Review

Advances and Challenges in Bacterial Spot Resistance Breeding in Tomato (*Solanum lycopersicum* L.)

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Abstract: Bacterial spot is a serious disease of tomato caused by at least four species of *Xanthomonas*. These include *X. euvesicatoria* (race T1), *X. vesicatoria* (race T2), *X. perforans* (races T3 and T4), and *X. gardneri,* with the distinct geographical distribution of each group. Currently, *X. gardneri* and *X. perforans* are two major bacterial pathogens of tomato in North America, with *X. perforans* (race T4) dominating in east-coast while *X. gardneri* dominating in the Midwest. The disease causes up to 66% yield loss. Management of this disease is challenging due to the lack of useful chemical control measures and commercial resistant cultivars. Although major genes for resistance (*R*) and quantitative resistance have been identified, breeding tomato for resistance to bacterial spot has been impeded by multiple factors including the emergence of new races of the pathogen that overcome the resistance, multigenic control of the resistance, linkage drag, non-additive components of the resistance and a low correlation between seedling assays and field resistance. Transgenic tomato with *Bs2* and *EFR* genes was effective against multiple races of *Xanthomonas*. However, it has not been commercialized because of public concerns and complex regulatory processes. The genomics-assisted breeding, effectors-based genomics breeding, and genome editing technology could be novel approaches to achieve durable resistance to bacterial spot in tomato. The main goal of this paper is to understand the current status of bacterial spot of tomato including its distribution and pathogen diversity, challenges in disease management, disease resistance sources, resistance genetics and breeding, and future prospectives with novel breeding approaches.

Keywords: bacterial spot; genome editing; host resistance; resistance breeding; transgenics; *Xanthomonas*

1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is an important vegetable crop in the United States and worldwide. Tomato is also a model crop to study fleshy fruit development and plant-pathogen interactions. Every year, tomato is inflicted with several bacterial, fungal, and viral diseases, including bacterial spot limiting its production. Bacterial spot (BS) disease is one of the major threats to tomato production that affects both fresh-market and processing tomatoes. The disease also occurs in pepper and other crops of the Solanaceae family [1]. Depending on the stage of infection and favorable climatic conditions, the disease can cause yield losses up to 66% [2,3]. In Florida, the tomato yield loss due to bacterial spot was estimated to be $3000 per acre ($7413 per ha) [4]. Likewise, ~$4 million to $12.5 million tomato yield losses have been reported in the Midwest of the USA [5]. Unfortunately, there are no commercial resistant tomato cultivars to this disease. The disease management relies on

the spray of copper and antibiotics, although resistant bacteria to such chemicals have been reported from several regions. Host resistance is critical to manage the BS disease in tomato. Here, we review the diverse nature and species complex of the pathogen, available host resistance sources to BS disease, the genetic and breeding efforts deployed so far, including transgenic approach, and the challenges involved in BS disease resistance breeding. We also discuss the novel breeding strategies such as genomics-assisted breeding, effector-based breeding, genome editing, and high throughput phenotyping-based breeding that can be implemented to improve BS disease resistance in tomato. Our review will provide valuable information to tomato breeders and other researchers working in this area to improve tomato for BS disease resistance.

2. Causal Pathogen, Race Structure, and the Distribution

Bacterial spot of tomato is caused by at least four species of *Xanthomonas*: *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* and four pathogenic races (T1, T2, T3, and, T4) are identified so far [6]. The T1 race has been reported in *X. euvesicatoria*, T2 race in *X. vesicatoria* and in *X. gardneri*, while T3 and T4 races are reported in *X. perforans* [6]. Mutation in effector genes *avrXv3* and *avrXv4* of races T3 and T4 belonging to *X. perforans* have also been identified and speculated to be race T5 [7]. However, isolation of race T5 under field conditions has not been reported yet. These pathogenic races are discovered based on hypersensitive responses (HR) on tomato differentials that include HI 7998 (T1), HI 7981 (T3), and LA716 (T4). However, no differential tomato germplasm is available showing HR response to race T2. This is why race T2 has been reported in both *X. vesicatoria* and *X. gardneri*.

Xanthomonas euvesicatoria was the only species, present as race T1, in Florida until 1991 before the *X. perforans* race T3 strain was reported. *Xanthomonas perforans* race T4 strain evolved in 1998 [7,8], and thereafter has been detected at higher numbers in field surveys in Florida. In a recent survey, only *X. perforans* race T4 were detected in the samples collected from different sites of Florida, and 94% of the sampled strains were *X. perforans* race T4 in North Carolina [3,9]. *Xanthomonas perforans* race T4 strains have also been reported in Louisiana [10]. *Xanthomonas gardneri* was found on a contaminated seed lot in 1991 and has since become widespread in the Midwest USA and Ontario Canada [11]. Both *X. gardneri* and *X. perforans* are predominant in Ontario, Canada [12]. *Xanthomonas gardneri* has been reported from tomato fields in Pennsylvania [13], and was responsible for the epidemics in the Midwest [5].

3. Plant-Pathogen Interactions and BS Disease Resistance

Host resistant *R* proteins mediate a response to effectors and activate plant defense responses such as effector-triggered immunity (ETI), which is characterized by localized cell death, termed as a hypersensitive response (HR) [14]. At least, 45 effectors have been reported in the bacterial spot. Among them *AvrBs2, XopD, XopF1, XopK, XopL, XopN, XopQ, XopR, XopX, XopZ1,* and *XopAD* represent the core effectors required during pathogenesis [1]. Tomato contains 355 *R* genes encoding the nucleotide-binding site-leucine-rich repeat (NB-LRR) proteins, and the role of each NB-LRR in the disease resistance is being studied [15]. Although only a few *R* genes against bacterial spot have been identified in tomato, these genes have not been fully utilized in the breeding program either because of a lack of markers or the evolution of new pathogen races.

Both the qualitative resistance and quantitative resistance have been identified in several tomato species in response to bacterial spot disease. The qualitative resistance characterized by HR has been identified to race T1 in *S. lycopersicum* accessions HI 7998 [16]; to race T3 in *S. lycopersicum* accessions HI 7981 and *S. pimpenellifolium* accessions PI128216 and PI 126932 [17,18]; to race T4 in *S. pennellii* accession LA716 [8]; and to *X. gardneri* in *S. pimpenellifolium* accessions LA2533, LA1936, and PI 128216 [19] (Table 1). Similarly, quantitative resistance against bacterial spot has been identified in *S. lycopersicum var. cerasiformae* accession PI 114490 (all races) [20,21]; *S. pimpenellifolium* accessions PI 126428, PI 340905-S, and PI 155372 to race T3 [17]; and *S. pimpenellifolium* accessions PI 128216 and PI

126932 to race T4 [17,18,20]. The availability of sources of resistance offers an opportunity to develop tomato cultivars resistant to existing races of these pathogens.

