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REVIEW

Biotechnological approaches for enhancing the resistance of tomato plants to phytopathogenic bacteria

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Abstract

Bacterial diseases of vegetable crops cause significant losses of yield and substantially decrease food quality. For sustainable development of agriculture, it is highly important to use the most effective strategies for the protection of vegetable crops from bacterial diseases which allows the creation of resistant cultivars and their introduction in regions with an increased risk of damage by phytopathogenic bacteria. This paper reviews the most widespread bacterial diseases of tomatoes, the mechanisms of interaction of plants with phytopathogenic bacteria, and the advantages of the biotechnological strategies over traditional and marker-associated breeding for creation of the resistant tomato cultivars. The current research progress on the use of biotechnological approaches such as cell selection, genetic engineering, genome editing, and gene silencing is summarized, with a special emphasis on the advantages and limitations of these methods.

Keywords: bacterial diseases, biotechnology, plant-microbe interaction, resistance to bacteria, tomatoes.

Introduction

Resistance to biotic stresses is one of the major requirements for new cultivars and hybrids of vegetable crops. Bacterial phytopathogens have a substantial impact on the yield of vegetable crops, in particular, tomatoes. Despite the successes of classical breeding in obtaining tomato genotypes with increased resistance to certain diseases, the problem of complex resistance to the most dangerous diseases has not been solved yet (Kolomiiets *et al.* 2019). The reasons for this are the genetic complexity of the trait, continuous microevolutionary changes occurring in the 'host-pathogen' system, the emergence of highly resistant biotypes of pathogens as the result of large-

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Abbreviations: AMP - antimicrobial peptides; EPS - exopolysaccharides; ET - ethylene; ETI - effector-triggered immunity; HR - hypersensitivity response; JA - jasmonic acid; LPS - lipopolysaccharides; NB-LRR - receptors with nucleotide-binding domains and leucine-rich repeats; NGT - new genomic techniques; PAMPs - pathogen-associated molecular patterns; PR proteins - pathogenesis-related proteins; PRR - pattern-recognition receptor; PTI - PAMP-triggered immunity; ROS - reactive oxygen species; SA - salicylic acid; SAR - systemic acquired resistance; STTM - short tandem target mimic RNAs.
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scale non-controlled use of chemical pesticides (Khaliluev and Shpakovskii 2013), an average daily temperature rise and sharp temperature fluctuations during the day, planting genetically homogeneous monocultures over a large area, intensive development of world trade, *etc*. (McDonald and Stukenbrock 2016).

To combat these challenges of the 21st century, the application of integrated plant protection approaches is crucial. These approaches facilitate the obtaining of high crop yields while minimizing detrimental impacts on the environment, biodiversity, and human health (Mostovjiak 2019, Deguine *et al.* 2021). The main strategies of integrated plant protection include the management of phytopathogen populations, pests, and weeds, along with the implementation of effective agrotechnological approaches, including scientifically based treatments with chemicals and cultivation of resistant cultivars (Mostovjiak 2019, Deguine *et al.* 2021). The appearance and rapid development of new genomic techniques (NGT) opens opportunities to use novel methods for the creation of tomato cultivars resistant to phytopathogens. These strategies rely on methods that allow high-throughput and accurate genome modifications of the target organisms (Zimny 2022). The methods of NGT include marker-assisted breeding, cell selection, genetic transformation, genome editing, and gene silencing, which are discussed here.

The continuous search for new sources of resistance to bacterial diseases is quite necessary for providing breeding programs with high-quality genetic stock material. Such an approach should enhance the process of selection of new cultivars with increased resistance or tolerance to certain phytopathogens (Wang *et al.* 2018). Compared to classical and marker-associated selection, advanced biotechnological methods such as cell selection, genetic engineering, and genome editing are much more effective methods that allow the development of cultivars resistant to bacterial diseases under controlled conditions within a short period of time (Garfinkel *et al.* 2019, Buziashvili *et al.* 2020, Buziashvili and Yemets 2023).

The cell selection approach is a promising method for obtaining plants resistant to bacterial diseases through co-cultivation of plants with phytopathogens or their toxic metabolites and further selection of the most resistant genotypes under controlled conditions (Lebeda and Švábová 2010, Girhepuje and Shinde 2011). The selective factors can be inorganic compounds, the biomass of weakened pathogen, organic substances with different structures and biochemical properties, such as lipopolysaccharide (LPS) molecules, flagellin, cell wall components, specific proteins secreted by the pathogen when contacting a plant, *etc*. (Švábová and Lebeda 2005). Plants that survive under the pressure of selective agents are potential sources of genes for resistance or tolerance to certain phytopathogens (Ivchenko *et al.* 2021).

The genetic engineering approach involves a transfer of foreign resistance genes into plant genomes using genetic transformation methods, or targeted editing of the plant's own genes involved in the immune responses to the disease using genome editing technologies. Therefore, this approach requires fundamental knowledge about the genetic determinants of resistance to phytopathogenic bacteria (Horvath *et al.* 2012). The successful production of transgenic tomato plants relies on using highly effective methods of transformation with the resistance genes, *in vitro* selection of transformed cell lines, and regeneration of transgenic plants carrying the following gene of interest. The main advantage of genetic transformation is the possibility of transferring one or more genes which could provide either narrowly specific resistance to a certain phytopathogen or non-specific long-term resistance to a wide range of bacterial or fungal phytopathogen species (Razzaq *et al.* 2021, Varshney *et al.* 2021, Buziashvili and Yemets 2023).

Nowadays, the areas of application of genome editing technologies quickly emerge from various fields of medicine to biotechnology and agriculture. The gene editing technologies could be used to modulate the desired phenotype of the target organism by precise deleting or modifying the sequences of the own genes, their promoters, or signal sequences (Gaj *et al.* 2016, Borrelli *et al.* 2018, Yin and Qiu 2019, Li *et al.* 2020, Barka and Lee 2022, Wang *et al.* 2022a, Akram *et al.* 2023, Ijaz *et al.* 2023). There is evidence of using gene silencing technology to generate transgenic plants with enhanced resistance to insects, plant viruses, and phytopathogenic fungi. These plants are usually transformed with the fragments of the genes encoding sense or antisense short RNAs that silence the target genes associated with phytopathogens or pests (Hou and Ma 2020, Halder *et al.* 2022).

Therefore, to select an effective strategy for protecting plants from phytopathogens, in-depth knowledge of the molecular mechanisms of interaction in the 'host-pathogen' system is required. This paper reviews the molecular mechanisms of interaction between the bacterial pathogen and the plant. We also focus on the advanced NGT for obtaining tomato cultivars resistant to bacterial diseases, such as traditional and marker-assisted breeding, cell selection, genetic transformation, genome editing, and short tandem target mimic (STTM) RNA-mediated silencing, considering the advantages and disadvantages of these methods and future prospects for their use in agriculture.

Mechanisms of interaction between phytopathogenic bacteria and host plant

The most common bacterial pathogens of tomatoes in the Mediterranean countries over the last 10 years are *Clavibacter michiganensis* subsp. *michiganensis* causing bacterial canker, *Pectobacterium* (*Erwinia*) *carotovora* subsp. *carotovora* causing soft rot, *Pseudomonas syringae* pv. *tomato* causing bacterial speck, *Pseudomonas corrugata* causing tomato pith necrosis, *Ralstonia solanacearum* the causative of bacterial wilt, and *Xanthomonas* spp., the causatives of bacterial spot (Blancard 2013, Panno 2021). Here we consider the biotechnological approaches of the management of tomato bacterial diseases caused by *Xanthomonas* spp. (*X. euvesicatoria*, *X. vesicatoria*,

X. perforans, and *X. gardneri*), *P. syringae* pv. *tomato*, *P. syringae* pv. *syringae*, usually causing bacterial speck diseases of trees, but sometimes occurring on tomatoes, *Erwinia amylovora*, which is a post-harvest pathogen infecting different vegetables and fruits, especially of *Rosaceae* family, *C. michiganensis* subsp. *michiganensis*, *P. carotovorum* subsp. *carotovorum*, and *R. solanacearum.*

The successful development and application of effective methods of controlling bacterial plant diseases strictly depend on the deep understanding of mechanisms of interaction between bacterial pathogens and the host plant organism at the tissue, cellular, and molecular levels. During colonization of infected plant, phytopathogenic bacteria secrete hydrolytic enzymes, toxins, phytohormones, exo- and lipopolysaccharides (EPS and LPS), *etc*. (Savidor *et al.* 2012). Some of the virulence factors of phytopathogenic bacteria that play a key role in the interaction with the host plant are LPS, EPS, type III secretion systems, transcription factors, *etc*. (Newman *et al.* 2000, Thieme *et al.* 2005, Scheibner *et al.* 2017, Islamov *et al.* 2021, Buziashvili and Yemets 2023). Lipopolysaccharides cover almost 80% of the cell surface of gram-negative bacteria (Erbs and Newman 2012). LPS molecules consist of lipid A, integrated into the lipid cell membrane, core oligosaccharide, and polysaccharide composed of repeating residues of the O-antigen. The main function of LPS is to inactivate the plant hypersensitive response (HR). In plants, LPS induce the expression of genes encoding the PR proteins such as β-1,3-glucanase (Newman *et al.* 2000). EPS play an important role in providing quorum sensing of phytopathogenic bacteria, which promotes the formation of biofilms, rapid reproduction, and colonization of the vascular system of plants (Islamov *et al.* 2021). In addition, complex exopolysaccharides produced by *C. michiganensis* subsp. *michiganensis* and *R. solanacearum* not only protect their cells from harmful environmental factors but also cause irreversible damage to plant cell membranes, which leads to loss of pressure potential and dysfunction of the vascular system (Milling *et al.* 2011, Imada *et al.* 2016).

