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General Defense Response under Biotic Stress and Its Genetics at Pepper (*Capsicum annuum* L.)

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Abstract: Since the beginning of resistance breeding, protection of plants against pathogens has relied on specific resistance genes encoding rapid tissue death. Our work has demonstrated in different host–pathogen relationships that plants can defend themselves against pathogens by cell growth and cell division. We first demonstrated this general defence response (GDR) in plants by identifying the *gds* gene in pepper. Subsequently, the existence of a genetic system for tissue defence became apparent and we set the goal to analyse it. The *gdr 1 + 2* genes, which operate the complete GDR system, protect plant tissues from pathogens in a direcessive homozygous state in both host and non-host relationships. The inheritance pattern of the two genes follows a 12:3:1 cleavage of the dominant epistasis. With the knowledge of the *gds* and *gdr 1 + 2* genes, the role of tissue-preserving (GDR) and tissue-destructive (HR) pathways in disease development and their relationship was determined. The genes encoding the general defence response have a low stimulus threshold and are not tissue-destructive and pathogen-specific. They are able to fulfil the role of the plant immune system by providing a general response to various specific stresses. This broad-spectrum general defence system is the most effective in the plant kingdom.

Keywords: pepper; general defence response; tissue retention; hypersensitivity response; resistance breeding

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1. Introduction

Since the beginning of our half-century of work on resistance breeding, experiments reported in publications of interest to us have been repeated to help understanding. We also took photographs of pathologies described in other authors' publications but not shown in pictures. With these and with photographs of our own work, we aim to make the knowledge of the symptoms in this speciality more visible and clearer.

The century-old history of plant resistance breeding has been based on the rapid destruction of the attacked tissue of the host plant, the hypersensitive response (specific HR).

In the relationship between cereals and *Puccinia graminis*, Ward [1] and Stakman [2], in the interaction between *Nicotiana glutinosa* and TMV, Holmes [3,4], in the host–pathogen relationship between *Capsicum annum* and *Xanthomonas vesicatoria*, Cook and Stall [5] described the local lesion in the *Bs1* gene, and Cook and Guevara [6] described the local lesion in the *Bs2* gene (Figure 1).

In addition to the study of HR induced by specific resistance genes in the host–pathogen relationship, the observation of the non-host–pathogen relationship has also started. The leaves of the *Nicotiana tabacum* L. *White Burley* plant were infiltrated with inoculum concentration of 10^5, 10^6, 10^7, 10^8 cells/mL of *Pseudomonas tabaci* being in a host–pathogenic relationship with tobacco, and of *Pseudomonas* species as non-host–pathogenic plant pathogen. Inoculum concentration of 10^6, 10^7, 10^8 cells/mL of bacteria being in a non-host–pathogenic relationship with tobacco induced rapid tissue destruction in 24–48 h for all *Pseudomonas* species. The 10^5 inoculum concentration induced partial tissue destruction only in *P. tabaci*, whereas in the other pathogenic *Pseudomonas* species, small local lesions

were formed in the area of infected leaf spots. The authors found that phytopathogenic *Pseudomonas* species can also multiply in the intercellular ducts of non-host plants, inducing (nonspecific) HR, and that this is considered a naturally occurring phenomenon, but the authors overlooked the fact that this phenomenon can only occur if the pathogen has entered the intercellular ducts in very large quantities. A very important aspect of the experiment, but not interpreted by the authors, is that the dilution series of *Pseudomonas* pathogen inoculum, the reaction type switch, was in all cases triggered by increasing the inoculum concentration from 10^5 cells/mL to 10^6 cells/mL, thus setting the limit of the overall tissue retention capacity of *White Burley* [7].

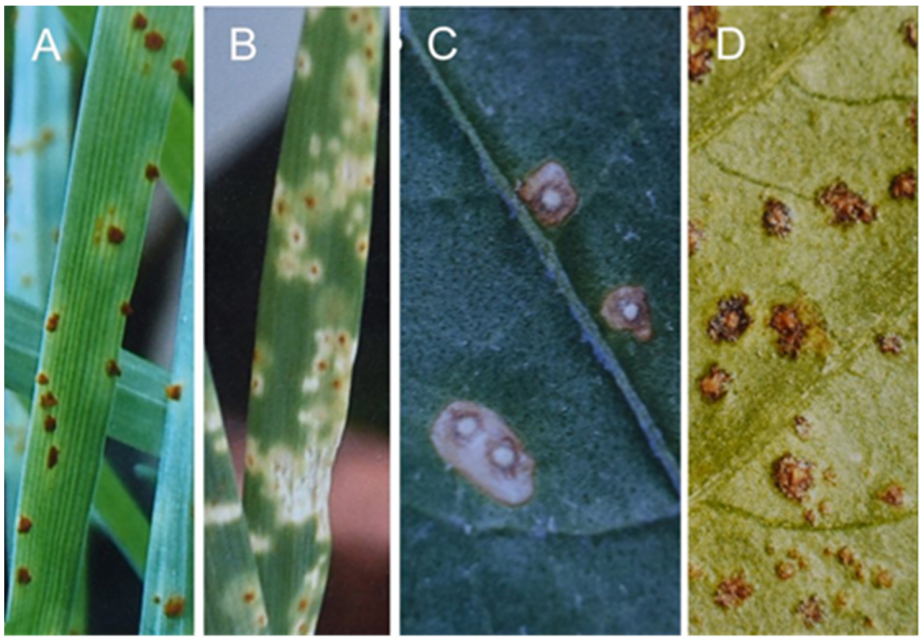

Figure 1. Local lesions in wheat-*Puccinia graminis* (**A**)—susceptible, (**B**)—resistant, *Nicotiana glutinosa*-TMV (**C**), *Capsicum annuum* (*Bs2* gene)-*Xanthomonas vesicatoria* (**D**).

The relationship between susceptibility and HR in host–pathogen and non-host–pathogen relationships was studied by Szarka et al. [8]. *Nicotiana tabacum cv. Pallagi* leaves were infiltrated with 10^1-10^9 cells/mL inoculum of *P. tabaci* (Figure 2A), and *P. phaseolicola* (Figure 2B) bacteria (Figure 2), irrespective of the host–pathogen or non-host–pathogen relationship.

Figure 2. Reaction exhibited by *Nicotiana tabacum* cv. *Pallagi* after infiltration with 10^7, 10^8 and 10^9 cells/mL concentrations of the bacteria *Pseudomonas tabaci* (**A**) and *Pseudomonas phaseolicola* (**B**).

The 10^9 cells/mL inoculum induced nonspecific HR in both bacterial species in the same way. The 10^8 cells/mL inoculum of *P. tabaci* induced the typical pathological symptoms of the disease on its host plant, tobacco, and 10^1–10^7 cell/mL inoculum of *P. tabaci* and 10^1–10^8 cells/mL inoculum of *P. phaseolicola* induced at most a slight bulging towards the front surface of the leaf and a chlorotic spot with indeterminate margins on infiltrated tissue sections. In other words, the tissue retention capacity of the *Pallagi* tobacco variety was able to tolerate stress caused by a bacterial suspension of *P. tabaci* at a concentration of 10^7 cell/mL and of *P. phaseolicola* at a concentration of 10^8 cell/mL. From this observation, conclusions were drawn on the tissue retention capacity of tobacco [8].

In evaluating the results, the term General Defence Reaction (GDR) was used to describe the tissue retention capacity of the plant in host–pathogen and non-host–pathogen relationships, i.e., the specific HR counterpoint, and its interpretation was described in 1995 [9].

The 10^8 cells/mL inoculum of *Pallagi* and *P. tabaci* formed a host–pathogen relationship in which a delicate equilibrium was established between the stress of the pathogen and the tissue retention capacity of the host plant, which was upset by the stress effect of the pathogen's increased proliferation. The plant tissue began to die, and this balance of power was eventually manifested in the susceptible disease. The *P. tabaci* suspension at concentration of 10^7 cells/mL showed the dominance of the GDR of the plant, while the rapid tissue death caused by the suspension at concentration of 10^9 cells/mL showed the destruction of the GDR. Since susceptibility as a physiological state is intermediate between the two, it was likely that GDR levels play a dominant role in the susceptibility of plants to pathogens [8].

To study susceptibility status and specific resistance gene activation, pepper cell lines with similar GDR levels, containing susceptible and *Bs2* resistance genes, were tested using a non-host–pathogen relationship, using *X. vesicatoria inocula* at concentrations of 10^1–10^9 cells/mL [8]. Leaf spots of susceptible and *Bs2* resistance gene-containing peppers infiltrated with suspensions of *X. vesicatoria* at concentrations of 10^1–10^7 cells/mL were at most chlorotic. The inoculum at concentration of 10^8 cells/mL produced a greasy spot on the susceptible cell line and a maroon discolouration on the leaf containing the *Bs2* gene, which did not dry out and caused resistance symptoms. Since pepper cell lines with similar tissue retention were tested, it was concluded that the *Bs2* gene is only activated when one of the stress levels represented by the *X. vesicatoria* dilution series breaks through the plant's general defence system. On the basis of this observation, it is likely that the general defence response is activated first in the defence process, and that its insufficiency leads to a state of susceptibility, which induces a physiological disturbance that triggers the specific resistance gene, which, thus, only becomes active after the development of the disease.

Contrary to the "resistance = HR" theory, the "destruction is not resistance" position could not be proven until a gene encoding a resistant response without tissue death was found.

The non-hypersensitive, nonspecific recessive gene found in cell line PI 163,192 of *Capsicum Annuum* became applicable for resistance identification in 1995. Based on the characteristics it encodes, it is a proposed marker of monogenic recessive trait: general defence system—*gds* [9]. In contrast to the *Bs2* gene (Figure 3), the response regulated by the *gds* gene (Figure 4) is manifested by cell growth, cell division, tissue compaction, and bulging of the infected leaf plate.

Unlike *X. vesicatoria* (Figure 5A) being in a host–pathogen relationship with pepper, *P. phaseolicola* (Figure 5B) and *X. Phaseoli* (Figure 5C) being in a non-host relationship, and the saprophytic *P. Fluorescens* (Figure 5D) bacteria, the *gds* gene responds with the same tissue lesions.

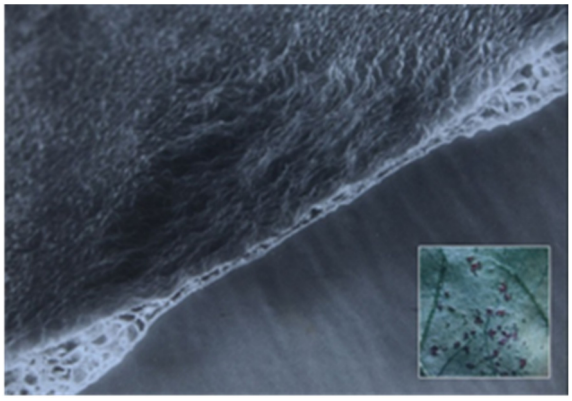

Figure 3. Pathology and section of a pepper leaf infected by *Xanthomonas vesicatoria*, containing the HR-inducing *Bs2* gene [8].

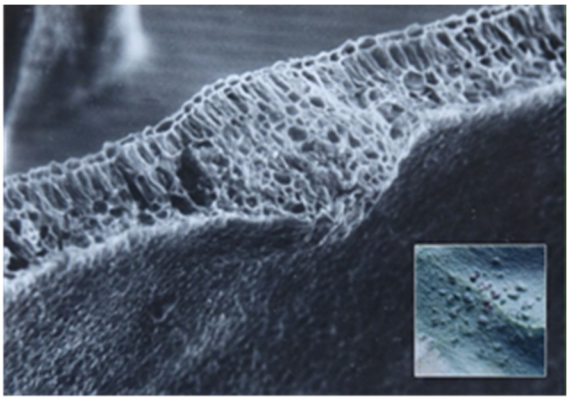

Figure 4. Pathology and section of a pepper leaf infected with *Xanthomonas vesicatoria*, containing the *gds* gene encoding tissue retention [10].

