Kamel A. Abd-Elsalam
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We'd like to dedicate this book to Prof. Aly A. Aly, my father in agricultural research, for his guidance, direction, and pearls of wisdom, but more importantly, for putting up with my panic attacks and questions while providing amazingly timely feedback and encouragement precisely when needed, without which it would have been nearly impossible to produce this piece of work.

Kamel A. Abd-Elsalam and Heba I. Mohamed

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About the Editors

Kamel A. Abd-Elsalam, Ph.D., is currently a Research Professor at the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Dr. Kamel's research interests include developing, improving, and deploying plant biosecurity diagnostic tools; understanding and exploiting fungal pathogen genomes, plant genome editing using CRISPR technique; and developing eco-friendly hybrid nanomaterials for controlling toxicogenic fungi, plant diseases, and nanobiotechnology applications in agroecosystems. He published 20 books related to nano-biotechnology applications in agriculture and plant protection, which were published by the world's major publishing houses such as Springer. He has published more than 160 scientific research articles in international and regional specialized scientific journals with a high impact factor and has an h-index of 36 and an i-10 index of 95, with 5206+ citations. In 2014, he was awarded the Federation of Arab Scientific Study Councils Prize for excellent scientific research in biotechnology (fungal genomics) (first ranking). In addition, according to Stanford University's worldwide database rating in 2021, Kamel A. Abd-Elsalam has been listed among the top 2% of the world's most influential scientists by Stanford University. Dr. Kamel earned his Ph.D. in Molecular Plant Pathology from Christian Albrechts University of Kiel (Germany) and Suez Canal University (Egypt), and in 2008, he was awarded a postdoctoral fellowship from the same institution. Dr. Kamel was a visiting associate professor at Mae Fah Luang University in Thailand, the Institute of Microbiology at TUM in Germany, the Laboratory of Phytopathology at Wageningen University in the Netherlands, and the Plant Protection

Department at Sassari University in Italy and Moscow University in Russia. **He was ranked within the top 2% of the most influential scientists in the world in nanobiotechnology for the year 2020 by Stanford University.**

Heba I. Mohamed is a Professor of Plant Physiology, Faculty of Education, Biological and Geological Sciences Department, Ain Shams University. Dr. Heba completed her M.Sc. and Ph.D. in Plant Physiology, Faculty of Education, Ain Shams University. Dr. Heba's research interests include biotic and abiotic stresses, plant biochemistry, use of eco-friendly compounds to alleviate plant stress, plant secondary metabolites, and genetic differences between different genotypes. She edited five books. She has published 24 book chapters, 5 review articles, more than 88 scientific research in international peer-reviewed journals, and has an h-index of 32 in Scopus. Dr. Heba is a reviewer of international peer-reviewed journals. She also is editor of the *Microbial Biosystems* journal. Dr. Heba has obtained a certificate of recognition in honor of achievement in international publication that supports Ain Shams University World. In addition, according to Stanford University's worldwide database rating in 2021, Heba I. Mohamed has been listed among the top 2% of the world's most influential scientists by Stanford University.

Part I

Identification and Diagnosis

An Introduction to Rice Diseases

1

Parteek Prasher and Mousmee Sharma

Abstract

Rice (*Oryza sativa*) represents the major food, feeding more than half of the world population every day. The dependence of such a large population to meet their daily dietary requirements on this tropical crop causes large-scale production in different parts of the world. Since the crop thrives comfortably in humid climates, the areas differing in such environmental conditions require the application of agrochemicals and require an extensive crop management programme to efficiently manage the diseases that hamper the crop's growth. The rice diseases, mainly caused by bacteria, fungi, and viruses, lead to significant damage and loss in the crop yield. The fungal diseases mainly attack stems, roots, grains, and foliage. The level of plant damage caused by these diseases depends on the innate capacity of the crop species to withstand the disease, severe environmental conditions, soil fertility and composition, the effect of agrochemicals, and the stage of plant growth. This chapter provides a concise discussion of the various diseases caused by bacteria, fungi, and viruses that impede rice crop growth.

Keywords

Oryza sativa · Diseases · Crop yield · Foot rot · Blast · Bacterial diseases · Fungal diseases

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1.1 Introduction

Disease is an abnormal condition of the plant species that deters the optimal functioning of its cells, tissues, enzymes, and biological and biochemical pathways (Nazarov et al. 2020). Disease in plants occurs via biotic factors or pathogens such as nematodes, bacteria, fungi, viruses, and mycoplasma. In addition, the abiotic or physical factors such as temperature, soil pH, nutrient deficiency, moisture content, presence of toxic elements in soil, water stress, heavy metal stress, and amount of light readily influence the plant's growth and development or the progression of diseased conditions (Hasan et al. 2020; Pautasso et al. 2012; Elad and Pertot 2014). The rice diseases cause an approximate 10% loss in annual production, with main diseases as 'blast' and 'helminthosporium' caused by *Pyricularia oryzae* Cav. and *Cochliobolus miyabeanus*, respectively (Asibi et al. 2019), while 'stem rot' and 'foot rot' diseases caused by *Leptosphaeria salvinii* Catt. and *Gibberella fujikuroi*, respectively, deter the production of rice adversely. The incidence of blast epidemic reportedly claims 60–70% loss in the rice production or even 100% crop loss in the individual fields (Nalley et al. 2016; Kihoro et al. 2013; Kirtphaiboon et al. 2021). Blast disease causes severe leaf infection, especially in the post-transplanting stage, causing total destruction of the foliage. Due to this disease, the half-filled rice earheads that form tend to break and fall off. The treatment of "foot rot" includes application of the seedlings with organo-mercurial fungicides (Kongcharoen et al. 2020), whereas the "blast" disease is much more widespread and requires immediate attention to prevent its spread. The popularisation of the breeding of resistant varieties of rice seedlings represents another desirable approach to prevent the outburst of diseases and to obtain a good yield (Laha et al. 2017; Miah et al. 2013; Dubina et al. 2020). Figure 1.1 illustrates the major diseases in rice. This chapter deals with a succinct discussion of the various diseases of rice and their causal pathogens.

1.2 Fungal Diseases in Rice

Nearly 20,000 fungal species reportedly cause plant diseases globally. These fungal species remain active or dormant on both living and dead tissues of the plants depending on the conditions favouring their growth and proliferation. Pathogenic fungi produce spores that, when dispersed by air, water, soil, invertebrates, and insects, may affect the whole crop. Certain fungi, such as mycorrhizae, provide significant benefits to plants by forming mutualistic relationships with their roots (Iqbal et al. 2021). Majority of the fungal species cause plant diseases, including rust, wily, blight, canker, leaf spot, anthracnose, mildew, and root rot. Fungal diseases such as rice blast serve as an alarming threat to global food security owing to their widespread distribution and destruction of the rice crop. *Magnaporthe oryzae* causes rice blast disease, which is the most devastating fungal disease, infecting the plant

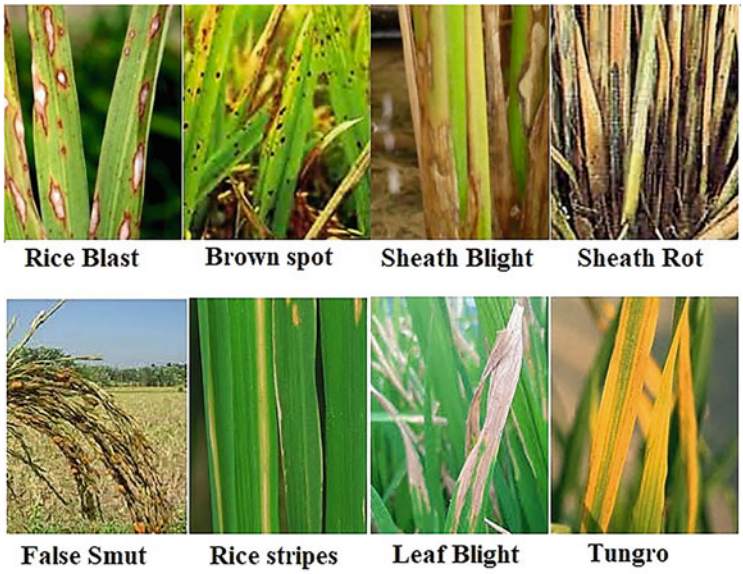

Fig. 1.1 Various diseases in rice crop that affect its yield

during all the growth stages and hampering the crop yield by 10–35%. Countering this pathogen encompasses cultural, biological, and molecular approaches that lead to the development of tolerant and resistant rice varieties by adopting effective breeding programs (Hirooka and Ishii 2013; O'Brien 2017; Sabri et al. 2020). Identification, isolation, and characterisation of several blast-resistant genes resulted in the emergence of allelic variants via molecular breeding and transgenic approaches such as miRNA and genome editing (Tabassum et al. 2021). Similarly, in the management of fungal resistance in rice, breeding techniques such as gene rotation, pyramiding, and multiline varieties proved highly profitable (Ramalingam et al. 2020). However, the co-evolution of the pathogens and their variable nature necessitate consistent research aimed at the advancement of sustainable, resistant cultivators. Table 1.1 presents the various fungal diseases in rice, their causal organisms, symptoms, and the affected plant parts.

1.3 Bacterial Diseases in Rice

Bacterial blight caused by gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* and bacterial leaf streak disease caused by the gram-negative bacteria *Xanthomonas oryzae* pv. *oryzicola* represent the deadliest bacterial disease in rice that affect the overall rice production worldwide (Yugander et al. 2017; Pradhan et al. 2020). Nevertheless, the rice plant too has adopted innate potency to counter the bacterial

Table 1.1 Symptoms and causal organism for the various fungal diseases in rice

Disease	Symptoms	Causing organism	Ref.
Rice blast	• Above ground parts of rice get effected • Elliptical or spindle-shaped legions occur on leaf blade • Lesions enlarge and coalesce eventually killing the leaf • Stem node turns blackish and becomes fragile • Brown lesions appear on branches of panicles and spikelets	*Pyricularia grisea*	Greer et al. (1997)
Sheath blight	• Ellipsoid or ovoid lesions appear on leaf sheath • Lesions coalesce and become bigger thereby causing leaf death • Waterline in the low land fields serve as favourable condition for fungal growth	*Rhizoctonia solani*	Li et al. (2021)
Brown spot	• Small, circular, brown coloured lesions on seedlings • Black discoloration of root occurs • Fungus causes dark brown to black oval spots on glumes • Black discoloration of grain occurs • Affected seedlings show stunted growth	*Bipolaris oryzae*	Shabana et al. (2008)
Leaf scald	• Zonate lesions, alternating light tan and dark brown spots starting from leaf edges • Enlargement and coalescing of lesions causes blight of leaf blade • Scalded appearance of leaf occurs	*Microdochium oryzae*	Manandhar (1999)
Narrow brown spot	• Short, linear, and brown lesions on leaf sheath, pedicels, and glumes • Net blotch-like pattern appears on leaf sheath as the cell wall turns dark brown, while intercellular areas turn tan to yellow • Diseases mainly appear in the mature stages of the rice crop	*Cercospora janseana*	Simanjuntak et al. (2020)
Stem rot	• Disease symptoms appear in the field after mild tillering stage • Small, blackish, irregular lesion appears on outer leaf sheath near water line • Fungus penetrates into the inner leaf sheath and causes partial/entire rotting • Fungus penetrates and rots the culm • Infection of the culm causes lodging, chalky grains, unfilled panicles, and death of the tiller • Infected stem contains dark greyish mycelium • Tiny, black sclerotia embed the diseased leaf sheath tissue	*Sclerotium oryzae*	Ghosh et al. (2020)