To date, the mechanisms of induction of plant defense reactions in response to various virulence factors have been thoroughly studied. In general, the plant immune response could be described by the 'zig-zag' scheme proposed by Jones and Dangl (2006). According to this scheme, the first phase of the plant immune response called PTI (PAMP-triggered immunity) is activated at the early stages of infection (Han 2019, Buziashvili and Yemets 2023).

At this phase, highly conserved pathogen-associated molecular patterns (PAMPs), such as oligosaccharides of LPS or lipid A of the bacterial cell wall, flagellin proteins, or elongation factor EF-Tu, are recognized by extracellular PRR receptors (pattern recognition receptors) which further transmit the signals inside the cell (Fig. 1). For example, the PRR receptor LORE (lipooligosaccharide-specific reduced elicitation) of *Arabidopsis* and other *Brassicaceae* plants can recognize the LPS of phytopathogenic *Pseudomonas* species and trigger a PTI response (Whitfield and Trent 2014, Ranf *et al.* 2015).

Although there is a wide diversity of currently known PRR receptors that recognize PAMPs and initiate PTI, some pathogens are able to bypass the primary defense reactions, in particular, due to the secretion of effector molecules. As a result, the recognition of PRR receptors becomes ineffective, which leads to the penetration of the pathogen into plant tissues and the further spread of the infection throughout the plant organism. The further development of infection triggers the next phase of the plant immune response - the effector-triggered immunity (ETI). The mechanism of ETI lies in the recognition of the effector

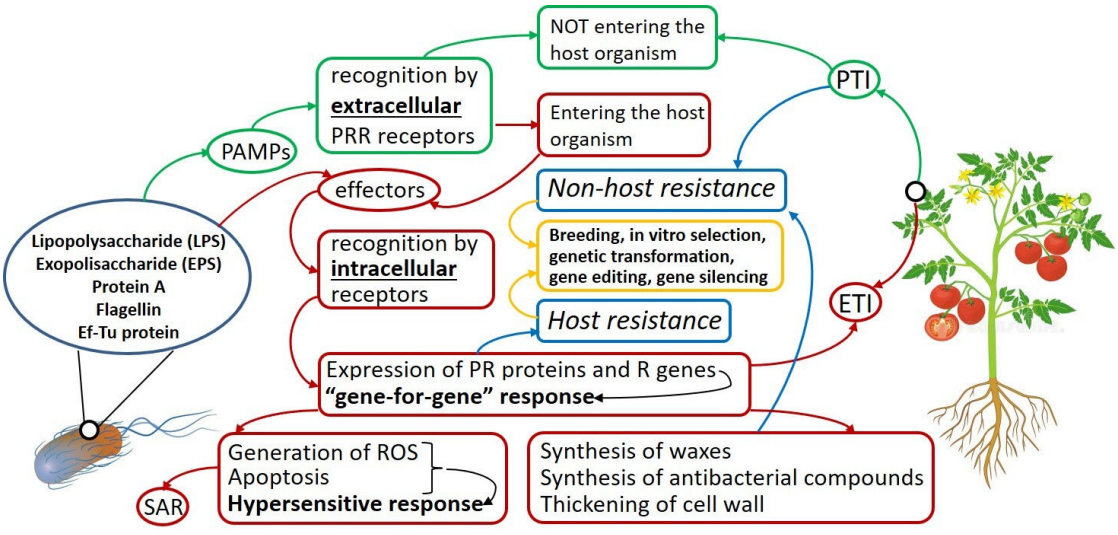

Fig. 1. Schematic presentation of various mechanisms of natural resistance or tolerance of plants to bacterial pathogens and the use of biotechnological approaches to increase plant resistance to phytopathogens. *Green* lines highlight the main stages of resistance formation by the PAMP-triggered immunity (PTI) mechanism, *red lines* - by the effector-triggered immunity (ETI) mechanism, *blue lines* - mechanisms of formation of the resistance such as host resistance and non-host resistance, *yellow lines* - mechanisms involved to the formation of resistance through the use of new genomic techniques considered in this paper.

(Avr protein) of bacteria by the corresponding R-protein of the plant according to the 'gene-for-gene' mechanism (Jones and Dangl 2006, Buziashvili and Yemets 2023). Most R-proteins are composed of a nucleotide-binding site and leucine-rich repeats (NB-LRRs). In addition to recognition of Avr protein, R-proteins participate in the formation of systemic acquired resistance (SAR), which is comprised by the HR that includes generation of reactive oxygen species (ROS) and apoptosis, and expression of the genes encoding PR (pathogenesis-related) proteins (Jones and Dangl 2006, Han 2019, Buziashvili and Yemets 2023). By the way, the LPS of phytopathogenic bacteria can also act as effectors and induce the plant defense responses by the ETI mechanism, such as production of reactive oxygen species (ROS) (Braun *et al.* 2005), synthesis of PR proteins (pathogenesis-related proteins) (Newman *et al.* 2000), and formation of systemic resistance (SAR) in various plant species (Erbs and Newman 2003, Dong and Ronald 2019, Köhl *et al.* 2019). The general mechanisms of PTI and ETI plant immune response, as well as the potential role of biotechnological approaches discussed in this paper in enhancing plant resistance to bacterial pathogens, are summarized in Fig. 1.

In addition, the mechanisms of resistance of host plants to phytopathogens could be classified into two types depending on the means of immune response and specificity range: (*1*) host resistance, which is carried out by the ETI or 'gene-for-gene' mechanisms and is typical to host plants having the immunity to a narrow range of phytopathogen species, and (*2*) non-host resistance (NHR), which provides the resistance to a wider range of phytopathogen species due to the genes to which the phytopathogen is not adapted. Nonspecific resistance is usually multi-component and is provided by several mechanisms, both PTI and ETI, in particular, by expression of PR genes, deposition of lignin, synthesis of antimicrobial compounds and secondary metabolites, such as phytoalexins, *etc*. (Gill *et al.* 2015, Sharma and Bhattarai 2019).

Therefore, the creation of plant cultivars with both host and non-host resistance to phytopathogens is an important task. The use of NGT opens opportunities for time-saving, environmentally safe, and low-energy-consuming genetic modification of agricultural plants to obtain new plant cultivars with desired characteristics which could further be used in the market after appropriate legislation (Zimny 2022). Taking into account the peculiarities of the molecular mechanisms of the interaction between phytopathogens and host plants, with the use of such NGT methods as breeding, cell selection, genetic engineering, gene silencing, and genome editing it is possible to create tomato plants with both host and non-host resistance to highly virulent bacterial phytopathogens, such as *Pseudomonas syringae* pv. *tomato*, various species of *Xanthomonas* (*X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri*), *Ralstonia solanacearum*, *Pectobacterium carotovorum*, *Erwinia amylovora*, *Clavibacter michiganensis* subsp. *michiganensis*, *etc*. A brief description of these approaches and underlying molecular mechanisms that enhance plant's resistance to phytopathogens is given in the next paragraphs.

Breeding for the resistance to bacterial pathogens of tomato

Over the last decades, with the global rise in the demands for high-quality products of agriculture, much efforts have focused on keeping the principles of integrated pest management which combine the use of a complex of all available means of disease and pest control, reducing the use of pesticides to an economically and ecologically justified level, minimizing the negative impact on the environment and human health (Mostovjiak 2019, Bigini *et al.* 2021, Deguine *et al.* 2021). Among the measures for the prevention and control of plant diseases, one of the most important is the cultivation of resistant cultivars (Mostovjiak 2019, Bigini *et al.* 2021, Deguine *et al.* 2021).

The list of cultivars and hybrids of tomato plants is extremely diverse and grows every year. The genetic diversity of cultivated tomato is relatively narrow, and a source of genes for valuable traits, particularly genes for resistance to bacterial, fungal, and viral diseases, can be found in wild species of *Solanum*, such as *S. pimpinellifolium*, *S. habrochaites*, *S. peruvianum*, *S. chilense*, *S. pennellii*, *S. galapagense*, *S. arcanum*, and *S. neorickii*. Numerous studies have been dedicated to the investigation of molecular markers such as RAPD, AFLP, RFLP, SSR, and candidate genes for tomato bacterial disease resistance (Sharma and Bhattarai 2019, Preston 2000, Lavale *et al*. 2022, Wang *et al*. 2022a).