Table 1. Summary of breeding efforts for resistance to different races of *Xanthomonas,* causing bacterial spot disease in tomato.

Race Type	Resistance Genotype	Species [a]	Resistant Genes [b]	Chromosome [c]	Bacterial Effector [d]	Markers [e]	References
T1 (before 1989)	HI 7998 (HR)	*S. lycopersicum*	*Rx3*	5	*avrRxv*	Rx3 -L1, SP5, TOM196 (SSR), TOM144 (SSR), COSOH57(SNP)	[16,18,22–26]
			Rx2	1			[16,22–24]
			Rx1	1			[16,22–24]
	PI114490	*S. lycopersicum var. cerasiformae*	quantitative	2,3,10,11			[21]
T2 (1989)	PI114490	*S. lycopersicum var. cerasiformae*	quantitative	2,3,10,11	deletion of 680 bp region of *avrRxv*		[20–22,27,28]
T3 (1992)	HI 7981 (HR)	*S. lycopersicum*	*Xv3*	11	*avrXv3*	cLEC-24-C3 (SNP), SL10029 (SNP)	[17,18,29,30]
	PI 128216 (HR)	*S. pimpenellifolium*	*Rx4*	11	*avrXv3*	pcc12	[17,31,32]
	PI 126932 (HR)	*S. pimpenellifolium*	*Rx4*	11	*avrXv3*		[18,33]
	LA716 (HR)	*S. pennelii*	*Xv4*		*avrXv4*		[8]
	LA 1589 (HR)	*S. pimpenellifolium*	*Rx$_{LA1589}$*	11	*avrXv3*		[34]
	PI114490	*S. lycopersicum var. cerasiformae*	quantitative	2,3,10	-	-	[21]
	PI 126428		quantitative	-	-	-	[17]
	PI 340905-S		quantitative	-	-	-	[17]
	PI 155372		quantitative	-	-	-	[17]
	Fla7600	breeding line	*Rx3* and *Xv3*		-	-	[18]
	Fla 8233	breeding line	quantitative				[35]
	Fla 8517	breeding line	quantitative				[35]
	Fla 8326	breeding line	quantitative				[35]
T4 (1998)	LA 716 (HR)	*S. penneli*	*RXopJ4*	6	*XopJ4*	J350 & J352	[8,36,37]
	PI114490	*S. lycopersicum var. cerasiformae*	quantitative	2,3,10,11	-	C2_Atlg10050 for QTL on chr 11	[21,38]
	PI 128216	*S. pimpenellifolium*	quantitative	-	-	-	[39]
	PI 126932	*S. pimpenellifolium*	quantitative	-	-	-	[39]
	Fla 8233	breeding line	quantitative	11	-	-	[35,38]
	Fla 8517	breeding line	quantitative	3, 11	-	-	[35,38]
	Fla 8326	breeding line	quantitative	11	-	-	[35,38]
X. gardneri	LA2533 (HR)	*S. pimpenellifolium*	-	-	-	-	[19]
	LA1936 (HR)	*S. pimpenellifolium*	-	-	-	-	[19]
	PI 128216 (HR)	*S. pimpenellifolium*	-	-	-	-	[19]

[a] Tomato species in which corresponding resistant genotype belongs to. [b] Resistant genes identified in a respective genotype against specific race. [c] Chromosomes in which bacterial spot resistance loci are located in tomato. [d] Bacterial effectors present in corresponding *Xanthomonas* race. [e] Molecular markers developed for the selection of respective resistance loci in tomato.

4. Genetics and Breeding Efforts to Improve the BS Disease Resistance So Far

Breeding tomato for resistance to BS disease has been challenging due to the evolution of new races of the pathogen limiting the effectiveness of known resistance genes, multigenic control of the resistance, and non-additive gene effects [38]. The genetics and breeding efforts to improve the BS disease resistance in tomato are summarized in Table 1. The HR-mediated-resistance in HI 7998 to race T1 is controlled by *Rx3* locus on chromosome 5 in multiple studies and is induced by the *avrRxv* effector of race T1 [22–25]. Initially, the marker Rx3-L1 was reported to be associated with the *Rx3* loci [25,40]. However, using a complex breeding population, Sim, et al., [26] identified SP5 associated with *Rx3* loci. The qualitative resistance to race T2 has not been reported so far. The HR-mediated-resistance in HI 7981, PI 126932, and PI 128216 to race T3 is controlled by a common locus *Rx4/Xv3* [33,41] that acted on a bacterial effector *avrXv3* [7,8]. The *Rx4* locus was fine mapped exactly at the pcc12 marker site and was of NBS-LRR class of resistance gene [32]. The resistance locus differed from the susceptible locus by 6-bp insertion/deletion (InDel) and eight SNPs. The HR-mediated-resistance in LA716 to race T4 is controlled by *RxopJ4* (*Xv4*) resistant locus [8,36,37]. The resistance locus *RXopJ4* was mapped to a 190 kb segment on the long arm of chromosome 6 between the markers J350 and J352 [37]. However, *RXopJ4* locus in LA 716 is linked with several negative traits, including autogenous leaf necrosis, low fruit yield, and small fruit size, and *RXopJ4* heterozygotes showed slow and weak HR along with inconsistent phenotypes, limiting the utilization of LA716 resistance in the breeding program [37].

Solanum lycopersicum var. cerasiformae PI 114490 conferred quantitative resistance to race T2, T3, and T4 [20]. The resistance to T2 race is controlled by two genes on chromosome 11 with additive gene effects and is different from those genes controlling resistance to race T3 [27]. In addition, three advanced breeding lines Fla. 8326 (large-fruited), Fla. 8233 (large-fruited), and Fla., 8517 (plum) with race T3 and race T4 resistance have also been developed [35]. These three lines have HI 7998 (in all three), PI128216 (Fla. 8233 and Fla. 8517), PI 114490 (Fla. 8517), and PI 126932 (Fla.8326) in their pedigree [35]. Hutton, et al. [38] identified a major QTL on chromosome 11 associated with the T4 resistance in PI 114490 derived inbred backcross (IBC) populations and confirmed this QTL in the populations derived from the three breeding lines Fla. 8517, Fla. 8233, and Fla. 8326. However, none of the PI114490 derived lines recovered full resistance of PI114490. The donor source for QTL on chromosome 11 was HI 7998 in all three breeding lines, suggesting epistatic gene interaction in the HI 7998 derived lines that is absent in HI7998 [38].