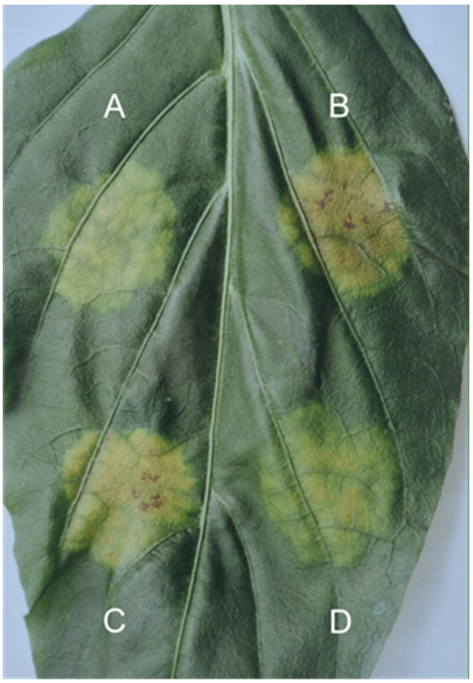

Figure 5. Tissue retention response of pepper leaves containing the *gds* gene to inoculation with a suspension of *Xanthomonas vesicatoria* (**A**), *Pseudomonas phaseolicola* (**B**), *Xanthomonas phaseoli* (**C**), and *Pseudomonas fluorescens* (**D**) at concentration of 10^8 cells/mL.

Thus, in addition to being non-hypersensitive, *gds* is also a non-specific gene, as it gives a generalised response (GDR) to specific stresses induced by different pathogenic species in peppers containing the *gds* gene.

The gene that protects pepper by producing a tissue retention defence response was published in 2002 [11]. The gene was given the name *bs5* and another recessive tissue retention gene, *bs6*, was also described at the same time.

Since the *bs5* gene was found in the same PI 163,192 cell line in which Szarka and Csilléry were the first to find and describe a tissue retention recessive gene in 1995 [9], the authors performed allele testing of *gds* and the *bs5* gene [12]. Based on the susceptibility and tissue retention pathogenicity of *X. vesicatoria*, the heritability tests confirmed that the two genes were identical. Pepper containing the *bs6* gene (ECW60) was shown to be susceptible in testing, which is presumably why it is not used in breeding today, unlike the *gds* (*bs5*) gene.

Another type of tissue retention response other than *gds* has also been reported [9]. In this experiment, pepper cell lines carrying the *Bs2* and *Bs3* genes were infected with *X. vesicatoria* and the infected leaves were detached from the plants and dried 24 h after inoculation (Figure 6). The leaves of the *Bs3* plant showed tissue damage indicative of HR already at the time of detachment, while the leaves of the *Bs2* plant showed only a slight purple discolouration indicating the location of the infected spots. For the *Bs3* gene, the dried infected tissue (Figure 6A) was only half as thick as the uninfected tissue, whereas for the *Bs2* gene, in addition to the slight purple discolouration, the infected tissue was twice as thick (Figure 6B) as the uninfected tissue.

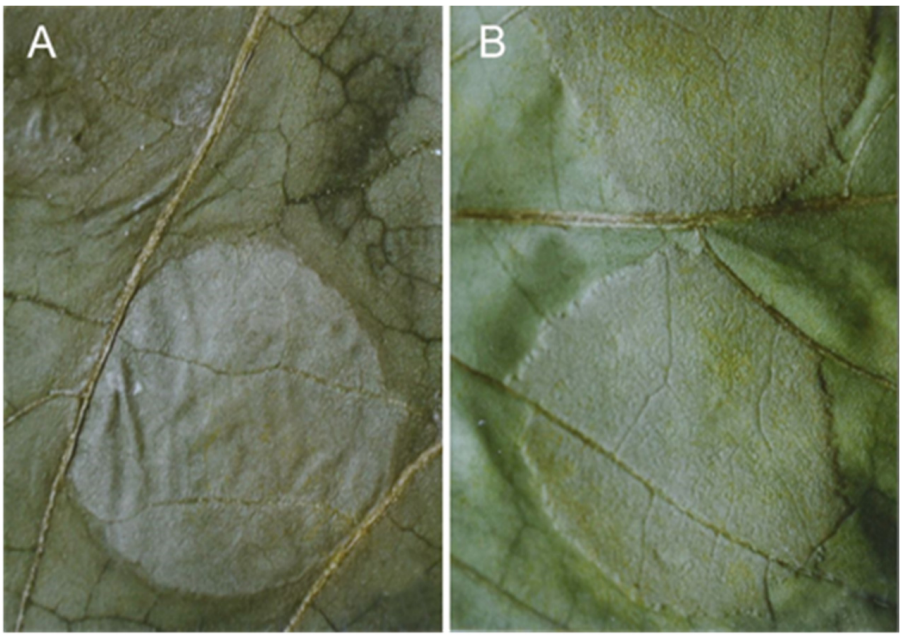

Figure 6. Tissue structural changes in leaves of pepper plants containing the *Bs3* (**A**) and *Bs2* (**B**) genes, detached at 24 h after infection with *Xanthomonas vesicatoria* and dried.

A similar tissue retention property was observed in the non-host–pathogenic relationship between pepper containing HR genes (*Bs2*, *Bs3*) and the bean pathogen *X. Phaseoli*.

The GDR phenomenon has also been described in several host–pathogen relationships where the host plant did not contain a known resistance gene and nonetheless developed tissue retention pathology suggestive of non-susceptibility (Figure 7). Such factors include: *Cucumis sativus–Pseudoperonospora cubensis* (Figure 7A,B), *Phaseolus vulgaris–Pseudomonas phaseolicola* (Figure 7C) [13].

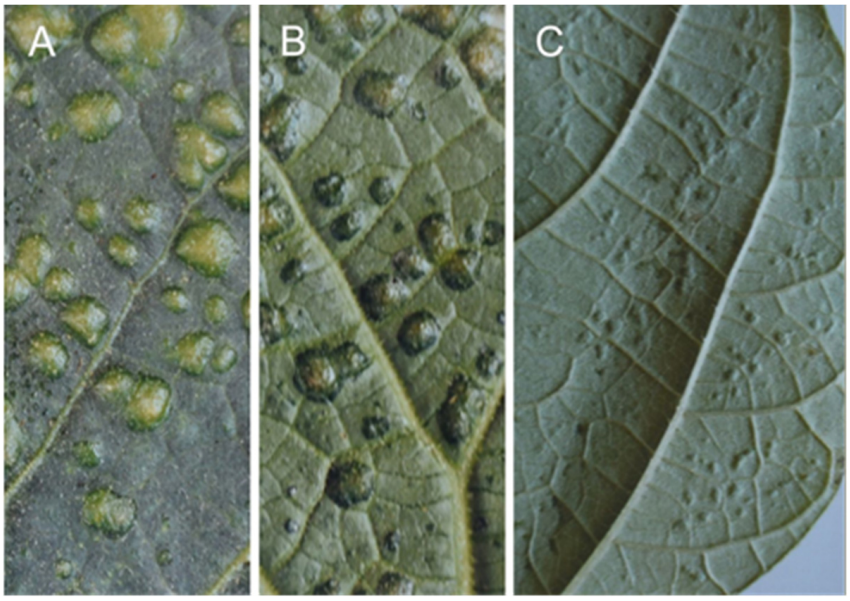

Figure 7. General Defence Reaction, GDR in Cucumber—*Pseudoperonospora cubensis*, (**A**)—adaxial leaf surface, (**B**)—abaxial leaf surface, and bean—*Pseudomonas phaseolicola* (**C**) host–pathogen relationship.

Hydrogen peroxide plays an essential role in the development of specific HR determined by specific resistance genes. H_2O_2 in plant-microbe or host–pathogen interactions, depending on its amount, either enhances or destroys host plant cells. The production of H_2O_2 by susceptible plants containing the *Bs2* and *gds* genes, upon the effect of *X. vesicatoria*, was as follows [10]. The author investigated the amount of H_2O_2 by infiltration of the entire leaf surface of the plants for 10 h after infection. In the susceptible host–pathogen relationship, the pathogen enters the plant without causing a stress effect, so no significant H_2O_2 change occurs. The pathogen only becomes detectable to the plant during its accumulation. In response to H_2O_2 as a signalling molecule for the initiation of death in the susceptible phase, the *Bs2* gene is activated, causing a specific HR characterised by H_2O_2 "burst". In the experiment, the H_2O_2 "burst" occurred within 30 min and after 8 h it was reduced to the control level, while the infected tissues were destroyed. H_2O_2 levels remained constant in plants containing the *gds* gene, which encodes a strong tissue retention capacity.

H_2O_2 induces lignin synthesis and cross-linking between phenolic compounds and cellular wall proteins. This results in increased resistance to the enzymes degrading the cellular wall [14]. The formation of cross-links between cellular wall proteins is very rapid, occurring within 2–5 min after a stimulus [15]. Non-host resistance is primarily based on general responses linked to the cellular wall. These include thickening, lignification of the cellular wall, accumulation of phenols, flavonoids, which are highly localized responses expressed at the point of pathogen entry [16].

The aim of our work is to describe the functional defects of specific resistance genes used in resistance breeding and to elucidate their causes. Furthermore, we will describe the pathophysiology of a previously unknown defence response without tissue destruction, its genetic analysis, and its application in resistance breeding.

2. Materials and Methods

The experiments were carried out at the Kecskemét site of Univer Product Plc. between 2017 and 2021.

A significant part of our observations on plant material for variety production were made in the framework of resistance breeding work. The plants were planted in the soil of the growing house, i.e., we worked with plants with strong roots and vigorous growth. The plants were inoculated before flowering, in the vegetative stage, which is characterised by strong growth. The leaves selected for this purpose were at 70–80% of their

development. Suspensions of different concentrations of *X. vesicatoria* and *P. phaseolicola* bacteria prepared from 48 h culture were used for inoculation. To form lesions, inoculum was applied to the abaxial surface of the leaf by brushing, mimicking the natural infection process. The primary criterion of evaluation in this case was the size of the lesion. For evaluation by tissue retention, intercellular ducts were flooded by injection. This method is less dependent on the environment and allows differentiation based on the quality of the infected tissue. The assessment of symptoms was performed on days 7 and 14, with occasional continuous evaluation.

For the genetic analysis, two infected leaves of a plant, directly above each other, were inoculated (Figure 8A,B).

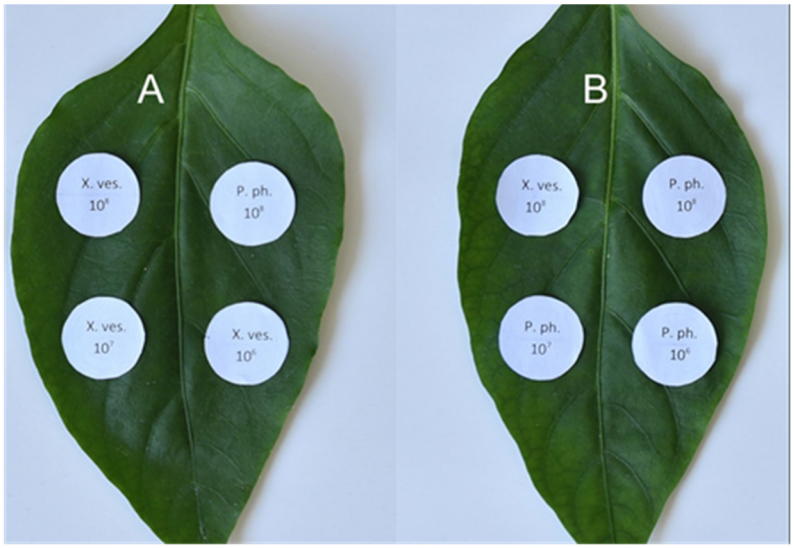

Figure 8. Location of tissue spots on leaves infected with a suspension of *Xanthomonas vesicatoria* (**A**) and *Pseudomonas phaseolicola* (**B**) at concentrations of 10^8, 10^7, 10^6 cells/mL for genetic analysis of the general defence response of pepper.