Among the wild species, the most important sources of resistance genes to *C. michiganensis* subsp. *michiganensis* are *S. arcanum*, *S. pennelii*, *S. chilense*, *S. habrochaites*, and *S. pimpinellifolium* (Sen *et al*. 2015, Khazaei and Madduri 2022). For instance, a study by Şanver *et al*. (2022) demonstrated a high degree of tolerance to *C. michiganensis* infection in the lines *S. habrochaites* LA1777 and *S. arcanum* LA2157, which could be used in breeding programs. Partial resistance to *C. michiganensis* was confirmed in the wild tomato species *Solanum hirsutum* (LA)407, which was comparable to the resistance of control samples from *S. peruvianum* LA2157. Resistance to *C. michiganensis* was also confirmed in lines obtained from backcrosses between *Solanum hirsutum* (LA)407 and *S. lycopersicum* (Francis *et al.* 2001). Koseoglou *et al.* (2023) showed that the tolerance of *Solanum arcanum* LA2157 to *C. michiganensis* is controlled not only by a single locus on chromosome 7 but also by two additional loci on chromosomes 2 and 4. These findings are crucial for consideration in breeding programs involving crosses of *S. lycopersicum* with wild *Solanum* species.

In the works of Sen *et al*. (2013), the resistance to bacterial canker was investigated in 24 lines of wild *Solanum* species. As a result, new tolerant lines were identified, such as *Solanum pimpinellifolium* GI.1554, *S. parviflorum* LA735, and *S. parviflorum* LA2072, and the tolerance of previously known lines of *S. peruvianum* LA2157, *S. peruvianum* PI127829, *S. peruvianum* LA385, *S. habrochaites* LA407, and *S. lycopersicum* cv. IRAT L3 was confirmed. It was also confirmed that there are hotspots on chromosome 7 of *S. lycopersicum* where introgression of resistance markers from *S. pimpinellifolium* GI.1554

or *S. arcanum* (LA2157) can occur. Additionally, certain cultivars of *S. lycopersicum* were found to be tolerant to *C. michiganensis* - the highest tolerance was demonstrated in Mexican cultivars Saher, Sv4401, Nápoles, and Súper óptimo in the study by Rivera-Sosa *et al.* (2022).

Numerous studies are also dedicated to exploring the molecular mechanisms of interaction between the pathogen *C. michiganensis* subsp. *michiganensis* and tomato plants. It has been shown that after infection with *C. michiganensis* subsp. *michiganensis*, both in resistant (*Solanum arcanum* LA2157) and susceptible lines and tomato cultivars (*Solanum lycopersicum* cv. Ailsa Craig and cv. Money Maker), the expression of genes induced by salicylic acid, as well as genes encoding receptors of the RLK family and transcription factors, polyphenol oxidase E, diacyl glycerol kinase, TOM1-like protein 6, and an ankyrin repeat-containing protein, was increased. This suggests their role in defense reactions (Pereyra-Bistraín *et al*. 2021, Yokotani *et al*. 2021).

In contrast to *C. michiganensis*, for which resistance genes have not been identified to date, resistance to *P. syringae* pv. *tomato*, the causal agent of bacterial speck in tomatoes, has been known since the 1980s. Early studies (Fallik *et al*. 1983, 1984) reported resistance to the bacterial speck in tomato cultivars Ontario 7710 and Rehovot-13, the wild *Solanum pimpinellifolium* P.I. 126927 line and its F1 and F2 progeny lines obtained from backcrosses with susceptible cultivars. Overall, Ontario 7710 is a standard for *P. syringae* pv. *tomato* resistance, with numerous studies conducted on the inheritance of its resistance to bacterial speck. It has been shown that the resistance to *P. syringae* pv. *tomato* of tomato cv. Ontario 7710 is conferred by a single gene *Pto* which is inherited in an incomplete dominance manner. Resistance to bacterial speck was maintained in the 1st and 2nd generations through backcrosses of tomato cvs. Luban and Rumba with good agronomic characteristics to the resistant cv. Ontario 7710. In these crosses, the progeny lines were heterozygous for the *Pto* resistance gene while retaining the favorable fruit characteristics of the parental cultivars (Kozik 2002, 2010). Additionally, in studies by Kozik (2002, 2010), resistance to *P. syringae* pv. *tomato* was demonstrated in two lines of wild *Solanum* species, *S. hirsutum* LA 1773 and LA 177b, and tolerance was observed in three tomato cvs. M 1812, Kujawski, and Warszawski. Pitblado and MacNeill (1983) reported resistance to *P. syringae* in cherry tomatoes Oregon Cherry, Early Cherry, Droplet, and Farthest North. All these cultivars carried the *Pto* resistance gene.

In another early study by Stockinger and Walling (1994), resistance to races 0 and 1 of *P. syringae* pv. *tomato* was confirmed in the lines of wild *Solanum* species *S. pimpinellifolium*, *S. peruvianum*, and *S. hirsutum* var. *glabratum*. The resistance of *S. hirsutum* var. *glabratum* to race 0 was mediated by the *Pto3* gene and exhibited incomplete dominance when crossed with *S. lycopersicum*. A resistance gene for race 1, *Pto4*, was identified in the *S. hirsutum* var. *glabratum* line, and it segregated independently of *Pto3*. Resistance to race 1 of *P. syringae* pv. *tomato*, conferred by the *Ptr1* gene, was also confirmed in *S. lycopersicoides* (Mazo-Molina *et al.* 2019). However, new strains of race 1 *P. syringae* pv. *tomato* are capable of overcoming the resistance conferred by the *Pto/Prf* gene cluster (Hassan *et al.* 2017). Notably, Stamova (2009) reported the loss of the resistance of cultivars Chico III and Ontario 7710, mediated by the *Pto1* gene. Furthermore, in a study by Sun *et al*. (2011), no lines resistant to race 0 of *P. syringae* pv. *tomato* were found among 29 Chinese tomato cultivars and hybrids analyzed. This highlights the need to search for new sources of resistance among tomato lines and their wild relatives.

In a study by Thapa *et al*. (2015), four QTL markers for resistance to *P. syringae* pv. *tomato* were identified among hybrid lines of *Solanum habrochaites* LA1777 and *S. lycopersicum* E6203. These markers, *bsRr1-1*, *bsRr1-2*, *bsRr1-12a*, and *bsRr1-12b*, were mapped to chromosomes 1, 2, and 12. Additionally, five resistant plant lines were discovered, including *S. peruvianum* LA3799, *S. peruvianum* var. *dentatum* PI128655, *S. chilense* LA2765, *S. habrochaites* LA2869, and *S. habrochaites* LA1777, which could be used in further research. Hassan *et al*. (2017) identified new *P. syringae* pv. *tomato* race 1-resistant lines from wild species, *Solanum neorickii* LA1329 and *S. habrochaites* 30 LA1253, which could contribute to the development of resistant cultivars. Stamova (2009) identified several *S. lycopersicum* lines - Rioli, Denali, Stella, lines 114, 99-22, and 774 - resistant to race 1 of California isolate A9 of *P. syringae* pv. *tomato*. Moreover, complex resistance to races 0 and 1 of *P. syringae* pv. *tomato* was found in *S. lycopersicum* lines with non-traditional fruit colors - L1078 and L1083 with brown-red fruits, L1130 with purple fruits, and L1088 and L584 with pink fruits (Ganeva and Bogatzevska 2017).

Numerous studies have focused on the molecular-genetic mechanisms of interaction between *P. syringae* pv. *tomato* and *S. lycopersicum* plants. In particular, Preston (2000) describes the functions of various virulence factors of the bacterium *P. syringae* pv. *tomato* - type III secretion system proteins and genes encoding them, including *HrpZ*, *HrpW*, *AvrA*, *AvrD*, *AvrE*, *AvrPto*, *AvrRpt2*, and *AvrRpm1*, as well as the roles of exopolysaccharides and coronatine in tomato disease development. Effector proteins encoded by these genes can be recognized by corresponding plant genes, serving as sources of resistance through the ‘gene-for-gene’ mechanism. In another study, Arofatullah *et al*. (2019) investigated the induction of *PR* genes, *chitinase*, and *glucanase* genes, in response to heat stress, highlighting their positive role in protecting tomato plants from *Pseudomonas syringae* pv. *tomato*.

Concerning resistance to bacterial wilt, the most well-known tomato cultivar resistant to race 3 biovar 1 of *Ralstonia solanacearum* is Hawaii 7996. Through backcrossing with the susceptible Indonesian cv. GM2, it was revealed that the resistance genes to *R. solanacearum* in the Hawaii 7996 are inherited in an additive-dominant manner (Maulida *et al*. 2019). The inheritance of resistance genes to *R. solanacearum* was also investigated in the study by Costa *et al*. (2019). It was found that the resistance of tomato cv. Yoshimatsu to *R. solanacearum* is controlled by recessive alleles of two genes with an additive effect. Overall, resistance to *R. solanacearum* is an unstable trait,

and very few lines of plants tolerant to bacterial wilt are known. For instance, in the study by Lebeau *et al*. (2011), the resistance of 30 tomato, eggplant, and sweet pepper lines to 12 different strains of *R. solanacearum* was investigated. None of the tested plants showed resistance to all *R. solanacearum* strains. Instead, partial resistance of tomato lines to races 1, 2B, and 3 was observed. In another study by Kim *et al*. (2016), out of 279 tomato lines tested, only 2 lines exhibited moderate resistance, and 4 lines showed high resistance to *R. solanacearum*. Microscopic examination of resistant lines inoculated with *R. solanacearum* revealed cell wall thickening and callose deposition in stem tissues.

