Chapter

Antimicrobial Resistance with Special Emphasis on Pathogens in Agriculture

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Abstract

Antibiotics have been used globally to manage the bacterial plant diseases irrespective of the expense involved. Although plant pathogenesis by bacteria is far lower than fungal counterparts, disrupted monitoring and surveillance for drug resistance with respect to human health raise serious concerns. The resistance derived by the plant as the host by the antibiotics used for many generations has now posed as a problem in phyto-systems. Although we currently lack the molecular understanding of the pathogens rendering antibiotic resistance to plants, robust resistance management strategies are critical to ensure management of critically important diseases that specifically target crops of high value and/or global agrarian importance. This chapter discusses evolution of plant-pathogenic bacteria, application of antibiotics and its repercussions on the microbiome of plant agricultural systems, and sustainable crop disease management by genetic engineering.

Keywords: agriculture, bacteria, fruit, genetic engineering, host, molecular biology

1. Introduction

Antibiotic resistance most commonly evolves in bacteria either through mutation of a target site protein, through the acquisition of an antibiotic-resistant gene that confers resistance through efflux or inactivation of the antibiotic, or through synthesis of a new target protein that is insensitive to the antibiotic [1]. An extensive body of knowledge has been gained from studies of antibiotic resistance in human pathogens and in animal agriculture. The ability of bacterial pathogens to acquire antibiotic-resistant genes and to assemble them into blocks of transferable DNA encoding multiple antibiotic-resistant genes has resulted in significant issues that affect successful treatment interventions targeting some specific human infections. The current global antibiotic resistance crisis in bacterial populations has been fuelled by basic processes in microbial ecology and population dynamics, engendering a rapid evolutionary response to the global deployment of antibiotics by humans in the millions of kilograms per year. What was not anticipated when antibiotics were discovered and introduced into clinical medicine is that antibiotic-resistant genes pre-existed in bacterial populations [2–4]. Furthermore, the extent to which antibiotic-resistant genes could be transferred between bacteria, and

even between phylogenetically distinct bacteria, was not understood 70 years ago but is becoming more apparent through a number of elegant studies identifying the microbial antibiotic resistome. The collection of all known antibiotic-resistant genes in the full-microbial pan-genome is defined as the antibiotic resistome [5].

2. Use of antibiotics in agriculture

Effective management of bacterial plant diseases is difficult and is exacerbated by factors such as the large size of bacterial pathogen populations on susceptible plant hosts and the few available bactericides. In the absence of durable and robust host disease resistance, antibiotics have represented the best option for bacterial disease control in many pathosystems because these materials provide the most efficacious means of reducing bacterial population size and limiting disease outbreaks. Although many new types of antibiotics were rapidly tested and then deployed in animal agriculture starting in the 1950s, antibiotic use for plant disease control was tempered by several factors, including lack of efficacy at lower doses, phytotoxicity problems at higher doses, and expense compared to other existing methods of disease control. Thus, although penicillin, streptomycin, aureomycin, chloramphenicol, and oxytetracycline were tested for plant disease control in the late 1940s [6, 7], only streptomycin and oxytetracycline were ultimately deployed in plant agriculture and only in specific disease pathosystems. Streptomycin is the main antibiotic currently in use for plant disease control around the world, targeting pathogens such as *Erwinia amylovora*, which causes fire blight of apple and pear; *Pseudomonas syringae*, which causes flower and fruit infection of apple and pear trees; and *Xanthomonas campestris*, which causes bacterial spot of tomato and pepper [8]. Oxytetracycline has been used as the primary antibiotic in specific disease control situations, including the control of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot of peach and nectarine [8]. In addition, oxytetracycline has been used as a secondary antibiotic for fire blight management in the United States, most prominently in situations in which streptomycin resistance has become a problem [9, 10].