In both leaves, the upper left spot shows the inoculation of 10^8 cells/mL of *X. vesicatoria* bacteria in a host–pathogenic relationship with pepper, and the upper right spot shows the inoculation of 10^8 cells/mL of *P. phaseolicola* bacteria in a non-host–pathogenic relationship with pepper. The lower two spots on the upper leaf show the result of inoculation by the bacterium *X. pesicatoria* at concentrations of 10^7 (left spot) and 10^6 (right spot) cells/mL. The lower two spots on the lower leaf show the spots resulting from the inoculation of *P. phaseolicola* at concentrations of 10^7 (left spot) and 10^6 (right spot) cells/mL. Both leaves were tested with a 10^8 cells/mL suspension of the two pathogens to account for possible differences due to the age of the leaves.

The reaction of the leaves was classified into pathogen groups according to whether the inoculation resulted in a green, tissue-retaining spot or a drying, necrotic spot (Figures 9–11), as required for genetic analysis.

Figure 12A shows the design of experiments to investigate the rate of response of genes controlling specific and general defence. This involved infecting a spot with 5 mm of diameter with one pathogen (a) and then, after absorption of the watery patch, re-infecting it with the other pathogen (b), knowing the location of the first spot (a).

Figure 12B illustrates the inoculation process to study the function of specific and general defence responses in damaged cells. This involved inducing a convex tissue spot infiltrated by mechanical pressure with an object that consequently damaged the cells to different degrees (c) and inoculating it with a bacterial suspension (d).

Of the hot peppers used in the experiments, *Unihot* is a commercial variety, the others are breeding lines (Table 1).

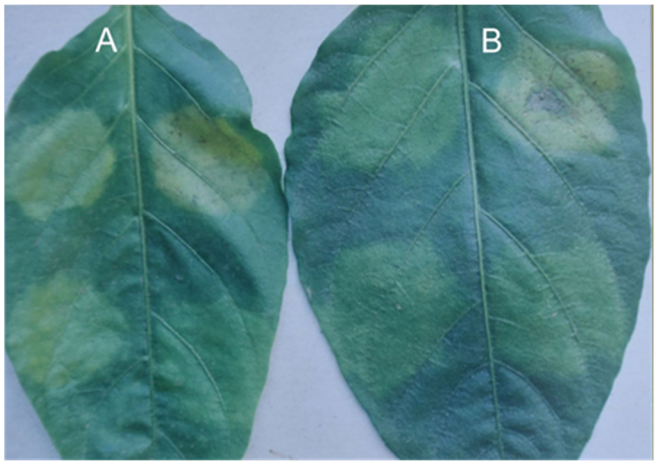

Figure 9. Pathogen symptom clusters induced by the inoculum of *Xanthomonas vesicatoria* (**A**) and *Pseudomonas phaseolicola* (**B**) at 10^8 cells/mL and their dilution series (10^7, 10^6 cells/mL). *X. vesicatoria*: Z-Z-Z-Z, *P. vhaseolicola*: Z-Z-Z-Z.

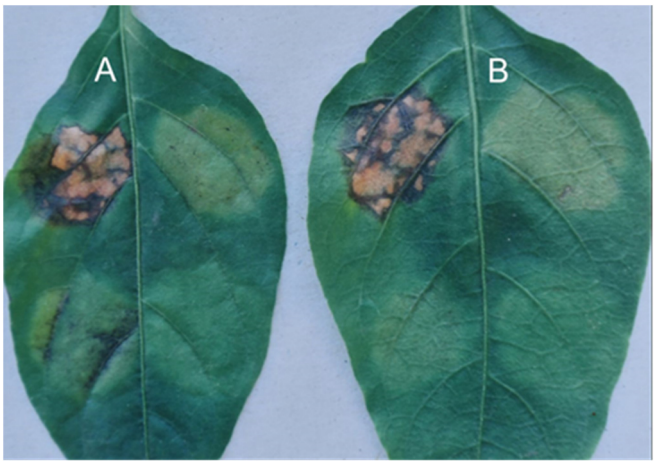

Figure 10. Pathogen symptom clusters induced by the inoculum of *Xanthomonas vesicatoria* (**A**) and *Pseudomonas phaseolicola* (**B**) at concentration of 10^8 cells/mL and their dilution series (10^7, 10^6 cells/mL). *X. vesicatoria*: L-Z-L_X-Z, *P. phaseolicola*: L-Z-Z-Z.

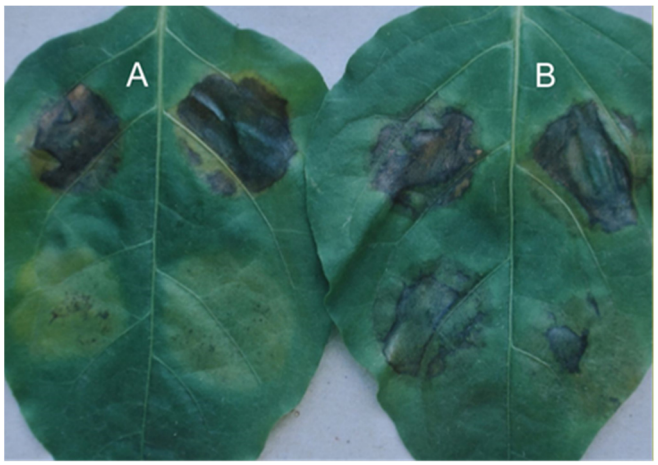

Figure 11. Pathogen symptom clusters induced by the inoculum of *Xanthomonas vesicatoria* (**A**) and *Pseudomonas phaseolicola* (**B**) at concentration of 10^8 cells/mL and their dilution series (10^7, 10^6 cells/mL). *X. vesicatoria*: P-P-Z-Z, *P. phaseolicola*: P-P-P-P.

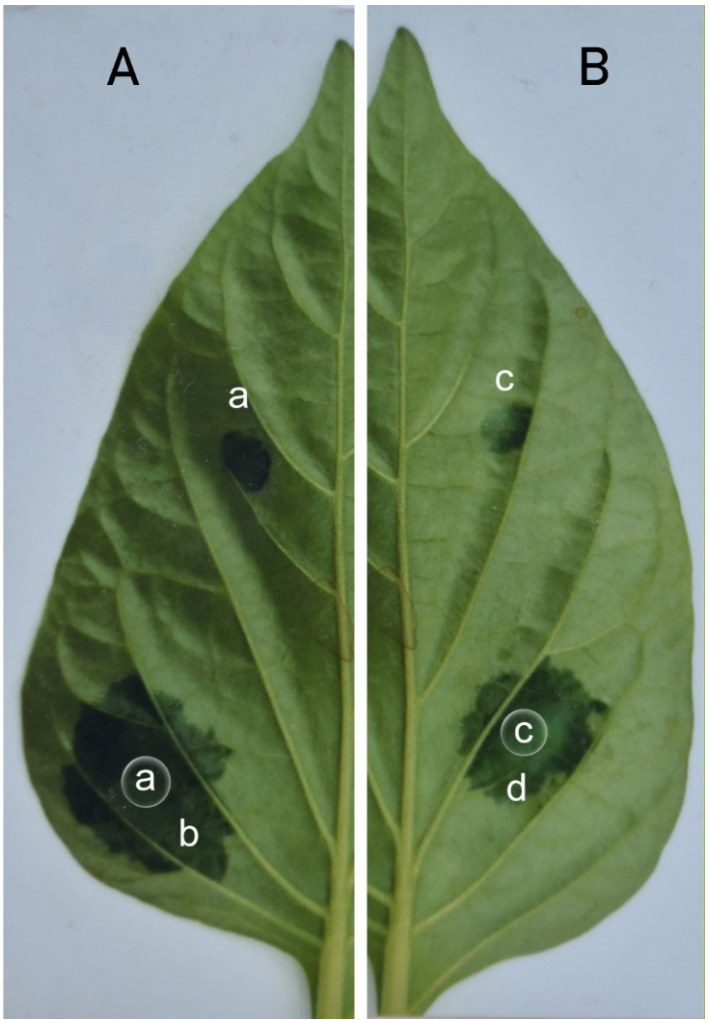

Figure 12. Pre-infection with a suspension of *Xanthomonas vesicatoria* in a spot with 5 mm of diameter (**Aa**), followed by super-infection with a suspension of *Pseudomonas phaseolicola* (**Ab**). Mechanical pressure-induced infiltrated tissue spot (**Bc**), followed by super-infection with a suspension of *Xanthomonas vesicatoria* (**Bd**).

Table 1. Reactions of the hot pepper lines used in hybridizations to inoculation with *X. vesicatoria* at concentration 10^8 cells/mL.

Pepper Materials Used	Reaction Type			
	S (P_x) [1]	**L_f** [2]	**L_x** [3]	**X** [4]
Unihot	+			
L330		+		
L1786			+	
L1710/2				+
L1713/2				+
L1715/3				+
L1716/4				+

[1] S (P_X): necrotic spot (susceptible); [2] L_F: rapidly dying slightly purple spot (*Bs2* gene); [3] L_x: green spot with purple vessels (*Bs2* gene); [4] X: uniform green tissue-retaining spots after inoculation with *X. vesicatoria* at concentration of 10^8 cells/mL and *P. phaseolicola* at 10^8 cells/mL.

The cleavages observed in F2 were subjected to Chi^2 test regression analysis. Our null hypothesis is that the observed genetic cleavage rates correspond to a given theoretical genetic cleavage.

There is no established terminology yet for plant traits and pathological processes related to tissue retention. Hence, we see the need for an interpretation of the terms and concepts we use.

Susceptibility (S): in the case of a host-pathogen relationship, plant tissue death characteristic of the pathogen. The rate of the process depends on the plant's capacity to retain tissue.

Hypersensitive response (HR): rapid tissue death in response to biotic stresses.

- Specific hypersensitive response (sHR): in a host–pathogen relationship, the destruction of the attacked plant tissue by a specific resistance gene.
- Nonspecific hypersensitive reaction (aHR): rapid tissue death in a non-host relationship due to stress induced by a pathogen, or rapid tissue death in a host–pathogen relationship without a specific resistance agent due to over-infection.

Tissue retention capacity: the genetically determined trait that protects plant tissues from destruction by biotic and abiotic stresses.

General defence response (GDR): a defence process under biotic stresses, in host–pathogen and non-host–pathogen relationships, based on the plant's tissue retention capacity to exclude pathogens in a general response to specific stresses.

- Weak/strong GDR: the degree of tissue retention manifested in pathogenicity without knowledge of the genotype.
- Complete GDR: the ability of a plant containing both genes (*gdr 1 + 2*) to retain tissue.
- Incomplete GDR: the degree of tissue retention in a plant containing either or neither of the *gdr 1* and *2* genes, under stress expressed as inoculum concentration.

3. Results

3.1. Insufficient Function of Specific HR

The observed disturbances in the function of specific HR genes used in resistance breeding under cultivation conditions were investigated in different host–pathogen relationships.

In tobacco-*TMV* and pepper-*ToMV-Ob* host–pathogen relationships, leaf viability functions are reduced by senescence and excision, respectively, and pathogens previously blocked in lesions are released. Despite the presence of resistance genes, the virus spreads unhindered in tissues, as indicated by tissue lesions characteristic of the pathogen (Figure 13A–C).

The function of the *Bs2* gene, which protects pepper against *X. vesicatoria*, is inhibited in cool, rainy weather. The bacterium escapes from the lesions already formed and infiltrates, showing the susceptibility of the flooded tissues. When weather conditions become more favourable (warming, reduction in humidity), the tissues invaded by the bacteria start to get purple. Leaf-drop as a self-destruction associated with the delayed onset of the *Bs2* gene function is an additional burden for the plant.

If plant life processes are weakened or inhibited due to senescence or adverse environmental factors, specific HR genes are unable to prevent the spread of the pathogen in the leaf, but only kill damaged cells already affected by the pathogen in a delayed, downstream manner. This characteristic results in their inadequacy and insufficiency in resistance inhibition.

Under conditions favourable to the plant, specific HR genes prevent the systemisation of pathogens in the plant by forming lesions. The smaller the lesion, the better the resistance efficacy, i.e., the less tissue loss. In turn, the pathogen releases and spreads further from the lesions formed on the leaves of plants whose life processes are inhibited. Based on this observation, it is clear that other plant properties besides specific HR genes are involved in the formation of lesions. This could be the plant's ability to retain tissue, which is

manifested as a general defence response (GDR) under biotic stresses. A strong correlation was found between small lesion diameter and high levels of GDR detected by infiltration.