Special efforts are focused on the search for new markers of resistance to *R. solanacearum* (Kunwar *et al*. 2020). Among 67 resistant tomato lines, 5 and 19 were homozygous for the Bwr6 and Bwr12 markers, respectively, and 6 were homozygous for both markers. It was found that the Bwr12 marker confers resistance to race 1, but not to race 2 of *R. solanacearum*, while Bwr6 provides resistance to both races 1 and 2. Line 94T765-24-79 did not carry the Bwr6 and Bwr12 markers but exhibited enhanced resistance to race 2, potentially indicating the presence of new markers of resistance to *R. solanacearum* (Kunwar *et al*. 2020).

The bacterial spot of tomatoes is caused by a complex of four species of *Xanthomonas*: *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri*. Tolerance loci have been identified in wild *Solanum* species and some tomato cultivars, which could be transferred to valuable cvs. through breeding methods. Notably, the resistance of cv. Hawaii 7889 to race 1 of *X. campestris* pv. *vesicatoria* is associated with a hypersensitivity reaction controlled by several loci located on the long and short arms of chromosome 1 and the long arm of chromosome 5 (Yu *et al*. 1995). Bhattarai *et al.* (2017) screened 63 tomato lines for resistance to *X. perforans* race T4 and identified 5 lines (74L-1W, NC2CELBR, 081-12-1X-gsms, NC22L-1, and 52LB-1) with enhanced resistance to bacterial spot. These lines were obtained through selection from *S. pimpinellifolium* L3707. In another study by Berrueta *et al.* (2016), the resistance of 12 tomato lines to race 2 of *X. campestris* pv. *vesicatoria* was evaluated, and the most resistant lines among them were Hawaii 7981, Loica, and Ohio 8245, which could serve as sources of resistance to *X. campestris* pv. *vesicatoria*. Additionally, 14 tomato lines were studied for resistance to races T1, T2, and T3 of *X. campestris* pv. *vesicatoria*. Resistance to race 3 of the pathogen was found in line 1168 with favorable morphological characteristics (indeterminate growth, large pink fruits); lines 1076 and L503 were resistant to races T1 and T2; lines L1080, L273, and L1260 were resistant to race T1, and line L1227 was resistant to race T3 (Ganeva *et al.* 2014). The comprehensive overview of resistant lines of *S. lycopersicum* and wild *Solanum* species to various races of *Xanthomonas* species, as well as the genes *Rx1*, *Rx2*, *Rx3*, *Rx4*, *Xv3*, *RXopJ4* conferring resistance to different races of *Xanthomonas* sp., was carried out by Sharma and Bhattarai (2019).

However, there are several challenges and complexities associated with breeding for resistance to bacterial diseases, including the polygenic nature of resistance, the influence of environmental factors, race- and pathotype-specificity of resistant cultivars, epistatic interactions of resistance genes, linkage of resistance traits with small fruit size, genetic variability of bacterial pathogens, and the absence of resistance loci to certain pathogens, such as *C. michiganensis* subsp. *michiganensis* (Preston 2000, Scott *et al*. 2005, Huet 2014, Sharma and Bhattarai 2019, Kolomiiets *et al*. 2019, Wang *et al*. 2022a). Therefore, the efforts should be focused on developing resistant cultivars, as tolerant plants can harbor bacterial cells and serve as pathogen reservoirs (Huet 2014). Moreover, the use of classical and marker-associated selection to create new cultivars resistant to certain diseases is limited by the natural genetic diversity of closely related wild species, and the process of development of new cultivars can last several years (Bigini *et al.* 2021). The use of modern biotechnological methods, such as cell selection, genetic transformation, genome editing, and STTM-mediated silencing allows the creation of new cultivars resistant to phytopathogens within a short period of time. This could be achieved by introducing various foreign disease resistance genes, high-precision controlled modifications of own immune response genes, and selection *in vitro* of the cell lines and regenerated plants on the resistance to phytopathogens. Altogether, these measures could provide complex long-lasting non-specific resistance to bacterial pathogens.

Biotechnological approaches for enhancing the resistance of tomato plants to bacterial diseases

Enhancing the resistance of tomato plants with the use of cell selection: In modern agricultural practice, a wide range of approaches is used to enhance the resistance of plants to adverse biotic or abiotic factors. Among them, cell selection is one of the most efficient methods (Slavov 2005, Anil *et al.* 2018, Ivchenko *et al.* 2021), which allows the selection of cell populations resistant to the selective factors, and then the regeneration of whole plants and evaluation of the genotypes for disease resistance. The co-cultivation of plants with phytopathogenic organisms has become a useful tool for the in-depth studying of the multiple factors that facilitate plant diseases (Švábová and Lebeda 2005). The use of different tissues and organs of plants, in combination with different types of selective agents under optimal conditions, can trigger reactions similar to those of the whole plant to the pathogen.

The cell selection *in vitro* allows obtaining regenerated plants with enhanced resistance to phytopathogens which preserve the important characteristics of the original sample. The procedure of cell selection *in vitro* for disease resistance usually includes the following components: (*1*) explants or promising cell variants with high-frequency regeneration isolated from genetically stable fertile plants, (*2*) an easily reproducible selective

agent, which causes similar immune reactions in the host plant, as does phytopathogen under natural conditions, and (*3*) confirmation of resistance of selected cell lines and regenerated plants under artificial infectious dose and natural disease pressure using control genotypes (sources of disease resistance) (Rao and Sandhya 2016).

With the use of cell selection, significant progress has been achieved in creating plant lines resistant to different pathogens. For example, alfalfa and flax lines resistant to fusarium wilt, tomato and carrot lines resistant to alternariosis, potatoes resistant to late blight and bacterial rot, fodder and sugar beets resistant to bacteriosis were created with the use of this method (Rao and Sandhya 2016). The results of biotests with *Clavibacter michiganensis*, *Xanthomonas campestris*, as well as with phytopathogenic fungi *Plasmodiophora brassicae*, *Mycosphaerella musicallo*, *Alternaria alternata*, *Fusarium solani*, *Colletotrichum trifolii*, *Peronospora tabacina*, and *Phytophthora cinnamoni* confirmed the possibility of using living cells of these pathogens for screening of plant cell cultures for disease resistance *in vitro* (El Hadrami *et al.* 2005, Lebeda and Švábová 2010). However, in a number of studies, the living cells of various pathogens tested as *in vitro* selection agents were found to be too harmful to plant tissues/organs and thus with limited applications.

Therefore, due to these difficulties in the co-cultivation of phytopathogen cells and tissues of the host plant, most researchers prefer to work with cell-free selective agents, such as a culture filtrate or a purified toxin associated with the development of the disease. An important condition is a correspondence between the resistance to the selective agent *in vitro* and the field resistance of plants to the disease (Gupta and Acharya 2018, Khoshru *et al.* 2023). According to some authors (Slavov 2005), the mechanisms of interaction between the pathogen and the host plant are identical both *in vitro* and *in vivo*. Considering this, the use of cell selection allows studying the mechanisms of the plant's immune response at cellular and molecular levels, biochemical features of the infected plant, stages of the pathological process, and pathogen recognition.

An example of the successful application of this method for creating tomato plants resistant to *A. solani* was described in Pat. 62592 Ukraine: IPC A01P 1/04 (2006.01) No. u201014200 (https://uapatents.com/3-62592-sposib-stvorennya-stijjkikh-proti-alternariozu-vikhidnikh-selekcijjnikh-form-tomata.html). This method included a two-stage selection *in vitro* and a one-stage *in vivo* selection under the natural disease pressure in greenhouses. The plants were inoculated *in vitro* using 40% culture filtrate (CF) of extracellular metabolites of *Alternaria solani*. With the use of the suggested method of multi-stage selection *in vitro* and after biotests under a high infectious dose in field trials, the resistant plants and their F1 seeds were obtained within 1 year.

Therefore, the application of cell selection *in vitro* for the development of tomato lines with increased resistance against bacterial pathogens is a promising strategy for creating planting material with enhanced immunity (Anil *et al.* 2018). This promotes the creation of tomato cultivars resistant to phytopathogenic bacteria, such as *C. michiganensis* subsp. *michiganensis*, *P. syringae* pv. *tomato*, and *X. vesicatoria*, within limited time and space, and subsequent growing of these cultivars in farms and greenhouses (Kolomiiets *et al.* 2017).