Bhattarai, et al. [42] reported the moderate level of resistance against race T4 in *S. pimpinellifolium* derived breeding lines, including 74L-1W (2008), NC2CELBR, 081–12–1X-gsms, NC22L-1 (2008), and 52LB-1, in the field and greenhouse studies. Further work is needed to characterize the resistance observed in these lines and to evaluate if the observed resistance in these lines is sufficient to manage the disease before integrating the resistance in the cultivar. Three *S. pimpinellifolium* accessions PI 128216, LA2533, and LA1936 showed both HR-mediated-resistance in the greenhouse and field resistance to *X. gardneri* and *X. perforans* race T3 [19]. HR to *X. perforans* was observed before the HR to *X. gardneri.* Another study identified quantitative resistance to *X. gardneri* in two varieties- IZK Alya (cherry type) and Nikolina F_1 (determinate large fruit type) [43]. Two genes, *Bs7* and *Bs3*, have been reported to confer resistance to *X. gardneri* avirulence genes *avrBs7* and *AvrHah1*, respectively in pepper, another member of the Solanaceae family [44,45].

5. Transgenic Resistance to Tomato BS Disease

Transgenic resistance is considered a promising tool because it eliminates linkage drag observed in the tomato cultivars developed from backcrossing. The pepper gene *Bs2* targeting the core effector *avrBs2* of *Xanthomonas* strains has been deployed in tomato through the transgenic approach. The *Bs2* transgenic tomato conferred resistance to all field strains of *Xanthomonas* in FL and increased the yield compared to non-transformed lines without any adverse effects in the transgenic tomatoes [46]. *Bs2* gene is safe for human consumption because *Bs2* protein belongs to one of the largest families of naturally occurring plant proteins (NB-LRR family), which has no known adverse effects on human

health and has been widely consumed [3]. Therefore, *Bs2* transgenic tomatoes should be safe as long as they are selectable marker-free.

However, a mutation in *avrBs2* effector has been observed in rare *Xanthomonas* strains that could overcome the resistance conferred by the *Bs2* gene in the future [47]. This suggests that multiple targets for such core effectors are necessary to achieve durable resistance. Two recessive genes in pepper *bs5* and *bs6* conferred resistance to all races of BS in peppers [48]. Such recessive genes are predicted more durable than the dominant resistance genes as these genes do not involve in specific gene-for-gene interactions [48]. Identifying and cloning tomato homologs of *bs5* and *bs6* genes will enhance the tomato breeding efforts for BS disease resistance and provide targets for future transgenic or gene editing approaches.

Transgenic approaches could also allow integration of *Bs3* and *Bs7* genes from pepper into a tomato to achieve resistance against *X. gardneri*. Moreover, transgenic expression of elongation factor-Tu (EF-Tu) receptor (*EFR*), a pattern recognition receptor (PRR) from *Arabidopsis thaliana* also conferred broad-spectrum bacterial resistance, including *X. perforans* in tomato. However, *EFR* resistance was less effective compared to that conferred by the combination of *Bs2* and *EFR* genes or *Bs2* gene alone [49]. Therefore, deployment of *Bs2* gene in combination with other novel resistance genes would provide durable resistance to BS disease in tomato. Although the effectiveness of *Bs2* transgenic tomatoes to manage BS disease is successfully demonstrated in multiple field trials, the *Bs2* transgenic tomatoes could not be commercialized because of public concerns towards genetically engineered food products.

6. Novel Breeding Strategies to Enhance Tomato BS Disease Resistance

Despite several breeding efforts, no commercial tomato cultivars resistant to BS are available yet. This emphasizes the necessity of novel strategies in breeding and biotechnology to achieve durable disease resistance in tomato for BS. In recent years, genomics and effectoromics have opened the pathway to identify *R* genes targeting the core effectors of the pathogens. For instance, effector genomics was employed to identify late blight *R* genes and pathogen recognition receptors in potato [50,51]. Similarly, comparative genomics has enabled the capacity to compare the genomes of closely related species and study the conserved genes among different genera within the same family. For example, comparative genomics allowed to isolate *R3a* gene in potato against the late blight pathogen [52]. Additionally, genome editing technology has enabled precise modification of the plant genome to improve disease resistance in crop plants such as in wheat to enhance powdery mildew disease [53], in citrus to improve citrus canker resistance [54], and in rice to improve bacterial blight [55]. High-throughput phenotyping (HTP) is another strategy to accelerate the resistance breeding, which enables accurate scoring and continuous monitoring of crop diseases. For instance, the quantitative virulence of *Z. tritici* that causes Septoria tritici blotch on wheat was assessed using the HTP-based automated image analysis [56]. In this study, HTP not only allowed to analyze the leaf disease symptoms but also quantify the pycnidia size and density. This kind of disease assessment is beyond the visual assessment alone. In summary, with all these new technologies available, it is expected that tomato breeding programs in the future will succeed in developing BS disease-resistant tomato cultivars.

6.1. Exploiting Tomato Genomic Resources

Tomato whole genome sequence was made available in 2012, which has been a great resource for the study of several traits, including bacterial diseases in tomato [57]. Recently, a pangenome of tomato has been made available and has added 4,873 genes not present in the reference genome [58]. Currently, sequences of several tomato genotypes are publicly available, which is useful for exploring potentially useful genes and the development of molecular markers (www.solgenomics.net). With the reduction of sequencing costs, there are reports of sequencing individual lines as per their necessity for the development of SNP markers or the identification of genes of interest. Sequencing of tomato genotypes and landraces and exploiting sequence polymorphisms in different tomato genotypes and landraces will shed new light on the genetics and molecular basis of BS resistance in tomato. For example,

molecular markers (SNP and InDels) were developed based on whole-genome re-sequencing of two tomato lines (Caimanta and LA0722) and using those markers in a segregating population; eventually, BS disease resistance lines were developed [59]. Menda et al. [60] have reviewed the various genomics resources that can be used in tomato improvement.

6.2. Genomics-assisted Breeding Approaches (GBA)

Genomic-assisted breeding approaches (GBA) include marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), marker-assisted backcrossing (MABC), and genomic selection (GS). Most of the genetic studies (QTL mapping) underlying BS disease resistance in tomato were based on the controlled crosses with limited recombinant events. Although association mapping has a higher mapping resolution to dissect the genetic basis of several quantitative traits, including disease resistance, the inbred nature of the species, low level of molecular diversity, and high genomic linkage disequilibrium (LD) have limited association mapping studies in tomato [61]. In recent years, GWAS has been adopted in tomato to dissect genetic variation of agronomical traits and fruit quality in part due to the availability of whole-genome sequence data, low sequencing cost allowing resequencing analysis, and availability of high-density SNP markers covering the whole genome [62,63]. The GWAS studies, in combination with the next-generation sequencing technologies, provide higher mapping resolution to detect the genetic region underlying the BS disease resistance and sequence variation underlying the region associated with the resistance. The markers associated with the disease-resistance can then be used in a breeding program via MAS and MABC. For instance, the use of InDel markers to select for the QTL region and a panel of SNP markers assayed on the KASP platform to select background genome selection allowed for the rapid development of NILs that are 95%–99% genetically identical, except for the QTL on chromosome 11 [64–66]. Also, cost-effective genotyping methods and high-density SNP markers facilitate genomic predictions of breeding values and selection via GS approach. Duangjit et al. [67] studied the potential of GS in tomato breeding programs to improve fruit quality traits in tomato. In the case of BS disease resistance, Liabeuf et al., [68] observed higher prediction accuracy through the GS model for resistance to *X. euvesicatoria* compared to phenotypic selection.

