3.2. Relationship between the general defense system and the hydrogen peroxide

Changes of the hydrogen peroxide (H_2O_2) concentrations during the responses of susceptible plants and the plants with the *Bs-2* or the *gds* gene shows well the basic differences between the different reactions.

As we expected we did not observe remarkable changes in the quantity of H_2O_2 in susceptible plants during the 10-hour-long experiment.

In plants carrying the *Bs-2* gene the H_2O_2 concentration reached the maximum value in the 30th minute which was a four-fold increase compared to the control value. Following this, the H_2O_2 content continuously decreased and reached the initial value in 8 hours. So, according to the present knowledge we could follow the H_2O_2 accumulation characteristic of hypersensitive specific resistance genes.

Contrary to this the quantity of H_2O_2 did not show any changes after the infection in the plants containing the *gds* gene compared to the control plants in the given time frame of the experiment. Thus during the defense ensured by the *gds* gene, the oxidative burst mediated by the H_2O_2 did not occur.

In different plant-microbe and host-pathogen rekations H_2O_2 takes part in strengthening or destroying the plant cell depending on its quantity in the given case. These different roles of H_2O_2 can be followed well along the evolutionary process of plant disease resistance. By pathological experiments (Szarka *et al.* 2002) it was already proven that the general defense reaction, the susceptible stage and the specific defense reaction constitute an evolutionary succession.

In the case of the *gds* gene great quantities of H_2O_2 are not produced during the effective defense. But in susceptible host-pathogen relation, as a consequence of destruction of the pathogen which overcomes the general defense reation of the plant, a slow H_2O_2 production begins. To ward off the specific stress caused by the pathogen a specific defense reaction developed in the host plant in the course of the evolution. This reaction which is evolutionaryly the youngest, starts operating only in the case of presence of H_2O_2 as a signal molecule which refers to destruction in the susceptible phase. Thus the specific defense reaction becomes the failure-correcting unit of the general defense system and so the two reactions, built on each other, constitutes the integral whole of the disease resistance.

3.3. Role of SA in the general defense system

Salicylic acid (SA) is a general signal molecule in several plant-pathogen relations which induces defense responses. We know the correlation between the SA concentration in plants and the level of disease resistance. The external SA treatment increases the resistance of plants which is manifested in smaller necrotic lesions. Knowing all these we have set ourselves to study the relation between the *gds* gene, encoding a reaction without tissue destruction, and the biologically active free SA.

While in the pepper line carrying the *Bs-2* gene we have observed a significant decrease (0.61-0.001 µg/FW) in the level of SA compared to the control plants (1.19 µg/g FW) the quantity of SA was below the sensitivity of HPLC in the plants carrying the *gds* gene during the whole peroid of the experience and we could not detect SA either in the non-infected control or in the water-infiltrated control leaves. On the basis of the SA experiments we can conclude that the SA, as a signal molecule, is not involved in the regulation of defense in the case of the *gds* gene.

It is known that SA-deficient transgenic plants that carry the *NahG* gene, encoding the salicylate hydroxylase enzyme, become more susceptible to pathogens. Compared to this, the phenomenon that the *gds* gene operates perfectly in case of quite a low SA level or in the absence of SA is a novelty.

It is also known that the *Rx* gene (Köhm *et al.* 1993) in potato and the *dnd1* gene (Yu *et al*. 1998) in *Arabidopsis* ensure symptomless, broad-spectrum resistance beside high concentrations of SA. It is noteworthy that in contrast with this, the *gds* gene ensures broad-spectrum, symptomless resistance beside a fairly low free SA level or in the absence of it.

3.4. Characterization of the general defense system on the basis of peroxidase enzyme activity

We did not observe changes in the quantity H_2O_2 during the general defense reaction which is encoded by the *gds* gene and is accompanied by preserving cells and tissues. In our experiments we wanted to know that what role the peroxidase enzyme (POD), having an antioxidant effect, can have in this reaction accompanied by preserving cells.

