

General defense system in the plant kingdom

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Summary: The goal of plant breeders is to improve the resistance of crops against virus, bacterium and fungus pathogens was easiest to achieve by selection for phenotypes displaying the hypersensitive reaction. The resistant plant of that type keeps its health by preventing or delaying the systemization of the pathogen by *destruction* of cells and tissues of variable size or *amputation* of the contaminated organs. The faster the reaction of the host plant is the more efficient and economical is the defense, since the extent of tissue destruction decreases proportionally with the speed of reaction.

During a breeding program for resistance carried out on several plant species, mainly vegetables over thirty years, also an alternative defense reaction has been experienced, which fundamentally differs from the hypersensitive reaction. In that reaction the cells and tissues of the host plant being exposed to the pathogen do not die, on the contrary they hinder systemization of the pathogen by tissue thickening. An additional significant difference is that on the contrary to hypersensitive reaction this reaction is less host- or pathogen-specific and works excellently even at high temperature (over 40 °C).

Material and method

First experiences have been derived from the practice of current resistance breeding of vegetable crops: mainly of pepper to bacterial leaf spot, *Xanthomonas campestris* pv. *vesicatoria* and of cucumber to downy mildew, *Pseudoperonospora cubensis*. The experiments to check the hypothesis referring to the mechanism of the defense mechanism were carried out on the breeding populations under greenhouse conditions.

Some 8–9 leaf blades of identical age were selected having active metabolism from intensely growing pepper and cucumber plants planted in the soil of the greenhouse.

The inoculation of pepper leaves was performed with 48-hour cultures diluted to 10⁸ cell/ml concentration of either of the following cultures: *Xanthomonas campestris* pv. *phaseoli*, *Pseudomonas syringae* pv. *phaseolicola*, the highly aggressive race 1 of *Xanthomonas* pv. *vesicatoria* isolated in Hungary and *Pseudomonas fluorescens* bacterium species. (Culture medium: 10 g/l extract of yeast powder, 20 g/l glucose, 20 g/l CaCO₃, 20 g/l Agar, 1000 ml water). The inoculum was injected into the intercellular ducts of the leaf on the abaxial surface with syringe or with brush.

Conidium suspension of *Pseudoperonospora cubensis* necessary for the inoculation of cucumber was prepared by washing from the leaves of infected cucumber and was sprayed on the abaxial surface of the leaf.

Observations

In addition to the application of genes registered as *Bs-1*, *Bs-2* and *Bs-3* in pepper genetics conferring different degrees of resistance against *X.c.pv. vesicatoria* bacterium species (Cook, A.A. & Stall, R.E. 1963; Kim, B.S. & Hartmann, R. W. 1985), we tried to find resistance types providing more efficient defense in pepper. In this way, we have detected the reaction of some specimens of the pepper line *PI 163 192* basically differing from the well-known resistance symptoms. Inoculated parts of the leaf blade have become yellowish green in 5–7 days and this pathological process got stuck on that level. This was proved by the fact that the inoculated tissue part has remained unchanged even after 40 days, did not become yellow, discolored or desiccated.

Pepper lines representing different degrees of susceptibility and resistance as carrying the genes *Bs-1*, *Bs-2*

and *Bs-3* and the new type of isogenic pepper lines carrying the newly discovered resistance were compared after having been inoculated by different means. The prognostic symptoms on the susceptible plants as a consequence of brush inoculation were infiltrated lesions of 3–4 mm diameter. Inoculation with injection caused similar changes in the tissues in 4–5 days. 48 hours after inoculation no changes were observed on the living leaves, but the infected tissues of the leaves were picked and dried and in the 48th hour became slightly chlorotic (*Figure 1/1–4*). Resulting from inoculation with brush, *Bs-1* resistance gene reacted with lesions of 2–3 mm diameter. Prognostic symptom resulting from injected inoculation is similar to the susceptible reaction and in 48 hours the tissues of neither the living nor the dried leaves showed difference compared with the susceptible one (*Figure 1/5–8*). Hypersensitive reaction typical to *Bs-3* resistance is expressed in lesions of 1 mm diameter, its tissues dried and became white. Injection resulted in similar changes within 2 days. In the 48th hour following the inoculation, tissues began to dry but still kept their green colors. The healthy tissues of dried leaves were thicker than the inoculated tissues (*Figure 1/9–12*). Phenotypes of *Bs-2* resistance reacted to inoculation by brush with violet lesions not larger than 0.5 mm. Inoculation injected into the leaf-blade made the affected leaf parts wine-red in 24 hours but began to dry only in 10–14 days. The inoculated tissues of leaves picked in the 24th and 48th hours kept their color and even after drying were twice as thick as the healthy tissues (*Figure 1/13–16*). The extraordinary speed of reaction in *PI 163 192*-type produced tiny lesions, which could not be exactly determined after inoculation by using the brush. Leaf blade inoculated with injection showed no sign of change in the 48th hour and slight chlorosis appeared on the 5th–10th day only. In accordance with the different levels of the newly discovered resistance, dried leaves reacted with different thickness of the tissues. Tissue thickening is directly related to the extent of chlorosis (*Figure 1/17–20*).

