**Abstract:** Bacterial spot (BS), caused by four species of *Xanthomonas*: *X. euvesicatoria*, *X. vesicatoria*, *X. perforans* and *X. gardneri* in tomato (*Solanum lycopersicum* L.) results in severe loss in yield and quality by defoliation and the appearance of lesions on fruits, respectively. The combined industry standard for BS control (foliar applications Actigard® rotated with copper plus mancozeb) does not offer sufficient protection, especially when weather conditions favor disease spread. Development of tomato cultivars with BS resistance is thus an important measure to minimize losses. Hypersensitive and non-hypersensitive resistance has been identified in different wild accessions and cultivated tomato relatives and has been transferred to cultivated tomato. However, complete resistance is yet to be obtained. With the advent of next generation sequencing and precise genome editing tools, the genetic regions that confer resistance to bacterial spot can be targeted and enriched through gene pyramiding in a new commercial cultivar which may confer higher degree of horizontal resistance to multiple strains of *Xanthomonas* causing bacterial spot in tomato.

**Keywords:** Tomato; bacterial spot; *Xanthomonas* spp.; resistance
However, with the species of Xanthomonas evolving from susceptibility to developing resistance to copper has led to the use of copper-based chemicals inefficacious [2,4,8].

Developing genetic resistance in tomato for BS seems to offer a relatively long-term and effective solution. There are resistances available to various strains of Xanthomonas in various wild relatives, plant introductions (PI) and relatives of tomato. Breeding efforts have been made in the past and undergoing to transfer the resistance from these wild relatives and accessions into advanced elite lines with varying level of success. In order to confer complete control, development of horizontal resistance towards multiple species of Xanthomonas is imperative. There have been continuous efforts around the globe to develop tomato that is resistant to different species of Xanthomonas causing BS. Although, there has been a lot of progress made towards this direction, a commercial tomato variety exhibiting complete resistance to BS is yet to be seen in the tomato industry. In this review, we discuss the various breeding approaches undertaken to develop resistance to BS in tomato.

2. Non-Host Resistance

There are numerous microbes/pathogens in the environment to which plants are exposed to. Out of numerous microbes present in the environment, relatively a few are capable of causing pathogenic infection in plants. Most of the plants exhibit resistance to a broad range of pathogens known as non-host resistance (NHR) [9,10]. Pathogen infection during NHR is restricted by a combination of many mechanisms involved at different layers of defense in plants. During plant-bacterial interaction at the surface level when a bacterium comes in contact with a host cell, it can be restricted by a defense known as preformed defense system [9]. This includes restriction of bacteria by the presence of structural components like trichomes, cuticular wax layer, antimicrobial compounds [11]. Some of the physiological activities also help in pathogen restriction like stomatal closure. When bacterium is able to invade the preformed defense system, a second line of defense kicks off in the plant system to restrict the pathogen known as induced defense [12]. Induced defense involves numerous chain activities to restrict the pathogen from being able to get established inside the plant system, multiply and spread. This includes de novo synthesis of antimicrobial compounds, reinforcement of host cell wall by callose, lignin and suberin deposition [11]. Production of secondary metabolites like phytoalexins, glycoproteins, phenolic compounds and organosulfur compounds are known to be synthesized in the host cell to arrest the bacterial infection. Induced defense also includes enhanced production of reactive oxygen species (ROS) and downstream activation of various defense related genes are known to be involved in NHR [13].

Plants have developed a unique ability to identify the pathogenic microbes, able to cause infection, by recognizing conserved microbial molecules known as pathogen-associated molecular patterns (PAMPs) or identify the non-pathogenic conserved elements known as microbe-associated molecular patterns (MAMPs) [13,14]. These conserved molecules include flagellin proteins present in bacteria that is used for bacterial mobility. PAMPs are identified by the inherent receptor molecules in the plant cell known as pattern recognition receptors (PRRs) [15–17]. Identification of the PAMPs or MAMPs by PRRs triggers myriad of induced defense responses known as PAMP triggered immunity (PTI) [13]. Conserved microbial elements are present in both pathogenic and non-pathogenic microbes and hence PTI is also observed in both resistant and susceptible plants through PAMP recognition and plays an important role during NHR [17]. PAMP recognition overlaps in both basal defense and NHR in initiating the defense response by the host [14]. PAMP has been used to study the response of various tomato lines of diverse genetic background in relation to BS resistance [18–20]. In this study, the lines with higher resistance produced higher amount of ROS when a PAMP, Xcc22, was used [18].