To compare the time-dependent changes of enzyme activiy characteristic of the given pepper lines we inoculated the leaf blade only in a small patch. Depending on the reaction type the POD activity reaches the maximum value in different points of time: in the first hour and in the third hour in the case of the *gds* gene and the *Bs-2* gene, respectly. In the susceptible pepper line the enzyme activity decreased after the inoculation and in the 6th hour it reached only the control value. This order corresponds to the order of efficiency of the reactions which can be determined on the basis of symptoms as well.

To assess the antioxydant capacity of the three different plant types, we applied an unnaturally high dose of the inoculum. As an effect of this infiltration which extended to the whole leaf blade the POD activity increased six-fold in the 24th hour in the plants containing the *gds* gene, while it increased only two- or three-fold in the plants carrying the *Bs-2* gene and in susceptible plants compared to the control values.

In the starting point of the increase of POD activity but especially in the rate of the increase, whose collective effect may have a fundamental strategic importance in the effective defense, we could observe great differences concerning the studied types of reaction. In the plants carrying the *gds* gene and having an outstanding antioxydant capacity the POD, scavenging reactive oxygen species, contributes considerably to the protection of infected tissues against the necrosis.

3.5. The effect of stress on methylated compounds and on the generation of formldehyde in the general defense system

Transmethylation, which is removal and addition of methyl groups, makes possible a fast and reversible regulation which has a low energy-demand. We investigated the process of demethylation as an effect of stress caused by infection in the three pepper lines representing the three types of reaction, namely the quantitative changes of methylated compounds and formaldehyde connected with each other.

During our experiments we set out from the fact that as a consequence of demethylation of different methylated compounds methyl groups are liberated in the case of a stress which are immediately formed into formaldehyde molecules. On this, the measurable quantity of methylated compounds starts to decrease and the concentration of free formaldehyde is increasing parallel with this.

We could separate well the three reaction types on the basis of different dinamism in correlative quantitative changes of choline, one of the methylated compounds detected in pepper leaves, and formaldehyde.

In the pepper lines being susceptible or carrying the *Bs-2* gene there was no strong correlation between changes in the quantities of choline and formaldehyde. In plants containing the *gds* gene and characterized by low stimulus threshold and high reaction speed as a consequence of the demethylation, starting at the moment of inoculation, the quantity of choline and formaldehyde changed in the opposite direction in strong correlation. Due to the fast demethylation of choline, the quantity of formaldehyde started to increase immediately and reached the maximum value in one hour. On this the main step of the defense is completed. Following this the quantity of formaldehyde was continuously decreasing and the quantity of choline started to increase slowly.

During quantitative changes of choline the minimum value observed already in the first hour and the maximum value of antimicrobial formaldehyde at the same time suggest that the *fast conversion* may have an important role when plants have to be protected against stresses.

3.6. Genetics of interaction of the *gds* and *Bs-2* gene

After we became acquainted with the specific *Bs-2* and the *gds* gene separately, we started to investigate the interaction of the two genes in order to utilize them in the pepper resistance breeding (Csillléry *et al.* 2007). We studied the inheritance of the *gds* and *Bs-2* gene on the same DH lines that we used for the biochemical experiments.

The F1 hybrids (*Bs-2/Bs-2*$^+$ *gds/gds*$^+$), prepared by crossing DH lines carrying the recessive *gds* gene (*Bs-2*$^+$*/Bs-2*$^+$ *gds/gds*) and the dominant *Bs-2* gene (*Bs-2/Bs-2 gds*$^+$*/gds*$^+$) respectively, strikingly did not show the phenotype that we expected according to the dominance of the *Bs-2* gene. Instead of the dark purple colour, referring to the *Bs-2* gene, the infected tissues showed only a slight purple colouration. At the same time, the tissues remained green and in some cases became thick. This shows that in the F1 progeny the phenotype of the recessive gene appeared beside that of the dominant gene in spite of the fact that the *gds* gene was in heterozygous constellation. We continued the investigation of the interaction of the two genes in the F2 generation and the symptom of the double-homozygous individuals (*Bs-2/Bs-2 gds/gds*) of the F2 generation gave us the answer for the symptom of the F1 progeny.