(continued)

Table 1.1 (continued)

Disease	Symptoms	Causing organism	Ref.
Sheath rot	• Leaf sheath containing young panicles gets rotted • Whitish powdery growth occurs inside affected sheath • Panicle fails to emerge as they remain inside the sheath • Grains become sterile, shriveled, and discoloured • Panicles that fail to emerge become rot • Florets turn red brown to dark brown	*Sarocladium oryzae*	Ayyadurai et al. (2005)
Bakanae	• Hypertrophic effect or abnormal elongation of plant occurs • Affected plants produce adventitious roots at the lower nodes of the culm • Affected plants contain very few tillers, and leaves dry quickly • Diseased tillers die quickly even before reaching maturity • Surviving infected plants bear empty panicles	*Fusarium fujikuroi*	Singh et al. (2019)
False smut	• Individual grains of the panicle turn into greenish spore balls with velvety appearance • Membrane around the spore balls eventually bursts as the spore grows while being enclosed in the floral parts • The outermost layer of the ball contains mature spores and the remaining fragments of the mycelium	*Ustilaginoidea virens*	Fan et al. (2020)

attack. The plant's immune response consists of dual mechanisms to counter the bacterial attack. Cell surface-localized pattern recognition receptors play a central role in the detection of pathogen-associated molecular patterns, including the highly conservative flagella of bacteria essential for sustaining the life of the pathogen (Mendes et al. 2018; Yuan et al. 2021a, b; Kim et al. 2020). These microbial components cause a variety of responses, including reactive oxygen species (ROS) generation, increased calcium ion concentrations, callose aggregation in the cell wall, activation of mitogen-activated protein kinases (MAPKs), and production of antimicrobial components such as phytoalexins (Jeandet 2015). Mainly, the broad-spectrum resistance shown by plants overcomes the intruding pathogens. It comprises a defence mechanism chiefly localised within the plant cell based on polymorphic resistance proteins that identify the specific virulence effectors secreted by the pathogens within the host cells, thereby prompting effector-triggered immunity (ETI) (Meng et al. 2020; Wang et al. 2016). ETI represents a robust resistance mechanism associated with cellular senescence at the infection site (Liu et al. 2013;

Table 1.2 Symptoms and causal organism for the various bacterial diseases in rice

Disease	Symptoms	Causing organism	Ref.
Bacterial blight	• Water-soaked lesions appear at the leaf margin • Increase in the size of affected region • Yellowish border appears between dead and green areas of the leaf • Withering of leaves or entire young plant occurs • Leaves become pale yellow at later stage of growth	*Xanthomonas oryzae*	He et al. (2010)
Bacterial leaf streak	• Water-soaked streaks appear between the leaf veins • Later, these become longer and translucent and become light brown coloured • Large areas of leaf become dry due to numerous streaks	*Xanthomonas oryzae*	Jiang et al. (2020)
Foot rot	• Infected plants become taller • Plants become thin, with yellowish green leaves • Seedlings dry at an early tillering • Partially filled grains	*Dickeya zeae*	Pu et al. (2012)
Grain rot	• Wilting and rotting of leaves • Discoloration of panicle • Shrivelled leaves • Lesions on seeds • Lesions on glumes	*Burkholderia glumae*	Zhou et al. (2016)
Sheath brown rot	• Appearance of necrotic areas on leaves • Discolouration of seeds occurs • Leaves show abnormal colours • Spikelets of emerging panicles become discoloured	*Pseudomonas fuscovaginae*	Razak et al. (2009)

Yuan et al. 2021a, b). This hypersensitive response serves as the strongest immune retort against the invading pathogen. Nonetheless, the approaches to mitigating the bacterial blight of rice present only trivial effectiveness. Chemical disease control is generally discouraged due to its environmental and human toxicity (Zhai et al. 2002; Amoghavarsha et al. 2021). The development of resistance among the pathogenic bacterial strains further questions the chemical methods of disease control (Ellur et al. 2016). Breeding of rice varieties with sturdy genes against the bacterial infection presents a viable option to ensure a healthy crop (Tao et al. 2021; Kumar et al. 2020a, b). The introduction of these genes to the genomes of commercial rice strains presents a highly desirable strategy to counter bacterial infection in the tropical countries that produce huge yields of rice every year (Wang et al. 2020; Oliva et al. 2019). Table 1.2 presents the various bacterial diseases in rice, their causal organisms, symptoms, and the affected plant parts.

1.4 Virus Diseases in Rice

In India, four virus types primarily affect the rice crop, with tungro being the most widespread virus disease affecting the rice crop in more than ten Indian states (Sharma et al. 2017; Nguyen et al. 2021). The virus diseases such as grassy stunt and strains such as GCV4 are confined to the southern part of the country (Ta et al. 2013; Zhao et al. 2021). Virus diseases like ragged stunt and necrotic mosaic are among the most damaging to rice production in India (Ghosh 1980; Bhattacharya et al. 2020). The majority of rice disease-causing viruses thrive in Asian and American continents, but rice stripe necrosis furovirus, maize streak germivirus, African cereal streak virus, rice yellow mottle sobemivirus, and rice crinkle disease persist in Africa and neighbouring countries (Awodero 1991; Liu et al. 2020). Intensified rice cultivation and the application of high-yield varieties, mechanical contamination, unregulated use of pesticides, fertilizers, and practise of crop monoculture serve as the determining factors for the evolution of virus diseases in rice (Ichiki et al. 2013; Rybicki 2015; Chen et al. 2020). The japonica rice varieties in the Americas and the Asian continents show vulnerability to the virus diseases, while the indica rice varieties show susceptibility to the virus-borne diseases (Cho et al. 2013; Orasen et al. 2020). Breeding and screening resistant rice varieties, plant quarantine, integrated pest management strategies, and the development of genetically engineered resistant rice varieties are all important approaches for effective disease management in rice (Savary et al. 2012; Chatterjee et al. 2021). Table 1.3 presents the various virus diseases in rice, their causal organisms, symptoms, and the affected plant parts.

1.5 Nematode Diseases in Rice

Nematodes predominantly cause a huge economic loss, mainly to two crops, maize and rice. The nematodes cause significant cellular changes inside the root-knot nematode-induced feeding sites upon interaction with the rice crop (Kyndt et al. 2014). The transcriptome analyses, exogenous hormone application, and mutant analyses suggested comprehensive models depicting the interactions of plant hormone pathways, such as jasmonate, in response to the innate defence adopted by rice against nematodes (Zhou et al. 2020; Gheysen and Mitchum 2019; Wang et al. 2014a, b). The nematodes represent soil-borne pathogens that pose a threatening loss to rice cultivators due to the emergence of new cultivation practises that include less water usage for growing the rice crop (Khan and Ahamad 2020). Reportedly, the nematode pathogens cause an alarming 10–25% loss to the rice crop worldwide (Kumar et al. 2020a, b). The havoc of pathogenic nematodes is mainly confined to tropical and subtropical regions with a large variety of species (Porazinska et al. 2012; Reddy 2021). In addition, the lack of proper resources for effective crop management and control and the ideal conditions for the thriving of nematode populations serve as determining factors for the nematode diseases in the rice crop grown in these areas (Prasad et al. 1987; Khan et al. 2021). Table 1.4 presents the

Table 1.3 Symptoms and causal organism for the various virus diseases in rice

Disease	Symptoms	Causing organism	Ref.
Tungro	• Affected areas exhibit stunted growth and reduced tillering • Leaves become orange-yellow coloured and develop rust-coloured spots • Leaf become discoloured starting from the tip that extends till the lower part of leaf blade • Young leaves display mottled appearance • Old leaves show rust-coloured specks of various sizes • Affected plants show a delayed flowering • Panicles bear partially filled grains covered with dark brown specks • Transmitted by green leafhoppers	*Rice tungro bacilliform virus* and spherical virus	Zarreen et al. (2018)
Grassy stunt	• Plant show severe stunting • Plants show excessive tillering • Leaves may be mottled or striped • Transmitted by brown plant hopper	Rice grassy stunt virus	Satoh et al. (2013)
Ragged stunt	• Plant show severe stunting • Plants show reduced tillering • Leaves show ragged appearance • Leaf blades twist to form a spiral • Vein swelling appear on leaf sheath, leaf blade, and culm • Transmitted by brown plant hopper	*Rice ragged stunt virus*	Wang et al. (2014a, b)

Table 1.4 Symptoms and causal organism for the various nematode diseases in rice

Disease	Symptoms	Causing organism	Ref.
Ufra or stem nematode	• Affected seedlings and plant show chlorosis • Stunted plant growth • Deformation and twisting of leaves occur • Exserted panicles, with unfilled grains	*Ditylenchus angustus*	Ali and Ishibashi (1996)
White tip	• Chlorosis of leaf occurs • Infected leaf dries up and shreds • Flag leaf becomes twisted • Panicles do not emerge • If panicles emerge, they display high sterility, distorted kernels, and distorted glumes	*Aphelenchoides besseyi*	Ou et al. (2014)
Root knot	• Infected plants display stunted growth and become yellow • Infected plants show reduced tillering • Infected plants show the appearance of root galls • Disease mostly occurs in upland, as compared to the lowland rice	*Meloidogyne graminicola*	Tian et al. (2018)

various virus diseases in rice, their causal organisms, symptoms, and the affected plant parts.

1.6 Conclusion

Oryza sativa, grown mainly as an annual plant, survives as a perennial crop in tropical areas. The rice crop in these areas faces vulnerability to a variety of diseases caused by bacteria, fungus, and nematodes, mainly due to the climatic conditions. Excessive use of fertilizers and chemicals to increase crop production and yield in order to feed the world's population exacerbates the crop's susceptibility to disease. The overuse of chemicals increases the incidence of microbial resistance and causes biomagnification in the higher trophic levels. However, plant breeding techniques, integrated pest management, biological methods of crop management, genome culturing, and the cultivation of resistant varieties of rice have contributed towards the effective management of rice disease. The production of flood-resistant rice, drought-resistant rice, and salt-tolerant rice also made sure there was enough of the crop to go around.

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Bacterial Disease of Rice

2

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Abstract

Rice bacterial infections are a serious stumbling block to long-term output, and they are quite important on a worldwide scale, particularly in Asian countries. The management of these diseases, particularly bacterial diseases, has included extensive research, including infection and disease development and chemical therapy. By employing proper disease management approaches, bacterial infection can be overcome. Farmers are increasingly using chemical management strategies to prevent output loss. When the disease condition and infection rate in the infected area are out of control, chemical management is required. The appropriate use of selective antibiotics and combinations of antibiotics will help manage bacterial infections and prevent yield loss. Excessive and poor antibiotic combination selection may disrupt the natural system's balance, posing a health risk to humans and animals. Chemicals used for longer periods may cause disease-causing germs to develop resistance. Natural and chemical management

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strategies should be used together in a controlled way to achieve eco-friendly results, so this is what you should do.