Plants demonstrate resistance to most of the pathogens and do not produce compatible reaction with them. But some of the pathogens are able to produce compatible reaction with the host. Although there are resistance sources identified which is conferred by R genes, this type of resistance is frequently overcome by pathogen evolvement [21]. New races have emerged in tomato for bacterial spot before resistance for the existing race could be deployed in commercial cultivars. NHR has been around for
long time and if transferred from non-host to host plants could confer broad spectrum, durable and enhanced basal resistance [22]. Genes conferring NHR are indispensable for the plants and can be more effective in recognizing and restricting the pathogen. Since, these genes for PAMPs like flagellin are required for pathogen survival, mutations in these genes are not common and hence the resistance developed for these genes can potentially last longer [10]. This might offer a sustainable approach for tomato production in future if the complete control of the disease can be achieved.

Several studies have also focused towards identifying genes involved in cell death and effector triggered immunity (ETI) regulators in NHR. Transferring the genes contributing NHR in tomato can be used to increase resistance for BS. Enhancing NHR by genetic engineering or breeding is gaining increased interest because of broad-spectrum resistance offered by NHR in comparison to R-gene mediated resistance to particular race of a pathogen [10]. In non-host pathogen like bean, soybean, cowpea, alfalfa and cotton, X. campestris pv. vesicatoria is not able to establish compatible reaction hence develop hypersensitive response (HR) [23]. A PRR gene, elongation factor thermo unstable receptor (EFR) from Arabidopsis when transferred to tomato reduced pathogen growth in the host [18]. Transfer of R gene, Bs2, from pepper to tomato has also shown increased resistance to bacterial spot in tomato [24]. Avirulence gene, AvrBs2, is required by the pathogen, X. campestris for its fitness hence it is difficult for the pathogen to survive with developing mutations in this gene [25]. So, transfer of Bs2 is expected to confer broad spectrum resistance to bacterial spot caused by multiple races of Xanthomonas in tomato and other solanaceous crops [24,25]. Based on previous studies of gene conferring NHR, the resistance has not been completely controlled or silenced by the use of single gene [10]. Hence, the nature of NHR is expected to be quantitative. Therefore, identification and transfer of many genes might be required to achieve complete resistance to bacterial spot.

3. Host Resistance

Host resistance in tomato against BS is conferred by both qualitative and quantitative genes via HR or non-HR reactions. Various sources of resistance to BS has been identified in wild accessions and relatives of tomato. Transferring resistance from these sources has been ongoing since the disease was diagnosed and varying level of success has been achieved. Although, the sources of BS resistance are identified, and the efforts to introgress the resistance in cultivated tomato has been performed, the quest for a hybrid with complete resistance is yet to be achieved owing to the complex host-plant interaction, plant-pathogen evolution and complex resistance mechanism. Major breeding efforts performed for developing BS resistance are discussed in this review.

3.1. Hypersensitive Resistance

In some instances, plant cells detect the pathogen at cell surface or in the cytoplasm due to the presence of plant immune receptors encoded by major disease resistance genes known as R genes. Effectors are the molecules present in race specific pathogen that are identified by the receptors encoded by R genes in monogenic inheritance fashion. This is known as gene-for-gene model or effector-triggered immunity (ETI) [13]. This type of immunity results in incompatible reaction and HR restricting the pathogen by localized cell death. Numerous avirulence genes (Avr) are present in Xanthomonas causing bacterial spot in tomato. These Avr genes produce effectors that are required during pathogenesis. There are R genes identified in tomato that encode nucleotide-binding site leucine rich repeats (NBS-LRR) which identify the effectors from pathogen and produce incompatible reactions restricting the pathogen and causing localized cell death [26]. Although there are 355 NBS-LRR genes identified in tomatoes, only few are known to act upon bacterial spot resistance [27].