The problem of antibiotic resistance is not limited to the Indian subcontinent only, but is a global problem. To date, no known method is available to reverse antibiotic resistance in bacteria. The discovery and development of the antibiotic penicillin during the 1900s gave a certain hope to medical science, but this antibiotic soon became ineffective against most of the susceptible bacteria. The antibiotic resistance in bacteria is generally a natural phenomenon for adaptation to antimicrobial agents. Once bacteria become resistant to some antibiotic, they pass on this characteristic to their progeny through horizontal or vertical transfer. The indiscriminate and irrational use of antibiotics these days has led to the evolution of new resistant strains of bacteria that are somewhat more lethal than the parent strain. More recently, in 2016, a Section 18 emergency exemption was granted by the US Environmental Protection Agency for the use of streptomycin and oxytetracycline on citrus trees in Florida for management of citrus Huanglongbing (HLB) disease [11–13]. Regarding other antibiotics, gentamicin has been used in Mexico for fire blight control and in Chile, Mexico, and Central American countries for vegetable disease control, while oxolinic acid (OA) has been used only in Israel for fire blight management [14, 15]. Lastly, kasugamycin is used in Japan and other Asian countries to control the fungal disease rice blast and bacterial seedling diseases of rice [16] and has recently been registered for use in the United States and Canada for managing fire blight [17]. Concerns regarding the use of antibiotics in plant disease control and potential impacts on human health have led to the banning of antibiotic

use by the European Union. However, streptomycin is still utilized for fire blight management in Austria, Germany, and Switzerland under strict control parameters.

3. Evolution of plant-pathogenic bacteria

3.1 Resistance to streptomycin

The lack of effective bactericide alternatives in several plant disease systems has resulted in a decade-long dependence or overdependence on streptomycin. As streptomycin has been used the longest, over the largest geographic area, and for treatment of the largest variety of crops, streptomycin resistance is relatively widespread among plant-pathogenic bacteria. Although the first streptomycin-resistant (SmR) plant-pathogenic bacteria detected were strains of *E. amylovora* harboring a chromosomal resistance mutation, the majority of SmR plant pathogens encode the transmissible SmR transposon Tn5393 [8]. Tn5393 is a Tn3-type transposon originally isolated from *E. amylovora* that harbors *strAB*, a tandem resistance gene pair that confers streptomycin resistance through covalent modification of the streptomycin molecule [18]. The Tn5393 transposon is composed of genes required for the transposition process (*tnpA* and *tnpR*), a central site that contains outwardly directed promoters for expression of both *tnpA* and *tnpR* as well as the *strAB SmR* genes. Expression of the *strAB* genes from Tn5393 in *E. amylovora* is driven by a promoter present in the 3 prime end of the insertion sequence IS1133 that is inserted directly upstream of the *strA* gene [19]. Two closely related variants of Tn5393 have also been found in plant pathogens: Tn5393a, an element that does not contain IS1133, has been detected in *P. syringae* and in a group of *E. amylovora* strains from California exhibiting a moderate level of resistance, and Tn5393b, an element that does not contain IS1133 but instead contains an insertion of IS6100 within the *tnpR* gene, has been characterized in *X. campestris* [19, 20].

There are two other reports of additional genetic mechanisms of streptomycin resistance in plant pathogens; these include the occurrence of the small, nonconjugative but mobilizable broad-host-range plasmid RSF1010 in some strains of *E. amylovora* isolated in California [21]. This observation carries further significance because RSF1010 has been distributed globally among a number of bacterial genera and also occurs in some human-pathogenic bacteria [22]. A recent report detailing an analysis of streptomycin-resistant *X. oryzae* subsp. *oryzae* from China indicated that four strains harbored the *aadA1* gene associated with class 1 integron sequences [23]. This observation is significant because of the importance of integrons in both the transfer of antibiotic resistance in human and animal pathogens and the accumulation of antibiotic resistance genes within one multiresistance element. To date, streptomycin resistance mediated by Tn5393 or the closely related variants has been reported in *E. amylovora*, *P. syringae*, and *X. campestris* isolated from North and South America and Asia [19, 20, 24–30]. The location of essentially the same genetic element in different genera of plant pathogens isolated from distinct crop hosts and from different continents is confirmatory evidence of the role of horizontal gene transfer (HGT) in the dissemination of antibiotic resistance in these pathosystems.