On the basis of the genetic analyses it was concluded that the *Bs-2* gene, being either in homozygous or in heterozygous constellation, causes only a slight purple colouration if the recessive *gds* gene is present even in heterozygous constellation. Because the ability of the *gds* gene to preserve tissues is effective even in heterozygous constellation. If the recessive *gds* gene is in homozygous constellation, it almost completely covers the phenotype of the dominant *Bs-2* gene and the purple colour refers to it only along the leaf veins.

In the course of our experiments we concluded that the two genes are inherited independently, there is no genetic linkage between them. But there is a linkage in respect of their functions. In this case the stress caused by the pathogen creates the relationship between the *gds* and the *Bs-2* gene. In the case of a natural infection the two reactions can not operate at the same time for the same stress because the two genes need different level of stimulus threshold for their operation. Under natural circumstances first the general defense reaction starts to operate because it has a low stimulus threshold and high reaction speed. This also explains the preserving of tissues. The specific defense reaction starts only in the case of the inefficiency of the general defense reaction.

3.7. The biochemical background of symptoms encoded by the *gds* and the *Bs-2* gene

We used double-homozygous plants for biochemical experiments to prove the different role of the *gds* gene, encoding the general defense system, and the *Bs-2* gene, responsible for the specific defense reaction, to examine the order of operation of the two genes and their interaction which manifests itself in the symptoms as well. By preparing double-homozygous plants we consciously created the integral whole of disease resistance. From the symptoms and main biochemical features of the parental lines we concluded the biochemical background of symptoms observed in the F1 and F2 progeny.

As a consequence of infection in the individuals of the F1 progeny, despite the dominance of the *Bs-2* gene, H_2O_2 was not produced in such quantity that could have led to tissue necrosis. The tissue thickening based on cell enlargement refers to the recessive *gds* gene being in heterozygous constellation while the purplish colouration refers to the dominant *Bs-2* gene being in heterozygous constellation as well. From the morphological changes of the infected tissues a strong antioxydant effect can be concluded but as an effect of the high dose infection such quantity of H_2O_2 was still produced that was sufficient for the activation of the *Bs-2* gene.

In the double-homozygous plants the reaction regulated by the *gds* gene, which ensures the operation of the general defense system in homozygous constellation perfectly, is so fast that such quantity of H_2O_2 was not produced that would have been enough for the operation of the *Bs-2* gene even in spite of the strong stress caused by the infection. The purplish colouration referring to the operation of the *Bs-2* gene develops only on tissue parts injured by the inoculation.

Studying the effect of injuries of plant cells on the operation of the *gds* and *Bs-2* gene we got known with new relations concerning the roles of the two genes in disease resistance. The *gds* gene protects healthy cells against infections thus the general defense reaction is preventive. In healthy cells the *Bs-2* gene is completely inactive beside the *gds* gene. Injured cells are not able to give the general defense reaction. The specific defense reaction, determined by the *Bs-2* gene, is the reaction of cells attacked and diseased by pathogens.

On the basis of the above the general defense reaction is able to fulfill the role of the plant immune system while specific defense reactions serve for correcting deficiencies of the general defense reaction in the system of plant disease resistance. The general defense sytem and the specific defense reactions constitute the integral whole of the plant disease resistance together. By restoring a high level general defense reaction in plants we can create a genetic background that ensures such an effective operation for the specific resistance genes that it is accompanied by tissue destruction to a minimal degree or no tissue destruction at all. By a resistance breeding strategy, based on this recognition, cultivars with effective and durable disease resistance can be produced.

4. Summary of the new scientific results

I examined the special genetical and pathophysiological features of the *gds* gene, encoding the general defense system, identified in pepper and considered a new scientific discovery, on the basis of biochemical processes. As we still know little about the general defense system of pepper, as well as about the general defense system of plants, I performed the presentation of this so far unknown form of resistance, ensured by the *gds* gene, in comparison with the *Bs-2* gene which is responsible for specific defense against the *X. c.* pv. *vesicatoria* bacterium.