3.1.1. Race T1

There are HR resistance identified for multiple races of Xanthomonas in tomato. S. lycopersicum accession Hawaii7998 (HI7998) confers HR to race 1 [28]. Effector avrRcxo in the T1 strain interacts
with the resistant proteins to confer HR in HI 7998. The HR resistance is controlled by multiple non-dominant and independent genetic genes [29]. Three genes Rx1, Rx2 and Rx3 were identified from the backcross 1 (BC1) population derived from HI 7998 and L. pennellii (LA716) [30]. Genes Rx1 and Rx2 are located in the opposite arms of chromosome 1 and Rx3 in chromosome 5 of HI7998 [29]. Defense response from Rx1 is highest between 16–32 h post inoculation whereas the effect of Rx2 and Rx3 is at peak during 24–32 h post infection. However, effect of Rx2 and Rx3 is epistatic and stronger in comparison to Rx1 [30]. Polymorphic markers identified in the BC1 population inherited from LA716 were linked to susceptibility. In a separate study, a locus linked to bacterial spot susceptibility was identified on chromosome 4 of HI7998 [31]. Additional polymorphic markers, mainly in Rx3 region, were identified from a F2 population derived from a cross between HI7998 and an elite breeding line Ohio (OH) 88119. However, the role of Rx1 in conferring resistance to bacterial spot in the field condition was not identified in the population studied and no polymorphic markers were identified to be linked with Rx2. The correlation between the HR and field resistance was relatively low between 0.31 and 0.37 in different populations [28,31]. A novel QTL in chromosome 1 was identified where Rx-2 was previously identified [31]. However, the source for this QTL was not HI 7998 but Florida (FL) 7600 or OH9242. In a study involving selected progenies generated from the crosses between individuals: FL8233 and OHMR13 (fresh-market) and OH7536, OH8614, OH03-6439, and OH03-7463 (processing) derived from HI7998, HI7981, PI128216, or PI114490 resistance to T1 strain was identified in chromosome 4 [32] where one segment conferring resistance was inherited from OH germplasm and another from PI114490. This indicates the involvement of multiple genes in conferring the resistance in the field. There have been estimations that three to five loci might be involved in field resistance [33]. In addition, the role of HR transitioning into disease resistance in field condition is not completely understood. T1 strain has been replaced by the T3 strain of Xanthomonas in different tomato growing regions with T3 appearing to suppress the growth of T1 [34]. With this trend, the importance of developing T1 resistance is crucial for building durable horizontal resistance in tomato by pyramiding the resistance genes into the germplasm.

3.1.2. Race T3

Race T3 appeared in various regions of the world before resistance to T1 had been transferred to commercial cultivars [35]. HR resistance to race T3 has been identified in HI7981 through incompatible interaction between host plant single gene, Xc3, and an effector gene avrXc3, in X. perforans [36,37]. Although HR is conferred by a single gene, the field resistance, however, is quantitatively controlled by Xc3 and other modifier loci [37]. Another source of HR resistance to T3 was identified in S. pimpinellifolium PI128216 [37]. HR response was also observed in some of the PI selections that exhibited strong resistance to T3, however, HR and field resistance were not completely correlated. An IBC population developed from the cross of PI128216 and OH88119 and non-parametric single-marker analysis identified two locations on chromosome 11 and chromosome 6 [38]. Locus on chromosome 11 was associated with completely conferring HR by itself however genomic regions on chromosome 6 was not identified to be related to HR indicating the single dominant gene on chromosome 11 conferring the resistance to T3. Chromosome 11 locus also increased field resistance to T3 [38].

Other HR resistance against race T3 has been identified as RxLA1589 in S. pimpinellifolium accession LA1589 and RxopJ4 in S. pennellii accession LA716 [39]. PI114490 do not confer HR however confers high resistance in field. There are five QTLs identified conferring the resistance in field. Most of the differentially expressed genes involved in T3 resistance in PI114490 are the genes in plant hormone signal transduction, plant-pathogen interaction and phenylalanine metabolism [40].