The source of Tn5393 to the plant pathogens was likely not from the antibiotic preparations themselves as a study of 18 available agricultural streptomycin formulations revealed no contamination with the *strA SmR* gene [31]. Instead, the acquisition of Tn5393 by bacterial plant pathogens was likely from commensal co-occurring epiphytic bacteria via HGT. For example, Tn5393 was thought to have been acquired by *E. amylovora* on the plasmid pEa34 from *Pantoea agglomerans*, a common orchard epiphyte [18]. The transfer event most likely occurred on the

apple flower stigma, a surface where *E. amylovora* grows to high population densities and where *Pantoea agglomerans* can also grow. *Pseudomonas syringae* and *X. campestris* pv. *vesicatoria* both have epiphytic phases where the pathogens grow on leaf surfaces, providing opportunities for HGT with other epiphytes. It should be noted that high-level streptomycin resistance, conferred by a spontaneous mutation within the *rpsL* gene that encodes the ribosomal target protein for streptomycin, does occur in some populations of *E. amylovora*, particularly within populations from the western United States as well as in a small number of strains isolated in Michigan and New Zealand [32, 33]. The minimal inhibitory concentration (MIC) of streptomycin in these highly resistant spontaneous mutants is greater than 4096 μg/mL [32]. In contrast, SmR strains of *E. amylovora* harboring Tn5393 exhibit MICs of streptomycin ranging from 512 to 1024 μg/mL [32]. Streptomycin solutions used for fire blight management are typically applied at 100 μg/mL; thus, it is unclear whether the increased level of resistance exhibited by the spontaneous mutants provides a survival advantage in streptomycin-treated orchards.

3.2 Resistance to tetracyclin

Tetracycline resistance has been reported in a few plant-pathogenic bacteria, including *P. syringae* [34, 35] and *Agrobacterium tumefaciens* [36]. Other studies have reported on sensitivity; for example, in one study, 138 strains of *E. amylovora* from the Pacific Northwest, USA, were all determined to be sensitive to oxytetracycline [37]. Although there are few reports of resistance, multiple tetracycline resistance genes homologous to *tetA* and *tetM* are present within the genomes of many different plant-pathogenic bacteria. Efflux pump proteins that belong to the same protein family as TetA have been identified in *Ralstonia solanacearum*; *Erwinia piriflorinigrans*; multiple *Xanthomonas* species, including *Xanthomonas citri*, *Xanthomonas phaseoli*, *Xanthomonas perforans*, *and X. campestris*; multiple *Pseudomonas* species, including *P. syringae*, *Pseudomonas aeruginosa*, and nonpathogenic *Pseudomonas putida* and *Pseudomonas fluorescens*. However, even though putative tetracycline-resistant proteins have been annotated in the NCBI database for plant-pathogenic bacteria such as *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Agrobacterium*, and *Ralstonia*, their function in tetracycline resistance remains to be characterized.

3.3 Resistance to oxolinic acid and kasugamycin

There are a few reports documenting resistance to other antibiotics used in plant disease management. OA was introduced in 1997 for fire blight management in Israel as a replacement for streptomycin, and OA resistance in *E. amylovora* was first detected in 1999 [38] and expanded in range by 2001 [39]. However, populations of OA-resistant *E. amylovora* fluctuated, with OA-resistant strains becoming undetectable in orchards where they previously occurred. Laboratory analyses of OA-resistant strains suggested that these strains were reduced in fitness compared to OA-sensitive strains [40]. Analysis of OA-resistant strains of *Burkholderia glumae* also showed that the strains were reduced in fitness, as these strains could not survive in rice paddy fields [41]. Kasugamycin was discovered in Japan and has been used since the 1960s in Asia for the control of rice blast caused by the fungus *Magnaporthe grisea* and for the control of bacterial grain and seedling rots of rice. This antibiotic has also been used to control diseases of sugar beet, kiwi, and Japanese apricot in at least 30 countries [42]. More recently, kasugamycin has been utilized for management of the blossom blight phase of fire blight disease in Canada and the United States. Resistance to kasugamycin was reported for two bacterial rice pathogens in Japan, *Acidovorax avenae* subsp. *avenae* and *Burkholderia glumae* [43, 44]. Kasugamycin resistance in *A.*

avenae subsp. *avenae* and *B. glumae* was conferred by a novel aac(2)-IIa acetyltransferase gene located within an IncP genomic island and likely acquired by HGT [45]. A promoter mutation that resulted in a fourfold increase in expression of the aac(2)-IIa gene was found to confer an increased level of kasugamycin resistance in strain 83 of *A. avenae* subsp. *avenae* [46]. Kasugamycin resistance has not been reported in *E. amylovora*; one study assessing the potential for spontaneous resistance revealed that a two-step mutational process was required and that spontaneous kasugamycin resistant mutants were substantially reduced in fitness [17].