6.3. Effectors-based Breeding Strategy

Effectoromics is a powerful tool to exploit core effectors from diverse groups of *Xanthomonas* and their matching target *R* genes in tomato [69]. Core effectors are crucial for pathogenicity and virulence. Therefore, the pathogens have a fitness cost to evolve the core effectors [70]. The detection of such core effectors and deployment of the resistance genes targeting those core effectors will improve the disease resistance and increase the resistance durability. The availability of pangenome assists in identifying multiple NLRs to recognize core effectors of *Xanthomonas,* causing BS in tomato. Advancement in the computational tools will facilitate the identification of candidate effectors, while resistance gene enrichment sequencing (RenSeq) will identify multiple NLRs that recognizes core pathogen effectors to achieve the disease resistance [53]. Both core effectors and NLRs can be transiently expressed in the tomato plant, and the HR phenotype indicates the recognition of effector genes by NLR. High throughput functional screening will further accelerate the identification of promising NLRs to recognize the core effectors and inform *R* gene deployment before the pathogen evolution [53].

Tomato breeding programs should, therefore, aim to identify and utilize tomato NLRs, recognizing the core effectors of *Xanthomonas spp*. Although pepper gene *Bs2* targeting *AvrBs2*, a core effector conserved among *Xanthomonas* species and races infecting tomato, has been deployed in tomato, it could not be utilized widely due to public concerns of transgenic tomatoes [46]. However, the other effectors such as *avrBsT*, *T3SS* belonging to *XopJ* effector family with a role in pathogen fitness under field conditions were detected among *X. perforans* and some strains of *X. euvesicatoria* and *X. vesicatoria* [71]. Therefore, *avrBsT* might be a suitable candidate to target *X. perforans* in the regions where this species

is predominant. The breeding approaches targeting evolutionary conserved bacterial effector in combination with resistant gene pyramiding could provide durable resistance against BS disease.

6.4. Genome Editing

Genome editing (GE) is a great tool that accelerates the trait improvement in a precise way in a breeding program if the genome sequences of the particular crop are available. The GE allows precise single gene introgression, gene knock out or gene modification to obtain the desired trait in the crops. GE offers two advantages over traditional breeding and transgenic methods in BS resistance breeding in tomato, which are i) GE overcomes the problem of linkage drag associated with the resistance genes such as in *RxopJ4* gene as observed in the traditional breeding method, and ii) GE avoids the random gene insertion and any foreign gene insertion in the crop genome unlike in transgenic approach. Unlike GM technology, GE techniques have not been regulated by the United States Department of Agriculture (USDA) and thus avoids regulatory issues. The plant community has three genome editing tools-zinc-finger nucleases (ZFNs), transcription activator-like effectors (TALE) nuclease system (TALEN), and clustered regularly interspaced short palindromic repeats/cas9 (CRISPR/Cas9). The GE has been utilized to improve disease resistance in several crops to many diseases, such as blast and bacterial blight in rice [55,72–74]. The potential of GE to insert multiple genes into a single locus was demonstrated by stacking two herbicide tolerance genes in maize [75]. Therefore, such technology can also be implemented in BS disease resistance breeding to integrate multiple BS resistance genes such as *Bs2*, *EFR*, *Bs3*, *bs5*, *bs6*, and *Bs7* from the pepper. This will allow simple inheritance of stacked genes in the breeding program.

More recently, CRISPR/Cas9 has gained more popularity than other GE systems because of simplicity, efficiency, and low off-target sites [76]. The potential of CRISPR/Cas9 to generate stable, highly specific, and heritable mutations in tomato plants without any off-targets has been demonstrated [77]. Also, CRISPR/Cas9 has been adopted in tomato to improve several traits including disease resistance such as powdery mildew [78] and bacterial speck [79]. Therefore, CRISPR/Cas9 could provide an alternate option to classical transgenic methods to develop BS disease resistance in future tomato breeding. The CRISPR/Cas9 technique would be ideal for integrating NLRs from wild species into cultivated tomato [53]. Another application of the CRISPR/Cas9 system in BS resistance breeding could be editing the *Bs2* and *EFR* syntenic genes in tomato to make it functional as the *Bs2* gene and *EFR* gene, so that tomato lines with these genes could be commercialized, unlike the transgenic *Bs2* tomato lines. In a study, CRISPR/Cas9 was used to inactivate the *Sl-DMR6–1* gene in tomato, which conferred resistance to multiple important pathogens of tomato, including *X. perforans* and *X. gardneri* [80]. Therefore, integrating *Bs2*, *EFR*, and *Sl-DMR6–1* mutation in tomato cultivars could provide a durable resistance to BS disease. To conclude, the potential of GE in improving BS disease resistance in tomato cannot be neglected.

6.5. Phenomics-Assisted Breeding

Although much progress has been achieved in the genomics area, phenotyping is still lagging behind. Precise phenotyping is critical in the case of breeding for complex traits such as disease resistance, and the phenotypic data are the primary data in case of association studies. The inaccurate phenotyping might give false-positive results during QTL analysis and genomics-assisted breeding. The phenotyping of BS disease is usually done visually using a traditional numeric scale, which is subjective and might not be precise and accurate.

In recent years, several high throughput phenotyping tools and phenomics platforms have been developed, which allow the collection of accurate and correct phenotypic data for genomics-assisted breeding and the identification of gene function [81]. The resources or projects for the phenomics studies in plants include International Plant Phenotyping Network (IPPN), European Plant Phenotyping Network (EPPN), German Plant phenotyping Network (DPPN), The Jülich Plant Phenotyping Centre (JPPC), PHENOME, the Australian Plant Phenomics Facility (APPF), Laboratory of Plant

Ecophysiological Response to Environmental Stresses (LEPSE), Green Crop Network (GCN), mutant genotype and phenotype dataset, PhenoPhyte, and SciNetS [82,83].

For the quantification of diseases, images-based methods that rely on the reflectance of waves have been developed, such as analyzing the images in the visible spectrum, hyperspectral images, thermographic images, and chlorophyll fluorescence images [84]. Likewise, several automated and versatile disease phenomics platforms based on imaging techniques have been developed. For instance, the multi-sensor platforms Scanalyser^{3DHT} and Scanalyser FIELD are developed by the company LemnaTec (http://www.lemnatec.com) for the high-throughput phenotyping in the greenhouse and field, respectively. The American company Qubit Systems (http://qubitsystems.com/portal/) developed PlantScreen™ Conveyor Systems and PlantScreen™ Field Systems for greenhouse and field studies, respectively [84]. The image-based methods have been implemented to examine the diseases caused by the genus *Xanthomonas* in citrus [85] and bean [86].