3.1.3. Race T4

In the early 2000s, the HR responses conferred by the differential lines HI7981, PI128216 and PI 126932 for T3 was overcome by a new race T4 [41,42]. XopJ4 effector was recognized to be conserved
in most of the field isolates in Florida which belonged to T4. This effector is recognized by a single dominant gene, \textit{RxopJ4} (previously known as \textit{Xv4}) and mapped on 20 cM segment of chromosome 3 [41] in the wild accession \textit{S. penellii} LA716. However, later study mapped \textit{RXopJ4} to the 190 kb region of the long arm of chromosome 6 based on the introgressed lines of \textit{S. penellii} in \textit{S. lycopersicum} [43].

Three lines FL8233 (PI1128216 and HI7998 in its pedigree), FL8517 (PI114490, PI128216 and HI7998 in its pedigree) and FL8326 (PI126932 and HI7998 in its pedigree), developed at the University of Florida showed moderate to high level of resistance to T4 but do not confer HR [44]. This indicates that these lines may contain multiple genes controlling the resistance rather than traditional single R gene [44]. It was reported that the resistance in these three lines are controlled by dominant effects along with significant contribution of epistasis and additive effects [44]. Resistance to T4 has also been identified in the population derived from \textit{S. pimpinellifolium} L3707 in breeding lines 74L-1W (2008), NC2CELBR, 081-12-1X-gsms, NC22L-1 (2008) and 52LB-1 in both field and greenhouse studies from North Carolina State University tomato breeding program [45].

Despite the availability of resistance in the tomato accessions like LA716, transferring the resistance to the elite lines is not trivial due to linkage drag. \textit{RXopJ4} introgressed lines showed low fruit yield, small fruit size and autogenous leaf necrosis [43]. This may cause a disadvantage when compared to the performance of elite tomato lines in the field.

3.1.4. \textit{Xanthomonas gardneri}

\textit{Xanthomonas gardneri} infects tomato to cause bacterial spot through \textit{avrHah1} effector that triggers Bs3-dependent HR in pepper [46]. Resistance to \textit{X. gardneri} is also known to be conferred by HR in controlled conditions in tomato and multiple loci in field. \textit{S. pimpinellifolium} LA2533, LA1936, PI1128216 conferred HR in greenhouse while showed resistance in the field without HR [47]. From the segregating population studies developed using the resistant accessions (IBC of OH 88119 and PI 128216 and two \textit{F2} of selected IBCs and OH8245 cross, BC1 of LA2533 and OH2641), the resistance conferred by LA2533 may be controlled by 2–4 loci, from PI 128216 conferred by 1-2 loci and that of \textit{F2} may be conferred by 2–4 loci [47].

4. Non-Hypersensitive Resistance

Race 2

Non-hypersensitive resistance for bacterial spot has been reported in tomato relative \textit{S. lycopersicum} var. \textit{cerasiforme} accession PI114490 [48]. PI114490 was also identified to be resistant to races T1 and T3 [49]. However, the resistance is stronger for T1 and T2 compared to that of T3 [50]. In a segregating \textit{F2} population developed from PI114490 and FL7600, the resistance was observed to be likely controlled by two-genes with additive nature, requiring all four alleles to confer maximum resistance [50]. From the inbred backcross population of (PI114490 X FL7600) cross with OH9242, identified seven markers linked to T2 resistance corresponding to five chromosomal regions in PI114490 of which two were linked with susceptibility to T2 and one with T2 resistance [31]. However, limited phenotypic variation (<15%) was explained due to each QTLs owing to the incomplete coverage of the genome (60%) by the markers or presence of other loci conferring the resistance by independent smaller effects or interaction between them [31]. This might be the reason that complete resistance for T2 has not been obtained yet in a new elite line.

5. Broad-Spectrum Resistance

Some host plants are able to confer non-race specific resistance to specific pathogens known as broad-spectrum resistance (BSR). [50] reported that PI114490 conferred field resistance to multiple races and is considered to offer BSR to BS in the field conditions. A QTL on chromosome 11 from PI114490 was identified to confer partial resistance (14.6%–63.8%) to multiple races T1–T4 [31]. However, this locus was not able to restore the level of resistance in the population as compared to that conferred
by PI114490 indicating the presence of other loci playing role in BSR. Another major QTL was also identified in chromosome 1 of PI114490 conferring 3%–11% resistance to T2–T4 [44]. There were also minor QTL reported conferring BSR to T1–T4 [44].