4. Application of antibiotics and its repercussions on the microbiome of plant agricultural systems

All of the antibiotics applied to trees in orchard systems using conventional air blast spraying systems does not reach the desired target; thus, the effects of antibiotic usage are potentially more complex than simply studying effects on the target pathogen and commensals co-located in the target plant habitat. Antibiotics reaching the target sites in the tree canopy impact the phyllosphere microbiome and flower microbiomes if applied during the bloom phase. Insects feeding within the tree canopy could also ingest the antibiotic, which could impact the insect gut microbiota. A portion of the antibiotic spray applied to trees will not reach the target because of spray drift or could be lost by runoff during spraying or runoff owing to rain events. It has been estimated that as much as 44–71% of spray solutions applied by air blast sprayers is lost into the environment [47]. Whether it hits the target or not, once the antibiotic solution has been released into the environment, the material is negatively affected by environmental parameters, including rainfall, sunlight (visible and ultraviolet radiation), and temperature, and other specific aspects of the plant leaf environment that may affect adsorption. For example, oxytetracycline residues are lost relatively rapidly from peach leaf surfaces because of weather parameters [48]. Any antibiotic lost from the tree target by spray drift may land on other plant surfaces, such as the leaves of grasses or weeds, and thus impact the microbes inhabiting the phyllosphere of those plants. There is also the possibility of drift offsite to nontarget plants, and insect or animal may feed on the nontarget plants and potentially consume the antibiotic, which could impact the gut microflora of these animals. We are aware of one study in which the percentage of streptomycin-resistant *E. coli* isolates from feces of sheep feeding in a pasture that was sprayed with streptomycin was shown to increase (from 14.7 to 39.9% compared to 15.8 to 22.3% in a control group) [49]. However, this study did not simulate actual conditions in commercial orchards as the streptomycin solution was sprayed directly onto the pasture grass and sheep were grazed in the pasture for 12 h immediately following application. Neither of these situations occurs in commercial orchards.

Two studies have been published examining the effect of antibiotic application in apple orchards on phyllosphere bacteria. In one study using both culture-based and culture-independent approaches, Yashiro and McManus [50] examined phyllosphere bacteria from apple orchards that either had received streptomycin applications in spring for fire blight management for up to 10 previous years or had not been sprayed. The percentage of culturable isolate resistant to streptomycin was actually larger from the non-sprayed orchards. An examination of community structure using 16S rRNA clone libraries indicated that streptomycin treatment did not have long-term effects on the diversity or phylogenetic composition of the phyllosphere bacterial community in the examined apple orchards [50]. A separate

cultural study evaluated the effect of weekly applications of streptomycin (for 0, 3, 5, and 10 weeks) beginning at 80% bloom on specific components of the phyllosphere community. Testing of orchard epiphytes for streptomycin resistance indicated that 76.2, 94.5, 95.5, and 98.5% of the bacterial isolates were resistant to streptomycin on trees receiving 0, 3, 5, and 10 applications within one season, respectively [51]. Further microbiome studies have also been conducted examining the effect of antibiotic usage on soil microbiomes in apple orchards. For example, Shade et al. [52] determined that streptomycin application to apple trees did not result in any observable difference in soil bacterial communities (soil collected beneath trees 8–9 days after streptomycin application). The authors concluded that application of the antibiotic had minimal impact on nontarget bacterial communities [52]. A second microbiome study of apple orchard soil collected 14 days after streptomycin application also failed to detect any influence of the antibiotic on the soil bacterial community [53].