To develop tomato cultivars resistant to biotic stress using either cell selection, genetic transformation, or genome editing, an effective system of regeneration and micropropagation *in vitro* is required using certain types of plant explants (Rai *et al.* 2011, Buziashvili *et al.* 2020). The choice of explants depends on the goal of the study. In general, experiments on cell selection and genetic transformation are usually carried out on primary or sub-cultivated callus, which does not lose the ability to regenerate during a series of passages (Bhatia *et al.* 2004, 2005; Ikeuchi *et al.* 2013, Bidabadi and Jain 2020). Since the hormonal composition of the nutrient medium has a large impact on callus formation, embryogenesis, and regeneration, a comprehensive study of the influence of these factors is essential for high efficiency of cell selection (Bhatia *et al.* 2005, Pérez-Clemente *et al.* 2013, Anil *et al.* 2018, Buziashvili *et al.* 2020).

In general, cell selection for resistance to bacterial pathogens is usually carried out with the use of bacterial LPS as selective agents. The use of LPS as a selective factor is favorable because of its diverse biological activity, moderate toxicity to plant cells, and ability to induce the immune response of the host plant. In the case of Gram-positive bacteria that do not have LPS in their cell wall (Erbs and Newman 2012), their exopolysaccharides (EPS) can be used as selective agents that induce defense reactions (Erbs and Newman 2003).

It was established that treatment of tomatoes and cucumbers with *P. syringae* LPS increased their resistance to bacterial diseases (Zdorovenko and Zdorovenko 2010). Also, it was shown that pre-treatment of *Arabidopsis* plants with *Pseudomonas aeruginosa* LPS modulated their sensitivity to bacterial infection (Shilina *et al.* 2017) which correlated with the origin of the LPS (from a saprophytic or phytopathogenic strain), the physiological state of the phytopathogen (native or phenol-treated), and the genotype of the plant (Shilina *et al.* 2017). It was also shown that an increase in the concentration of the selective factor in the culture medium forced a gradual increase in the deposition of callose responsible for strengthening the cell walls (Emel'yanov *et al.* 2008).

However, the results of our studies showed the ability of LPS of *P. syringae* pv. *atrofaciens* to induce chromosomal aberrations in the cells of the apical meristem of *Allium cepa* (Butsenko 2016). An increase in the frequency of chromosomal aberrations induced by LPS of *P. syringae* pv. *atrofaciens* can be considered as a negative consequence of cell selection, which might lead to the loss of valuable properties of cultivated plants. On the other hand, the mutagenic properties of LPS of phytopathogenic bacteria can increase the genetic diversity of the original forms, which can become the source of increased resistance to phytopathogens. In the process of further selection, the cell lines and regenerated plants possessing this feature are picked up. In our studies, using the multi-step selection *in vitro* we obtained cellular variants that were able to

grow on a selective medium with LPS and maintained a stable resistance during 4 passages. The produced lines can serve as the initial material for the further selection of tomato cultivars resistant to bacterial diseases (Kolomiiets *et al.* 2017, 2019; Anil *et al.* 2018).

Increasing the resistance of tomato plants to bacterial diseases with the use of genetic transformation: New innovative biotechnologies have a high priority in modern agriculture. There are various strategies for increasing the resistance of tomato plants to phytopathogens using transgenic technologies, which include: (*1*) the transfer of genes that activate the own defense mechanisms of tomato plants, (*2*) the transfer of genes responsible for the synthesis of secondary metabolites, (*3*) the transfer of R-genes from systematically distant species of plants that recognize Avr proteins of bacteria and ensure the resistance by the 'gene-for-gene' mechanism, and (*4*) transfer of the genes encoding antimicrobial peptides (AMP) (Table 1). Each of these strategies effectively protects the transgenic tomato plants against bacterial diseases.

One of the strategies to protect tomato plants from bacterial diseases is the transformation with genes that stimulate their own mechanisms of protection against phytopathogens - enhance the expression of *PR* genes, induce an HR, or activate non-canonical resistance mechanisms.

One such example is the transformation of tomato plants with the *CBF1* gene of *Arabidopsis thaliana* (Li *et al.* 2011). As known, the expression of the *CBF1* gene activates plant defense mechanisms such as the expression of *PR* genes. In this study, authors showed that the increased expression of *CBF1* correlated with the activation of the constitutive expression of the transcription factor RAV (related-to-ABI3/VP1), genes of the *ERF* family (ethylene-responsive factor) and some *PR* genes [*PR3* (chitinase), *PR5* (thaumatin-like protein), *PR7* (endoproteinase), *PR9* (peroxidase), and *PR10* (RNase-like protein)] and represses the proliferation of *R. solanacearum* in the vascular system thus enhancing the resistance of transgenic plants to bacterial wilt (Table 1).

Another example is the transformation of tomato plants with the genes of the subunits of the elongator complex (*ELP3*, *ELP4*) of *A. thaliana* (Pereira *et al.* 2018). The elongator protein complex is involved in many cellular processes - exocytosis, histone modification, synthesis of tRNA and miRNA, α-tubulin acetylation, DNA demethylation in the zygote, transcription of genes involved in plant immune response mechanisms, in particular, stomatal closure in response to effectors of *P. syringae* pv. *tomato*. Increased expression of *PR* genes *PR1b1*, *PR-5x*, *DES*, and *ER1* was noted in transgenic tomato plants with overexpression of *ELP3* and *ELP4* genes of *A. thaliana*. It was also shown that transgenic tomato lines expressing *ELP3* and *ELP4* genes were more resistant to *P. syringae* pv. *tomato* than control ones, which may indicate the involvement of stomata in the development of resistance (Table 1).

Increased resistance of tomato plants to *P. syringae* pv. *tomato* was achieved by transformation with the *YODA* kinase gene of *A. thaliana* (Téllez *et al.* 2020). The gene of YODA kinase regulates various cellular processes, such as the development of stomata and modulation of the resistance to phytopathogens through non-canonical (independent of jasmonate, ethylene, and salicylic acid) signaling cascade. In the transgenic tomato plants carrying the *YODA* kinase gene, a reduced number of stomata was noted compared to control ones, while the transpiration rate did not differ from the control. Also, constitutive activation of the immune response genes was noted in transgenic plants in the absence of the infection (Table 1).

In order to enhance the resistance to bacterial pathogens, tomato plants were also transformed with the *NPR1* gene of *A. thaliana* (Lin *et al.* 2004). The *NPR1* gene encodes a protein that activates the expression of *PR* genes in response to salicylic acid. The *NPR1* protein regulates the defense reactions by the SAR mechanism. Tomato plants transformed with the *NPR1* gene showed increased resistance to *R. solanacearum* and *X. campestris* pv. *vesicatoria*. Also, constitutive enhancing expression of *PR* genes, such as *GLUa*, *GLUb*, and *CHI3* was observed in transgenic plants (Table 1).

Although ferredoxin-1 is an important component of photosynthetic reactions that transfer electrons from photosystem I (PS I) to the enzyme Fd:$NADP^+$ reductase and does not directly affect plant immune responses, it was shown that transformation of tomato plants with the *PFLP* gene encoding ferredoxin-1 from sweet pepper (*Capsicum annuum*) enhances the resistance of transgenic tomato plants to *R. solanacearum* and *Erwinia amylovora* (Huang *et al*. 2007). However, the expression of *PFLP* had a negative impact on the height of transgenic plants which were lower than the control ones, possibly due to the constitutive production of ROS (Table 1).

Thus, the transformation of tomatoes with genes that activate their own defense mechanisms allows obtaining transgenic plants with increased resistance to *P. syringae* pv. *tomato*, *R. solanacearum*, *Erwinia amylovora*, and *X. campestris* pv. *vesicatoria*. However this approach has several limitations, such as minor changes in the morphology of transgenic plants due to the activation of defense mechanisms (Huang *et al.* 2007, Téllez *et al.* 2020), and the possibility that phytopathogen will bypass the defense mechanisms provided by the transgene (Pereira *et al.* 2018).

Transformation with genes of the synthesis of secondary metabolites: Transformation of plants with genes encoding the enzymes that catalyze the synthesis of secondary metabolites could also be successfully applied to increase their resistance to bacterial diseases.

For example, tomato plants transformed with the polyphenol oxidase (*PPO*) gene from potato (*Solanum tuberosum*) showed enhanced resistance to *P. syringae* pv. *tomato* (Li and Steffens 2002). Polyphenol oxidase catalyzes the oxidation of phenols into quinones, which are highly reactive molecules that covalently modify

Table 1. Examples of the application of genetic engineering methods to increase the resistance of tomato plants to bacterial pathogens.