Therefore, plant disease phenomics will not only allow us to collect BS disease data accurately or quantify the variation precisely, but also generate the reproducible data. This allows the accurate dissection of the underlying genetic architecture of BS disease resistance during QTL mapping and association mapping studies. This provides a better understanding of the mechanism of BS disease resistance in tomato and guides tomato breeders accordingly to improve BS disease resistance in tomato.

7. Conclusions

One of the current challenges in the BS disease resistance breeding program is to achieve durable resistance. Despite the continuous efforts to identify and introgression of resistance, it has been challenging to develop a BS disease-resistant cultivar for a long time because of the emergence of new races and species of the pathogen. The negative correlation between fruit quality and disease resistance has made the introgression of resistance even more challenging. In recent years, we have ample opportunities, as explained in the above sections, and can go beyond the classical breeding approaches to obtain broad-spectrum BS disease resistance against multiple species and races of the pathogen. The goal of improving disease resistance to BS disease in tomato can be achieved through the integration of available tomato genome resources, genomics tools, gene or genome editing tool, effectoromics, and plant disease phenomics tools. Employing multidisciplinary approaches seeks not only to improve disease resistance, but also to increase yield and improve fruit quality in tomato.

References

1. Potnis, N.; Timilsina, S.; Strayer, A.; Shantharaj, D.; Barak, J.D.; Paret, M.L.; Vallad, G.E.; Jones, J.B. Bacterial spot of tomato and pepper: Diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Mol. Plant Pathol.* **2015**, *16*, 907–920. [CrossRef]
2. Pohronezny, K.; Volin, R.B. The effect of bacterial spot on yield and quality of fresh market tomatoes. *HortScience* **1983**, *18*, 69–70.
3. Horvath, D.M.; Stall, R.E.; Jones, J.B.; Pauly, M.H.; Vallad, G.E.; Dahlbeck, D.; Staskawicz, B.J.; Scott, J.W. Transgenic resistance confers effective field level control of bacterial spot disease in tomato. *PLoS ONE* **2012**, 7. [CrossRef] [PubMed]
4. Vallad, G.E.; Timilsina, S.; Adkison, H.; Potnis, N.; Minsavage, G.; Jones, J.; Goss, E. A recent survey of Xanthomonads causing bacterial spot of tomato in Florida provides insights into management strategies. In Proceedings of the 2013 Florida Tomato Institute, Naples, FL, USA, 4 September 2013; Ozores-Hampton, M., Snodgrass, C., Eds.; University of Florida: Naples, FL, USA, 2013; p. 25.

5. Ma, X.; Ivey, M.L.L.; Miller, S.A. First report of *Xanthomonas gardneri* causing bacterial spot of tomato in Ohio and Michigan. *Plant Dis.* **2011**, *95*, 1584. [CrossRef] [PubMed]

6. Jones, J.B.; Lacy, G.H.; Bouzar, H.; Stall, R.E.; Schaad, N.W. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. *Syst. Appl. Microbiol.* **2004**, *27*, 755–762. [CrossRef] [PubMed]

7. Minsavage, G.; Balogh, B.; Stall, R.; Jones, J.B. New tomato races of *Xanthomonas campestris* pv. *vesicatoria* associated with mutagenesis of tomato race 3 strains. *Phytopathology* **2003**, *93*, S62.

8. Astua-Monge, G.; Minsavage, G.V.; Stall, R.E.; Vallejos, C.E.; Davis, M.J.; Jones, J.B. Xv4-vrxv4: A new gene-for-gene interaction identified between *Xanthomonas campestris pv. vesicatoria* race T3 and the wild tomato relative *Lycopersicon pennellii*. *Mol. Plant Microbe Interact.* **2000**, *13*, 1346–1355. [CrossRef] [PubMed]

9. Adhikari, P.; Adhikari, T.; Timilsina, S.; Meadows, I.; Jones, J.B.; Panthee, D.R.; Louws, F.J. Phenotypic and genetic diversity of Xanthomonas perforans populations from tomato in North Carolina. *Phytopathology* **2019**, *109*, 1533–1543. [CrossRef]

10. Ivey, M.L.L.; Strayer, A.; Sidhu, J.K.; Minsavage, G.V. Bacterial leaf spot of tomato (*Solanum lycopersicum*) in Louisiana is caused by *Xanthomonas perforans*, tomato race 4. *Plant Dis.* **2016**, *100*, 1233. [CrossRef]

11. Cuppels, D.A.; Louws, F.J.; Ainsworth, T. Development and evaluation of PCR-based diagnostic assays for the bacterial speck and bacterial spot pathogens of tomato (vol 90, pg 451, 2006). *Plant Dis.* **2006**, *90*, 969. [CrossRef]

12. Abbasi, P.A.; Khabbaz, S.E.; Weselowski, B.; Zhang, L. Occurrence of copper-resistant strains and a shift in *Xanthomonas* spp. causing tomato bacterial spot in Ontario. *Can. J. Microbiol.* **2015**, *61*, 753–761. [CrossRef] [PubMed]

13. Kim, S.H.; Olson, T.N.; Peffer, N.D.; Nikolaeva, E.V.; Park, S.; Kang, S. First report of bacterial spot of tomato caused by *Xanthomonas gardneri* in Pennsylvania. *Plant Dis.* **2010**, *94*, 638. [CrossRef] [PubMed]

14. Stukenbrock, E.H.; McDonald, B.A. Population genetics of fungal and oomycete effectors involved in gene-for-gene interactions. *Mol. Plant Microbe Interact.* **2009**, *22*, 371–380. [CrossRef] [PubMed]

15. Piquerez, S.J.M.; Harvey, S.E.; Beynon, J.L.; Ntoukakis, V. Improving crop disease resistance: Lessons from research on *Arabidopsis* and tomato. *Front. Plant Sci.* **2014**, 5. [CrossRef] [PubMed]

16. Scott, J.W.; Jones, J.B. Sources of resistance to bacterial spot in tomato. *HortScience* **1986**, *21*, 304–306.

17. Scott, J.W.; Jones, J.B.; Somodi, G.C.; Stall, R.E. Screening tomato accessions for resistance to *Xanthomonas-campestris pv. vesicatoria*, race T3. *HortScience* **1995**, *30*, 579–581. [CrossRef]

18. Wang, H.; Hutton, S.F.; Robbins, M.D.; Sim, S.C.; Scott, J.W.; Yang, W.C.; Jones, J.B.; Francis, D.M. Molecular mapping of hypersensitive resistance from tomato 'Hawaii 7981' to *Xanthomonas perforans* race T3. *Phytopathology* **2011**, *101*, 1217–1223. [CrossRef]

19. Liabeuf, D.; Francis, D.M.; Sim, S.C. Screening cultivated and wild tomato germplasm for resistance to *Xanthomonas gardneri*. In Proceedings of the IV International Symposium on Tomato Diseases, Orlando, FL, USA, 24–27 June 2013; Paret, M.L., Vallad, G.E., Zhang, S., Jones, J.B., Eds.; University of Florida: Naples, FL, USA, 2015; Volume 1069, pp. 65–70.