Keywords

Bacterial leaf blight · Bacterial leaf streak · Bacterial panicle blight · Antibiotics · Management

2.1 Introduction

The paddy crop (*Oryza sativa*) is widely grown in India and Asian countries. Rice is one of the world's most significant cereal crops, providing nutrition to the vast majority of people in Africa, Asia, and Latin America (US Department of Agriculture 2021; Kadu et al. 2015). Rice consumption has increased marginally in recent crop years when compared to previous crops. India consumed roughly 504.3 million metric tonnes of rice in the years 2020 and 2021 compared to the worldwide average. In 2008/2009, 437.18 million metric tonnes of crop were consumed globally. After China, India is the world's second-largest rice producer. According to the area, rice comprises about 23.3% of farmed land. It contributes 43% to food grain output and 46% to cereal production in the United States. Rice refers to a different number of grain species. There are about 40,000 different varieties of *Oryza sativa* worldwide, divided into four broad categories: indica, japonica, aromatic, and glutinous. Increasing rice consumption means expanding rice-growing areas and increasing rice production, both of which have made rice more vulnerable to disease (Fargette et al. 2013; Dai et al. 2010).

Droughts, weather fluctuations, floods, and illnesses are among the variables that affect rice yield. Rice is susceptible to a range of diseases caused by bacteria, viruses, or fungi; the most devastating are bacterial infections, resulting in yield losses of up to 50% depending on the rice variety, growth stage, geographic location, and environmental conditions.

By implementing eco-friendly measures at an early stage, we can decrease or eliminate the use of carcinogenic and toxic chemicals and reduce or eliminate the discharge of such chemicals in agricultural land. When the condition has progressed beyond the point where it is cost-effective, chemicals should be used. It will support and promote the natural competitors of harmful bacteria in the ecosystem.

Bacterial leaf blight (BLB), bacterial leaf streak (BLS), and bacterial panicle blight (BPB) are three main bacterial diseases of rice caused by Gram-negative bacteria of the *Xanthomonas oryzae* genus: *X. oryzae* pv. *oryzae* (Xoo), *X. oryzae* pv. *oryzicola* (Xoc), and *Burkholderia*. The bacterial leaf blight (BLB) disease can cause yield losses of up to 50% in favourable situations (Ou S. Rice Infections). In 1884, farmers in the Fukuoka district of Kyushu, Japan, discovered BLB illness (Tagami and Mizukami 1962) (Tagami and Mizukami 1962). Tagami and Mizukami, the first cases of bacterial leaf streak (BLS), were discovered in Mali in 2003 and Burkina Faso in 2009 (Wonni I) (Wonni II) (Wonni III). BPB was first

Table 2.1 Major bacterial diseases which are causing maximum yield loss in the rice crop

Bacterial disease name	Pathogenic bacterial species	First reported year
Leaf blight	*Xanthomonas oryzae* pv. *oryzae*	1884
Leaf streak	*Xanthomonas oryzae* pv. *oryzicola*	2003
Panicle blight	*Burkholderia glume*	1967

identified as the principal cause of grain rotting and a seedling blight on rice in Japan in 1967 and was dubbed bacterial grain rot. These illnesses are now considered emergent diseases that can result in a significant decrease in gross rice yield. Different species of *Pantoea* (Doni et al. 2019) and *Sphingomonas* (Kini et al. 2017) genera reduced the rice gross yield.

Xanthomonas oryzae has been identified as being responsible for bacterial leaf blight-like symptoms in rice. *Pantoea*, *P. stuartii*, *P. ananatis*, and *P. agglomerans* have all been identified as BLB disease-causing pathogens in different nations. (HB Lee and AD Gonza'lez) Only a few isolates of *Sphingomonas* species have been identified as plant pathogens and have been linked to BLB disease symptoms (Kini et al. 2017).

Extensive research studies are on the *Pantoea* and *Sphingomonas* species to establish the information and documentation of well-known *Xanthomonas* bacteria. In rice, the bacterial sheath brown disease is caused by *Pseudomonas fuscovaginae* through seed transmission. However, recently other pathogens like *Sarocladium oryzae* and *Fusarium* spp. also showed similar sheath rot symptoms. Based on the reported evidence in this chapter, we focused on the three bacterial diseases causing divesting in the yield of rice. Table 2.1 contains the list of major bacterial diseases and Table 2.2 the other stains of the bacterial species.

2.2 Rice Leaf Blight Disease

2.2.1 Disease Development

Xanthomonas oryzae infects the rice plants by invading through the water pores and taking advantage of newly formed wounds (Mukoo et al. 1957). The pores for water percolation in the plant can be found on the edges of the leaf's upper section. Lesions usually begin on the upper section of the leaf, at the leaf margins. The water-soaked lesions became yellowish-white in hue, spreading from the square form's equal sides to form elongated circular to uneven lesions. The wavy margins of the leaf blades were plainly visible on the leaves, which are an indication of the condition. Under humid conditions, the lesions usually begin on both leaf margins and can even be seen on newly infected leaf veins. The disease's progression and the emergence of symptoms in the rice field are both influenced by the environment. The illness can be divided into two different phases, the leaf blight phase and kresek phase, which is the harmful phase of the epidemic (Reddy and Ou 1976; Ou 1985) (Fig. 2.1).

Table 2.2 List of the various bacterial stains

Species	Type of bacteria	Strain
X. oryzae pv. *oryzae*	Gram-negative bacterium	OS225
		OS198
		OS86
		Z173
		JS158-2
		CJO13-1
		BAI3
		ABB27
		ABB37
X. oryzae pv. *oryzicola*	Gram-negative bacterium	BAI10
		BAI119
		BLS256
		AHB4-75
		JSB3-22
		YNB10-32
		GXB3-14
		SCB4-1
		CJOC13-1
B. glumae	Gram-negative bacterium	CU-1
		CU-2
		CU-3
		LMG2196
		NCPPB3923
		CFBP3831
		3252–8
P. ananatis	Gram-negative bacterium	ARC22
		ARC315
		ARC593
P. stewartii	Gram-negative bacterium	ARC903
		ARC10
P. agglomerans	Gram-negative bacterium	ARC982
		ARC1000
		ARC282
		ARC933

2.2.1.1 Leaf Blight Phase

After the tillering stage reaches its maximum peak position, the leaf blight phase of the rice leaf blight disease becomes more obvious in the tropical and temperate zones, with the initial symptoms on the leaf blades. Bacterial infection normally begins in the lower sections of the plant and expansions up to the leaf upperside portion (Goto 1992; Cha et al. 1982). The upper half or entire portion of the leaf

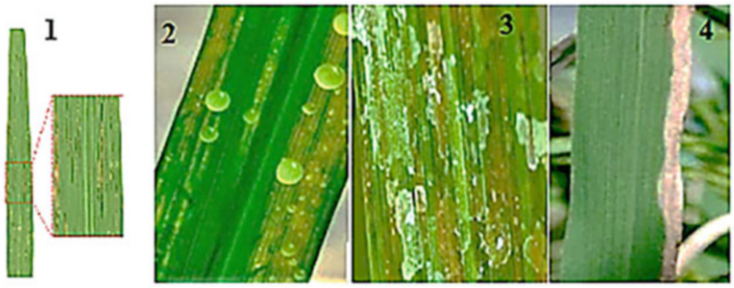

Fig. 2.1 1. Bacterial light caused by *Xanthomonas oryzae* pv. *oryzae*, 2. yellow droplets of bacteria on the leaf, 3. dried bacteria on the leaf, 4. dried leaf margins (Jiang et al. 2020)

blade acquires a pale yellow colour before drying up depending on the severity of the disease (Mizukami and Wakimoto 1969).

2.2.1.2 Kresek Phase

The word "Kresek", which means "the sound of dead leaves," were rubbed together (Wakimoto 1969). This stage of the disease was originally documented in Indonesia in the twentieth century in the context of a separate bacterial rice disease (Reitsma and Schure 1950). The "Kresek" phase of the bacterial leaf blight illness appeared 1–2 weeks after the nursery plants were transplanted into the field. The plant's leaves turn grey-green to yellowish in extreme conditions. The disease's "Kresek" phase can also emerge in mature plant stages (Goto 1992; Watanabe 1975). Symptoms on the foliar sections of the plant are similar to those on younger plants during the Kresek phase, but the rotting of the stem also reaches the upper part of the leaf.

In the roots of the weed "*Leersia hexandra*", the bacteria *Xanthomonas Oryzae* will be detected. Bacteria infiltrate rice nursery beds during the growing season and spread across the beds and channels as water is watered to the immature plants. The pathogen might enter the rice nursery by infected straw or infected seeds in the field (Mizukami and Wakimoto 1969). The pathogen accumulates on the surface of the roots and travels towards the crown once it reaches young plants. The bacterium begins to multiply by consuming the plant compounds released by the roots (Mizukami 1957, 1959, 1961) (Mizukami 1957, 1959, 1961) (Mizukami). Under moist conditions, the pathogen entered the stomata of coleoptiles and leaf sheath, where it multiplied in the intercellular spaces of parenchyma. It was also speculated and reported that the infection could be spread by insects or bugs, such as *Leptocorisa acuta*, which was found in the rice crop (Mohiuddin et al. 1976).

2.2.2 Rice Bacterial Leaf Blight Management

Rice leaf blight can be controlled through hygienic conditions, the selection of resistant seed variants, the application of appropriate pesticides, and the use of biological control approaches. Cultural management methods such as using healthy

seeds, removing old and diseased straws and stubbles, maintaining an appropriate water level, using nitrogenous compounds properly, and adopting a proper water drainage system can aid in pathogen control.

2.2.3 Chemical Management

According to the literature and documented evidence, substantial research has been conducted to establish the efficacy of various pesticides in controlling and managing bacterial leaf blight in rice and reducing yield loss. Varying combinations of antibiotics in various strengths have been recommended for disease inhibition by various workers over the years. Table 2.3 lists some of the medications and combinations used to treat BLB.

2.3 Bacterial Leaf Streak

2.3.1 Disease Development

Bacterial leaf streak is a seed-borne disease and is mainly infected by the pathogen *Xanthomonas oryzae* pv. *oryzicola*. It can spread or infect through seed and physical contact of the infected plant to other plants. The presence of moisture content around the seed as a film facilitates the release of bacteria from the diseased seed and attacks the inside tissue where it grows further as colonies. The bacterium enters through the

Table 2.3 Antibiotics and combination of antibiotics used in the chemical management of BLB disease

S. No	Antibiotics/combination of antibiotics	Reference year
1	Streptomycin sulphate 200 ppm + copper oxychloride (0.25%)	Kumar et al. (2009)
2	Streptocycline + copper sulphate were effective at 1000 ppm concentration	Patel et al. (2009)
3	Amistar at 0.1%	Mustafa et al. (2013)
4	Streptomycin + copper oxychloride highly effective under in vitro condition at 4%	Singh et al. (2015)
5	Azoxystrobin 25 SC	Swati et al. (2015)
6	Blitox 0.3% + streptocycline at 250 ppm	Ashish et al. (2016)
7	Mancozeb 500 ppm + streptocycline 100 ppm	Kamble et al. (2017)
8	Trifloxystrobin 25% + tebuconazole 50% at 50 ppm	Bala et al. (2017)
9	Nativo 75 WG at 0.65%	Qudsia et al. (2017)
10	Streptomycin at concentration of 0.03% and 0.05% was effective	Prasad et al. (2018)
11	Carbendazim at 500 ppm concentration	Jadhav et al. (2018)
12	Mancozeb at 500 ppm	Jadhav et al. (2018)
13	Streptocycline at 250 ppm	Jadhav et al. (2018)

openings on the leaf's surface, stomata, and the wounds on the leaf. Under favourable warm conditions, the bacteria multiply in parenchyma tissue and starts spreading from bottom to top of the plant.