During the breeding program, the great number of hybrid combinations prepared with the selected line of *PI 163 192* provided good possibility to know the whole spectrum of prognostic symptoms of the newly discovered resistance type. This ranged from the symptom bordering susceptibility to symptoms referring to reaction more susceptible than the original source. The changes observed as prognostic symptoms ranged from different degrees of chlorosis and thickness of the tissues exposed to inoculation on the leaf blades. Those spots become solid and shiny, crack easily, and the tissues become swollen towards the adaxial surface of the leaf.

In order to explore the new type of host pathogen relation the identical phenotype, isogenic pepper lines of the susceptible, the *Bs-2* resistance gene and the resistance found in *PI 163 192* were inoculated with the following bacteria: *Xanthomonas campestris* pv. *vesicatoria* (bacterial leaf spot); *X. c. pv. phaseoli* (common blight of beans); *Pseudomonas syringae* pv. *phaseolicola* (halo-blight of beans) and the saprophytic *P. fluorescens* bacterium (*Figure 2*).

The *X. c. pv. vesicatoria* caused typical symptoms on the susceptible pepper plants corresponding to a compatible host-pathogen relation. The pathogen *P. s. pv. phaseolicola* produces an exotoxin, which is incompatible with pepper causes hypersensitive tissue destruction (Klement & Lovrekovich, 1961). *X. c. pv. phaseoli* is also incompatible and the saprophyte *P. fluorescens*, both cause chlorotic patches with blurred edges, slight tissue thickening and sometimes swollen leaf blade.

Pepper lines armed with *Bs-2* reacted as inoculated with *X. c. pv. vesicatoria* by showing typical prognostic hypersensitive symptoms referring to resistance, whereas *X. c. pv. phaseoli* and *P. fluorescens* caused identical symptoms on resistant as well as on susceptible plants. *P. s. pv. phaseolicola* being incompatible in host-pathogen relation with pepper also containing *Bs-2* and could not induce hypersensitive reaction on inoculated plants. Instead, similarly to inoculation with *X. c. pv. phaseoli* and *P. fluorescens*, tissues became yellowish green and slightly thickened.

Resistance from *PI 163 192* reacted with slight chlorosis to inoculation by all the four types of bacteria. Differences were shown only in the thickness of the inoculated tissues and the separation of the borderline of the healthy and inoculated tissues. The tissue inoculated with *X. c. pv. vesicatoria* became two-three times thicker than the healthy one and as a result of this process swelled towards the adaxial surface of the leaf blade. Resulting from inoculation with *X. c. pv. phaseoli*, *P. s. pv. phaseolicola* and *P. fluorescens*, the plants reacted with milder changes of the tissues as the provocation was identical in character but weaker compared to *X. c. pv. vesicatoria*.

The relation of the susceptible pepper and *P. fluorescens* saprophytic bacterium just as the resistant of the *PI 163 192* type pepper and *X. c. pv. vesicatoria* pathogen represent greatly different host-pathogen relations. However, the tissue changes were identical in character but different in extent (*Figure 3*). Symptoms of both are characterized by chlorosis of which the extent remained unchanged for long weeks following infection, as tissues became thick and solid to an extent which is necessary for defense and it may lead to swollen leaf blades.