The microbiome studies detailed above have provided information that show limited impacts of antibiotics on the selection of antibiotic resistance at a period of time after application. However, there are no published studies to date assessing the resistome of crop plants and in particular the resistome of crop plants that have been treated with antibiotics. Interestingly, the application of struvite ($MgNH_4PO_4 \cdot 6H_2O$), which has been used as a plant fertilizer, alters the antibiotic resistome in the soil, rhizosphere, and phyllosphere [54]. This might have resulted from the fact that struvite usually contains ARGs, antibiotic-resistant bacteria, and antibiotic residues [50]. The need for knowledge of the antibiotic resistome in plant agricultural systems and especially in plant agricultural systems in which antibiotics are applied is critically important because we need to understand whether the use of antibiotics in plant agriculture has the potential to select ARGs that could impact human health. This issue regarding potential impacts to human health is highly significant, with current implications for the use of antibiotics in animal agriculture [55, 56]. Identification of particular ARGs, and the organisms harboring these genes, is important for risk assessments of pathogen acquisition of resistance based on close phylogenetic relationships with coinhabiting antibiotic-resistant commensals. If ARGs of importance in clinical medicine are identified in the resistome of plants sprayed with antibiotics, it is critical to determine whether their frequency and/or bacterial host range changes based on antibiotic exposure.

5. Knowledge gaps in plant-pathogen system

One of the gaps involved in the understanding of the host-plant-environment interaction is the attributes involved with respect to the change in climatic conditions. Changes brought about by the pathogen populations to the host are influenced by cultural practices, control methods, introduction of new cultivars or varieties, and climatic variability in equal measure. A majority of these studies are often hindered due to the difficulty in obtaining the information or evidence with respect to the presence of the pathogen throughout the said period, genetic composition and its associated changes before and after interaction with the host, climatic requirements for the host and pathogen during the said period and arrive at a convincing trend without background noise with respect to the disease pattern.

Similar to the pathogen-human interaction, the challenge and attack by pathogenic organisms are halted by the defense mechanisms of the plants. This mechanism is often trespassed by the evolution and emergence of newly faced pathogens that have evolved in response to evolution or agricultural practices and

colonization strategies in native communities with no prior evolutionary history [57–59]. It is well-known that the ecosystem, frequency, and evolution of both host and pathogens are largely dependent on catastrophic outbreaks that have a direct involvement of the human population. Added to this is the development of a new species, migration of humans, speciation, susceptibility of the plants, divergence, and climate change [58]. With a positive association between the emergence of new pathogens and extinction of crop production being rendered by many researchers, understanding and identification of emerging pathogens is a necessary strategy to counter them [60, 61].

Understanding the emergence of new pathogens has largely been a challenge for scientists as the host-pathogen interaction is a complex process. Global distribution and diversity of plant pathogens is also dependent on trade, human migration, plant ecosystem, and distribution of plant-based products. An additional indirect way to gauge pathogens and their associated effects is the elucidation of migration pathways [62]. The ever-increasing investment by the researchers in analyzing genome sequences has revealed another world of improvement in understanding the adaptability of pathogens to plant disease [63–65], and any changes in pattern of pathogenicity may thus arise. Horizontal gene transfer and interspecific hybridization have been the two mechanisms that have been comprehensively reviewed [58, 63, 66–69]. Along with strategies such as population genomics study for development of improved disease management, awareness of agricultural heterogeneity and management or restriction of movement of plant materials aids have also been integrated. Further a cumulative effort by plant epidemiologists, ecologists, pathologists, and academic researchers facilitates successful management of emerging phytopathogens.

6. Sustainable crop disease management by genetic engineering (GE)

In addition to a plethora of published GE strategies, ongoing research, and the wide expansion of genetic resources, conceivable applications are gaining momentum [70] that invests prospective for future generations. The dynamics of the adaptation of pathogen toward the host can be invested by GE strategies due to its selective efficacy against a group or particular target pathogens. Such a targeted advantage minimizes health concerns at the consumers' end with no risk of nontarget biota in an agrarian ecosystem. Some of the processes that occur naturally have also been undertaken in GE processes (**Table 1**). Although the futuristic potential of GE strategies with controlled disease conditions in the subsequent host generations is questionable in the present day, it is demonstrated that GE strategies that were initiated as a proof of concept are now well-established and have been marketed as commercially viable varieties.

6.1 Boosting plant recognition of infection

Similar to a human system, plants also trigger defense molecules on recognizing particular molecules of invading pathogens generally referred to as pathogen-associated molecular patterns (PAMPs; [71–73]) that illicit a PAMP-triggered immunity. Although PAMP receptor molecules differ among plant species, genes that encode PAMP receptor can be transformed into other crops for triggering immunity [73]. Such a method of transformation does not introduce a novel defense mechanism but rather introduces a receptor that helps the transformed plant recognize infection making it independently counter the infection by its natural immune system [74–77].