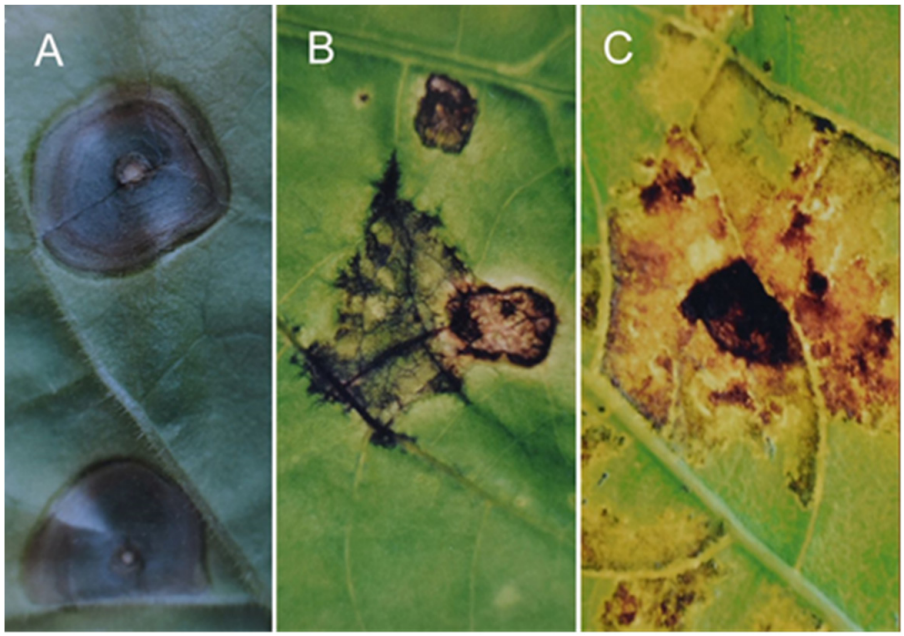

Figure 13. Local lesions of tobacco (**A**) and pepper (**B**,**C**) leaves as a consequence of senescence, excision, reduced life cycle, and unfavourable environmental conditions, leading to the release of the pathogen (*TMV*, *ToMV-Ob*, *X. vesicatoria*) and its spread in the plant.

3.2. Specific HR and GDR

The previous association, together with the fact that in a host–pathogen relationship, significant tissue thickening in the presence of a specific resistance gene (*Bs2*) inhibited rapid tissue death within 24 h, justified a detailed investigation of GDR (Figure 6).

We chose the *Bs2* gene for our work because the purple discoloration showing its function is a good indicator for pathological genetic studies. *Bs2*, a so-called specific resistance gene capable of destroying pepper tissue, induces different pathogenic symptoms that also accurately reflect the GDR levels of the plants (Figure 14).

The manifestations of increasingly higher GDR levels are shown in Figure 14A–C. Figure 14C also shows that in the presence of high levels of GDR, the specific HR gene could only be activated along the vessels and in the vessels and induce partial tissue death. These levels of tissue retention induced by *X. vesicatoria* at a concentration of 10^8 cells/mL were also confirmed by testing with a suspension of *P. phaseolicola* at the same concentration. The pathologies of the *Bs2* gene with different genetic structures in terms of tissue retention were designated L_F (Figure 14A), LL (Figure 14B), and L_X (Figure 14C).

3.3. Inheritance of GDR

3.3.1. Finding Plants X

For the genetic work, we selected plants marked L_X (Figure 15A), which were hybridized to the susceptible (S) variety *Unihot* (Figure 15B). Individuals of the F_1 generation were infected with *X. vesicatoria* inoculum at a concentration of 10^8 cells/mL, resulting in purple and desiccated uniform L_L disease symptoms (Figure 15C).

For accurate genetic analysis, the F_2 generation was also tested with inoculum at concentrations of 10^6, 10^7, 10^8 cells/mL and a stress band of appropriate width was established to assess the spectrum of tissue retention.

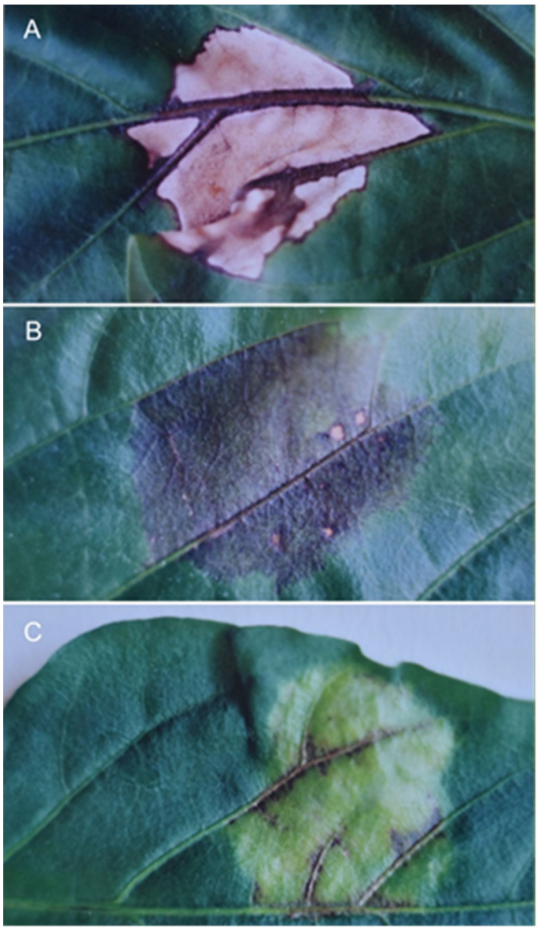

Figure 14. Pathogenicity induced by *Xathonomas vesicatoria* inoculum at concentration of 10^8 cells/mL in pepper lines carrying the *Bs2* gene. (**A**) L_F—rapid tissue death (HR), (**B**) L_L—purple-coloured tissue, (**C**) L_X—tissue retention (GDR), vessels getting purple.

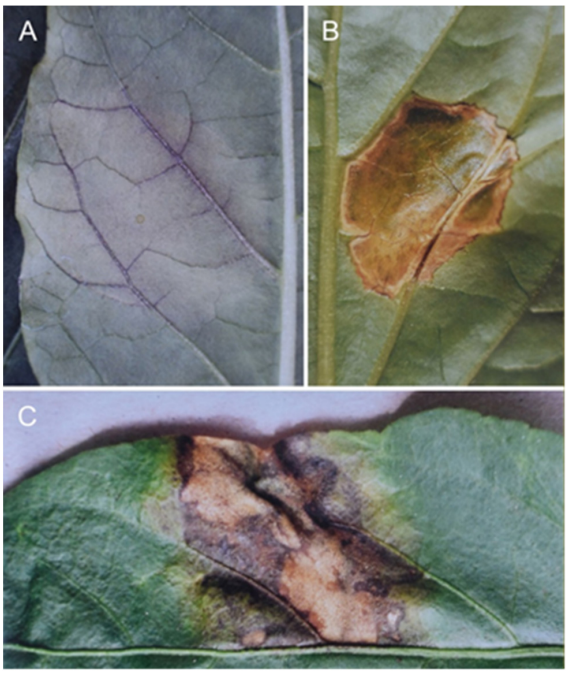

Figure 15. Response of *Bs2* gene-containing peppers with L_x pathotype (**A**) and susceptible (S) *Unihot* variant (**B**) and F_1 generation (**C**) to *Xanthomonas vesicatoria*.

Two directly overlapping infected leaves of each plant were inoculated (Figure 8). This allowed us to characterise the tissue retention capacity of the plants by pathogen symptom clusters (Figures 9–11). Based on these experiments, individual plants of the F_2 generation were infected with *X. vesicatoria* inoculum at concentration of 10^8 cells/mL and *P. phaseolicola* inoculum at concentration of 10^8 cells/mL. Individual plants with both infected spots green in colour and a thickened leaf plate were selected from the fission population. These two characteristics always occurred together. The resulting plants with a high level of tissue retention were marked X. The non-X marked individuals of the fission population were not yet divided into phenotypic categories and were treated as a single whole (Σ Non-Z_x + Z_p) (Table 2).

Table 2. Hybridization of L_x and susceptible (S) lines (2017/1).

Hybridization	Phenotype Categories				
	S (P_x) [1]	**L_L [2]**	**L_X [3]**	**Z_x [4] + Z_p [5] (X)**	**Σ Non-Z_x + Z_p**
P1 L1786			+		
P2 Unihot	+				
F1		+			
F2				33	451

[1] S (P_x): a necrotic susceptible spot formed by inoculation of *X. vesicatoria* at a concentration of 10^8 cells/mL. [2] L_L: uniform purple spot formed by inoculation of *X. vesicatoria* at a concentration of 10^8 cells/mL. [3] L_X: green patch with purple vessels formed by inoculation of *X. vesicatoria* at a concentration of 10^8 cells/mL. [4] Z_X: a green tissue-retaining spot formed by inoculation of *X. vesicatoria* at a concentration of 10^8 cells/mL. [5] Z_P: a green tissue-retaining spot formed by inoculation of *P. phaseolicola* at a concentration of 10^8 cells/mL.

The results of this experiment, despite its shortcomings, suggested that it is a dihybrid (two-gene) inheritance system in which two recessive genes must be homozygous for the Z_x + Z_p symptom, i.e., the complete tissue retention GDR system, to manifest. This can occur in 9:3:3:1, 9:6:1, 12:3:1, and 15:1 dihybrid cleavages, and based on the available data, we started with the 15:1 possible cleavage as a working hypothesis, which became our null hypothesis and on which we performed the Chi^2 test regression analysis: $\sum (Y_i - X_i)^2/X_i = (451 - 454)^2/454 + (33 - 30)^2/30 = 0.0198 + 0.3 = 0.3198 < 3.84$, which shows that the calculated Chi^2 value is lower than the value in the contingency table for the given degree of freedom (1), and, therefore, the null hypothesis for the 15: 1 cleavage was accepted, i.e., the observed cleavage may correspond to the trait inherited by the two recessive genes (double recessive homozygotes) at $p = 0.05$ significance level.

X (Z_x + Z_p) plants selected from the F2 generation were strictly self-fertilized and their progeny were tested with inoculum of *X. vesicatoria* at concentration of 10^8 cells/mL and *P. phaseolicola* at a concentration of 10^8 cells/mL. It was found that the progeny of the X lines highlighted in generation F2 in generation F3 also all responded to infection with both bacteria with green, non-necrotic spots, so the lines did not show any symptomatic cleavage. However, it was still necessary to exclude the possibility that the X-labelled plants also contain the *Bs2* gene in a repressed, hypostatic state. For this, X-labelled individual plants were hybridized with a variety (*Unihot*) susceptible to the *X. vesicatoria* pathogen. The F1 generation gave susceptible symptoms when tested with inoculum of *X. vesicatoria* at a concentration of 10^8 cells/mL, thus, making it clear that the *Bs2* gene is not present in the X plants even in a repressed state. This demonstrates that *gdr* genes can act without the presence of the *Bs2* gene and are, therefore, not helper, intensifier, or modifier genes, which can only act in the presence of resistance genes.

3.3.2. Hybridization of Plants X with Line L330

In further studies, the pepper line L330 was used as a hybridization partner for the X lines. This line contains the *Bs2* gene and develops a very fast, 24 h, drying out and dying HR under infection by *X. vesicatoria* (Figure 16(A1)) and *P. phaseolicola* (Figure 16(A2)), and its symptom type belongs to the L_F category.