Another intercellular pathogen, *Pseudoperonospora cubensis* also offered an opportunity to clarify the role of tissue thickening and the resulting swelling of leaf blades in the defense mechanism of that peculiar, newly discovered resistance to pathogens. The cucumber type used for pickling and resistant to downy mildew appeared due to breeding for resistance the first time worldwide in Hungary (*Perez F1, Mohikán F1*). Resistant leaves reacted to infection with peronospora by fast tissue drying as a symptom of hypersensitivity (*Balogh P., Szarka J. & Ruskó J.*). In course of the breeding work segregates appeared that reacted to infection by a kind of "blistering" (*Figure 4*): humps appeared on the adaxial surface of the leaves keeping their color and became chlorotic only after some weeks.

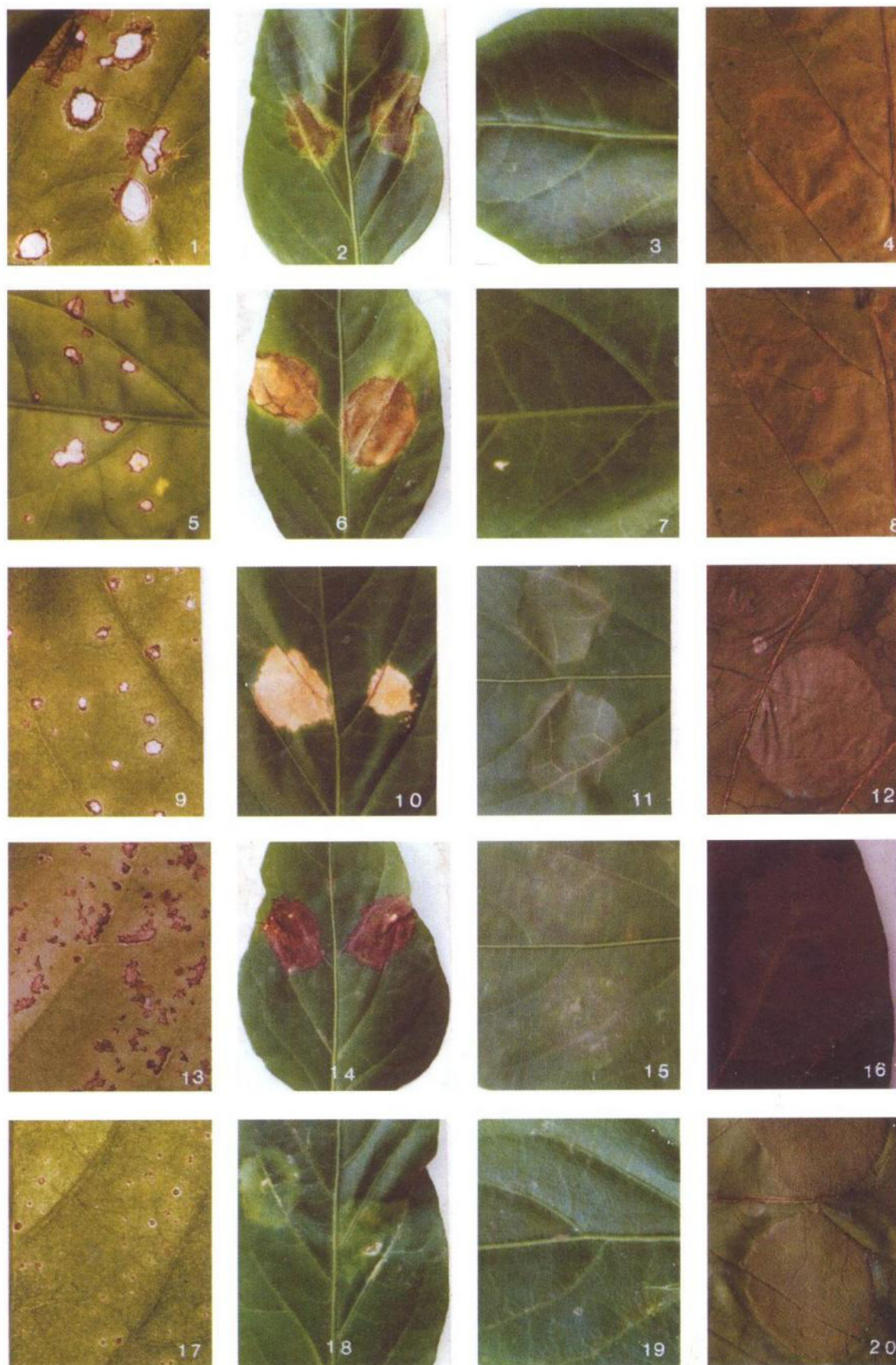


Figure 1 Reactions of pepper lines either susceptible or carrying one of the resistant genes: *Bs-1*, *Bs-2*, *Bs-3* as well as *gds* derived from *PI 163 192* conferring the new type of resistance to bacterial leaf spot, *Xanthomonas campestris* pv. *vesicatoria*