1.

The time-dependent and the quantitative changes of the glucose content as a consequence of infection were also different in plants containing either the *gds* or the *Bs-2* gene and these were in accordance with tissue alterations belonging to the given plant type.

The energy demand of uniform infected tissue parts in the case of the general defense reaction is greater than that of the specific defense reaction. But in the case of natural infections the reaction of the *gds* gene is restricted to only 10-15 cells and takes place just in a few hours so it is much more economical than the specific reaction which develops in 5-6 days and is accompanied by lesions of 4-5 mm in diameter.

2.

On the basis of our experiments it can be concluded that the plants containig the *gds* gene do not need the oxidative burst, mediated by hydrogen peroxid, to the defense in contrast with such plants that carry a specific resistance gene and react with fast tissue destruction.

3.

In the case of the general defense system, encoded by the *gds* gene, salicylic acid (SA) as a signal molecule does not take part in the regulation of defense. The *gds* gene ensures broad-spectrum resistance without cell destruction beside quite a low free SA level or in the absence of it.

4.

It is very likely that the outstanding antioxidant capacity of the plants carrying the *gds* gene is of basic strategic significance during the general defense reaction which is accompanied by preserving tissues.

5.

During the general defense reaction regulated by the *gds* gene the quantity of choline, considered a formaldehyde-generator, and formaldehyde changes fast in strong correlation with each other in the opposite direction.

The antimicrobial formaldehyde, produced by *fast* demethylation following the infection, may have a significant effect in protecting plants from stresses.

6.

During the genetical experiences it was proven that the *gds* and *Bs-2* gene are inherited independently but there is a strong functional linkage between them which is established by the stress caused by the pathogen. Investigation of biochemical processes elicited by the stress confirmed the pathological observation that the general defense reaction, characterized by a low stimulus threshold and high reaction speed, starts earlier and the specific defense reaction starts only in the case of its inefficiency.

7.

Despite the dominance of the *Bs-2* gene, in the F1 progeny of the *Bs-2/Bs-2* gds^{+}/gds^{+} x $Bs\text{-}2^{+}/Bs\text{-}2^{+}$ *gds/gds* combination the appearance of preserving tissues, the phenotype characteristic of the recessive *gds* gene, refers to the success of an antioxidant effect. On the other hand, in the case of the high dose infection a certain quantity of hydrogen peroxid (H_2O_2) is still produced which is sufficient to activate the *Bs-2* gene.

In the double-homozygous (*Bs-2/Bs-2 gds/gds*) individuals from the F2 progeny the *gds* gene being in homozygous constellation ensures the operation of the general defense system perfectly thus such strong antioxidant processes begin that these can efficiently prevent the destruction triggered by the pathogen. Tissue alterations referring to the operation of the *Bs-2* gene can be observed only at sites of possible injuries caused by the artificial infection where H_2O_2 was produced because of disturbance of the general defense reaction. At these sites H_2O_2 activates the operation of the *Bs-2* gene as a signal molecule.

Studying the effect of injuries of plant cells on the operation of the *gds* and *Bs-2* gene it can be concluded that the *gds* gene protects healthy cells against infections thus the general defense reaction is preventive. In healthy cells the *Bs-2* gene is completely inactive beside the *gds* gene. Injured cells are not able to give the general defense reaction. The specific defense reaction, determined by the *Bs-2* gene, is the reaction of cells attacked and diseased by pathogens.

Literature cited

Csillery, G., Mityko, J., Szarka, E. and Szarka, J. (2007): Genetics of interactions between general and specific plant defense reactions. XIIIth Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant. Warsaw, Poland. Proceedings 59-69.

Gersbeck, N., Schönbeck, F. and Tyihák, E. (1989): Measurement of formaldehyde and its main generators in *Erysiphe graminis* infected barley plants by planar chromatographic techniques. *J. Planar Chromatogr*. 2. 86-89.