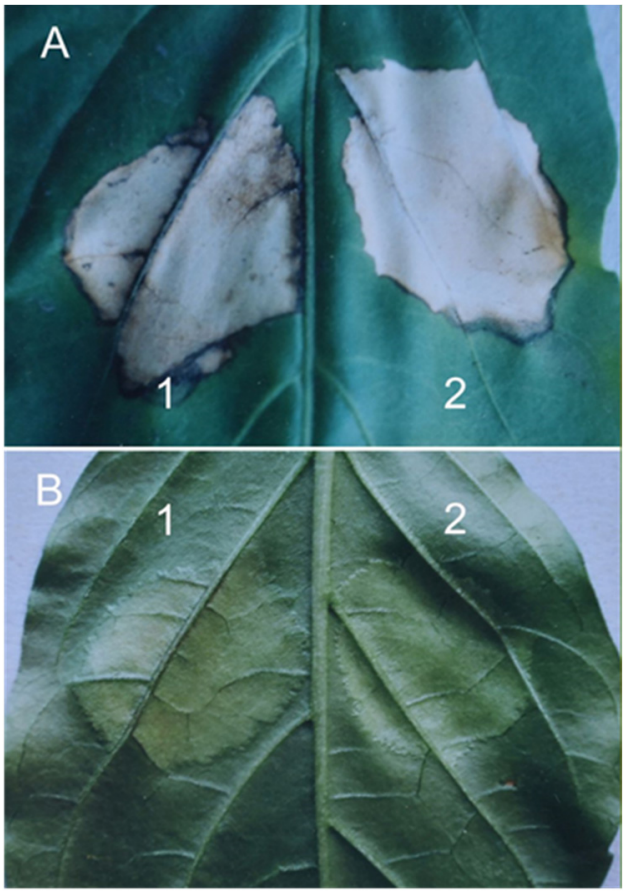

Figure 16. Pathological history of pepper line L330 (**A**) containing the *Bs2* gene and line X–(**B**) with strong tissue retention capacity induced by inocula of *Xanthomonas vesicatoria* (**1**) and *Phseudomonas phaseoicola* (**2**) at a concentration of 10^8 cells/mL, used for genetic analysis of GDR.

Because of the extremely rapid tissue death, it was assumed that none of the tissue-retaining *gdr* genes were present and, therefore, it is suitable for determining the inheritance of *gdr* genes. The F_1 generation of hybridization between the X lines and the L330 line showed signs of purple discoloration and partial tissue death suggestive of *Bs2* in all hybridization, which we identified as the L_L symptom type.

Individual plants of the F_2 generation were infected with inoculum of *X. vesicatoria* at concentration of 10^8 cells/mL and inoculum of *P. phaseolicola* at a concentration of 10^8 cells/mL, and individual plants of the fission population were phenotyped. This made it clear that, from a genetic point of view, the symptoms can be classified into three distinct phenotype categories, which at first sight assume 12:3:1 cleavage with the null hypothesis being that this distribution exists. Our assumption was subjected to Chi^2 test regression analysis.

The contingency table shows a value of 5.99 at a probability level of $p = 0.05$ at degree of freedom 2, the calculated Chi^2 value is less than this, so in all cases, the null hypothesis that the empirical frequencies are consistent with 12:3:1 cleavage of the dominant epistasis is accepted (Table 3). The genes carried by the direcessive homozygotes were named *gdr 1 + 2* genes and identified as manifestations of the complete GDR system. The *gdr 1 + 2* genes protect the plant tissue from pathogen destruction in both host and non-host relationships. The cleavages also show that one of the *gdr* genes can eradicate *P. phaseolicola* on its own (phenotype category $P_X + Z_P$) but can only defend against *X. vesicatoria* attack in combination with the other *gdr* gene in the form of a tissue retention green spot (phenotype category $Z_x + Z_p$).

Table 3. F2 population of X × L330 hybridizations.

Hybridization	Empirical Frequency			Theoretical Frequency 12:3:1			Chi [2]	Level of Significance
	P_X [1] + P_P [2]	P_X + Z_P [3]	Z_x [4] + Z_p	P_X + P_P	P_X + Z_P	Z_x + Z_p		
L1710/2 × L330	640	131	42	610	152	51	5.964	$p = 0.05$
L1713/2 × L330	260	51	23	251	63	21	2.799	$p = 0.05$
L1715/3 × L330	142	22	11	132	33	11	4.516	$p = 0.05$
L1716/4 × L330	368	91	20	359	90	30	5.034	$p = 0.05$

[1] P_X: necrotic spot formed by inoculation of *X. vesicatoria* at a concentration of 10^8 cells/mL.[2] P_P: necrotic spot formed by inoculation of *P. phaseolicola* at a concentration of 10^8 cells/mL. [3] Z_P: a green tissue-retaining spot formed by inoculation of *P. phaseolicola* at a concentration of 10^8 cells/mL. [4] Z_X: a green tissue-retaining spot formed by inoculation of *X. vesicatoria* at a concentration of 10^8 cells/mL.

3.4. Morphological and Histological Characterisation of GDR and Comparison with Other Resistance Genes

3.4.1. GDR and Bs2 Gene

Recognition and identification of GDR helps to interpret pathophysiology based on microscopic observation (Figure 17).

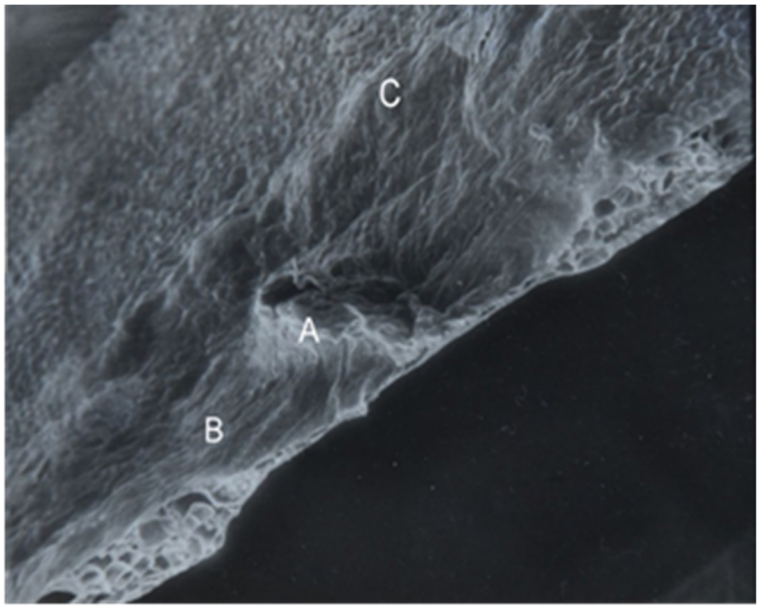

Figure 17. Lesion induced by *Xanthomonas vesicatoria* on a pepper line containing the *Bs2* gene with an incomplete GDR system.

In the presence of incomplete GDR, the tissue compaction at the infection point (Figure 17A) is not sufficient to block the pathogen. Therefore, the *Bs2* gene, which senses pathogen-induced physiological disturbances (susceptibility state), is activated and induces specific HR in the tissues surrounding the infection site with tissue compaction (Figure 17B). However, the HR-induced stress activates distant tissues, resulting in a cell enlargement-mediated tissue compaction barrier around the necrotic tissue spot (Figure 17C).

With the knowledge of the GDR, macroscopic modelling of the processes observed in the lesions was also performed. The design of the experiment is shown in Figure 12A. Susceptible pepper leaves were infiltrated with a suspension of *X. vesicatoria* at concentration of 10^8 cells/mL in a spot of 5 mm in diameter. After 10 min, when the inoculum squeezed into the intercellular passages was absorbed, it was superinfected with a suspension of *P. phaseolicola* at concentration of 10^6 cells/mL. In this way, we activated the incomplete GDR in a non-host relationship (Figure 18).

Figure 18. Leaves of susceptible peppers with incomplete GDR infiltrated with *Xanthomonas vesicatoria* (at a concentration of 10^8 cells/mL) in a spot of 5 mm, followed by super-infection with a suspension of *Pseudomonas phaseolicola* (at a concentration of 10^6 cells/mL), the border of which is marked by scarring.

The super-infection also resulted in further drift of *X. vesicatoria* bacteria in the intercellular passages, as indicated by yellowing of the area. Due to dilution and the activation of the GDR, this area of tissue has not dried out as the 5 mm spot in the centre. Scar tissue at the border of the spot infiltrated by *P. phaseolicola* indicates that the distal part of the leaf plate has been involved in the exclusion of the pathogen (Figure 19).

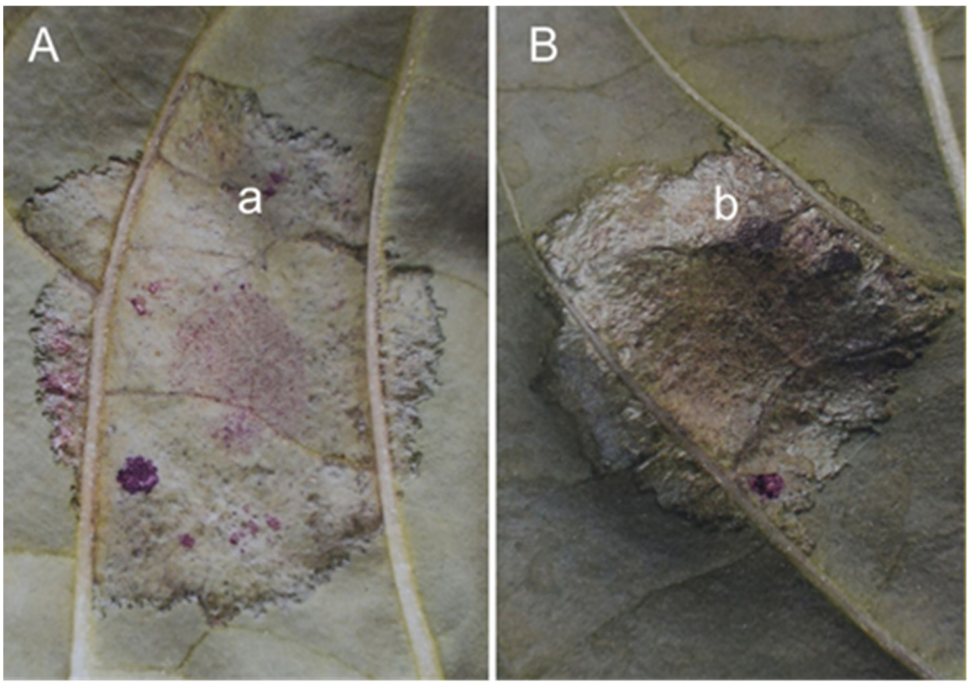

Figure 19. Tissue changes after infiltration of pepper lines having the *Bs2* gene and incomplete (**A**) and complete (**B**) GDR system (*gdr 1 + 2 genes*) with *Xanthomonas vesicatoria* bacteria (at a concentration of 10^8 cells/mL) in a spot of 5 mm and subsequent super-infection with a suspension of *Pseudomonas phaseolicola* at concentrations of 10^6 (**a**), 10^8 (**b**) cells/mL.

As described above, testing of pepper lines having incomplete (Figure 19A) and complete (Figure 19B) GDR system (*gdr 1 + 2 genes*) carrying the *Bs2* gene was also performed.

Suspension of *P. phaseolicola* at concentration of 10^6 cells/mL was used for superinfection of plants with incomplete GDR, and at concentration of 10^8 cells/mL for plants with complete GDR.

In the presence of incomplete GDR (19/A), the *Bs2* gene was activated by *X. vesicatoria*, although its role in defence is questionable. Complete GDR (19/B) formed a solid, shiny tissue spot in a non-host relationship, with no discolouration indicative of *Bs2* gene activation.

3.4.2. GDR and *gds* Gene

The *gds* and *gdr 1 + 2 genes* are indistinguishable on the basis of pathological symptoms induced by natural infection, as they induce cell enlargement and cell division that are common to plants. However, when unnatural inoculation is used, the two tissue retention mechanisms show distinctive differences. The *gds* gene induces a pronounced convexity in the leaves of young plants due to the 'accelerated' growth of infected spots (Figure 20A), whereas the *gdr 1 + 2* gene leaves the leaf plate flat upon emergence of the pathogenic symptom (Figure 20B).