20. Scott, J.W.; Miller, S.A.; Stall, R.E.; Jones, J.B.; Somodi, G.C.; Barbosa, V.; Francis, D.L.; Sahin, F. Resistance to race T2 of the bacterial spot pathogen in tomato. *HortScience* **1997**, *32*, 724–727. [CrossRef]

21. Scott, J.W.; Hutton, S.F.; Shekasteband, R.; Sim, S.C.; Francis, D.M. Identification of tomato bacterial spot race T1, T2, T3, T4, and *Xanthomonas gardneri* resistance QTLs derived from PI 114490 populations selected for race T4. In Proceedings of the IV International Symposium on Tomato Diseases, Orlando, FL, USA, 24–27 June 2013; Paret, M.L., Vallad, G.E., Zhang, S., Jones, J.B., Eds.; University of Florida: Naples, FL, USA, 2015; Volume 1069, pp. 53–58.

22. Whalen, M.C.; Wang, J.F.; Carland, F.M.; Heiskell, M.E.; Dahlbeck, D.; Minsavage, G.V.; Jones, J.B.; Scott, J.W.; Stall, R.E.; Staskawicz, B.J. Avirulence gene *avrRxv* from *Xanthomonas campestris* Pv *vesicatoria* specifies resistance on tomato line Hawaii-7998. *Mol. Plant Microbe Interact.* **1993**, *6*, 616–627. [CrossRef]

23. Wang, J.F.; Stall, R.E.; Vallejos, C.E. Genetic analysis of a complex hypersensitive reaction to bacterial spot in tomato. *Phytopathology* **1994**, *84*, 126–132. [CrossRef]

24. Yu, Z.H.; Wang, J.F.; Stall, R.E.; Vallejos, C.E. Genomic localization of tomato genes that control a hypersensitive reaction to *Xanthomonas-campestris pv. vesicatoria* (Doidge) dye. *Genetics* **1995**, *141*, 675–682. [PubMed]

25.   Yang, W.; Miller, S.A.; Francis, D.M.; Sacks, E.J.; Lewis Ivey, M.L. Resistance in *Lycopersicon esculentum* intraspecific crosses to race T1 strains of *Xanthomonas campestris pv. vesicatoria* causing bacterial spot of tomato. *Phytopathology* **2005**, *95*, 519–527. [CrossRef] [PubMed]

26.   Sim, S.C.; Robbins, M.D.; Wijeratne, S.; Wang, H.; Yang, W.C.; Francis, D.M. Association analysis for Bacterial spot resistance in a directionally selected complex breeding population of tomato. *Phytopathology* **2015**, *105*, 1437–1445. [CrossRef] [PubMed]

27.   Scott, J.W.; Francis, D.M.; Miller, S.A.; Somodi, G.C.; Jones, J.B. Tomato bacterial spot resistance derived from PI 114490; Inheritance of resistance to race T2 and relationship across three pathogen races. *J. Am. Soc. Hort. Sci.* **2003**, *128*, 698–703. [CrossRef]

28.   Wang, J.F.; Jones, J.B.; Scott, J.W.; Stall, R.E. A new race of the tomato group of strains of *Xanthomonas campestris* pv. vesicatoria. *Phytopathology* **1990**, *80*, 1070.

29.   Scott, J.W.; Stall, R.E.; Jones, J.B.; Somodi, G.C. A single gene controls the hypersensitive response of Hawaii 7981 to race 3 (T3) of the bacterial spot pathogen. *Rep. Tomato Genet. Coop.* **1996**, *46*, 23.

30.   Jones, J.B.; Stall, R.E.; Scott, J.W.; Somodi, G.C.; Bouzar, H.; Hodge, N.C. A 3rd tomato race of *Xanthomonas-campestris* pv *vesicatoria*. *Plant Dis.* **1995**, *79*, 395–398. [CrossRef]

31.   Robbins, M.D.; Darrigues, A.; Sim, S.C.; Masud, M.A.T.; Francis, D.M. Characterization of hypersensitive resistance to bacterial spot race T3 (*Xanthomonas perforans*) from tomato accession PI 128216. *Phytopathology* **2009**, *99*, 1037–1044. [CrossRef]

32.   Pei, C.C.; Wang, H.; Zhang, J.Y.; Wang, Y.Y.; Francis, D.M.; Yang, W.C. Fine mapping and analysis of a candidate gene in tomato accession PI128216 conferring hypersensitive resistance to bacterial spot race T3. *Appl. Genet.* **2012**, *124*, 533–542. [CrossRef]

33.   Hutton, S. Inheritance and Mapping of Resistance to Bacterial spot race T4 (*Xanthomonas perforans*) in Tomato, and its Relationship to race T3 Hypersensitivity, and Inheritance of race T3 Hypersensitivity from PI 126932. Ph.D. Thesis, University of Florida, Florida, FL, USA, 2008.

34.   Sun, H.; Liu, X.; Li, W.; Yang, W. Preliminary mapping of a gene in tomato accession LA1589 conferring resistance to race T3 of bacterial spot. *J. Agric. Univ. Hebei* **2011**, *6*, 12.

35.   Hutton, S.F.; Scott, J.W.; Jones, J.B. Inheritance of resistance to bacterial spot race T4 from three tomato breeding lines with differing resistance backgrounds. *J. Am. Soc. Hort. Sci.* **2010**, *135*, 150–158. [CrossRef]

36.   Stall, R.E.; Jones, J.B.; Minsavage, G.V. Durability of resistance in tomato and pepper to *Xanthomonads* causing bacterial spot. *Annu. Rev. Phytopathol.* **2009**, *47*, 265–284. [CrossRef] [PubMed]

37.   Sharlach, M.; Dahlbeck, D.; Liu, L.; Chiu, J.; Jimenez-Gomez, J.M.; Kimura, S.; Koenig, D.; Maloof, J.N.; Sinha, N.; Minsavage, G.V.; et al. Fine genetic mapping of *RXopJ4*, a bacterial spot disease resistance locus from *Solanum pennellii* LA716. *Appl. Genet.* **2013**, *126*, 601–609. [CrossRef] [PubMed]

38.   Hutton, S.F.; Scott, J.W.; Yang, W.; Sim, S.-C.; Francis, D.M.; Jones, J.B. Identification of QTL associated with resistance to bacterial spot race T4 in tomato. *Appl. Genet.* **2010**, *121*, 1275–1287. [CrossRef] [PubMed]

39.   Scott, J.W.; Hutton, S.F.; Jones, J.B.; Francis, D.M.; Miller, S.A. Resistance to bacterial spot race T4 and breeding for durable, broad-spectrum resistance to other races. *Rep. Tomato Genet. Coop.* **2006**, *56*, 33–36.