The obvious signs appear as elongated streaks running down the leaf's veins. The disease's primary symptom is the formation of thin water-soaked transparent veinal streaks that can range in length from 1 to 10 cm. The veins constrict and restrict the streaks, which turn yellow or orange-brown. The streaks become rough and dry as they progress, coalescing to form huge patches that occasionally cover the entire leaf surface. The infection has been found to spread to the leaf sheath and seed coat in some instances, although the symptoms are unclear (Ou 1985). The presence of water or dew on the leaf surface enhances the number of lesions by easily spreading bacteria over the large surface area. The bacteria overwinter in the soil, other perennial plants, and some other weed plants (Tillman et al. 1996) (Fig. 2.2).

2.3.2 Management of Bacterial Leaf Streak

The proper application of required fertilisers can manage the BLS. Proper distance needs to be maintained during plantation to avoid or minimise the physical contact of the plants. Selection of resistant seed varieties may reduce the intensity of the infection and the loss of the yield. Before seeding, the seeds are treated with chemicals and hot water to remove the spores of the bacteria. By practising field sanitation, removing rattons and straws from the field will minimise the inoculum at the beginning of the season. Providing a good drainage system for seedbeds,

Fig. 2.2 1. Initial water-soaked lesions, 2. enlarged and merged lesions (Shrvan Kumar et al. 2017)

growing nurseries in isolated upland areas, and avoiding clipping seedlings during transplantation may reduce the risk of bacterial infection to a large number of plants.

2.3.3 Chemical Management

Chemical control techniques for BLS have been studied and reported on in several research. However, many of them have almost the same characteristics as the bacterial leaf blight infection.

2.4 Bacterial Panicle Blight Disease

2.4.1 Disease Development

The Gram-negative bacteria *Burkholderia glumae* causes panicle blight disease (BPB), also known as rice bacterial grain rot (BGR). *Pseudomonas glumae*, the bacteria that causes bacterial panicle blight disease, was discovered in 1967 (Kurita and Tabei 1967). In 1992, the non-fluorescent bacteria in Pseudomonas were assigned to the genus *Burkholderia*. The bacteria's optimum temperature for growth is 30 degrees Celsius; however, it may still grow at tropical temperatures (Saddler 1994).

BPB is mostly a seed-borne disease, with inoculums that have persisted in the soil. The inoculum can survive in the leaf sheath and panicle of adult rice plants, and it can develop epiphytically from the booting stage (Goto and Ohata 1956; Uematsu et al. 1976; and Sayler et al. 2006). Physical contact is also a way for it to spread from one plant to another. Night-time temperatures are higher than humidity climatic conditions, which encourage bacteria to proliferate rapidly, increasing the risk of crop loss (Cha et al. 2001) (Cha et al. 2001). Cha et al. found that *B. glumae* can cause sterility in spikelets as well as discolouration in growing seeds. The bacteria on the leaf cause an initial infection, which then spreads to the sheath, infecting the growing panicle (Tsushima 1991; Tsushima et al. 1996). The bacteria penetrate the lemma and paleae through the stomata, where they multiply in parenchymatous intercellular gaps before spreading to the plant's surrounding healthy tissues (Tabei et al. 1989). In some situations, bacteria have been found in the parenchyma, epidermis, and sclerenchyma of glumes (Hikichi 1993). The entire disease cycle hasn't been studied yet, but a lot of research is going on to figure it out.

2.4.2 Management of Bacterial Panicle Blight Disease

The primary inoculum in the seeds is destroyed by soaking them in hot water for 10 min at 57 °C (Tagami and Mizukami 1962). Appropriate field cleanliness, such as eliminating old rice straws and alternate, collateral, weed, and volunteer plants, is critical to avoid or prevent infection. When planting, it's important to keep enough

Fig. 2.3 Panicle blight caused by bacterial *B. glumae* (1. infection in sheath 2. panicles) (Ortega and Rojas 2021)

spacing between the plants to avoid direct contact. When transplanting, avoid trimming the seedling's tip. The contact period of the inoculum can be reduced by maintaining good irrigation and drainage of water. Disease rate can be minimized by using resistant types and (IR-20, IRBB21, IR-36, Sasyasree, Govind, Pant Dhan-4, Pant Dhan-6, and Saket-4) correct nitrogen fertiliser (Fig. 2.3).

2.4.3 Chemical Management

Before seeding, the seeds can be soaked overnight in 100 ppm streptomycin solution (Devadath and Padmanabhan 1970) which can eradicate the primary inoculum from the seeds. *P. fluorescens* applied to wet seed treatment at a rate of 10 grammes per kilogramme of seeds also aids in eradicating the main inoculum. Spraying of neem oil 60 EC at 3% or NSKE @5% in the field can control the disease spreading to healthy plants. Some other antibiotics used in BPB disease management are listed in Table 2.4.

2.4.4 Molecular Diagnosis of Bacterial Disease of Rice Disease

The presence of *X. oryzae* and *B. glumae* in infected rice may cause damage the crop; many studies have listed the three bacteria as quarantined organisms. Both conventional and real-time PCR have been widely used to detect or verify the presence of

Table 2.4 Antibiotics used in the disease management of BPB

S. No	Antibiotics	Reference year
1	Streptocycline at 100 µg	Banerjee et al. (1984)
2	Agrimycin 100 at 100 µg	Banerjee et al. (1984)
3	Oxolinic acid at 300 µg	Shtienberg et al. (2001)
4	Streptomycin sulphate at 100 µg	Shtienberg et al. (2001)
5	Glycoside B at 700 µg	Shtienberg et al. (2001)
6	Kasugamycin at 80 µg	Shtienberg et al. (2001)

Table 2.5 Primer used for the detection of bacterial disease of rice

Pathogen	Primer	Sequence	Reference
Xanthomonas oryzae pv. *oryzae*	Xo3756F	CATCGTTAGGACTGCCAGAA	Bangratz et al. (2020)
	Xo3756R	GTGAGAACCACCGCCATCT	
Burkholderia glume	toxB-F	GCATTTGAAACCGAGATGGT	
	toxB-Rd	TCGCATGCAGATAACCRAAG	

X. oryzae and *B. glumae* in recent decades. These molecular-based methods are rapid, accurate, and sensitive for detecting pathogens. However, they can detect only one pathogen each. Several methods have been developed to distinguish highly similar pathovars of *X. oryzae* and *X. oryzae* pv. *oryzicola* using multiplex or real-time PCR.

2.4.4.1 Sample Collection

Rice-infected bacterial samples were collected from the paddy field and cultured in selective culture media Luria-Bertani medium for 24 h.

2.4.4.2 Bacterial DNA Isolation

Rice-infected bacterial DNA was extracted with a genomic DNA isolation kit following the manufacture protocol. Quality of extracted DNA was checked using NanoPhotometer UV/VIS spectrophotometer. The optical density 260/280 of the isolated DNA was approximately 1.8 and diluted in double distilled water (Table 2.5).

The bottleneck for PCR-based diagnostic or detection tools has been the availability of pathogen-specific primers. Sequence polymorphisms of 16S ITS are often observed in strains of different species. In previous studies, specific DNA primers and probes have been designed based from 16S.

2.5 Conclusion

The major challenges for farmers in the production and cultivation of rice are overcoming natural disasters and various microbial diseases. It's not possible to take steps to overcome natural disasters against Mother Nature. But the other microbial diseases can be overcome by practising the proper disease management

techniques. Nowadays, farmers are adopting chemical management techniques to reduce the loss of yield. Chemical management needs to adapt when the disease condition and the rate of infection of the infected area are not under control. The proper application of selective antibiotics and a combination of antibiotics will control bacterial diseases and help to reduce the loss of yield. Excessive and poor antibiotic mixture selection may disturb the standard system's balance, posing a hazard to humans and livestock. Further, prolonged usage of chemicals may cause resistance in the disease-causing bacteria. So, it is advisable to adopt both natural and chemical management techniques in a controlled and interdependent way to achieve eco-friendly results. Molecular techniques will help to detect the bacterial disease caused by the rice pathogen.

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Viral Diseases of Rice

3

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Abstract

Rice, a fundamental means of life for millions, is the second most widely consumed staple food globally. It is mainly consumed by people living in Latin America, Asia, and Africa. With the gradual increase in the world's population, it has become essential to increase rice cultivation as well. Rice is harvested on roughly 159 million hectares around the world. Rice is produced in excess of 700 million tons per year, with Asia accounting for nearly all of it. Rice has a significant impact on the global food security, human nutrition, as well as the economy. Rice has a vast amount of nutritional value as it provides about 15% of global human per capita protein and 20% of the per capita energy (International Rice Research Institute, 2002. Rice Almanac, Third Edition). It can also be used for medicinal purposes, and its hulls can be used for fuel and in many industrial processes. Being one of the most cultivated crops worldwide, it is infected by several fungal, bacterial, and viral diseases. Among other things, rice production and yield are seriously threatened by viral diseases. In the Asia-Africa area, about 30 distinct rice viruses have been documented. Each year, an estimated 37% of all rice crops are lost to pests and diseases. Insect vectors such as leafhoppers, aphids, and whiteflies are commonly agents that transmit these viruses. Pale and discontinuous yellow stripes, blotches, and dead tissue streaks on the leaves are typical symptoms of viral infections in rice crops. Some of the management strategies involve using integrated pest management, screening, and breeding for varietal resistance, virus-free propagation material, etc.

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Keywords

Viral diseases · PCR · Vector · Yield losses · CRISPR · RNAi

3.1 Introduction

Rice is one of the most widely consumed cereals and cash crops in the world, and it is grown all over the world (Sangeetha et al. 2020). It is grown in almost all Asian countries, some of eastern and central Africa and Central and South America (Mitrofanova et al. 2018). Asia alone consumes about 650 million tons of rice which accounts for 90% of the global population (Ali et al. 2018). The first viral disease of rice was recorded in Japan in 1883 (Milošević et al. 2012). Since then, viruses have been infecting and causing damages to rice crops in several Asian, African, and American countries. Over 30 rice viruses have been reported in these countries through experimental tests and in nature. The last few decades have shown how greatly can yield, quality, and production of rice be compromised by viral diseases (Tang et al. 2020). Twenty-five viruses have a direct economic threat to rice growth and production. These viruses are majorly transmitted by vectors whose host range is limited to gramineous plants, usually leafhoppers and plant hoppers, that can migrate over large distance (Sangeetha et al. 2020), while some are seed or soil-borne (Tang et al. 2020). These viruses as well as their vectors favor hot and humid climatic conditions. Rice virus disease management techniques include varietal resistance screening and breeding, chemical treatment, plant quarantine, efficient cultural practices, integrated pest management, disease management training, and bioengineered resistance (Sangeetha et al. 2020) and ongoing collaboration with other laboratories pursuing the same disease-management goal. This chapter includes some important viral pathogens and diseases of rice crop as well as describes their taxonomic position and nucleotide sequence, particle morphology, diseases symptoms and host range, virus transmission, economic significance, purification, diagnostic techniques, as well as management and control measures to better understand the viruses and the diseases they cause on rice crop and manage their disease problem (Table 3.1).