Gene	Source	Plant response to phytopathogens	Reference
Activation of the plant's own mechanisms of resistance			
CBF1	*Arabidopsis thaliana*	Transgenic lines did not show signs of bacterial wilt after inoculation with *R. solanacearum*	Li *et al.* 2011
ELP3 (Elongator), *ELP4*	*A. thaliana*	Absence of symptoms on transgenic plants after inoculation with *P. syringae* pv. *tomato*	Pereira *et al.* 2018
YODA kinase	*A. thaliana*	Higher resistance of transgenic plants to *P. syringae* pv. *tomato*	Téllez *et al.* 2020
NPR1 (nonex-pressor of PR genes)	*A. thaliana*	After inoculation with *R. solanacearum* and *X. campestris* pv. *vesicatori*a, transgenic lines exhibited much less symptoms of bacterial diseases than control	Lin *et al.* 2004
PFLP (ferredoxin-1 of sweet pepper)	*Capsicum annuum*	The damage of transgenic plants by *R. solanacearum* and *Erwinia amylovora* was lower than of control	Huang *et al.* 2007
Transformation with genes of synthesis of the secondary metabolites			
PPO (polyphenol oxidase)	*Solanum tuberosum*	Transgenic lines with overexpression of PPO were more resistant to *P. syringae* pv. *tomato* than control	Li and Steffens 2002
THT (tyramine N-hydroxy-cinnamoyl-transferase)	*Solanum lycopersicum*	The enhancement of the resistance of transgenic plants to bacterial speck disease caused by *P. syringae* pv. *tomato* was observed	Campos *et al.* 2014
Formation of non-host resistance by the 'gene-for-gene' mechanism			
Roq1 (recognition of XopQ1)	*Nicotiana benthamiana*	The resistance of transgenic plants to the bacteria *X. perforans*, *X. euvesicatoria*, and *P. syringae* was greatly enhanced compared with non-transgenic control. Transgenic plants did not show any symptoms of bacterial wilt after inoculation with *R. solanacearum*	Thomas *et al.* 2020
EFR (EF-Tu receptor) *FLS2* (flagellin-sensing 2) and *BAK1* (BRI1-associated receptor kinase 1)	*A. thaliana*	Increased resistance of transgenic plants to *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *Clavibacter michiganensis* subsp. *michiganensi*s	Plancarte-De la Torre *et al.* 2016
Xa21	*Oryza sativa*	All transgenic plants carrying the *XA21* gene were resistant to *P. solanacearum*	Afroz *et al.* 2011
Bs2	*Capsicum* sp.	The sensitivity to bacterial spot caused by different races of *Xanthomonas* sp. was lower in transgenic lines than in control	Horvath *et al.* 2012
ERF (ethylene-response factor)	*A. thaliana*	Transgenic tomato lines expressing EFR showed a noticeable decrease in the symptoms of bacterial wilt caused by *R. solanacearum* and bacterial spot caused by *X. perforans*	Lacombe *et al.* 2010
Prf	*S. lycopersicum*	Increased resistance of transgenic plants to the highly virulent strain *P. syringae* pv. *tomato* T1, and also to *X. campestris* pv. *vesicatoria* and *R. solanacearu*m	Oldroyd and Staskawicz 1998
Transformation with genes encoding APM			
TLP (thaumatin)	*Actinidia deliciosa*	Most of the transgenic lines had increased resistance to *X. vesicatoria* compared to the control	Korneeva *et al.* 2011
Thi2.1 (thionine)	*A. thaliana*	More than 80% of transgenic plants carrying the *Thi2.1* gene were resistant to *R. solanacearum*	Chan *et al.* 2005
GLU (β-1,3-glucanase), *AFP* (defensin)	*N. tabacum*, *Medicago sativa*	Enhanced resistance of transgenic T1 plants to bacterial wilt after inoculation with *R. solanacearum*	Chen *et al.* 2006
CecB (cecropin B)	*Hyalophora cecropia*	Increase of the resistance of transgenic plants to *R. solanacearum* and *X. campestris* pv. *vesicatori*a	Jan *et al.* 2010
SlP14a-PPC20	*S. lycopersicum*, *Helianthus annuus*	After inoculation with *R. solanacearum*, 92.3% of transgenic plants were viable	Morais *et al.* 2019
LL-37 (cathelicidin)	*Homo sapiens*	After inoculation with *Pectobacterium carotovorum* subsp. *carotovorum* and *X. campestris* pv. *vesicatoria*, the symptoms in transgenic plants carrying *LL-37* were much lower than in control plants	Jung 2013
hLf (human lactoferrin)	*H. sapiens*	44 - 55% of transgenic plants infected with *R. solanacearum* were resistant to bacterial wilt	Lee *et al.* 2002
hLf (human lactoferrin)	*H. sapiens*	Inhibition of the growth of *R. solanacearum*, *C. michiganensis* subsp. *michiganensis*	Buziashvili *et al.* 2020
Lys (endolysin)	bacteriophage CMP1	After inoculation with *C. michiganensis* subsp. *michiganensis*, most of the transgenic plants were resistant to bacterial cancer	Wittmann *et al.* 2016

intracellular compounds with the formation of a brown color. The expression of the *PPO* gene is induced by wounding, biotic, and abiotic stresses, and is regulated by salicylic acid, jasmonate, and ethylene. Moreover, quinones are also characterized by antibiotic and cytotoxic activity directly affecting pathogens and pests. For these reasons, tomato plants expressing the *PPO* gene of *S. tuberosum* show increased resistance to *P. syringae* pv. *tomato* (Table 1) (Li and Steffens 2002).

Another gene involved in the synthesis of secondary metabolites which was successfully used to increase the resistance of tomato plants to bacterial speck is the *THT* gene (Campos *et al.* 2014). This gene encoding N-hydroxycinnamoyl transferase enzyme catalyzes the synthesis of amides of hydroxycinnamic acid, which are synthesized in response to injury or infection by pathogens and play an important role in the processes of plant development. These phenolic compounds are biologically active substances found in flowers, seeds, and pollen. They have antioxidant, antibacterial, fungicidal, and insecticidal activities. Transgenic tomato plants with an increased *THT* expression were more resistant to *P. syringae* pv. *tomato* than the control ones (Campos *et al.* 2014). In addition, transgenic plants showed higher concentrations of hydroxycinnamic acid amides in flowers and fruits, as well as a three-fold increased content of salicylic acid in leaves and a 1.5-fold enhanced expression of *PR-1* gene 2 d after inoculation (Table 1).

Therefore, with the use of this approach, a significant increase in the resistance of transgenic tomato plants to *P. syringae* pv. *tomato* has been achieved. However, this method also has some drawbacks, especially the high content of secondary metabolites in fruits which can negatively affect their marketable qualities such as taste and appearance (Li and Steffens 2002).

Induction of non-host resistance by the 'gene-for-gene' mechanism: The other approach for increasing the resistance of tomato plants to phytopathogens is the transformation by *R*-genes, which products inhibit phytopathogen toxins and induce hypersensitive response (HR) (Glazebrook 2005, Khaliluev and Shpakovskii 2013, Boddy 2016).

For this purpose, the *Roq1* gene of *Nicotiana benthamiana* was transferred into the tomato genome. *Roq1* gene encodes a Toll/Interleukin-1 receptor (TIR) with NB-LRR, which provides resistance *via* the ETI mechanism. This gene recognizes the highly conserved XopQ1 effector protein of *Xanthomonas* bacteria, a homologue of the HopQ1 protein of *P. syringae* pv. *tomato* and RipB1 of *R. solanacearum*. XopQ/HopQ1 proteins are highly conserved virulence factors of phytopathogenic bacteria that affect cytokine levels and interact with 14-3-3 proteins of sensitive plant species. Thomas *et al.* (2020) showed an increased resistance of tomato plants transgenic for the *Roq1* gene against *X. perforans*, *X. euvesicatoria*, *P. syringae* pv. *tomato*, and *R. solanacearum*. However, transgenic plants inoculated by the *R. solanacearum* mutant strain with deletion of the *RipB* gene were sensitive to bacterial wilt, indicating the possibility of a loss of the resistance of transgenic lines to phytopathogens as a result of mutations in *HopQ1* gene homologues (Table 1).

Also, the transformation of tomato plants with three *Arabidopsis thaliana R*-genes was carried out (Plancarte-De la Torre *et al.* 2016). These *R*-genes were *EFR* encoding a receptor of the RLK (receptor-like kinase) family, which recognizes a fragment of the bacterial elongation factor EF-Tu, *FLS2* encoding an RLK receptor, which recognizes a highly conserved 22-amino acid epitope of bacterial flagellin, and a *BAK1* gene which product is an FLS2 and ERF co-receptor and a member of the SERK (somatic embryogenesis-related kinase) family of kinases. The *ERF* and *FLS2* genes encode PRR receptors that recognize highly conserved bacterial proteins and provide resistance by the PTI mechanism preventing the penetration of bacteria into the plant organism and the development of systemic infection. This contributes to the formation of long-term non-specific resistance of plants to various bacteria. It is important to note that tomatoes have orthologs of *EFR*, *FLS2*, and *BAK1* genes, thus, the transfer of genes with other amino acid sequences from other plant species can increase the resistance of tomatoes to virulent races of phytopathogens. The results of this study showed the increased resistance of transgenic tomato plants to *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *Clavibacter michiganensis* subsp. *michiganensis* (Table 1).

To increase the resistance to bacterial speck, tomato plants transformed with the O*ryza sativa Xa21* gene, which encodes RLK with NB-LRR, recognizes the corresponding avirulence genes of bacteria of the genus *Xanthomonas* and provides resistance to a wide range of phytopathogenic bacteria *Xanthomonas* sp. by the 'gene-for-gene' mechanism (Afroz *et al.* 2011). In this work, the transgenic plants were resistant to bacterial speck caused by *P. syringae* pv. *solanacearum*, while the controls were completely wilted (Table 1).