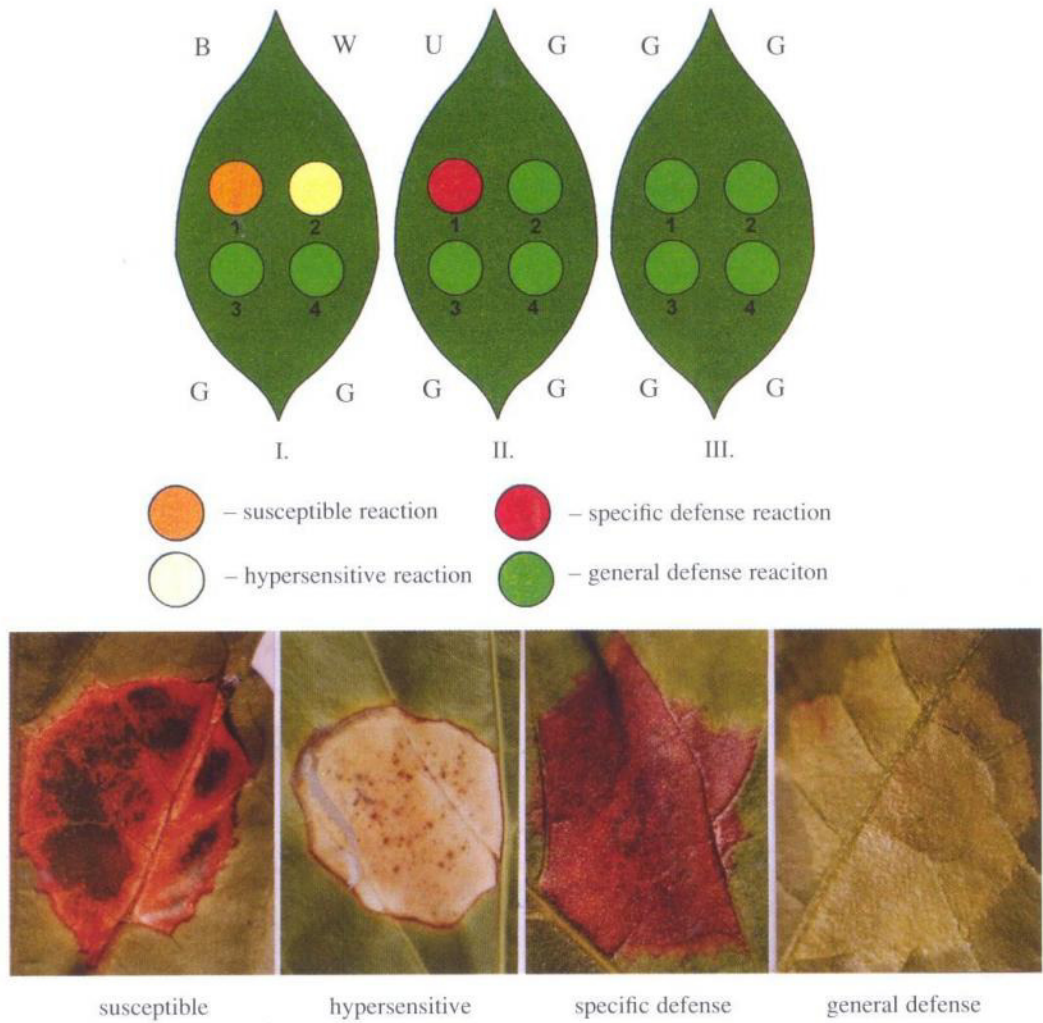


Figure 2 Reactions of pepper lines either susceptible (I) or carrying resistance gene *Bs-2* (II) or the new type of resistance (*gds* gene) of *PI 163 192* (III) to inoculation with *Xanthomonas campestris* pv. *vesicatoria* (1), *Pseudomonas syringae* pv. *phaseolicola* (2), *Xanthomonas campestris* pv. *phaseoli* (3) and *Pseudomonas fluorescens* (4) bacteria.