3.2 Black-Streaked Dwarf Virus

Rice black-streaked virus, a member of the *Fijivirus* genus, mostly affects rice and maize crops, causing diseases such as maize rough dwarf disease and rice black-streaked dwarf disease. It usually occurs in East Asian countries and causes major yield losses (Tang et al. 2020). RBSDV was originally identified in Japan in the early 1950s, where it impacted rice crops. It was later discovered in Korea and China. This disease is associated with a plant hopper *Laodelphax striatellus*.

Table 3.1 Describe the common symptoms, host range, and mode of transmission of viral diseases of rice

Serial no.	Viral diseases	Host range	Symptoms	Transmission
1	Black-streaked dwarf virus	RBSDV usually infects hosts that belong to the *Poaceae* family including *Zea mays*, *Oryza sativa*, and *Triticum aestivum* (Tang et al. 2020)	Normal symptoms include wrinkled leaves that are dark green, white tumor-like protrusions endings, incomplete tassels, upgrowth of rootlets, and formation of the tiller on the upper part of the plant (Mohammed et al. 2012)	This virus is transferred from one place to another by an insect vector *Laodelphax striatellus* and sometimes by *Unkanodes sapporona* and *Unkanodes albifascia* (Mangli et al. 2012)
2	Rice yellow mottle virus	Confined to species in the family *Poaceae*, predominantly in the tribes Oryzeae (*Oryza sativa L.* and *Oryza glaberrima Steud.*) and Eragrostideae (Adkins et al. 2003; Adkins et al. 2003; Adkins et al. 2006; Lan and Lu 2020)	Yellow or orange leaf discoloration, necrosis, stunted growth, yellow-green streaking, leaf mottling, reduced tillering, and empty spikelets. Unsynchronized flowering and eventually plants' death or discoloration of the grains (Lan and Lu 2020; Odongo et al. 2021)	Leaf debris and spikelet contaminants (Kamenova and Adkins 2004; Cagno et al. 2018), insect vectors, i.e., beetles (Adkins et al. 2003; Himeno et al. 2014; Sõmera et al. 2015; Zeng et al. 2016) or wounds (Odongo et al. 2021)
3	Rice tungro disease	Cultivated rice, some wild rice varieties, and other grassy weeds often found in rice paddies (VIRUS; Matsuura et al. 2004)	Stunted plants (Kumar and Dasgupta 2021), yellow to orange discoloration, and interveinal *chlorosis* (Lawson et al. 1995; Goto et al. 2015; Brindhadevi et al. 2021). Mottled young leaves, rusty spots on older leaves, reduced tillering (Asjes and Blom-Barnhoorn 2001; Matsuura et al. 2004; Goto et al. 2015; Brindhadevi et al. 2021)	Leafhopper vector transmission (green leafhopper is considered to be most efficient) (Bautista et al. 1995; Hirayama et al. 2017)
4	Rice dwarf virus	This virus affects 28 species of 15 genera including *Oryza sativa*, *H.distichum* var. *nudum*, *H. vulgare*, *Triticum aestivum*, *Secale cereale*, *Avena sativa*, *Zea mays saccharata*, *Setaria italica*, *Paspalum thunbergii*, *Polypogon fugax*, *Leersia japonica*, *Eragrostis ferruginea*, *Lolium multiflorum*, *O. australiensis*, *O. barthii*, *O. brachyantha*, *O. latifolia*, *O. nivara*, *O. glaberrima*, and *Beckmann taerucaeformis* (Ara et al. 2012)	One of the major symptoms that are found in the rice crops infected by RDV through which they can be characterized is stunting of the crop. (Kumar and Dasgupta 2021). Other symptoms include increased tillering, dark-green discoloration, and white chlorotic spots on the leaves (Haxim et al. 2017)	Rice dwarf virus is transmitted by *Nephotettix cincticeps*, *N. nigropictus*, and *Recilia dorsalis*

3.2.1 Taxonomic Position and Nucleotide Sequence

RBSDV is a member of the *Fijivirus* genus and the *Reoviridae* family. The viral particle contains a double-layered capsid with a 50-nm core and double-stranded genomic RNA (Tang et al. 2020). It has ten genomic segments of dsRNAs that are designated in the order of increasing electrophoretic mobility in the polyacrylamide gels as S1-S10 (Abdul-Samad and Mat 1995). These segments range in sizes from 4.5 to 1.4 kb. Segments have a low content of GC (32.0–38.3%) and have a conserved 5′–3′ terminal sequence (Zhang et al. 2016).

3.2.2 Particle Morphology of the Causal Virus

The particle of its casual virus is of an icosahedral shape that ranges in diameter approximately 70–80 nm (Eyvazi et al. 2021). The infected cells from the insect vector or the plant host contain two different kinds of particles; large particles range from 75 to 85 nm in diameter, whereas small particles are 55 nm in diameter (Lin et al. 2000).

3.2.3 Purification

The diseased samples are extracted in 0.2 M phosphate buffer at a pH of 7.5. 0.01 M EDTA is also used. It is centrifuged at 20,000 rpm (34,500 g) for 1 h as well as 8000 rpm (5000 g) for 20 min. Phosphate buffer (0.01 M) that contains M EDTA at the pH of 7.0 (Niu et al. 2014) is used to keep the virus suspended The isolation of DNA strands of RBSDV along with its PCR amplification can be achieved by using the FastQuant RT Kit (TIANGEN, Beijing, China) and KOD-Plus-Neo enzyme with relevant primers (TOYOBO, Osaka, Japan) (Zhang et al. 2019).

3.2.4 Disease Symptoms

The symptoms of the black-streaked dwarf virus vary with the age of the crop. Normal symptoms include wrinkled leaves that are dark green, white tumor-like protrusion endings, incomplete tassels, upgrowth of rootlets, and formation of the tiller on the upper part of the plant (Blystad et al. 2015). Twisted leaf blades appear, and when the heads are formed, they contain dark-brown blotches (Kainana 1976). In the *Poaceae* family, it is responsible for extreme restricted and rigid and darkened leaves of the crop (Tang et al. 2020) (Fig. 3.1).

Fig. 3.1 Symptoms of diseases caused by the rice black-streaked dwarf virus (RBSDV) and the vector *Laodelphax striatellus* (small brown plant hopper) (**a**) Rice nursery, (**b**) Infected twisted leaves leaves (**c**) Stem Showing symptoms (**d**) formation of tiller on the upper part of plant, (**e**-**g**) wrinkled leaves (**h**) Vector (Cheong et al. 2003)

3.2.5 Diagnostic Techniques

The infection is detected by the use of RT-PCR and confirmed by gel electrophoresis using the relevant primers (Matsukura et al. 2019). The dot-ELISA process is simple as well as a rapid method to screen many samples at the same time (Lin et al. 2000).

3.2.6 Control/Management of the Disease

The management of this disease can be obtained by screening and the development of varieties as well as the use of insecticides (Kim et al. 1988). If resistant varieties

are not present, integrated pest management is used for inhibiting the viral transmission by insect vectors (Tang et al. 2020). Different combinations of cultural practices can also be used. Escaping the peak periods of insect attack by sowing the seeds early can also prevent the onset of disease. Infection can also be reduced by planting the crop at the spacing of 30 × 10 cm (Cheong et al. 2003).

3.2.7 Economic Significance

The plant that is infected with RBSDV has fewer panicles and a lower percentage of ripe grains. Infection can result in losses in yield of up to 60%. RBSDV is also responsible for a substantial yield loss in maize (Zhao et al. 2018).

3.2.8 Host Range and Transmission

RBSDV usually infects hosts that belong to the *Poaceae* family including *Zea mays*, *Oryza sativa*, and *Triticum aestivum* (Tang et al. 2020). Other hosts include oats, barley, rye and species of *Digitaria*, *Echinochloa*, *Eragrostis*, *Glyceria*, *Lolium*, *Agrostis*, *Alopecurus*, *Panicum*, and *Poa* in the *Gramineae*. This virus is transferred from one place to another by an insect vector *Laodelphax striatellus* and sometimes by *Unkanodes sapporona* and *Unkanodes albifascia.* For L. *striatellus*, there's about a 30% proportion of active transmitters. This insect breeds on wheat, rice, and barley. *Unkanodes sapporona* breeds on wheat, barley, and maize. The only means of transmission for this virus is through an insect vector; hence the efficiency in the migration and transmission is essential for the successful development of disease (Tang et al. 2020).

3.3 Rice Yellow Mottle Virus

Rice Yellow Mottle Virus; Taxonomic position and nucleotide sequence.

3.3.1 Taxonomic Tree

Domain	Virus
Kingdom	*Orthornavirae*
Phylum	*Pisuviricota*
Class	*Pisoniviricetes*
Order	*Sobelivirales*
Family	*Solemoviridae*
Genus	*Sobemovirus*
Species	*Rice yellow mottle virus*

3.3.2 Nucleotide Sequence

RYMV is 4450 nucleotide long and its genomic RNA contains 5 ORFs (Sõmera et al. 2015). ORF1 (nt 80–553) encodes proteins having 157 amino acids. ORF2 (nt 608–3607) codes a polyprotein containing 999 amino acids. ORF3 is said to encode a polypeptide containing 127 amino acids and is also enclosed in ORF2. ORF4 (nt 3447–4166) protrudes the 3′ terminus of OFR2, encodes a protein of 26 KDa, and is considered as coat protein of RYMV (Odongo et al. 2021).

3.3.3 Economic Significance

RYMV has importance economically (Brunt et al. 1980) and can put the development and extension of rice production in a region at a possible risk because it is a key biotic hurdle to rice cultivation in Africa (Séré et al. 2013; Blystad et al. 2015).

3.3.4 Disease Symptoms

Its symptoms consist of yellow/orange leaf discoloration, necrosis, and stunted growth during the vegetative state and yellow-green streaking, leaf mottling, reduced tillering, and empty spikelets. RYMV can also cause unsynchronized flowering and eventually plant death or discoloration of the grains (Brunt et al. 1980; Lan and Lu 2020; Odongo et al. 2021).

3.3.5 Host Range

It has a confined host range, limiting to *Poaceae* species, predominantly in the tribes Oryzeae (*Oryza sativa L.* and *Oryza glaberrima Steud.*) and Eragrostideae (Adkins et al. 2003; Matsui et al. 2005; Adkins et al. 2006; Lan and Lu 2020).

3.3.6 Transmission

RYMV cannot be transmitted through rice seeds, but it can be transmitted through leaf debris and spikelet contaminants (Kamenova and Adkins 2004; Cagno et al. 2018). It can be transmitted through insect vectors, i.e., beetles (Adkins et al. 2003; Himeno et al. 2014; Sõmera et al. 2015; Zeng et al. 2016) or wound (Odongo et al. 2021).