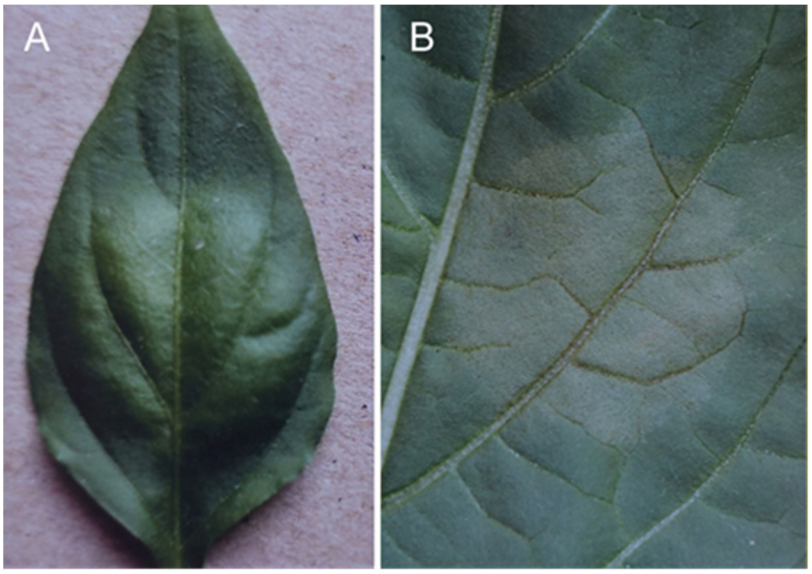

Figure 20. Leaf bulging induced by the *gds* gene in young pepper leaves (**A**) and the absence of bulging with *gdr 1 + 2* genes (**B**) in response to *Xanthomonas vesicatoria* infection.

In the case of complete infiltration of the leaf plate (Figure 21), the *gds* gene develops its characteristic convexity (Figure 21A), whereas the leaf plate containing the *gdr 1 + 2* genes remains flat (Figure 21B), the latter with purple vessels indicating that the *Bs2* gene is also present in this genome. In the presence of weak GDR, the leaf containing the *Bs*2 gene responded with complete destruction (Figure 21C).

Differences in the structural changes of infected tissues between *gds* and *gdr 1 + 2* genes can also be observed (Figure 22).

The *gdr 1 + 2* genes responded to stress induced by *P. Phaseolicola* (at concentration of 10^8 cells/mL) by forming a shiny compacted tissue (Figure 22A). The *gds* gene in an incomplete GDR background induces strong cell division and proliferation in response to *X. Vesicatoria* infection (at concentration of 8 cells/mL) (Figure 22B). The *gds* gene-induced cell proliferation is strongest in spongy parenchymal tissue (Figure 23).

However, such cell proliferation only occurs in plants with weak GDR. A characteristic difference between the *gds* and *gdr 1 + 2* genes for tissue retention includes the change in leaf vessels in response to infection (Figure 24).

Figure 21. Response to whole-leaf infiltration with *Xanthomonas vesicatoria* bacteria in the presence of *gds* (**A**), *gdr 1 + 2* (**B**) and *Bs2* gene in an incomplete GDR background (**C**).

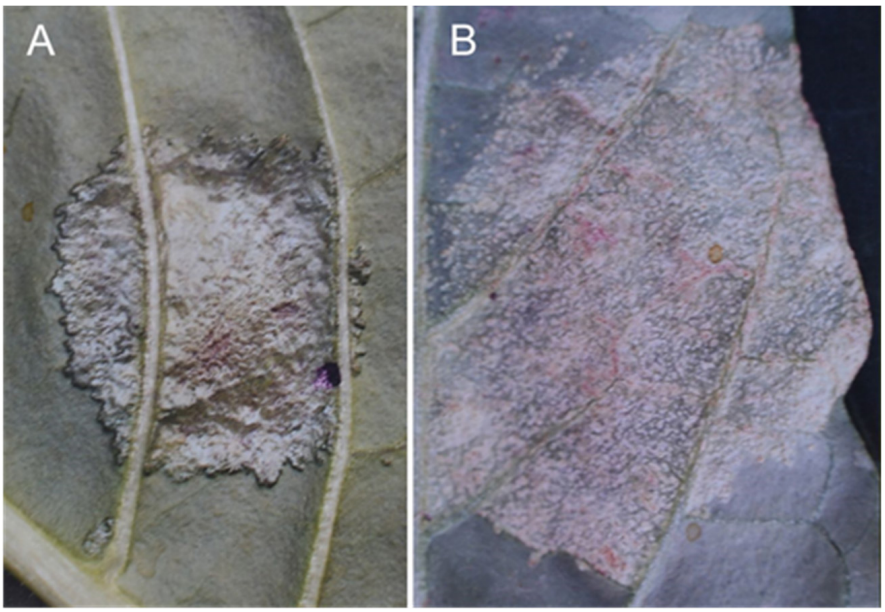

Figure 22. The *gdr 1 + 2* genes induced a shiny compacted tissue spot (**A**), and the *gds* gene resulted in uncontrolled cell division in an incomplete GDR background, upon *Xanthomonas vesicatoria*-induced stress (**B**) in pepper leaves.

Vessels passing through *X. vesicatoria*-infected spots rupture along the transport routes due to cell division induced by the *gds* gene (Figure 24A). Veins in infected spots of leaves containing *gdr 1 + 2* genes, as shown in Figure 19B, Figure 20B, and Figure 22A, show no change. If the *Bs2* gene is also present in the plant, the vessels may turn slightly

purple (Figure 24B). (In susceptible plants, whitening of the vessels is the first indication of damage.)

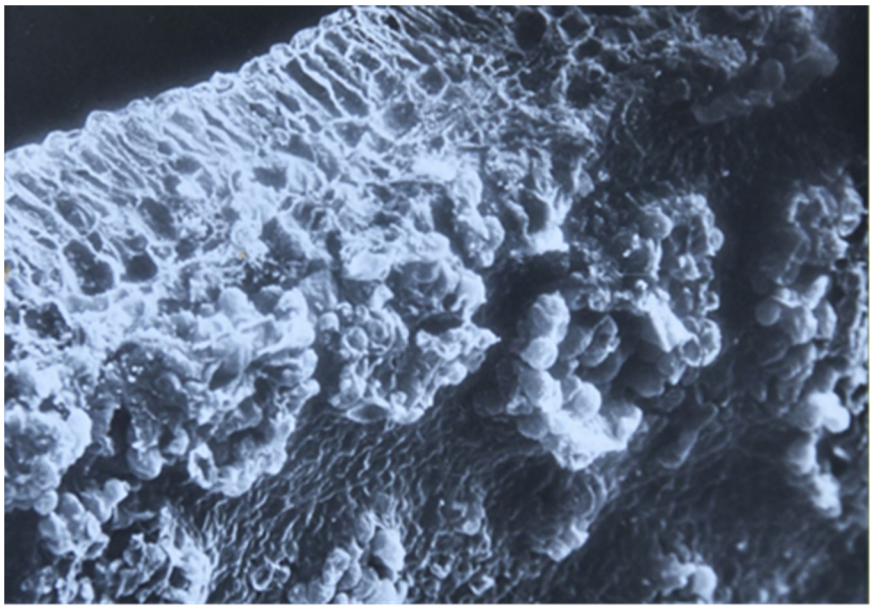

Figure 23. Cell proliferation in spongy parenchymal tissue of pepper leaves containing the *gds* gene, in response to *Xanthomonas esivcatoria* infection.

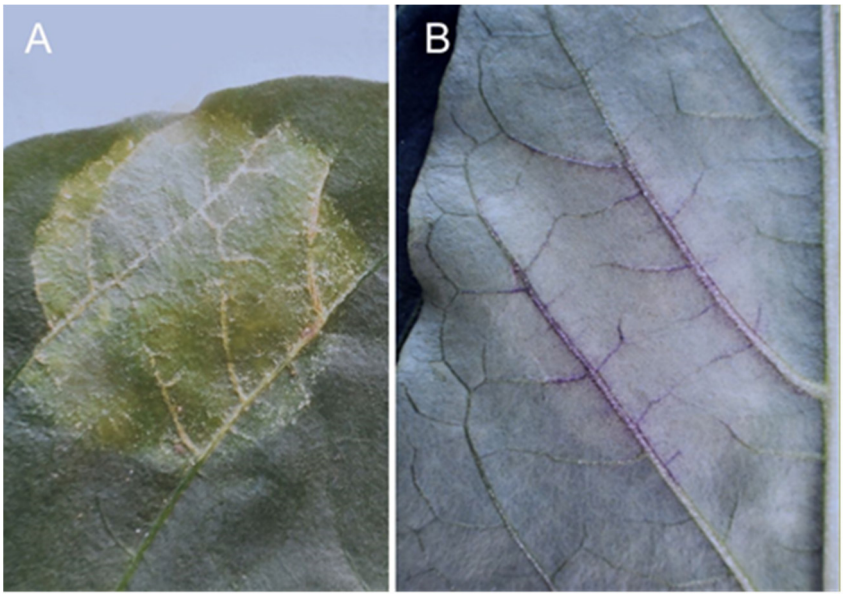

Figure 24. Vessel lesion caused by *Xanthomonas vesicatoria* on pepper vessels containing the *gds* gene (**A**) and mild purple discolouration of vessels of an infected spot also containing the *Bs2* gene, which responds in a manner characteristic of *gdr 1 + 2* genes (**B**).

The difference between the processes controlled by the *gds* and *gdr 1 + 2* genes is also reflected in the relationship to the *Bs2* gene. When a pepper line containing both the *gds* and *Bs2* genes in the homozygous state was infected with *X. vesicatoria*, a pathological symptom characteristic of the *gds* gene was observed, but the purple discolouration characteristic of the *Bs2* gene was not seen.

A pepper line homozygous for both the *gdr 1 + 2* and *Bs2* genes also shows lesions characteristic of the *gdr 1 + 2* gene, with the difference that the vessels may be slightly purple.

This may be due to, among other things, the compression on or damage of protruding vessels on the abaxial surface during infiltration.

In an incomplete GDR background, the vessels show strong purple discolouration (Figure 14C).

Mechanical damage to tissues has given rise to the idea of investigating the role of genes encoding tissue retention or tissue destruction in the pathogenesis.

In this experiment, a spot was infiltrated by applying strong mechanical pressure on the leaves of double homozygous pepper lines containing only the *gds* gene or both the *gds* and *Bs2* genes, and the affected spot and its environment were infiltrated at these points with a suspension of *vesicatoria* at concentration of 10^8 cells/mL. The inoculation procedure is shown in Figure 12B. In the case of the *gds* gene, the inoculated tissue spot responded in a manner showing susceptibility, while tissue retention characteristic of the *gds* gene was observed in the surrounding tissue (Figure 25A). The centre of the point of the double homozygous line injured by pressure dried out, but the surrounding ring was purple, indicative of the *Bs2* gene. The *Bs2* gene was, therefore, only activated in the damaged tissue. Surrounding tissues showed a tissue retention response of the *gds* gene (Figure 25B).

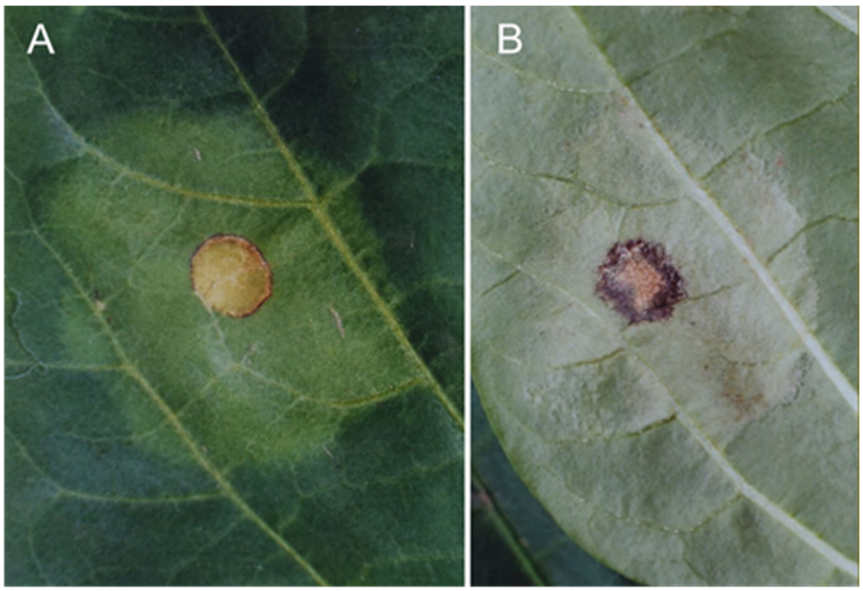

Figure 25. Healthy and pressure-stressed tissue of a pepper line containing the *gds* gene inoculated with *Xanthomonas vesicatoria* responding with tissue compaction, indicative of susceptibility (**A**) and the specific defence response of healthy and pressure-stressed tissue of a plant carrying *gds* and *Bs2* genes, respectively, indicative of the *Bs2* gene (**B**).