brown – susceptible reaction
white – hypersensitive reaction

violet – specific defense reaction
green – general defense reaction



Figure 3 Reaction of susceptible pepper line to inoculation with *Pseudomonas fluorescens* bacterium and reaction of *PI 163 192* pepper line to inoculation with *Xanthomonas campestris* pv. *vesicatoria* bacterium

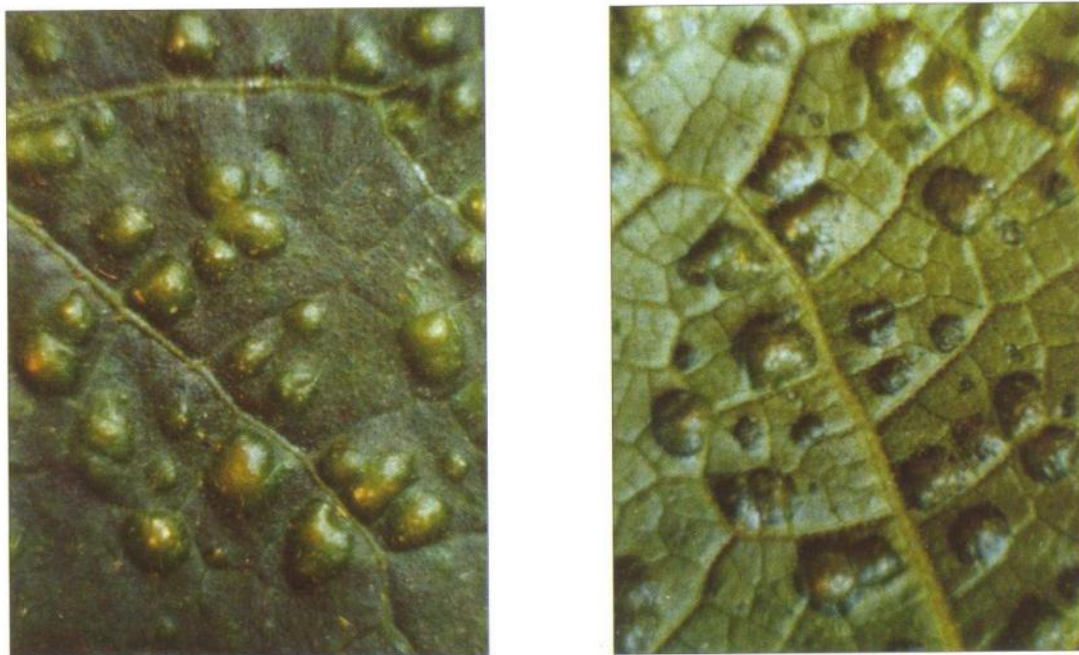


Figure 4 To the effect of the infection of the *Pseudoperonospora cubensis* the cell walls of the leaf blades of the U-28 resistant cucumber lines inhibit the intercellular air ducts by pressing cell to cell around the infection point. That way, the tissues appear to be infiltrated and on the adaxial surface of the leaves there are humps while on the abaxial surface pits developed

On the abaxial surface as negatives of the humps, pits developed.

Since the texture of leaf tissues of cucumber is much looser than the pepper's, deformations causing swelling leaf blades lead to pressure around the infection point that pressed cell to cell and reduced the intercellular air ducts. Consequently, the affected tissue became infiltrated. Infiltration-like deformation was not caused by pathogens multiplying in the intercellular space but by packing of the tissues as a reaction to infection. That way, growth of the pathogen is inhibited. Growing pressure among the cells caused the emerging humps keeping balance with the pressure. As an analogy, the texture of pepper leaf is more tight, with the diminishing space of intercellulars the tissues appear to be infiltrated and the leaf blade swollen.