Transformation of tomato plants with the *Bs2* gene from *Capsicum annuum* was carried out to enhance their resistance to bacterial spot (Horvath *et al.* 2012). The Bs2 protein recognizes the corresponding avirulence genes (*avrBs2*) of bacteria of the genus *Xanthomonas* and provides resistance by the 'gene-for-gene' mechanism. Tomatoes transgenic for the *Bs2* gene were resistant to *X. perforans* infection in the field. Moreover, depending on weather conditions and the level of infection load, transgenic plants had 1.5 - 10 times higher yield than control plants (Table 1).

Another gene encoding the PRR-receptor of *A. thaliana* was transferred into genome of tomato plants to enhance their resistance to phytopathogenic bacteria (Lacombe *et al.* 2010). In this work, tomato plants were also transformed with the *ERF* gene from *A. thaliana*, which is a component of the first line of plant immune defense and is important in providing PTI. The ERF protein is a PRR-receptor, which recognizes the bacterial elongator Ef-Tu protein. Most PRRs are highly conserved (such as the FLS2 flagellin receptor), but the ERF receptor is only found in some members of the *Brassicaceae* and

Solanaceae families, and some rice cultivars. In this work, it was shown that the expression of *ERF* in transgenic tomato plants causes an HR in response induced by elf18 peptide, a fragment of the Ef-Tu protein. As a result, transgenic plants showed an increased resistance to *R. solanacearum* and *X. perforans*. Bacterial wilt and bacterial spot symptoms were significantly less in transgenic plants than in controls. The authors also note that constitutive expression of defense genes, or ROS production, was not observed in transgenic plants (Table 1).

Transgenic tomato plants with enhanced expression of their own *Prf* gene were also created to increase the resistance to different bacterial phytopathogens (Oldroyd and Staskawicz 1998). The results of the previous studies showed that only co-expression of the *Pto* and *Prf* genes can provide resistance to *P. solanacearum* pv. *tomato* by the gene-for-gene mechanism. It was shown that the protein encoded by the *Prf* gene acts downstream of the Pto protein in the HR signaling cascade, and mutations in the *Prf* gene lead to a loss of avrPto-induced resistance to *P. solanacearum* pv. *tomato*. The *Prf* gene is a member of the family of resistance genes encoding receptors with the NB-LRR, Pto encodes a serine-threonine protein kinase. The work (Oldroyd and Staskawicz 1998) showed an increase in the resistance of transgenic plants not only to *P. solanacearum* pv. *tomato*, but also to *X. campestris* pv. *vesicatoria* and *R. solanacearum* compared to the control. The authors note that the enhanced resistance to bacterial pathogens was implemented through the SAR mechanism because an increased salicylic acid content and an enhanced expression of *PR1* and *PR2* genes were noted in transgenic plants. At the same time, the constitutive activation of SAR mechanisms in transgenic plants did not affect their phenotype (Table 1).

Thus, the transformation of tomatoes with *R*-genes from systematically distant plant species resulted in a significant increase in the resistance of transgenic plants to phytopathogenic bacteria - the number of CFU (colony-forming units) of *R. solanacearum* pv. *tomato*, *X. campestris* pv. *vesicatoria*, *X. perforans*, *X. euvesicatoria*, and *R. solanacearum* in the tissues of transgenic plants transformed with *Roq1* and *Prf* genes was more than 100 times lower than in the control (Oldroyd and Staskawicz 1998, Thomas *et al.* 2020). At the same time, the transformation of tomatoes with the *Bs2* gene resulted in a 1.5 - 10-fold increase in the yield under high disease pressure (Horvath *et al.* 2012). Thus, we can conclude that transformation with *R*-genes is more effective than transformation with genes that activate the plant's own defense mechanisms. However, the disadvantage of this method is the possibility of a loss of resistance as a result of mutations in the *Avr* genes of pathogens (Thomas *et al.* 2020).

Transformation by genes encoding AMP: Since phytopathogens are able to overcome ‘gene-for-gene’ resistance mechanisms, a promising strategy for increasing resistance to phytopathogens is the transformation with 2 the genes of antimicrobial peptides (AMP) - short peptides of 12 - 50 amino acids in length, which are widely represented among all living organisms, including PR (pathogenesis-related) plant proteins. All AMPs are specific to bacterial cells, which differ from eukaryotic cells by the presence of negatively charged phospholipids on the extracellular surface. The main mechanism of antibacterial activity of AMPs relies on breaking the integrity of the cell membrane of bacteria due to the formation of pores (López-García *et al.* 2012, Jung and Kang 2014).

Plant PR proteins are small peptides with a molecular mass of 5 - 75 kDa, which were grouped into 17 families according to their activity. Examples of some PR proteins are chitinases, glucanases, thaumatin-like proteins (TLPs), proteinase inhibitors, peroxidases, ribonuclease-like proteins (RLPs), defensins, thionins, lipid transport proteins (LTPs), oxalate oxidases (OXOs), *etc*. (López-García *et al.* 2012, Moosa *et al.* 2017).

To increase the resistance of tomato plants to *R. solanacearum*, transformation with the *Thi2.1* gene of *A. thaliana* was carried out (Chan *et al.* 2005). *Thi2.1* gene encodes the cysteine-rich AMP thionine. Due to the toxicity of thionine to mammalian cell cultures and laboratory animals, the *Thi2.1* gene was placed under the control of the tobacco tissue-specific RB7 promoter, which inactivates gene expression in fruits. Transgenic plants carrying the *Thi2.1* gene were more resistant to bacterial wilt than the control ones, as less than 20% of transgenic and more than 40 - 50% of control plants inoculated with *R. solanacearum* became wilted (Table 1).

A similar approach was used by Chen *et al.* (2006). Tomato plants were transformed with a construct containing the fused *GLU* and *AFP* genes, which encode the tobacco AMP glucanase and defensin of *Arabidopsis thaliana*, respectively (Chen *et al.* 2006). Transgenic plants carrying the fusion *GLU-AFP* gene inoculated with *R. solanacearum* were resistant to bacterial wilt (Table 1).

In order to enhance the resistance to bacterial speck, tomato plants were transformed with the *TLP* gene of *Actinidia deliciosa* (Korneeva *et al.* 2011). The *TLP* gene encodes thaumatin, an AMP from the PR-5 group, which is expressed in response to infection by phytopathogens. Thaumatin is known to have a sweet taste (1 000% sweeter than sucrose), and its expression may affect fruit taste, but the authors note that this effect is negligible compared to the protective properties of the peptide. The obtained results (Korneeva *et al.* 2011) confirmed the increased resistance of transgenic plants to *X. vesicatoria* (Table 1).

An equally effective strategy for increasing the resistance of plant crops to phytopathogens is transformation with genes encoding AMP of non-plant origin (Osusky *et al.* 2000, Dahleen *et al.* 2001, Marcos *et al.* 2008, Patil *et al.* 2016). In the study of Jan *et al.* (2010), tomato plants were transformed with the *CecB1* gene encoding the α-helical antimicrobial peptide in *Hyalophora cecropia*, which has lytic activity against most Gram-negative and some Gram-positive bacteria. In this research, tomatoes transformed with the *CecB1* gene had increased resistance to *R. solanacearum* and *X. campestris* pv. *vesicatoria* (Table 1). However, there is evidence of cecropin B toxicity for mammalian intestinal cells (Jan *et al.* 2010).

An alternative approach was used by Morais *et al.* (2019) using *CecB* homologues with reduced toxicity to animal cells. In this work, tomato plants were transformed with a chimeric gene *SlP14a-PPC20*, which was created by the fusion of the *P14a* gene of *S. lycopersicum* and the *PPC20* gene of *Helianthus annuus*. The search for gene sequences, encoding functionally active α-helical AMPs, was carried out using *in silico* approaches. As a result, the *P14a* and *PPC20* genes were selected for further studies. The *P14a* encodes a tomato PR protein, a putative protease that disrupts *E. coli* cell membrane protein A, whereas the *PPC20* gene encodes a fragment of sunflower phosphoenolpyruvate carboxylase with a similar amino acid sequence and antibacterial activity to *CecB*. In this study, low toxicity of SlP14a and PPC20 peptides for the human intestinal epithelial cell line (SK-CO15) was shown. Moreover, the significant antibacterial activity of the SlP14a-PPC20 protein obtained from transgenic plants against *R. solanacearum* was noted, as this protein inhibited bacterial growth by 84%. Also, a remarkable increase in the resistance of transgenic plants to bacterial wilt was shown: 92.3% of inoculated transgenic plants were alive (Table 1).

Another gene of the AMP used for tomato transformation was the *LL-37* gene, which encodes the active form of human cathelicidin (Jung 2013). Cathelicidin is an antimicrobial peptide which is present in the lysosomes of human immune cells. The *LL-37* gene was fused to the fragment of the *vc-2* gene of *Pisum sativum*, which directs secretion into the extracellular space. Transgenic plants transformed with the *LL-37* gene were more resistant to *Pectobacterium carotovorum* subsp. *carotovorum* and to *X. campestris* pv. *vesicatoria*. At the same time, the expression of *PR* genes (encoding PR proteins AT4G25780, AFP1, LTP, and AGP) was increased in transgenic plants 6 and 12 h after inoculation with *P. carotovorum* subsp. *carotovorum* compared with controls (Table 1) indicating the enhancement of the plant's immune response independently of *LL-37* expression.