Köhm, B.A., Goulden, M.G., Gilbert, J.E., Kavanagh, T.A., and Baulcombe, D.C. (1993): A potato virus X resistance gene mediates an induced, nonspecific resistance in protoplasts. *Plant Cell* 5. 913–920.

Raskin, I., Turner, I. M. and Melander, W. R. (1989): Regulation of heat production in the inflorescences of an *Arum* lily by endogenous salicylic acid. *PNAS*. 86. 2214-2218.

Sárdi, É., Velich, I., Hevesi, M. and Klement, Z. (1996) The role of endogenous carbohydrates in the *Phaseolus-Pseudomonas* host-plantage interaction. 1. Bean ontogenesis and endogenous carbohydrate components. *Hort. Sci. Hung*. 28. 65-69.

Shannon, L. M., Kay, E. and Lew, J.Y. (1966): Peroxidase Isozymes from Horseradish Roots. *J. Biol. Chem.* 241. 2166-2172.

Szarka, J. and Csilléry, G. (1995): Defence systems against *Xanthomonas campestris* pv. *vesicatoria* in pepper. IXth Eucarpia Meeting on Genetics and Breeding on *Capsicum* and Eggplant. Budapest, Hungary. Proceedings 184-187.

Szarka, J., Sárdi, É., Szarka, E. and Csilléry, G. (2002): General defense system in the plant kingdom III. *Int. Jour. Hort. Sci.* 8 (3-4) 45-54.

Toldi, O., Renfors, E., Kulmala, I. and Sorvari, S. (2008): The endophyte *Pseudomonas fluorescens* provides resistance to different fungal pathogens by activating defense mechanisms in host plants through H2O2 signalling. In: Sorvari, S. és Pirttilä, A. M. (szerk.) 'Prospects and Applications for Plant-Associated Microbes. A Laboratory Manual, Part A: Bacteria' (in press)

Yu, I-ching, Parker, J. and Bent, A. F. (1998): Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *PNAS*. 95. 7819-7824.

List of publications related to the subject of dissertation

Articles in journals

Journals with IF-worth:

É. Sárdi, E. Szarka, G. Csilléry and J. Szarka. Biochemical Examination of the General Defense System of Plants by OPLC. Journal of Planar Chromatography, Vol. 19. May/June 2006. 233-237.

Journals without IF-worth:

Szarka, J., Sardi, E., Szarka, E. and Csillery, G. General defense system in the plant kingdom III. International Journal of Horticultural Science, 8 (3-4) 2002. 45-54.

J. Szarka, O. Toldi, E. Szarka, J. Remenyik and G. Csilléry. General Defense Reaction in the Plant Kingdom. Acta Agronomica Hungarica, 54 (2), 2006. 221-232.

Szarka, J., Szarka, E. and Csilléry, G. 2007. A New Form of General Disease Resistance Associated with Host Cell Proliferation. Acta Phytopathologica et Entomologica Hungarica, 42 (2), 2007. 273-277.

Szarka, E., Sárdi, É., Csilléry, G. and Szarka, J. Transmethylation and the general defense reaction of plants. International Journal of Horticultural Science, 13 (4) 2007. 35-40.

Publications in conference reviews

Hungarian conferences, full paper

Szarka E., Csilléry G., Szarka J. és Sárdi É. A növénynemesítés és környezeti hatásai. Proceedings of the 11th Symposium on Analytical and Environmental Problems. Szeged, Hungary, 27 September 2004. 335- 338.

Hungarian conferences, abstract

Sárdi É., Szarka E., Csilléry G., Szarka J. A paprika általános védekezési rendszerének biokémiai vizsgálata. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. Budapest, 2003. november 6-7. 112-113.

Szarka E., Stefanovits-Bányai É. and Sárdi É. A növényi betegségellelnállóság egységes egésze II. X. Növénynemesítési Tudományos Napok. Budapest, 2004. febr. 18-19. 157.