40.   Yang, W.; Francis, D.M. Marker-assisted selection for combining resistance to bacterial spot and bacterial speck in tomato. *J. Am. Soc. Hort. Sci.* **2005**, *130*, 716–721. [CrossRef]

41.   Baimei, Z.; Haipeng, C.; Junjie, D.; Wencai, Y. Allelic tests and sequence analysis of three genes for resistance to *Xanthomonas perforans* race T3 in tomato. *Hortic. Plant J.* **2015**, *1*, 41–47.

42.   Bhattarai, K.; Louws, F.J.; Williamson, J.D.; Panthee, D.R. Resistance to *Xanthomonas perforans* race T4 causing bacterial spot in tomato breeding lines. *Plant Pathol.* **2017**, *66*, 1103–1109. [CrossRef]

43.   Aleksandrova, K.; Ganeva, D.; Bogatzevska, N. *Xanthomonas gardneri*–characterization and resistance of Bulgarian tomato varieties. *Türk Tarım Ve Doğa Bilimleri* **2014**, *7*, 1540–1545.

44.   Potnis, N.; Minsavage, G.; Smith, J.K.; Hurlbert, J.C.; Norman, D.; Rodrigues, R.; Stall, R.E.; Jones, J.B. Avirulence proteins AvrBs7 from *Xanthomonas gardneri* and AvrBs1.1 from *Xanthomonas euvesicatoria* contribute to a novel gene-for-gene interaction in pepper. *Mol. Plant Microbe Interact.* **2012**, *25*, 307–320. [CrossRef]

45.   Schornack, S.; Minsavage, G.V.; Stall, R.E.; Jones, J.B.; Lahaye, T. Characterization of AvrHah1, a novel AvrBs3-like effector from *Xanthomonas gardneri* with virulence and avirulence activity. *New Phytol.* **2008**, *179*, 546–556. [CrossRef] [PubMed]

46. Tai, T.H.; Dahlbeck, D.; Clark, E.T.; Gajiwala, P.; Pasion, R.; Whalen, M.C.; Stall, R.E.; Staskawicz, B.J. Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14153–14158. [CrossRef] [PubMed]

47. Dangl, J.L.; Horvath, D.M.; Staskawicz, B.J. Pivoting the plant immune system from dissection to deployment. *Science* **2013**, *341*, 746–751. [CrossRef] [PubMed]

48. Vallejos, C.E.; Jones, V.; Stall, R.E.; Jones, J.B.; Minsavage, G.V.; Schultz, D.C.; Rodrigues, R.; Olsen, L.E.; Mazourek, M. Characterization of two recessive genes controlling resistance to all races of bacterial spot in peppers. *Appl. Genet.* **2010**, *121*, 37–46. [CrossRef]

49. Kunwar, S.; Iriarte, F.; Fan, Q.R.; da Silva, E.E.; Ritchie, L.; Nguyen, N.S.; Freeman, J.H.; Stall, R.E.; Jones, J.B.; Minsavage, G.V.; et al. Transgenic expression of *EFR* and *Bs2* genes for field management of bacterial wilt and bacterial spot of tomato. *Phytopathology* **2018**, *108*, 1402–1411. [CrossRef]

50. Vleeshouwers, V.; Rietman, H.; Krenek, P.; Champouret, N.; Young, C.; Oh, S.K.; Wang, M.Q.; Bouwmeester, K.; Vosman, B.; Visser, R.G.F.; et al. Effector genomics accelerates discovery and functional profiling of potato disease resistance and *Phytophthora infestans* avirulence genes. *PLoS ONE* **2008**, *3*, 8. [CrossRef]

51. Domazakis, E.; Lin, X.; Aguilera-Galvez, C.; Wouters, D.; Bijsterbosch, G.; Wolters, P.J.; Vleeshouwers, V. Effectoromics-based identification of cell surface receptors in potato. In *Plant Pattern Recognition Receptors: Methods and Protocols*; Shan, L., He, P., Eds.; Springer: Cham, Switzerland, 2017; Volume 1578, pp. 337–353.

52. Huang, S.W.; van der Vossen, E.A.G.; Kuang, H.H.; Vleeshouwers, V.; Zhang, N.W.; Borm, T.J.A.; van Eck, H.J.; Baker, B.; Jacobsen, E.; Visser, R.G.F. Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J.* **2005**, *42*, 251–261. [CrossRef]

53. Zhang, M.; Coaker, G. Harnessing effector-triggered immunity for durable disease resistance. *Phytopathology* **2017**, *107*, 912–919. [CrossRef]

54. Jia, H.G.; Zhang, Y.Z.; Orbovic, V.; Xu, J.; White, F.F.; Jones, J.B.; Wang, N. Genome editing of the disease susceptibility gene *CsLOB1* in citrus confers resistance to citrus canker. *Plant Biotechnol. J.* **2017**, *15*, 817–823. [CrossRef]

55. Li, T.; Liu, B.; Spalding, M.H.; Weeks, D.P.; Yang, B. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat. Biotechnol.* **2012**, *30*, 390–392. [CrossRef]

56. Stewart, E.L.; McDonald, B.A. Measuring quantitative virulence in the wheat pathogen *Zymoseptoria tritici* using high-throughput automated image analysis. *Phytopathology* **2014**, *104*, 985–992. [CrossRef] [PubMed]

57. Sato, S.; Tabata, S.; Hirakawa, H.; Asamizu, E.; Shirasawa, K.; Isobe, S.; Kaneko, T.; Nakamura, Y.; Shibata, D.; Aoki, K.; et al. Tomato Genome, C. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **2012**, *485*, 635–641.

58. Gao, L.; Gonda, I.; Sun, H.; Ma, Q.; Bao, K.; Tieman, D.M.; Burzynski-Chang, E.A.; Fish, T.L.; Stromberg, K.A.; Sacks, G.L.; et al. The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. *Nat. Genet.* **2019**, *51*, 1044–1051. [CrossRef] [PubMed]

59. Cambiaso, V.; Pratta, G.R.; da Costa, J.H.P.; Zorzoli, R.; Francis, D.M.; Rodriguez, G.R. Whole genome re-sequencing analysis of two tomato genotypes for polymorphism insight in cloned genes and a genetic map construction. *Sci. Hortic.* **2019**, *247*, 58–66. [CrossRef]

60. Menda, N.; Strickler, S.R.; Mueller, L.A. Advances in tomato research in the post-genome era. *Plant Biotechnol.* **2013**, *30*, 243–256. [CrossRef]

61. Ranc, N.; Muños, S.; Xu, J.; Le Paslier, M.-C.; Chauveau, A.; Bounon, R.; Rolland, S.; Bouchet, J.-P.; Brunel, D.; Causse, M. Genome-wide association mapping in tomato (*Solanum lycopersicum*) is possible using genome admixture of *Solanum lycopersicum* var. *cerasiforme*. *G3: Genes| Genomes| Genet.* **2012**, *2*, 853–864. [CrossRef]

62. Bauchet, G.; Grenier, S.; Samson, N.; Bonnet, J.; Grivet, L.; Causse, M. Use of modern tomato breeding germplasm for deciphering the genetic control of agronomical traits by genome wide association study. *Appl. Genet.* **2017**, *130*, 875–889. [CrossRef]

63. Sauvage, C.; Segura, V.; Bauchet, G.; Stevens, R.; Do, P.T.; Nikoloski, Z.; Fernie, A.R.; Causse, M. Genome-wide association in tomato reveals 44 candidate loci for fruit metabolic traits. *Plant Physiol.* **2014**, *165*, 1120–1132. [CrossRef]

64. Phan, N.T.; Sim, S.C. Genomic tools and their implications for vegetable breeding. *Hortic. Sci. Technol.* **2017**, *35*, 149–164.