3.3.7 Purification

Purification of RYMV is done using new or deep-frozen young rice leaves harvested 14 days after infection. Liquid nitrogen is used to grind the leaves, which are then homogenized in a 0.1 M phosphate buffer (pH 5.0). It can be further purified by centrifugation at 3000 × g for 2 h through a 10–40% sucrose gradient (Kamenova and Adkins 2004). The RNA is extracted by using GeneJET Plant RNA Purification Mini Kit followed by RT-PCR using RYMV CP specific primers. The PCR products are then sequenced by Sanger sequencing technology (Hébrard et al. 2020).

3.3.8 Diagnostic Techniques

The direct visual evaluation of rice for detection of RYMV is done based on Standard Evaluation System (SES) (Abou Ghanem-Sabanadzovic et al. 2012). Serological diagnostic techniques include the precipitation methods and the nitrocellulose test. Many variants of DAS-ELISA for detection of RYMV (Nagpal et al. 2005; Jia and Martin et al. 2008; Gautam 2019; Liu et al. 2020; Mabele et al. 2020; Marwal and Gaur 2020) utilizing a panel of polyclonal and monoclonal antibodies are used (Fekih-Hassan et al. 2003). Western and Northern immune blotting and PCR are used for the translation and expression of RYMV (Fekih-Hassan et al. 2003; Sicard et al. 2018). Electron microscopy (Caldwell and Robb 1962; Mokra et al. 2008) and RT-PCR amplification of the ceruloplasmin (CP gene) (Caldwell and Robb 1962) can also be used to identify RYMV.

3.3.9 Particle Morphology of the Causal Virus

RYMV is a positive-sense RNA virus with a single strand. Particles are distinctive, being spherical having a diameter of about 28 ± 3 nm and containing about 77% protein (Caldwell and Robb 1962).

3.3.10 Geographic Distribution, Epidemiology, and Yield Losses

The first case of RYMV was discovered in Kenya and East Africa in the 1960s (Caldwell and Robb 1962); after that in many East and West African countries, it has been reported on occasionally (Mokra et al. 2008; Issaka et al. 2021).

According to the estimations, RYMV can cause rice yield losses between 20% and 100% (Brunt 1971; Richins and Shepherd 1983; Larsen et al. 2005; Odongo et al. 2021) with important socio-economic effects for farmers.

3.3.11 Control/Management of the Disease

3.3.11.1 Prevention

- Resistant and tolerant varieties can be used (Pappu et al. 2005; Nerway et al. 2020; Odongo et al. 2021).
- Direct sowing or nursery areas that haven't been affected before can also assist prevent the virus from spreading (Larsen et al. 2005; Katsurayama et al. 2020).
- Regular weeding and clearance of shrubs around fields can help reduce RYMV by preventing the peak period of the virus's insect vectors.
- The use of prophylactic control measures or other sanitary techniques can also help to prevent the spread of RYMV (Harris and Eade 1963).

3.3.11.2 Chemical Control

To directly control or prevent the spread of this virus, no chemical control methods are available yet. However, in some countries, insecticides are present to manage insect vectors, thus inhibiting viral transmission (Hirayama et al. 2017).

3.4 Rice Tungro Disease

Tungro was reported to be caused by a combination of two morphologically unrelated viruses in 1979: the Rice tungro spherical virus (RTSV), an RNA virus, and the Rice tungro bacilliform virus, a DNA/RNA virus (RTBV) (De Haan et al. 1991; Sether and DeAngelis 1992; Günther et al. 2000).

3.4.1 Taxonomic Position and Nucleotide Sequence

3.4.1.1 Taxonomic Tree

	Rice tungro spherical virus (RTSV)	*Rice tungro bacilliform virus* (RTBV)
Domain	Virus	Virus
Kingdom	*Pararnavirae*	*Pararnavirae*
Phylum	*Artverviricota*	*Artverviricota*
Class	*Revtraviricetes*	*Revtraviricetes*
Order	*Ortervirales*	*Ortervirales*
Family	*Secoviridae*	*Caulimoviridae*
Genus	*Waikavirus*	*Tungrovirus*
Species	*Rice tungro spherical virus* (RTSV)	*Rice tungro bacilliform virus* (RTBV)

3.4.1.2 Nucleotide Sequence

RTBV, like other plant pararetroviruses, is transcribed asymmetrically with full coding capacity on the negative strand. RTBV has four ORFs, able to encode proteins of 24, 12, 194, and 46 kDa, respectively (Sether and DeAngelis 1992; Maloy and Murray 2001). ORF3 encodes a P194 polyprotein which consists of four

present domains: the coat protein of the virus (37 kDa), reverse transcriptase, aspartate protease, and ribonuclease H (Parrella 2003).

Only five RTSV complete genomes are currently accessible in the NCBI database (Kannan et al. 2020). RTSV assists the transmission of RTBV. The viral RNA has a 515-nucleotide leader sequence and encodes a single major ORF1 at the 5′ end, beginning after the leader sequence (Azzam and Chancellor 2002). ORF1 encodes for a polyprotein that is broken by a protease into three coat proteins (CPs) that are arranged next to each other (CP1–3) (Sharma et al. 2012; Kannan et al. 2020).

3.4.2 Disease Symptoms

Noticeably stunted plants (Kumar and Dasgupta 2021) yellow to orange discoloration, and interveinal *chlorosis* on the leaves (Lawson et al. 1995; Goto et al. 2015; Brindhadevi et al. 2021). At times, young leaves are mottled, while on older leaves, rusty spots appear. Virus reduced tillering with poor root system. In the initial stage of infection, there is no formation of panicles, if formed, remain tiny with some, deformed and chaffy grains (Asjes and Blom-Barnhoorn 2001; Matsuura et al. 2004; Goto et al. 2015; Brindhadevi et al. 2021) (Fig. 3.2).

Fig. 3.2 Symptoms of rice plant infected with Tungro infected plant (Credits: Knowledge bank)

3.4.3 Host Range

Tungro mostly infects cultivated rice, but certain wild rice types and other grassy weeds can also be found in rice fields (VIRUS; Matsuura et al. 2004; Sankarganesh et al. 2020).

3.4.4 Transmission

Transmission mainly occurs by the leafhopper vector, and the green leafhopper is considered to be the most effective vector for transmission (Bautista et al. 1995; Hirayama et al. 2017).

3.4.5 Purification

PCR products are usually purified using QIAquick® Purification Kit following the manufacturer's instructions (Qiagen, Valencia, CA) (Caguiat et al. 2020).

3.4.6 Diagnostic Techniques

Tungro can be detected by DNA amplification and visualization of the amplified DNA using electrophoresis. Tungro can be verified using serological methods when they are accessible (Rosello et al. 1999), such as ELISA, latex agglutination test, and rapid immunofilter paper assays (RIPA) (Brunt 1968). BLAST can be used to find local similarity in-between sequences from previous researches (Waing et al. 2020).

3.4.7 Particle Morphology of the Causal Virus

The bacilliform capsid of the Rice tungro bacilliform virus (RTBV) possesses a circular double-stranded DNA genome (Hull 1996), while *Rice tungro spherical virus* (RTSV) has spherical/isometric capsid with a diameter of 30 nm, single-stranded RNA genome (Kannan et al. 2020).

3.4.8 Geographic Distribution, Epidemiology, and Yield Losses

Tungro is one of the most destructive and devastating rice diseases in South and Southeast Asia (Gulati et al. 2016). In extreme cases, in varieties susceptible to tungro, if infected at an early stage of development, yield loss might be as high as 100% every year (Kannan et al. 2020).

3.4.9 Control/Management of the Disease

After a plant is infected, it is impossible to cure it; hence prevention is the best measure to manage or control the disease.

3.4.9.1 Cultural Control/ Biological Control

- Integrated management of tungro disease, i.e., field sanitation, rouging, or removing the hosts weed species of the virus as well as the vectors (Jochems 1930).
- Grow disease-tolerant cultivars or host/virus-resistant varieties (Johnson 1936).
- Biocontrol of insect vector by using natural enemies (i.e., ladybugs) (Bambaradeniya and Edirisinghe 2009).
- A technical culture like varieties inter-cropping and Legowo system (Rumanti et al. 2016).
- Practice synchronous planting with surrounding farms (Costa 1945).

3.4.9.2 Chemical Control

- The vectors can be controlled in the nursery by the application of carbofuran or isoprocarb (Thomas and Zaumeyer 1950; Martin et al. 1959; Brindhadevi et al. 2021) (Fig. 3.3).

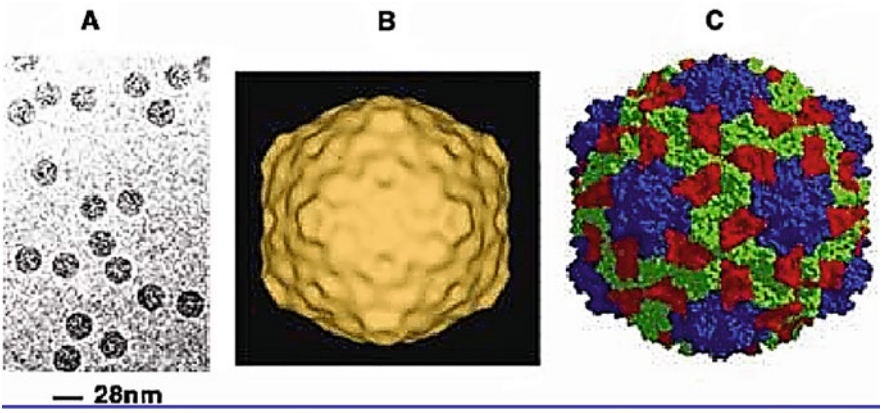

Fig. 3.3 Structure of Rice yellow mottle virus (RYMV) at different resolutions (Palukaitis et al. 1992): (**a**) electron micrograph of frozen-hybridized native RYMV (cryoelectron microscopy), (**b**) three-dimensional surface-shaded density maps of RYMV derived by cryo-EM (Gorovits et al. 2013), (**c**) space filling model of RYMV generated from X-ray crystallography data (Ravindra and Kalaria 2019)

3.5 Rice Dwarf Virus

Rice dwarf disease is induced by the rice dwarf virus. It infects crops, causing yield losses in production of rice throughout East Asian countries. It is transmitted by one of the most common leafhoppers in rice fields and the green rice leafhoppers, specifically *Nephotettix cincticeps* and two other species (Nakagawa et al. 2018). Green rice leaf hoppers that are free of RDV acquire it by feeding on diseased plant-parts (Xia et al. 2021). Virus multiplies in the insect body and hence is passed on from one generation to another. The virus induces white flecks on the leaves and causes dwarfing in graminaceous plants. It cannot be transmitted through the seeds of the host plant (Costa and Carvalho 1961).

3.5.1 Particle Morphology of the Causal Virus

The capsid of rice dwarf virus is a double shelled icosahedral. It is approximately 70 nm in diameter. This virus has several structural proteins that are called P1, P2, P3, P4, P5, P6, P7, P8, and P9 (Nakagawa et al. 2018). Its structure is determined by X-ray crystallography at a 3.5A ° resolution (Costa and Carvalho 1961). Miura and Fujii-Kawata were the first ones to analyze the genomic structure of RDV. They discovered that the virus's genome is made up of 12 dsRNA segments (Akiew et al. 1993).