Using various race-specific and non-specific resistant lines HI7998, PI114490, PI128216 and OH 9242, there were three lines developed: FL8233, FL8517, and FL8326 which conferred BSR to multiple races [51]. BSR in the population developed by using these three individuals indicated the presence of recessive epistatic suppression gene inhibiting the susceptibility of these lines to multiple races [44]. However, the epistatic gene is yet to be identified.

One of the potential method to enhance BSR is to transfer the race specific resistance gene/loci for multiple races from various sources into commercial line to enable its defense mechanism to counter all existing races of pathogen infecting the host and alleviate its resistance. Gene pyramiding is a popular method used in tomato breeding facilitated by marker assisted selection [52]. In order to perform this, uniformly distributed polymorphic markers covering the whole genome is essential along with gene/loci and their interaction information. The limitation of traditional breeding is the requirement of long time to successfully introgress the resistance from wild relatives to the advanced cultivated tomato. With the rapid evolving pathogen, linkage drag can slow down the progress and cause severe economic loss. Fusarium wilt race 3 resistance has been identified to be linked with increased susceptibility to BS due to linkage drag [49]. These type of linkage drags can hinder the pyramiding of multiple disease resistances and may take longer. Therefore, transgenic approach can expedite the development of elite lines with the resistance with minimized linkage drag. Transforming the resistance could also lead to solving the loss of resistance in heterozygotes due to incomplete dominance as observed for the Xo2 and Rx4 genes mapped in chromosome 11.

6. Genetic Engineering

6.1. Transgenic Approach

The transfer of a gene from one organism to another that do not naturally produce offspring when crossed is known as transgenic approach. Usually, genes conferring disease resistance are transferred from resistant to susceptible plant to enable the susceptible plant to stand against the disease. Bs2 gene conferring resistance to different races of bacterial spot in pepper is known to possibly confer durable resistance to BS if transferred into tomato. The Bs2 gene encodes motifs with nucleotide binding site-leucine-rich repeat class of resistance genes. The presence of Bs2 in tomato was confirmed by the co-expression of avrBs2 [24]. Transient expression of Bs2 gene in tomato lines conferred hypersensitive response and suppressed the growth of X. campestris in an avrBs2-dependent manner and functioning in a heterologous system. The fitness of X. campestris is regulated by avrBs2 and both virulence and avirulence in X. campestris is controlled by Bs2 [25]. The EFR gene from Arabidopsis in combination with Bs2 gene from pepper or separately had been transferred in tomato to control bacterial spot and bacterial wilt in tomato. The expression of EFR and Bs2/EFR genes in tomato lines reduced the severity of bacterial spot from 22 to 98% in the field compared to non-transgenic plants whereas marketable yield increased by 43 to 170% in Bs2/EFR plants compared to control plants [53].

The resistance of plant against diseases can be enhanced by integration of the genes producing antimicrobial peptides (AMPs) from other plant [54–57]. The AMPs help in building biological defenses against pathogens. The examples of AMPs are cecropins, magainins, and sarcotoxin IA [57–59]. Giant silk moth, Hyalophora cecropia produces a lytic peptide called Cecropins [59,60]. Gram-negative and gram-positive bacterial growth is inhibited using cationic peptide cecropin B (CB). A construct named pBI121-spCB was made by the fusion of CB gene to secretory signal peptide from the barley a-amylase gene [60]. The construct was transformed into tomato plants which showed increased resistance to bacterial spot that was confirmed by western blot analysis [60].

The transfer of non-expresser of PR genes (NPR1) from Arabidopsis to tomato helped to acquire broad spectrum resistance against various diseases [61]. NPR1 is one among the genes for systemic
acquired resistance (SAR). The tomato cultivar in which NPR1 was transferred was already resistant to tomato mosaic virus and heat tolerant. The transgenic tomato showed various levels of resistance for various diseases with a moderate degree of resistance for bacterial spot. The level of resistance was directly dependent on the amount of NPR1 protein accumulated by the transgenic lines where a higher level of NPR1 proteins showed broader and higher level of resistance against diseases and lower level of NPR1 protein showed low resistance against the diseases. Similarly, the NPR1 protein was also directly affecting the inheritance of the disease resistance [61].