Plant species	Disease	Pathogen species	Pathogen class	Gene product	Reference
Arabidopsis	Crown gall disease	*Agrobacterium tumefaciens*	Bacteria	Arabinogalactan protein	[78, 79]
	Crown gall disease	*Agrobacterium tumefaciens*	Bacteria	Mannan synthase	
	Root-knot nematode	*Meloidogyne incognita*	Nematode	Kelch repeat protein	[80, 81]
	Powdery mildew	*Erysiphe orontii*	Fungus	Receptor-like kinase	[82]
	Root-cyst nematode	*Heterodera schachtii*	Nematode	Ethylene response	[83, 84]
	Bacterial speck	*Pseudomonas syringae*	Biotrophic bacteria	Lectin receptor kinase	[22]
	Gray mold/rot; leaf spot	*Alternaria brassicicola; Botrytis cinerea*	Necrotropic fungus	Expansin	[85]
	Powdery mildew	*Golovinomyces orontii*	Biotrophic fungus	Membrane-attached protein	[86]
	Downy Mildew	*Hyaloperonospora arabidopsidis*	Biotrophic oomycete	ADP ribosylation factor—GTPase activating factor	[87]
	Bacterial wilt	*Ralstonia solanacearum*	Biotrophic bacteria	MAPkinase phosphatase	[88]
	Aphid	*Myzus persicae*	Insects	Fatty acid desaturase	[89]
Maize	Southern corn leaf blight	*Bipolaris maydis/Cochliobolus heterostrophus*	Necrotrophic fungus	Mitochondrial transmembrane protein	[90]
	Powdery mildew	*Blumeria graminis*	Biotrophic fungus	Long-chain aldehyde synthesis	[91]
Tomato	Gray mold/rot	*Botrytis cinerea*	Necrotrophic fungus	Polygalacturonase and expansin	[92]
	Soft rot, gray mold/rot	*Botrytis cinerea*, *Erwinia chrysanthemi*	Fungus, bacteria	ABA aldehyde oxidase	[93]
	Powdery mildew	*Leveillula taurica*	Biotrophic fungus	Membrane-anchored protein	[94]
	Aphid	*Macrosiphum euphorbiae*	Insects	Fatty acid desaturase	[95, 96]
	Fusarium wilt	*Fusarium oxysporum*	Hemibiotrophic fungus	Lipid transfer protein	[97]
Rice	Bacterial blight	*Xanthomonas oryzae*	Bacteria	MAPKKK	[98]
	Blight rot	*Burkholderia glumae*	Bacteria	MAP kinase	[99]
	Rice blast	*Magnaporthe oryzae*	Hemibiotrophic fungus	Transcription factor WRKY	[100, 101]
	Leaf blight	*Xanthomonas oryzae*	Bacteria	Stearoyl-ACP desaturase	[102]

Table 1.
Genes and their contributions to the plant-pathogen interaction studies.

6.2 Mining R genes

An intracellular receptor protein (R-protein) is produced as a mechanism of effector-triggered susceptibility which is banked on by a model of disease resistance [72, 103]. This protein is specifically detected in the presence or when an activity of a pathogen effectors is triggered resulting in effector-triggered defense [103]. However, these effectors may modify or change the defense response in the host in response to a new effector produced by the pathogen. With this production of specific R genes with respect to the pathogen effector, pools of resistance genes evolved can be made useful in breeding crops for disease resistance by producing cisgenics [104]. Exceptional efforts by conventional introgression of cisgenes undertaken in crops such as apple, banana, grape, and potato have established it to be labor intensive and time consuming [73, 104]. GE strategies offer a major advantage not only by making it easier and faster but also evading linkage drag [50, 74]. Further introgression of R genes can be made feasible between unrelated plant species among monocots and dicots [77, 105–108]. The tendency of the pathogen to overcome the resistance rendered by R genes can be circumvented by mining R genes from unrelated species by integrating GE strategies and breeding [109, 110].

6.3 Upregulating defense pathways

The activity of defense can be boosted by targeting molecules such as reactive oxygen species, pathogenesis-related genes involved in defense regulation, signaling, and associated processes activating acquired resistance. Such measures were profited to a great extent in enhancing resistance to diseases such as citrus greening and pathogens such as *Rhizoctonia solani* and *Magnaporthe oryzae* that utilizes the plant's own natural immune system without the introduction of new or novel metabolic pathways [111, 112].