3.5.2 Taxonomic Position and Nucleotide Sequence

RDV is from the family *Reoviridae* and genus *Phytoreovirus*. It contains a vast range of hosts including plants, animals, insects, and humans (Nakagawa et al. 2018). Their genome is transcribed inside the capsid that is intact. Before it is released from the capsid, the nascent mRNA is capped (Konishi et al. 2010). Genome analysis shows that every genome sequence of RDV has 4 3′-terminal and six 5′-terminal nucleotides that are conserved in all 12 segments of double-stranded RNA (S1-S12) (Akanda and Maino et al. 2004). These 12 segments of dsRNA encode structural and non-structural protein. Seven structural proteins encoded are P1, P2, P3, P5, P7, P8, and P9. The five non-structural proteins include Pns4, Pns6, Pns10, Pns11, and Pns12 (Zhao et al. 2020).

3.5.3 Purification

It can be purified from the infected leaves of the host (rice) plant. The diseased sample is places in phosphate buffer (pH 6.8) and 0.1% thioglycolic acid. The sample is then centrifuged in chloroform followed by the pallet being resuspended in the phosphate buffer solution (Milošević et al. 2015). RNA can be extracted by

using the TRIzol reagent. The further PCR amplification program is 94 °C for 3 min, 35 cycles, and 72 °C for 10 min (Ren et al. 2018).

3.5.4 Geographic Distribution, Epidemiology, and Yield

It is distributed mainly in Asian locations like China, Japan, Fujian, Honshu, Korea Republic, Shikoku, Zhejiang, Thailand, etc. (Barbosa et al. 2008). It is recently reported in the Philippines (Takahashi et al. 1993).

3.5.5 Control/Management of the Disease

Controlling the insect vector is a common methodology to minimize the damage caused by RDV. Another control method is by developing insect-resistant crops through genetic engineering (Xia et al. 2021). By using chemical fertilizers as well as synthetic insecticides, the cultivation of high-yielding varieties is feasible. Resistant varieties can also be used to control the disease. The control of RCV through elimination of weeds from fallow paddy fields is an idea that's been proposed by scientist but hasn't been practically implemented (Palukaitis et al. 1992).

3.5.6 Disease Symptoms

One of the major symptoms that are found in the rice crops infected by RDV through which they can be characterized is stunting of the crop (Kumar and Dasgupta 2021). Other symptoms include increased tillering, dark-green discoloration, and white chlorotic spots on the leaves (Haxim et al. 2017) (Fig. 3.4).

3.5.7 Host Range

This virus affects 28 species of 15 genera including *Oryza sativa*, *H. distichum* var.*nudum*, *H. vulgare*, *Triticum aestivum*, *Secale cereale*, *Avena sativa*, *Zea mays saccharata*, *Setaria italica*, *Paspalum thunbergii*, *Polypogon fugax*, *Leersia japonica*, *Eragrostis ferruginea*, *Lolium multiflorum*, *O. australiensis*, *O. barthii*, *O. brachyantha*, *O. latifolia*, *O. nivara*, *O. glaberrima*, and *Beckmann taerucaeformis* (Ara et al. 2012).

3.5.8 Transmission

Rice dwarf virus is spread by *Nephotettix cincticeps*, *Nephotettix nigropictus*, and *Recilia dorsalis* that are it's insect vectors. Most of these insect vectors transmit the virus till their death. RDV replicates and assembles its virions in the vector's salivary

Fig. 3.4 Rice dwarf virus causes chlorotic specks on the leaf (Palukaitis et al. 1992)

gland cells (Liu et al. 2021). *Nephotettix cincticeps* has the feeding periods of 5 min, and the incubation period of RDV in the insect ranges from 12 days at 29.2 °C to 17 days at 20 °C (Milošević et al. 2015). However, the period of continuous transmission is 5 days (Liu et al. 2021).

3.5.9 Diagnostic Techniques

RT-PCR can be used to detect the virus. Applications such as studying viral population dynamics, screening for viral resistance, virus-host interaction, and virus multiplication are feasible with the use of RT-PCR (Jacquemond 2012). Another method of detecting the presence of RDV is by extracting the RNA using TRIzol (Invitrogen, California, United States). By using the One-Step gDNA Removal kit and the cDNA Synthesis SuperMix Kit (TransGen Biotech, Beijing, China), reverse transcription reactions can be performed. The synthesized Cdna can be used as a template for PCR to confirm presence of RDV (Chen et al. 2021).

3.6 CRISPR and RNAi Tools for Managing Rice Virus Diseases

A technology that effectively targets and modifies a specific gene in plant has potential for interpretation the function of genes hence improving the quality of a crop. The present-day advance technology used for gene-editing that can be used for this purpose is based on the CRISPER or CRISPER-associated genes systems. These are said to be more effective, simpler, and flexible than transcription activator-like

effector nucleases and zinc finger nucleases, which are used in other gene-editing approaches. CRISPER technology offers a better alternative approach to the traditionally used methods for the breeding of plants to improve their traits. Since plant viruses bring about devastating losses to the crop production and its productivity and quality, there has been a rise of interest in using various CRISPER/Cas techniques and technologies to develop disease-resistant cultivars of plants which are less susceptible to viral attack (Green and Hu 2017; Borrelli et al. 2018).

There are two ways the CRISPER/Cas system that can be used in the production of resistant crop varieties against plant pathogenic viruses. First, by using CRISPER/Cas, we can induce targeted mutation in the genes of the relevant host plants that interfere with their ability to function as biosynthetic machinery which allows the successful viral replication and infection of the plant. Secondly, CRISPER/Cas can be used in plants to target the viral genome. For example, CRISPER/Cas systems that target DNA could be used to target the genome of DNA-containing viruses. Similarly RNA-cleaving CRISPER/Cas systems can target the RNA of RNA-containing viruses, for example, CAS9 from *Francisella novicida* (FnCas9) (Price et al. 2015) or Cas13a (previously known as C2c2) (Abudayyeh et al. 2016; Green and Hu 2017). CRISPER/Cas systems have promising potential in controlling viral infections and the general improvement of the crop. However, there is a notable issue in their distribution, which is the lack of efficacy through which the reagents of CRISPER/Cas are delivered into the cells of plants (Baltes et al. 2017). This limitation can be overcome by establishing suitable and efficient protocols of delivering CRISPER/Cas reagents into the plant tissues. Selecting the correct CRISPER/Cas editing system is also crucial. The eukaryotic mechanism of RNA interference (RNAi) refers to RNA-mediated sequence-specific gene silencing. This technique has also proved to be effective in increasing the viral resistance of crops (Dubrovina and Kiselev 2019).

3.7 Conclusion

In this chapter, we offer an overview of all the abovementioned viral diseases of rice including their taxonomy, transmission, host range, symptoms, purification, diagnostic techniques, and their control/management. In the present era, there is still a need for more detailed research on various viruses that cause devastating yield loss in the production of rice since it is one of the most significant cereals and cash crops that affects the livelihood of millions of people around the globe. It also has a significant part in the global food security and worldwide economy. These viruses infect the rice crop and cause several structural and physiological damages that decrease its quality and economical value. The viruses of rice are usually carried around by insect vectors. These viruses are found in various parts of the insect, for example, the sucking gland cells. When these insect vectors feed on susceptible crops, they transmit the virus. After the virus is transmitted, it becomes very difficult to prevent infection, and hence the disease is formed. Only by avoiding transmission of viruses and inhibiting secondary dissemination to other plants we can effectively

manage their spread. There are many strategies that can be used to prevent infection. One of the main techniques is by using integrated pest management to control the insect vectors, hence preventing the transmission. Another method is through the production of resistant varieties by genetic engineering.

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Etiology, Epidemiology, and Management of Maize Diseases

4

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Abstract

The production of maize (*Zea mays*) across the world is continually challenged by the development of a variety of diseases like rust, northern leaf blight, maize streak, and grey leaf spot. Developing host defences against these pathogens can

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be a critical aspect of integrative pest control. This chapter discusses ecological, conventional, and molecular breeding strategies, as well as techniques for improving resistance mechanisms. In collaboration with other scientists, we will be able to use molecular breeding methods to improve the quality of susceptibility factors by using appropriate experimentation methods.

Keywords

Maize · Bacterial diseases · Modern breeding technologies · Host resistance · Sustainability

4.1 Introduction

The maize (*Zea mays* L.) family of *Poaceae* is the world's most important annual cereal crop. According to the Taino language, Zea means "sustaining life", derived from an ancient Greek word, and may also mean "life giver". The word "maize" has the Spanish connotation "maiz", which is the best way to describe the plant. Different other names like zea, silk maize, makka, barajovar, etc. are useful to identify the plant (Kumar and Jhariya 2013). The crops are accepted as a staple food source throughout the entire world. After rice and wheat, maize is the third most important crop in the world (Sandhu et al. 2007). Maize has starch (72%), protein (10%), and fat (4%), providing energy at 365 kcal/100 g (Nuss and Tanumihardjo 2010). Maize contains a lower amount of protein than rice and wheat. Maize gives B vitamins and important minerals, including fibre content, but is poor in remaining nutrients, like vitamin B12 and vitamin C, and contains less calcium, folate, and iron. Daily food in the diet and other components, like vegetables, tea (e.g. oxalates), coffee (e.g. polyphenols), eggs (e.g. phosvitin), and milk (e.g. calcium), inhibit the absorption of nonheme iron content, which is present in maize (Nuss and Tanumihardjo 2010).

The maize crop is attacked by about 65 pathogens, including fungi, bacteria, and viruses (Pavan and Shete 2021). In 2001–2003, fungal, nematode, and bacterial diseases were reported to cause a 9% loss in maize crops worldwide (Oerke 2006). The yield loss in individual states of the United States and Ontario, Canada, was evaluated by university plant pathologists. It has been reported that 7.5–13.5% of grain production was from 2012 to 2015 (Mueller et al. 2016). The total yield loss in the United States is more than 2–15% in a year (Munkvold and White 2016). Maize is affected by many bacterial diseases. Some of the common bacterial diseases that are frequently observed in maize include stalk rot (creating deficiency in grain filling and lodging) and bacterial leaf blight, which are caused by *Pseudomonas avenae*, bacterial stalk rot caused by *Erwinia dissolvens*, bacterial leaf spot caused by *Xanthomonas campestris*, and *Erwinia chrysanthemi*, which is responsible for top rot and bacterial stalk and many more. In addition, maize productivity is also severely reduced by several fungal and viral pathogens and nematodes. Numerous methods have been recommended for the control and management of maize diseases

(Pavan and Shete 2021). The availability, cost-effectiveness, and feasibility of each method differ among production regions. Recommended practices for the control of fungal pathogens include agronomic practices like conventional tillage, intercropping, crop rotation, and fungicide application. Effective management of bacterial pathogens focuses on protection by limiting the source of primary inoculum through crop rotation and residue management and by reducing the rate of disease development. However, planting of resistant cultivars, developed through modern biotechnological approaches, can effectively reduce the incidence and disease index and is widely recommended. Therefore, the present chapter will help to focus on the etiology, epidemiology, and management of different maize diseases.