6.2. Genome Editing

Plant breeding technique, crossing resistant and susceptible cultivar to obtain F1 and backcrossing with the resistant cultivar is the classical method to obtain resistance to bacterial spot in tomato. However, it would take many years and is labor intensive to obtain the resistance. To overcome this problem of traditional breeding approach, various genome editing tools like TALENs (Transcription Activator Like Effector Nucleases) and ZFNs (Zinc Finger Nucleases) are being investigated. These genome-editing techniques use programmable nucleases to make double-strand breaks in the DNA at specific location that helps in genetic modifications in the gene or genome [62]. The DNA breaks help in insertion or deletion through non-homologous end joining whereas double strand breaks are repaired by homology directed repair using a homologous repair template [63,64]. However, high cost and complexity for designing these nucleases make these tools among the techniques that were not adapted widely for plant-based research. A new technology that substituted ZFN and TALENs is CRISPR/Cas9 system for gene editing. Cas9 is a bacterial CRISPR-associated protein-9 nuclease from Streptococcus pyogenes and CRISPR/Cas9 is a modified bacterial immune system for genome editing [64]. The system consists of Cas9 nuclease and single guide RNA (sgRNA) that is specific to nucleic acid sequence. The sgRNA target specificity depends on ribonucleotide-protein complex formation so, it is quite easy to design target specific guide RNA necessary for Cas9 recognition [64].

The application of CRISPR/Cas9 system was successful for transient expression and recovery of stable transgenic plants [64]. In tomato, SlDMR6-1 orthologue Solyc03g080190.2 helped in acquiring broad spectrum diseases resistance through CRISPR/Cas9 mediated mutagenesis [65]. The use of CRISPR/Cas9 helped in deleting few nucleotides in SlDMR6-1 gene leading to frameshift and premature truncation of the protein. The progeny (T1 plants) after deletion was infected with X. gardneri (Xg153) and X. perforans (Xp4b) where SlDMR6-1 mutants were resistant compared to wild types without affecting the plant growth and development. The upregulation of downy mildew resistance (DMR6) gene was observed during infection in Arabidopsis thaliana [66]. During the process of mutagenesis in DMR6 gene, increase in salicylic acid level was highly correlated with disease resistance in Arabidopsis because of which the orthologue of same gene was used in tomato to get broad spectrum disease resistance. DLO1 was also regulated with DMR6 and had synergic effects on plant disease resistance. However, a lower level of resistance was shown by dlo1 mutants and even the growth of plants was affected when double mutant (dmr6dlo1) was made. Thus, a single mutation on DMR6 gene producing dmr6 mutants was an effective tool in Arabidopsis to have broad spectrum resistance [66]. Thus, CRISPR/Cas9 would be an effective way to obtain broad spectrum resistance against various races of bacterial spot pathogen.

7. Conclusions

Next generations of sequencing technologies have facilitated the availability for reference genomes and sequencing and re-sequencing of advanced lines and wild accessions which are critical in identifying the resistance genes. On the pathogen side, identification of effector sequences and virulent components can be used to identify the genes conferring resistance to the pathogens. This can be extremely helpful to expedite the process of identifying and introgressing resistance in tomato in case when new race emerges or evolves from the existing one.
Progress made so far in developing bacterial spot resistance in tomato has primarily relied on traditional and marker assisted breeding with few instances of using genetic transformation. As the availability of genome sequences of tomato becomes more feasible, development of precise markers and approaches like genomic and genome-wide selections will be more effective and result in higher gain. Use of precision technology like targeted genome editing can facilitate the progress made by traditional and marker assisted breeding.

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References


29. 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]


32. Yang, W.; Sacks, E.J.; Lewis Ivey, M.L.; Miller, S.A.; Francis, D.M. 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]


47. Liabeuf, D.; Francis, D.M.; Sim, S.C. Screening cultivated and wild tomato germplasm for resistance to *Xanthomonas gardneri*. *Acta Hortic.* 2015, 1069, 65–70. [CrossRef]