It is difficult to observe the phenotypic characteristics of the *gds* and *gdr 12* genes because their appearance in the same plant is uncertain due to their interactions. Importantly, in the presence of weak GDR, as with HR genes, the *gds* gene does not appropriately function.

Since all plants have some level of general defence response, function, and expression of neither the specific resistance gene (*Bs2*), nor the *gds* gene can be studied without the influence of GDR. However, the GDR can be tested on its own. This fact also points to its fundamental role in the plant's defence mechanism.

In the case of a weak GDR system, the *gds* gene does not function properly in environmental conditions unfavourable to the plant, whereas the complete GDR (*gdr 1* + *2*) gene provides sufficient protection even in this case (Figure 26).

The *gds* gene (Figure 26A) was unable to protect infected spots from the stress effect of either *X. vesicatoria* (Figure 26(A1)) or *P. phaseolicola* (Figure 26(A2)) inoculum at concentration of 10^8 cells/mL, resulting in chlorosis of the entire leaf and subsequent shedding. In the case of *gdr 1* + *2* genes (Figure 26B), neither *X. vesicatoria* (Figure 26(B1)) nor the

bacterial suspension of *P. phaseolicola* (Figure 26(B2)), which induced a stronger stress due to exotoxin production, was able to cause tissue destruction and necrosis.

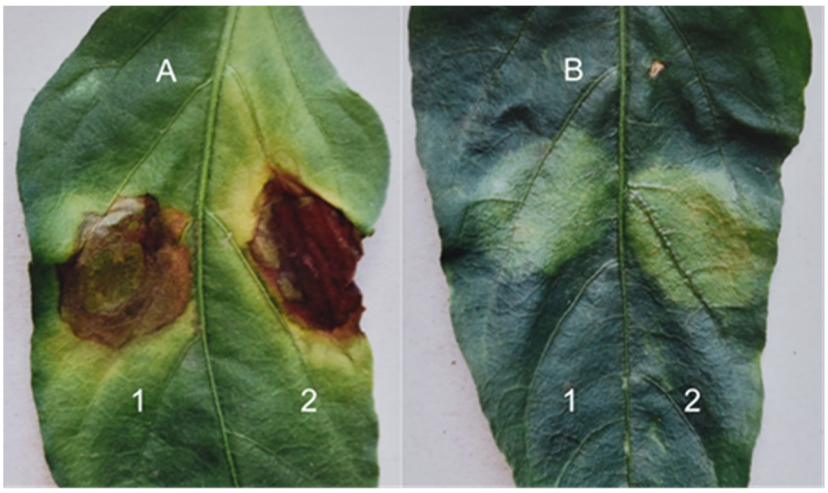

Figure 26. Protection provided by the *gds* gene (**A**) in a weak GDR structure and by the complete GDR (*gdr 1 + 2* genes) (**B**) under unfavourable environmental conditions against stress caused by *Xanthomonas vesicatoria* (**1**) and *Pseudomonas phaseolicola* (**2**) bacterial suspension at concentration of 10^8 cells/mL.

4. Discussion

4.1. Tissue Destruction and Tissue Retention in Resistance

The decades-long history of resistance breeding based on rapid tissue death has revealed several shortcomings. The findings hitherto considered as fundamental that a specific resistance gene prevents the spread of the pathogen in the plant by the development of specific HR, is only partially true.

4.1.1. Role of Specific Resistance Genes Encoding Hypersensitivity in Pathogenesis

A plant carrying a specific resistance gene suffers significant tissue loss due to the action of its tissue-destructive resistance gene when exposed to a pathogen that disrupts its life processes. This loss can be increased by breeding with a cluster of specific HR genes, which, in the event of an epidemic, may result in susceptible varieties producing more than those with more HR genes [17]. The strong dependence of HR gene function on environment also poses a high risk (Figure 13).

In our work we found that specific HR genes alone are not able to inhibit the pathogen even under optimal conditions. The general defence response (GDR), based on the tissue retention capacity of plants, plays a crucial role in inhibition. We have demonstrated that there is a strong correlation between small lesion diameter and high GDR. In general, this property determines the size of the lesions in the presence of specific HR genes, i.e., the effectiveness of the defence against the pathogen.

The practice in resistance breeding is to identify resistant plants with specific HR genes based rapid tissue death 24–48 h after inoculation. As a consequence, plants with poor GDR are the basis for resistance breeding work. Specific HR genes, on the other hand, are ineffective in weak GDR, as they attempt to prevent pathogen spread in a downstream way rather than in a preventive manner. This is a fundamental problem in their effectiveness.

4.1.2. Relationship between Susceptibility and the General Defence Reaction

The hypersensitivity of plants lacking specific resistance genes to host–pathogen and non-host–pathogen interactions was investigated in tobacco.

We determined the overall tissue resistance of the tested plants, which was characterized by stress levels expressed as inoculum concentrations. Since tissue retention was

present in both host and non-host relationships, we recognized this phenomenon as the general defence response, GDR.

We further found that in the host–pathogen relationship, a state of susceptibility was formed at the boundary of the GDR and the switch to nonspecific HR (Figure 2). We concluded that tissue retention is a fundamental determinant of plant susceptibility to pathogens.

4.1.3. Interaction between the General Defense Reaction and the Specific Defense Reaction

The role of GDR, susceptibility, and HR in the pathogenesis was investigated in the case of pepper-*X. vesicatoria* relationship.

In our experiment, susceptible pepper lines containing the *Bs2* gene had similar levels of GDR. The susceptible host–pathogen relationship is characterized by the fact that the pathogen enters the plant without causing stress and only becomes detectable to the plant during its accumulation. In the experiment, this stress effect was induced by the concentration of 10^8 cells/mL and the susceptible pathogenic symptom developed. The *Bs2* gene of the pepper line was also activated at the same stress level expressed as the inoculum concentration and developed its characteristic pathology. Thus, when the GDR is exceeded, the specific resistance gene is activated only after the development of the susceptible physiological state, in a sort of downstream manner.

The level of tissue resistance, therefore, determines the initiation of the susceptible pathological process. For the plant, the pathogenesis is the result of the general tissue retention of the GDR and the tissue destruction of the specific HR. The resulting balance between the plant and the pathogen determines the size of the lesion and, in the case of infiltration, the degree of tissue retention.

Electron microscopy images of the lesions also show that, in addition to the tissue-destructive capacity of the plant to produce HR, another property, GDR, which is involved in tissue retention, also participates in the process. Starting from the point of infection, cells infected by the pathogen are destroyed by HR in a downstream manner until the GDR prevents further spread of the pathogen by forming a ring of tissue compaction (Figure 17). The diameter of the lesion, and, thus, the effectiveness of pathogen inhibition, is determined by the GDR.

Examining the role of the GDR-labelled trait in the formation of lesions, it was confirmed that GDR forced to function in a non-host relationship significantly inhibited the destruction of tissue formed in a susceptible relationship (Figure 18).

In the case of infection by infiltration, the *Bs2* gene used as an indicator clearly shows the difference in GDR levels between pepper lines (Figure 14). The pathology demonstrates that GDR plays a crucial role not only in determining susceptibility but also in the development of pathological symptoms caused by the *Bs2* gene. Incomplete GDR provides partial protection against *Bs2* gene-driven tissue destruction, whereas complete GDR (*gdr 1 + 2*) provides complete protection against *Bs2* gene-driven tissue destruction (Figure 19).

4.2. Genetic Analysis

In order to apply the general defence response in resistance breeding, we started to investigate the heritability of GDR, assuming that we are likely to be dealing with multiple gene traits. A line with a complete GDR system, which gave a tissue-retaining green spot after leaf infection with the inoculum of *X. vesicatoria* at concentration of 10^8 cells/mL and *P. phaseolicola* at a concentration of 10^8 cells/mL for both pathogens, was marked with X. This line was hybridized with a line containing the *Bs2* gene, which, on the contrary, responded to the same two pathogens with extremely rapid and vigorous tissue destruction, and is, therefore, presumed to lack any tissue retention gene. Three distinct phenotypic categories were found in the F2 generations. The empirical cleavages followed well the 12:3:1 phenotypic frequencies of the dominant epistasis, which was confirmed by the Chi^2 statistical regression test for all four hybridizations. It was found that the complete GDR system is only established when both *gdr* genes (*gdr 1 + 2*) are present in the homozygous

state. Based on the observed symptoms and cleavages, it can be assumed that one of the *gdr* genes is able to form a tissue-retaining green spot in the homozygous state following infection with *P. phaseolicola* inoculum at a concentration of 10^8 cells/mL (Figure 10) and *X. vesicatoria* inoculum at a concentration of 10^8 cells/mL (Figure 10). However, both *gdr* genes must be in a homozygous state to form the tissue-retaining green spot (Figure 9). This clearly shows that the two *gdr* genes form one system in which one gene works independently and the other helps the other *gdr* gene to fight against *Xanthomonas* infection. We plan to explore these two processes in more detail in the future.

The F_1 generation of a hybridization performed by Csilléry et al., to study the genetic relationship between *Bs2* and *gds*, despite the inheritance pattern of the *Bs2* gene being found to be dominant, responded to infection with *X. vesicatoria* bacteria with green spots and purple vessels indicative of tissue retention, instead of purple tissue changes [18].

The pathological symptoms were correctly defined, but due to the incomplete knowledge of GDR at that time, the relationship between the two genes was not fully elucidated.

4.3. Phenotypical Deviation of Tissue Retaining Reactions

The known *gds* gene and the *gdr 1 + 2* genes, which also regulate tissue retention, are difficult to distinguish on the basis of their pathogenic symptoms, since both tissue retention mechanisms protect against microbial attack by inducing cell enlargement and cell division, which are fundamental to the plant. However, if excessive inoculation methods are used, the characteristics of the two traits can be distinguished (Figures 20–24).

4.3.1. Comparison of Pathogenic Symptoms Induced by *gdr 1* + 2 Genes and *gds* Gene on Pepper Leaves

In young leaves of plants containing the *gds* gene, the infected spot may bulge due to enlargement of columnar parenchyma cells (Figure 20A), whereas in more mature leaves, spongy parenchyma cells may respond to infection by meristematic cell proliferation (Figures 22B and 23). In *gdr 1 + 2* gene-directed defence, the leaf plate remains flat (Figures 20B and 21B), tissues may become shiny, and the leaf plate may thicken (Figure 22A). In both cases, infected spots on the leaf plate may be slightly chlorotic.

When the whole leaf is infiltrated (Figure 21), the *gds* gene is characterised by bulging (Figure 22A) and the *gdr 1 + 2* genes by flattening (Figure 21B), indicating a differential change in leaf tissue.

The vascular response induced for the *gds* gene is the rupture of vessels as a consequence of cell division. For the *gdr 1 + 2* genes, no visible change occurs in the vessels (Figure 20B), but if the *Bs2* gene is present in the plant, the vessels may turn slightly purple (Figure 21B).

Plants are protected against any microbial attack by the general defence response, as a primary defence front, by strengthening the cellular wall. Reactive oxygen species (ROS) play an important role in the immediate strengthening of the cell. Basic plant life processes, such as lignin synthesis for cellular wall formation and degradation processes, are also accompanied by specific amounts of H_2O_2 [14]. Since H_2O_2 is also released during the initiation of pathogen-induced tissue destruction [19–21]), it confuses the *Bs2* gene, which detects H_2O_2 as a signalling molecule and is triggered by it. The purple discolouration of vessels in the presence of the *gdr 1 + 2 gene* indicates excess *Bs2* gene activation.