Several works were carried out on the transformation of tomato plants with the human lactoferrin gene (*hLf*) (Lee *et al.* 2002, Buziashvili *et al.* 2020). This gene encodes a glycoprotein with Fe-binding, antibacterial, fungicidal, antiviral, antioxidant, and anti-inflammatory activities (Buziashvili and Yemets 2023). Lactoferrin is a component of the innate non-specific immunity that is present in human secretory fluids. Transgenic tomato plants inoculated with *R. solanacearum* had enhanced resistance to bacterial wilt (Lee *et al.* 2002). In our recent study (Buziashvili *et al.* 2020) it was shown that transgenic tomato plants expressing *the hLf* gene were resistant not only to *R. solanacearum* but also to *C. michiganensis* subsp. *michiganensis*, the causative agent of bacterial canker (Table 1).

An interesting approach based on the transformation of tomato plants with the endolysin (*lys*) gene of bacteriophage CMP1 was shown by Wittmann *et al.* (2016). Endolysin protein specifically binds murein B2γ of different subspecies of *Clavibacter michiganensis*. This approach allows the obtaining of transgenic plants resistant to certain phytopathogens without affecting neither their morphology nor the soil microbiome. Although some amount of bacterial cells was detected in xylem and leaf extracts of inoculated transgenic plants, the symptoms of bacterial cancer were not observed in these plants, and their seeds were not contaminated with *C. michiganensis* subsp. *michiganensis* (Table 1).

Therefore, the transformation of tomato plants with AMP genes can be used to increase their resistance to the dangerous bacterial pathogens *P. carotovorum*, *X. vesicatoria*, and, in particular, to the quarantine bacteria *R. solanacearum* and *C. michiganensis* subsp. *michiganensis* - actually, no tomato resistance genes to these phytopathogens have been identified so far. However, the expression of some AMPs in transgenic plants has minor 'side effects' - AMP toxicity to animals (Chan *et al.* 2005, Jan *et al.* 2010) or a change in the taste qualities of fruits (Korneeva *et al.* 2011), which could be resolved by using tissue-specific promoters. At the same time, the highest resistance was observed when genes of non-plant origin were used for transformation, such as human lactoferrin *hLf* (Lee *et al.* 2002, Buziashvili *et al.* 2020) and endolysin *lys* of the bacteriophage CMP1 (Wittmann *et al.* 2016) - in these studies, the resistance of the transgenic plants to phytopathogens was maintained for 56 and 30 d after inoculation, which is sufficient for obtaining of healthy fruits.

Application of genome editing methods and gene silencing to create resistant cultivars

Genome editing is a process of removal, insertion, and replacement of DNA fragments using programmed nucleases that bind to specific sites and make local DNA double-strand breaks (Mohanta *et al.* 2017, Li *et al.* 2020). The DNA breaks are then stitched together by intracellular repair mechanisms (usually non-homologous end joining, NHEJ). Currently, the best-known genome editing technologies are ZFN (zink-finger nucleases), TALEN (transcription activator-like effector nucleases), and CRISPR/Cas (clustered regularly interspaced short palindromic repeats) (Li *et al.* 2020). Given the simplicity and high accuracy of genome editing technologies, one of the promising areas of their application is the creation of transgene-free cultivars of vegetable crops with improved valuable properties, in particular, with increased resistance to phytopathogens (Borrelli *et al.* 2018, Yin and Qiu 2019, Barka and Lee 2022, Wang *et al.* 2022b, Ijaz *et al.* 2023). This strategy is aimed at editing the susceptibility (*S*)-genes that are functionally conserved among different plant species (Barka and Lee 2022).

Currently, quite a few articles have been published on obtaining bacterial disease-resistant cultivars of tomatoes using genome editing methods. For example, the genome-edited tomato plants with a 7-nucleotide deletion in exon 3 of the *DMR6-1* gene encoding Fe(II)-dependent oxygenase were created using CRISPR/Cas9-mediated method

Table 2. Application of genome editing and gene silencing methods to obtain tomato plants resistant to bacterial diseases.

Gene	Modification of expression	Plant response to phytopathogens	Reference
DMR6-1 (downy mildew resistance 6)	CRISPR-Cas9-mediated deletion of 7 nucleotides in the 3 exon	Increased resistance to *Xanthomonas gardneri*, *X. perforans*, and *Pseudomonas syringae* pv. *tomato*, less severe symptoms in transgenic plants	Thomazella *et al.* 2021
JAZ2	CRISPR-Cas9-mediated deletion of the C-terminal domain of JAZ2 receptor	The control plants inoculated by *P. syringae* pv. *tomato* showed the symptoms of bacterial speck whereas the gene-edited plants with *JAZ2* deletion were healthy	Ortigosa *et al.* 2019
STTM (short tandem target mimic) RNAs miR482/2118	Silencing	The resistance to *P. syringae* pv. *tomato* of transgenic plants carrying *STTM482.1* and *STTM2118b.5* were higher than in control plants	Canto-Pastor *et al.* 2019

(Thomazella *et al.* 2021). In this work, an increase in resistance to bacterial pathogens *Xanthomonas gardneri*, *Xanthomonas perforans*, and *Pseudomonas syringae* pv. *tomato* was shown for transgenic tomato plants. A slight decrease in the height of shoots of transgenic plants was also noted, which may be associated with the constitutive activation of defense systems (Table 2).

A CRISPR/Cas9-mediated deletion of the C-terminal domain of the *JAZ2* gene was also used by Ortigosa *et al.* (2019) to increase the resistance of tomatoes to phytopathogens. The *JAZ2* gene encodes the coronatine receptor. Coronatine is the effector of *P. syringae* pv. *tomato*, which induces the opening of stomata in the process of plant colonization. This approach is aimed at the activation of non-canonical defense mechanisms against phytopathogens and the prevention of the antagonism between signaling pathways of defense responses against biotrophic pathogens, mediated by salicylic acid, and necrotrophic, mediated by jasmonate. As a result, the obtained genome-edited tomato plants were resistant to bacterial speck after surface inoculation with *P. syringae* pv. *tomato*. At the same time, the authors note that the transpiration rate of transgenic plants and resistance to the necrotrophic fungal pathogen *Botrytis cinerea* remained unchanged (Table 2).

In the report of Canto-Pastor *et al.* (2019), genome editing technologies *per se* were not applied, but with the use of gene silencing by STTM (short tandem target mimic) miR482/2118, the transgenic tomato plants with increased resistance to bacterial spot were obtained. STTM miR482/2118 inactivates miR482/2118 in plant cells, which downregulates the synthesis of NB-LRR receptors by RNA interference mechanisms. As a result, a decrease in the titer of *P. solanacearum* pv. *tomato* cells was shown in the leaves of transgenic plants compared to the control (Table 2).

Thus, the use of CRISPR/Cas9 and STTMmiRNA technologies allows obtaining tomato plants with increased resistance to phytopathogens *Xanthomonas gardneri*, *Xanthomonas perforans*, and *Pseudomonas syringae* pv. *tomato*. However a possible negative consequence of these interventions is a slight decrease in the shoot height of transgenic plants as a result of the constitutive activation of defense mechanisms (Thomazella *et al.* 2021).

To sum up, the results of the previous studies comprehensively analyzed in this review show that the technologies of *in vitro* cell selection, genetic transformation, genome editing, and gene silencing are promising biotechnological approaches that ensure a sustainable increase in the resistance of tomato plants to bacterial pathogens and contribute to compliance with the principle of integrated pest management. Genome editing technologies are fairly new and powerful techniques that allow within a short period of time to perform a controllable change in the sequences of resistance genes and create new resistant alleles that are fixed in the genome and transmitted to the next generations. The potential of this method to create new resistant cultivars is extremely high, and it will be revealed by researchers in the nearest future.

Concluding remarks

Tomatoes are among the most important vegetable crops consumed all over the world, but they are subjected to highly virulent bacterial phytopathogens such as *R. solanacearum*, *C. michiganensis* subsp. *michiganensis*, *X. vesicatoria*, and *P. syringae* pv. *tomato*. The effective strategy for the management of tomato bacterial diseases is the cultivation of resistant cultivars. Obtaining new tomato cultivars resistant to bacterial pathogens with the use of classical and marker-associated breeding is limited by the complex polygenic nature of the resistance and the high variability of the bacterial pathogens. Application of advanced biotechnological methods is of high priority for obtaining genotypes with increased resistance to phytopathogenic bacteria because of their high precision and high throughput compared with breeding. An in-depth understanding of molecular mechanisms of interaction between bacterial phytopathogens and host plant and the processes underlying the formation of resistance is essential for the precise use of these strategies. Today, the most promising approaches to increase the resistance of cultivated tomato plants to phytopathogens is the use of cell selection, genetic engineering methods, genome editing, and gene silencing which can undoubtedly realize their full potential in the near future.

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