Szarka E., Sárdi É., Csilléry G. és Szarka J. A magyar fűszerpaprika új korszaka I. Általános és specifikus védekezési rendszerek a rezisztencianemesítésben. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. Budapest, 2005. október 19-21. 356-357.

Timár Z., Kapitány J., Szarka E., Mitykó J., Földi T., Balogh M., Gutermuth Á., Szarka J., Csilléry G. és Kiss Gy. B. A magyar fűszerpaprika új korszaka II. A hagyományos és mokekuláris alapú nemesítés eredményei és lehetőségei. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak. Budapest, 2005. október 19-21. 358-359.

Szarka J., Szarka E., Gutermuth Á. és Csilléry G. Gyors sejtpusztulásban és sejtosztódásban megnyilvánuló növényi reakciók szerepe a rezisztencianemesítésben. 52. Növényvédelmi Tudományos Napok. Budapest, 2006. február. 23-24. 59.

Timár Z., Mitykó J., Gutermuth Á., Szarka E., Balogh M., Földi T., Csilléry G., Szarka J. és Kapitány J. A magyar fűszerpaprika új korszaka. XII. Növénynemesítési Tudományos Napok. Budapest, 2006. márc. 7-8. 31.

Szarka E., Gutermuth Á., Timár Z., Földi T., Csilléry G. és Szarka J. Általános és specifikus védekezési rendszerek a rezisztencianemesítésben. XII. Növénynemesítési Tudományos Napok. Budapest, 2006. márc. 7-8. 33.

Szarka J., Szarka E., Nagy B. és Csilléry G. A növények általános védekezési rendszerének és specifikus védekezési rendszereinek kapcsolata. 53. Növényvédelmi Tudományos Napok. Budapest, 2007. február. 20-21. 21.

International conferences, full paper

Csillery, G., Szarka, E., Sardi, E., Mityko, J., Kapitany, J., Nagy, B. and Szarka, J. The unity of plant defense. Genetics, breeding and physiology. XIIth Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant. Noordwijkerhout, The Netherlands, 17-19 May 2004. 147-153.

Sárdi É., Szarka E., Stefanovits-Bányai É., Csilléry G. and Szarka J. Biochemical Examination of the General Defense System of Plants by OPLC Method. International Symposium on Planar Separations. Planar Chromatography. Visegrád, Hungary, 23-25 May 2004. 585-592.

Csillery, G., Mityko, J., Szarka, E. and Szarka, J. 2007. Genetics of interactions between general and specific plant defense reactions. XIIIth Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant. Warsaw, Poland, Sept. 5-7. 2007. 59-69.

International conferences, abstract

Sardi, E., Szarka, E., Csillery, G., Szarka, J., Stefanovits-Banyai, E. Possible role of formaldehyde cycle in the general defense system of plants. 6th International Conference on Role of Formaldehyde in Biological Systems. Methylation and Demethylation Processes. Pécs, Hungary, October 13-16, 2003. 64.

Sárdi, É., Szarka, E., Stefanovits-Bányai, É., Szarka, J., Csilléry, G. Study of the biochemical background of the general defense system by OPLC method. International Symposium for HPTLC. Lyon, France, October 15-18, 2003. 304-311.

E, Szarka, J, Szarka and G, Csilléry. The Role of the General Defense Reaction in the Resistance Breeding of Pepper against the *Xanthomonas vesicatoria* Bacterium. 18th International Pepper Conference. Palm Springs, California, May 21-23, 2006. 8-9.

Szarka, J., Szarka, E. and Csilléry, G. The General Defense Reaction of Plants. Plant Resistance Symposium. Non-specific and Specific Innate and Acquired Plant Resistance. Budapest, Hungary, August 31-September 3, 2006. 78.

Szarka, E., Kiss, Gy. B., Mitykó, J., Balogh, M., Földi, T., Szarka, J. and Csilléry, G. Breeding of multiresistance in vegetables. Indian-Hungarian Binational Seminar on „Biology of Biotic and Abiotic Stress in Higher Plants”. Szeged, Hungary, September 27-28, 2006.