4.3.2. Difference between the Processes Controlled by *gds* and *gdr* 1 + 2 Genes in Relation to the *Bs2* Gene

If a pepper line homozygous for both the *gds* and *Bs2* genes is infected with *X. vesicatoria*, pathological symptoms specific for the *gds* gene are obtained. We have never observed purple discolouration characteristic of the *Bs2* gene. This is confirmed by testing the ECW 1–6 pepper lines containing the *Bs1* (sHR), *Bs2* (sHR), *Bs3* (sHR), *Bs4* (sHR), *bs5* (*gds*), and *bs6* (S) genes.

If, in addition to the *gdr 1 + 2* genes in the homozygous state, the *Bs2* gene is also part of the plant genome, then purple vessels may occur, but this is not inherent to the disease

process, but is a mis-activation of the *Bs2* gene caused by excess H_2O_2 accompanying lignin synthesis in the transportation pathways. This was never observed for the *gds* gene.

When mechanical pressure was applied to a point on a leaf of a pepper line containing only the *gds* gene or double homozygous for the *gds* and *Bs2* genes, inoculated with the bacterium *X. vesicatoria*, and a disruption of life processes was induced, the first case showed susceptible disease, the second case showed purple lesions indicating the destruction by the *Bs2* gene. The *Bs2* gene only acted in a downstream manner upon detecting a disturbance in cell function (Figure 25). The unaffected tissues around the pressure points responded with a green tissue spot induced by *X. vesicatoria* bacterium, characteristic of the *gds* gene.

It applies for both *gds* and *gdr 1 + 2* genes that they only function in cells with complete integrity and that only intact tissues are capable of tissue retention responses, which are characterised by a low stimulus threshold and high reaction rate.

Our experiments demonstrate that specific resistance genes work most effectively in a background of strong general defence responses. So much so that in the case of a complete GDR (*gdr 1 + 2* genes), they remain in a latent, repressed state and, therefore, become redundant and represent only a genetic burden to the plant (Figure 19).

4.3.3. Application of the Tissue Retention *gds* and *gdr* 1 + 2 Genes in Resistance Breeding

The importance of specific HR genes in resistance breeding has been overestimated, as plant-microbe interactions are numerous, host–pathogen interactions are numerous, and host–pathogen interactions carrying specific resistance genes are few in number compared to these. The latter are not sufficient to protect different plant species against microorganisms.

The search for specific HR genes in resistance breeding goes back hundreds of years. Their widespread use has made resistance breeding a one-plane process, excluding the consideration of any other plant defence system in the selection work. The use of the known dominant resistance genes (*Bs1*, *Bs2*, *Bs3*) does not result in durable resistance because their use in field monocultures leads to the emergence of new pathogenic variants [22]. In contrast to specific HR responses involving different tissue destruction, for almost thirty years we have been investigating types of defence systems involving tissue retention, which are a different way for resistance breeding. We expressed this by not putting the tissue retention *gds* gene described in 1995 in the then next fourth position of the *Bacterial spot = Bs* gene sequence, because it would have narrowed the spectrum of action of the gene found. In fact, *gds* is not only a resistance gene against Xanthomonas, but also a non-pathogen specific gene with a broad spectrum of action (less sensitive to temperature). It is the first known resistance gene that protects pepper tissues and does not destroy them. Resistance defined by the *gds* gene is not associated with a hypersensitive response and no special effector or avirulence factor appears to be involved in the interaction of the host plant with the gene products in the development of resistance symptoms [23]. When the *gds* gene is used, the assimilation surface of the plant is not reduced during pathogen control.

In order to naturally incorporate the beneficial properties of genes conferring tissue retention into the breeding program, we needed to understand their physiological roles and interactions. In doing so, we looked at the role of tissue retention in manifestation of pathogenic symptoms in other plant species (Figure 7). Until then, the phenomenon that tissues of organs, including leaves, that have specialised from a meristematic state can regain their ability to divide in response to biotic stress, was unknown in plant pathology. This property has led to a significant increase in the efficiency of producing dihaploid plants from plants containing the *gds* gene and to initiatives to incorporate it into other plant species.

The discovery and practical application of the recessive resistance gene in pepper is undoubtedly a paradigm shift in resistance breeding. Following the description of the novel *gds* gene [9], only the *bs5* gene was reported [11], followed by the isolation of the *xcv-1* gene [24]. The equivalence of *gds = bs5 = xcv-1* has been demonstrated based on literature and test hybridization [12,25].

The last decade has witnessed a spread of practical applications of the *gds* gene. A clear sign of this spread is that since 2014, major breeding companies around the world have been acquiring the know-how to use the *bs5* gene from the 2Blades Foundation (Evanston, IL, USA) for their pepper breeding programmes (Figure 27) [26].

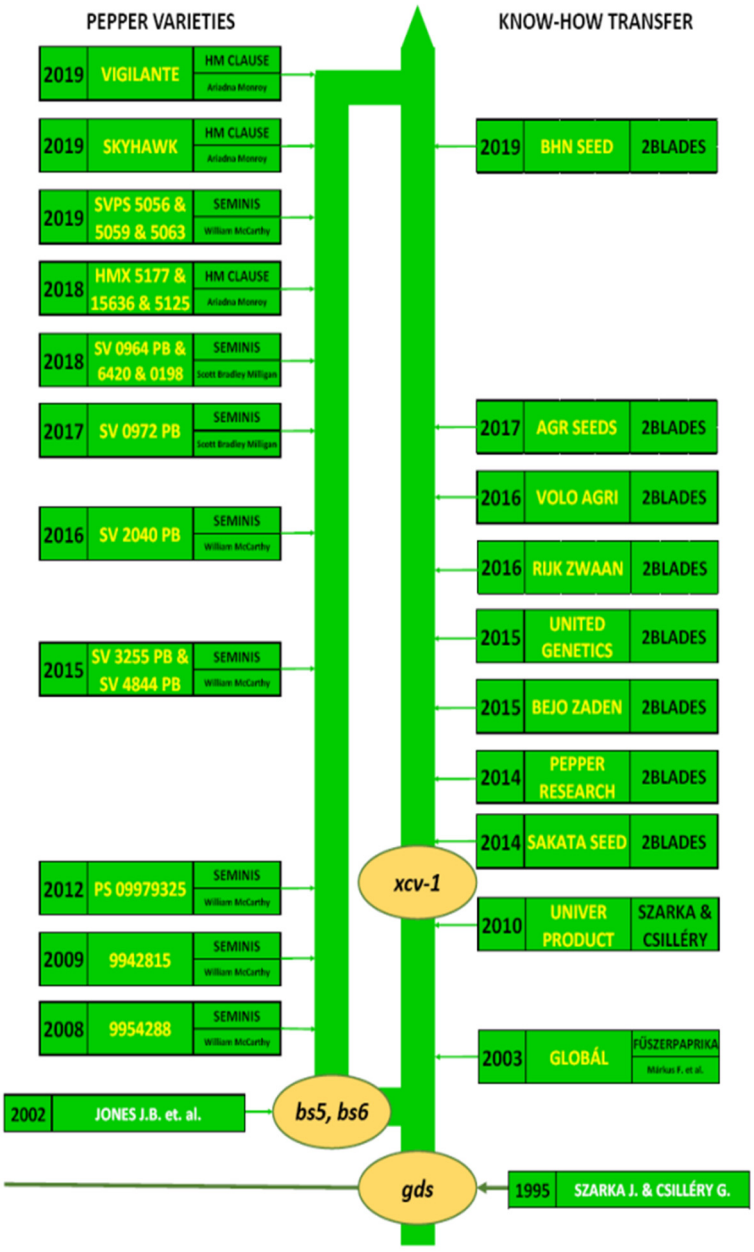

Figure 27. Twenty-five years of non-hypersensitive, non-specific recessive resistance in pepper [26].

In addition to pepper, the applicability of the *gds* (*xcv-1*) gene in other plant species has also been discussed. A patent application has been filed for the identification of the *xcv-1* gene by cloning based on gene mapping and for procedures to develop plants with resistance. The name *xcv-1* was derived from the renaming of *gds*. During the process, this right was also transferred from the Agricultural Biotechnology Research Centre (Hungary) to the 2Blades Foundation (USA) [24].

The complex trait system of tissue retention has been under continuous investigation since the discovery of *gds*. The result of this work includes the *gdr 1* + *2* gene and pathological experience with GDR function. The use of the *gds* gene in pepper breeding or its

incorporation into other plant species carries the risk that the tissue retention gene may not function properly in the presence of incomplete GDR (Figure 26).

The *gds* is a genetic code that is activated by various stresses and has so far only been found in one pepper line. Its efficient functioning and success in breeding is attributable to the GDR background, as is the case for the HR gene.

As for the relationship between the genes encoding tissue retention, the *gds* gene is dependent on GDR for its function, while the *gdr 1 + 2* genes can function completely independently of *gds*.

GDR is the basis of plant defence. It has been experimentally demonstrated to reduce susceptibility-induced mortality, to protect against self-destruction encoded by specific HR genes, to promote *gds* gene function, to aid adaptation to abiotic stresses and resistance to biotic stresses, to have a low stimulus level, and a high and rapid responsiveness. All these factors make it suitable for the role of the plant immune system.

The classification of the multitude of plant–microbe interactions into host–pathogen and non-host–pathogen or compatible and incompatible opposition pairs makes it difficult to understand the natural processes that we want to express.

On pepper, *X. campestris pv. vesicatoria* (compatible host–pathogen relationship) develops a susceptibility pathogenesis, but in the presence of *gds*, *gdr 1 + 2* genes, pepper defends against infection by *X. campestris pv. vesicatoria* with the same tissue retention response as against infection by the bean pathogen *P. phaseolicola* (incompatible non-host–pathogen relationship with pepper).

Consequently, the genes encoding tissue retention have transformed a host–pathogen relationship into a non-host–pathogen relationship. This provides us with a form of defence that has evolved over the evolutionary history of plants with exceptional efficiency.

The role of the newly identified *gdr 1 + 2* genes in disease processes downstream of the *gds* gene may point to a new direction in resistance breeding.

Half a century of experience in studying hundreds of thousands of pathogens provoked and studied in various plant–microbe relationships has guided us from specificity to generalised protection.

We have found confirmation of this in the observations and ideas of the founder of stress theory, János Selye, in his book entitled In vivo. Such observations include the "vigorous mitotic cell proliferation" observed in animal experiments in response to biological stressors and the summarizing formulation that, "All observable biological effects produced by various stimuli are the sum of two components, the specific effect and the nonspecific response. The latter may even mask the specific effect!" [27].

5. Conclusions

The inadequacies of the history of resistance breeding and our present work demonstrate that specific HR genes alone cannot safely protect the plant because they only act in a downstream manner in cells attacked by the pathogen, inducing cell death.

A decisive role in the defence of plants containing HR genes is played by the plant's tissue retention capacity, which is expressed in the general defence response (GDR). GDR pre-emptively excludes the pathogen without tissue loss.

Specific HR genes do not protect but destroy the cells affected by pathogens. GDR genes protect the plant by strengthening the cells. The difference in the stimulus thresholds of the two responses also determines the order and effectiveness of the response. The non-pathogen specific GDR plays the role of the plant immune system due to its low stimulus threshold and high reaction rate.

Tissue retention capacity can be increased by breeding to a level where the GDR alone can provide adequate protection for the plant. In this case, the presence of specific HR genes is unnecessary; sometimes it is even a burden to the plant.

In our work on tissue retention in pepper, we found that the GDR system we studied is regulated by two recessive genes (*gdr 1 + 2*) that are completely independent of the HR-conferring *Bs2* gene and the previously identified tissue retention *gds* gene.

Studies on pepper demonstrate that plant resistance can be made safer without the incorporation of specific HR genes, relying solely on GDR.

An important point is that while the function of specific HR genes is dependent on the environment, GDR is as independent as possible from environmental factors. It functions under the extreme conditions that the plant can tolerate, acting as a plant immune system.

Host plant–pathogen contacts carrying specific HR genes are very rare in nature, while there are a lot of host–pathogen contacts and plant–microbe contacts are countless. This is possible because all plants have GDRs. Consequently, if we want to integrate resistance breeding into the order of nature, our work must be based on the tissue retention capacity of plants.

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