6.4 Disarming host susceptibility genes

Some important genes that facilitate normal physiology in plants have been observed to be involved in facilitating pathogen colonization and infection. Changes induced in such susceptibility genes is an efficient strategy for disease resistance [113]. Disarming susceptibility genes may alter the pathosystems and many host factors that contribute to compatibility between the pathogen and host. Gaining a new function to replace the lost host factor is not a likely by the pathogen to overcome the activity of a disarmed susceptibility gene; therefore, this strategy does not leave any exogenous DNA [113].

6.5 Silencing essential pathogen genes

RNA interference is elicited in plants to silence genes that render pathogenicity by using genetic constructs with identical sequence of dsRNA. Such efforts directly trigger posttranscriptional gene silencing of the natural disease process. Such a process of silencing does not generate a biochemical pathway or produce a novel protein. Integrating the need of the hour with the potential of the strategy of RNA silencing proved profitable for the papaya industry in Hawaii [114, 115]. Such applications are observed in cases where severe strains of the virus can be reduced in case of an infection by a mild strain. Implementing a natural phenomenon for cross-protection as a means to manage disease conditions has practical drawbacks. These drawbacks were controlled by feeding insects with dsRNA constructs that can trigger RNAi [116, 117].

7. Engineering CRISPR/Cas immune system

Clustered regularly interspaced short palindromic repeats has been identified to be a prokaryotic defense system that combines with its associated proteins (Cas) to render an endonuclease activity that cuts the invading DNA at a particular target of interest. This specificity is determined by the sequence of DNA that matches the sequence of the RNA guide strand associated with the Cas protein. Some studies have engineered a Cas9/gRNA that targets the replicating DNA of Gemini virus that leads to agrarian crisis in tropical and subtropical climates [118–120]. Significant resistance to host can be achieved against a DNA virus by a targeted sequence-specific engineered complex of Cas9/gRNA, although the results are meant to be reproducible [121]. Long-term utilization of this strategy against a variety of genetic elements that hamper the host such as viruses can be successfully targeted [122–126].

Genome editing, brought about by *Agrobacterium*-mediated transformation or biolistic methods, gives way to a wide range of possibilities for genetic changes. Targeted modifications, specific mutagenesis, and /or modest changes can be brought about by targeting existing genes in live cells. By using CRISPR/Cas9, it is possible to create a non-transgenic gene edit that can be introgressed by conventional breeding and can yield a change that cannot be distinguished from a mutation [127]. Another application of CRISPR is that the genome editing is HDR-based that allows editing a gene from the crop's natural pool giving rise to cisgenic lines that can achieve outcomes stabilized by conventional breeding. HDR-based genome editing strategies also helps add a specific gene from an evolutionary distant organism therefore making the regulatory scrutiny mandatory similar to that of transgenics [128, 129]. Various research groups have validated CRISPR/Cas9 techniques to be straightforward, low cost, and efficient, but the accessibility of the applications of genome editing is largely dependant on democratizing genome editing, nonprofit organizations, and governmental regulations.

8. Conclusion

While recognizing the important benefits GE technologies offer, larger considerations merit attention, especially questions of public acceptability and of whether there are any long-term ecological risks different from those posed by conventional breeding. In considering such issues, it is important to remember that, not only do diverse GE strategies exist, but diverse GE manipulations are possible, ranging from very modest, targeted mutagenesis, through cisgenics and intragenics, to insertion of transgenes from other crops, from other (non-crop) plants, and from evolutionarily distant organisms. Thus, in considering socioeconomic and cultural perspectives of GE, it is important to bear in mind this diversity of strategies and applications: GE crops can differ markedly from one another. A useful GE construct may target one or a few pathogens of particular importance, but other breeding techniques still is important for tackling disease problems not targeted by available GE traits. Thus, GE should be understood, not as the best approach to addressing sustainability challenges, but as a suite of tools that capitalizes on the knowledge that biologists gain through our ongoing study of Nature. GE simply expands the breeding "toolbox," providing options to consider on a case-by-case basis for enhancing the sustainability of crop disease management.

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