Conclusions

In addition to the well known hypersensitive reaction ensuring the disease-resistance of pepper and cucumber plants (by fast auto-destruction of cells), another, less specific or rather general defense system has been observed. *This general defense system is inhibiting the invasion of microbes through the intercellular space by tissue thickening and not by fast tissue destruction.*

Regarding the reaction speed and the character of prognostic symptoms of susceptible and *Bs-1*, *Bs-3* resistance genes, no changes have been detected in using the inoculation method either by brush or by injection. *Bs-2* resistance gene made the infiltrated tissue violet in one day but began to dry only in 10–14 days. The infiltrated tissues

of pepper lines carrying newly discovered resistance became slightly chlorotic only in 5–10 days and began to dry only in weeks. The fact that chlorosis of the provoked tissues began only in 5–10 days make us conclude very fast and not slow reaction since insignificant tissue deformations caused by infection become visible only with the growth of the leaf blade. Small tissue deformations becoming visible with the growth of the leaf blade proved our hypothesis that the reaction of *the newly discovered resistance without tissue destruction is much faster than hypersensitive reaction leading to tissue destruction.*

In incompatible host-pathogen relation, resistance conferred by the *Bs-2* gene so far most efficient against *X.c.pv. vesicatoria* and resistance derived from *PI 163 192* behaved similarly although *Bs-2* comes from *Capsicum chacoense* species. By the way the line *PI 163 192* belongs to the *C. annum* species. It is particularly noteworthy that the defense system ensured by *Bs-2* did not react hypersensitively but with a reaction identical with the new type of resistance to *P.s.pv. phaseolicola*, which produces an exotoxin. Therefore we can assume that the specific (monogenic, dominant) resistance determined by *Bs-2* in *C. chacoense* is not based on the hypersensitive reaction. Further evidence is that the operation of *Bs-2* carrying specific resistance is not weakened even at high temperature (40 °C), no violet discoloration occurred, and the inoculated tissue did not dry out, but became chlorotic and thick. *The operation of the newly discovered resistance carried by PI 163 192 is not weakened by high temperature either.*

Genetic analysis of the pepper showed that *the newly discovered resistance selected from PI 163 192 is highly*

Table 1 Resistant and susceptible parents and hybrids as well as segregating populations segregating for the *gds* gene according to their symptoms observed after being inoculated with *Xanthomonas campestris* pv. *vesicatoria*

	Number of inoculated plants			Expected ratio	Chi2	P
	<i>gds</i> genotype	X.c.v Susceptible	X.c.v Resistant			
<i>C. annuum</i> cv. Táltos (P ₁)	<i>gds+/gds+</i>	480	0			
<i>C. annuum</i> PI. 163192 (P ₂)	<i>gds/gds</i>	0	970			
<i>C. annuum</i> cv. Táltos x <i>C. annuum</i> PI. 163192 F ₁	<i>gds/gds+</i>	1350	0			
<i>C. annuum</i> PI. 163192 x <i>C. annuum</i> cv. Táltos F ₁	<i>gds/gds+</i>	1360	0			
Selfed F ₂	942	301	3 : 1	0.088	0.766	
(P ₁ x P ₂) F ₁ x (P ₂) F ₁		521	486	1 : 1	1.216	0.270

efficient against *X.c.pv. vesicatoria* and is inherited fundamentally by a single, recessive gene (Table 1). It is important that after several back-crosses, depending on the genotype of the recurrent parents of a wide scale of genetic variability, resistant phenotypes appeared with even faster reaction, that means less extended deformation of tissues than that of the original resistance source itself. Similar strengthening of the defense system in the backcross generations with tissue thickening compared to the resistance source was experienced also in the breeding program for resistance to *Pseudoperonospora cubensis* of cucumber. This is evidence that susceptible phenotypes also may harbor genes, which interact with the main resistance genes improving the effect of the defense mechanism (cf. epistasy).

Our observations show that this system exists also in host plants which are susceptible to pathogens and have no specific resistance for excluding microbes incompatible with them but in accordance with the relation of plants and microbes on different levels. Therefore, the newly discovered defense system works efficiently even in not compatible plant-microbe relation.

Based on our resistance improving experiences, we can declare that the plant species have a non-specific, fundamental, old defense system widespread in nature in addition to specific defense systems based on the hypersensitive reaction which are perhaps younger regarding evolution. In our opinion, hypersensitive reaction

based on fast tissue destruction of plants and the general defense system based on the strengthening and thickening of tissues as a whole, complementing each other constitute the complex entity of disease resistance of plants.

Based on the above facts the newly discovered feature of plants suitable to defend the effects of microbe was called *general defense system* of which the gene symbol proposed is *gds* (Szarka & Csilléry, 1995).

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