65. Shirasawa, K.; Fukuoka, H.; Matsunaga, H.; Kobayashi, Y.; Kobayashi, I.; Hirakawa, H.; Isobe, S.; Tabata, S. Genome-wide association studies using single nucleotide polymorphism markers developed by re-sequencing of the genomes of cultivated tomato. *DNA Res.* **2013**, *20*, 593–603. [CrossRef]

66. Potnis, N.; Branham, S.E.; Jones, J.B.; Wechter, W.P. Genome-wide association study of resistance to *Xanthomonas gardneri* in the USDA pepper (*Capsicum*) collection. *Phytopathology* **2019**, *109*, 1217–1225. [CrossRef] [PubMed]

67. Duangjit, J.; Causse, M.; Sauvage, C. Efficiency of genomic selection for tomato fruit quality. *Mol. Breed.* **2016**, *36*, 29. [CrossRef]

68. Liabeuf, D.; Sim, S.C.; Francis, D.M. Comparison of marker-based genomic estimated breeding values and phenotypic evaluation for selection of Bacterial spot resistance in tomato. *Phytopathology* **2018**, *108*, 392–401. [CrossRef]

69. Du, J.; Vleeshouwers, V.G. The do's and don'ts of effectoromics. In *Plant-pathogen Interactions: Methods in Molecular Biology (Methods and Protocols)*; Birch, P., Jones, J., Bos, J., Eds.; Humana Press: Totowa, NJ, USA, 2014; pp. 257–268.

70. Swords, K.M.; Dahlbeck, D.; Kearney, B.; Roy, M.; Staskawicz, B.J. Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. vesicatoria avrBs2. *J. Bacteriol.* **1996**, *178*, 4661–4669. [CrossRef] [PubMed]

71. Abrahamian, P.; Timilsina, S.; Minsavage, G.V.; Kc, S.; Goss, E.M.; Jones, J.B.; Vallad, G.E. The type III effector AvrBsT enhances *Xanthomonas perforans* fitness in field-grown tomato. *Phytopathology* **2018**, *108*, 1355–1362. [CrossRef] [PubMed]

72. Wang, F.J.; Wang, C.L.; Liu, P.Q.; Lei, C.L.; Hao, W.; Gao, Y.; Liu, Y.G.; Zhao, K.J. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene *OsERF922*. *PLoS ONE* **2016**, *11*, e0154027. [CrossRef] [PubMed]

73. Belhaj, K.; Chaparro-Garcia, A.; Kamoun, S.; Nekrasov, V. Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* **2013**, *9*, 39. [CrossRef]

74. Belhaj, K.; Chaparro-Garcia, A.; Kamoun, S.; Patron, N.; Nekrasov, V. Editing plant genomes with CRISPR/Cas9. *Curr. Opin. Biotechnol.* **2015**, *32*, 76–84. [CrossRef]

75. Ainley, W.M.; Sastry-Dent, L.; Welter, M.E.; Murray, M.G.; Zeitler, B.; Amora, R.; Corbin, D.R.; Miles, R.R.; Arnold, N.L.; Strange, T.L.; et al. Trait stacking via targeted genome editing. *Plant Biotechnol. J.* **2013**, *11*, 1126–1134. [CrossRef]

76. Mushtaq, M.; Sakina, A.; Wani, S.H.; Shikari, A.B.; Tripathi, P.; Zaid, A.; Galla, A.; Abdelrahman, M.; Sharma, M.; Singh, A.K.; et al. Harnessing genome editing techniques to engineer disease resistance in plants. *Front. Plant Sci.* **2019**, *10*, 550. [CrossRef]

77. Pan, C.; Ye, L.; Qin, L.; Liu, X.; He, Y.; Wang, J.; Chen, L.; Lu, G. CRISPR/Cas9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations. *Sci. Rep.* **2016**, *6*, 24765. [CrossRef] [PubMed]

78. Nekrasov, V.; Wang, C.; Win, J.; Lanz, C.; Weigel, D.; Kamoun, S. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Sci. Rep.* **2017**, *7*, 482. [CrossRef] [PubMed]

79. Ortigosa, A.; Gimenez-Ibanez, S.; Leonhardt, N.; Solano, R. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of *SlJAZ2*. *Plant Biotechnol. J.* **2019**, *17*, 665–673. [CrossRef] [PubMed]

80. Wang, T.; Zhang, H.Y.; Zhu, H.L. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. *Hortic. Res.* **2019**, *6*, 77. [CrossRef] [PubMed]

81. Mir, R.R.; Choudhary, N.; Singh, B.; Khandy, I.A.; Bawa, V.; Sofi, P.; Wani, A.; Kumari, S.; Jain, S.; Kumar, A. Harnessing genomics through phenomics. In *Phenomics in Crop Plants: Trends, Options and Limitations*; Kumar, J., Pratap, A., Kumar, S., Eds.; Springer: New Delhi, India, 2015; pp. 273–283.

82. Bhat, J.A.; Salgotra, R.K.; Dar, M.Y. Phenomics: A challenge for crop improvement in genomic era. *Mol. Plant Breed.* **2015**, *6*, 1–11. [CrossRef]

83. Chen, D.; Chen, M.; Altmann, T.; Klukas, C. Bridging genomics and phenomics. In *Approaches in Integrative Bioinformatics*; Chen, M., Hofestädt, R., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 299–333.

84. Giglioti, É.A.; Sumida, C.H.; Canteri, M.G. Disease phenomics. In *Phenomics*; Fritsche-Neto, R., Borém, A., Eds.; Springer: Cham, Switzerland, 2015.

85. Bock, C.H.; Poole, G.H.; Parker, P.E.; Gottwald, T.R. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. *Crit. Rev. Plant Sci.* **2010**, *29*, 59–107. [CrossRef]

86. Xie, W.L.; Yu, K.F.; Pauls, K.P.; Navabi, A. Application of image analysis in studies of quantitative disease resistance, exemplified using common bacterial blight-common bean pathosystem. *Phytopathology* **2012**, *102*, 434–442. [CrossRef]