4.2 Etiology of Different Maize Diseases

Maize is responsible for the 20% of the calories consumed by the world, directly or indirectly (Sabagh et al. 2021). Maize is grown in the areas wider than any other crop because of its capability of growing under temperatures ranging from cool to very hot on wet to semi-arid lands and in many different types of soils. Shiferaw et al. (2011) reported that the total of 54% losses are estimated every year due to the insect pests, diseases, and microorganisms. After the insects, diseases play a major role in affecting maize quality and yield. Some common diseases that hold immense importance throughout the globe include southern corn leaf blight (*Bipolaris maydis*), common rust (*Puccinia sorghi*), southern rust (*Puccinia polysora*), northern corn leaf blight (*Exserohilum turcicum*), grey leaf spot (*Cercospora* species), kernel and ear rots (*Fusarium* and *Aspergillus*), stalk and ear rots (*Diplodia* and *Fusarium*), etc. These species contaminate seeds by producing mycotoxins that compromise human health, food quality, and safety. In developing countries like Asia and Africa, maize crops experience downy mildews, post-flowering stalk rot, sheath blight, maize streak virus, etc. These diseases affect the seed quality in the field as well as during storage. Etiological studies refer to finding out the causes of the diseases, the factors that the pathogens use, and the conditions that are favourable for the pathogens to attack the susceptible host (Jeffers 2004; Edmeades et al. 2017). There are different biological agents that are responsible for the diseases of maize in storage and in the field. The diseases and agents that are critical for the maize crop are discussed below.

4.2.1 Bacterial Diseases

Most of maize disorders are induced by fungus; others are affected by bacteria, posing a serious danger to maize yield and profitability (Rehman et al. 2021). Insect injuries and scrapes are the most common entry points for bacteria into the plant. A bacterium called *Pseudomonas syringae* pv. *syringae* causes holcus leaf spot, the most common bacterial disease (Shao et al. 2021). The symptoms appear on the lower leaves of the maize plant at first reproduction stage. Dark green, water-soaked

spots appear in an irregular pattern. Later, the lesions dry out to form a papery texture with no borders (Kour et al. 2019). The favourable conditions for *Pseudomonas syringae* to become active are temperature range of 77–86 °F. Along with the warm conditions, humid conditions also attract the bacterial agent to attack the maize crop (Jayapriya and Hemalatha 2020).

Stewart's wilt, which is a concern for sweet corn from over 100 years, is caused by, *Erwinia stewartii* (*syn. Pantoea stewartii*). *Erwinia stewartii* is a facultative anaerobic, Gram-negative, nonflagellate, nonspore-forming, rod-shaped bacterium. Intracellularly, polysaccharides produced by this pathogen contribute to the blockage of the vascular system, which responds to the appearance of symptoms (Ma et al. 2016). Stewart's wilt occurs in two phases: the seedling phase and the leaf blight. This bacterium does not travel to maize by itself but needs a vector to reach its target. When feeding on maize crops, the corn flea beetle acts as a vector, transferring bacteria to the plant (Stack et al. 2001; Cushatt 2020). Beetles feed on the leaves, and white marks are left behind on the leaves as a result of their scraping. The infected plants show pale green to yellow streaks initially and later become brown as the tissues die. There is a chance of plant wilting if the plant stalk is infected. If cut, the stalk will produce droplets of pale yellow bacteria (Doblas-Ibáñez et al. 2019). Leaf blight is the second stage of the infection. Along the leaf's length, water-soaked lesions emerge, and as they mature, they turn necrotic. The main culprit for this disease is the flea beetles, which emerge in the early spring, coinciding with the maize crop in the field. Beetles can carry pathogens all winter long if they feed on diseased plants, or they can receive the bacterium directly from eating those plants. The disease risk and corn flea beetle population are said to increase if the sum of average monthly temperatures during winters exceeds 90 °F. At the same time, lowering the average monthly temperature to 80 °F reduces the risk of disease and beetle survival (Duong et al. 2018).

The Goss's wilt is another disease with similar symptoms to Stewart's wilt. This disease first appeared in 2009 and now is widespread in the corn-producing states (Cooper et al. 2018). It has two phases, just as Stewart's wilt: seedlings wilt (which causes a systemic infection) and leaf blight (which kills the leaves). It is caused by bacterial pathogen, *Clavibacter michiganensis* subspecies *nebraskensis*. It stays in the infected corn debris for a whole winter season and can be transmitted at a very low level. While systemic wilt is not as common as leaf blight, if it does emerge, the plants are fated for the rest of the growing season if it persists. Plant yield and survival is highly compromised in this infection. The seedling wilt phase infects the vascular bundles of the plant and travels along the xylem of the plant. Symptoms show the discoloration of the xylem to plant wilting or death (Jackson et al. 2007, 2009).

The primary symptoms of leaf blight phase appear as the elongated grey to light yellow lesions with irregular margins extending parallel to the veins. Shiny patches of the dried bacterial discharge appear on the lesions (Cooper et al. 2018). Diseased plant residues on the surface of the soil transmit bacteria, causing Goss's wilt to occur. Wind- or hail-induced wounds allow the pathogen access to the plant. Hot dry weathers can inhibit disease development except for fields with overhead irrigation (Cooper et al. 2018; Harding et al. 2018).

Erwinia dissolvens is another bacterial pathogen that causes threats to maize yield and survival. The disease caused by this pathogen is called bacterial stalk rot, but the symptoms appear at the whorl stage of the maize crop. This disease causes decay to the first internode above the soil. The pith becomes soft and water soaked. The plant remains green, but the stalk twists and falls over. Because the vascular tissue is still intact, the plant's green colour will not fade for several weeks (Jackson et al. 2009). Bacteria cause degradation of the tissues, and the destroyed tissues form a slimy substance that becomes the reason for the foul odour from the infected plants. When the decay occurs prior to tasseling, the upper leaves of the plant that form the whirl die and can be easily removed from the plants. However, the bottom leaves are still in good condition. Viruses and other pathogens can infect a plant at any node throughout its whole length, up to and including its leaves and tassel. However, this disease affects the individual plants only and does not spread to neighbouring plants (Kumar et al. 2017). High humidity and high temperatures (88–95 °F) following pollination favour the development of this pathogen. There are many other bacterial pathogens that cause diseases and damage to the maize crop. Some of them are mentioned in Table 4.1.

Table 4.1 Bacterial diseases their casual agents and favourable conditions for disease development in maize

Disease	Causal organism	Favourable conditions	Reference
Bacterial leaf spot	*Xanthomonas campestris* pv. *holcicola*	Temperatures between 65 and 68 °F, high humidity, plant injury, and excessive fertilization	Mbega et al. (2012)
Bacterial stalk and top rot	*Erwinia carotovora* subsp. *carotovora* *Erwinia chrysanthemi* pv. *zeae*	High-temperatures and high-relative humidity	Kumar et al. (2017)
Bacterial stripe	*Pseudomonas andropogonis*	High relative humidity, leaf wetness, continuous corn, and minimum tillage. Heavy rainfall and overhead irrigation also may favour bacterial infection	Saha et al. (2015)
Chocolate spot	*Pseudomonas syringae* pv. *coronafaciens*	Mild, damp conditions with high humidity and temperature between 0 and 30 °C—the optimum temperature around 15–20 °C	Guo et al. (2020)
Purple leaf sheath	*Hemiparasitic bacteria*	Abundant rainfall and temperature range between 73 and 90 °F	Li et al. (2018)
Seed rot-seedling blight	*Bacillus subtilis*	Wet soils, in low-lying areas in a field or in soils that have been compacted or remain wet for an extended period of time. Low soil temperatures (50–55 °F) and wet soil conditions especially are favourable	Lanza et al. (2016)

4.2.2 Fungal Diseases

Maize is an important cereal crop feeding a large population around the world. Although important, maize is attacked by more than 60 diseases that affect its quality and yield. Fungi are among the principle causes of maize deterioration during storage and in field that could cause 50–80% losses of maize if the conditions are favourable for fungi growth. A larger number of maize diseases are due to different fungi. *Penicillium*, *Fusarium* spp., and *Aspergillus* spp. cause the major diseases, which cause grain loss of about 11% of the total production (Mueller et al. 2016; Mannaa and Kim 2017). The development of fungi is expected when the temperature and moisture content of maize is high during storage. *Aspergillus* spp. becomes systemic and produces harmful toxins in seedlings and contaminates seeds in storage by producing aflatoxins. *Fusarium* invades seeds before harvest and produces mycotoxins which are damaging for maize as well as humans (Tsedaley and Adugna 2016).

Common smut caused by *Ustilago maydis* produces light green galls containing black masses of spores. These swollen galls later turn white and infect corn ears through silks. These galls can appear at any time in the growing season anywhere in the above-ground part of the plant. The fungus survives on corn debris and is transmitted when the conditions are favourable. Smut spores can survive in the soil for the whole winter season and are spread by wind and rain. Actively growing young tissues are more susceptible (Neupane and Ghimire 2020). Head smut is another fungus species that is responsible for head smut, a soilborne disease. The pathogen enters systemically, and symptoms appear at the time of tasseling and silking. Symptoms appear in the form of conspicuous galls that replace ears or tassels. These galls are covered by fragile membranes containing masses of dark brown spores, vascular bundles, and teliospores. The presence of vascular bundles and a membrane makes it distinct from common smut. Once the membrane is broken, wind and rain spread the teliospores to soil where it stays viable for a long time. The favourable conditions for *S. reiliana* are dry and warm weather at temperature 70–80 °F (Simón et al. 2021). *Aspergillus flavus* and *A. parasiticus* are the most important species of fungi that produces cancer causing aflatoxins. They affect the seed quality as well as the health of the consumers. Aspergillus infections and green fungal growth are common in the ear. When the seedlings are damaged by insects or scars appear on plants due to hail, aspergillus ear rot infections are more likely to occur. Infected ears produce a greenish-gold fluorescence when viewed with a black light at a wavelength of 365 nm. The disease worsens in drought conditions (Rehman et al. 2021).

Gibberella ear rot, caused by *Gibberella zeae*, which affects the tip of the ears and produces pink to red fungus growths, which moves towards the end of the ears. Infection occurs during silking. This disease is more prevalent when the weather is cool and wet during first 21 days of the silking. The fungus survives in the soil debris during winter seasons and enters the plant as soon as it is possible. The fungus produces two mycotoxins that are vomitoxin (or DON) and zearalenone, both of which are harmful for livestock. Extended periods of rain and fall increases the

severity of the disease (Dalla Lana et al. 2021). *Stenocarpella maydis* is the fungi species that causes *Diplodia* ear rot in maize. The symptoms start at the base of the ear but can appear on any part of the plant. The fungus produces white growth between kernels which later appears grey with black pycnidia. The fungus transmits to new plants by splashing water. The injured plants due to birds and insects invite the pathogen for infection. Dry conditions during early vegetative growth stages followed by warm, wet weather within the first 3 weeks after silking favour the development of *Diplodia* ear rot (Atenya 2016; Mabuza 2017). *Puccinia polysora*, responsible for southern corn rust, is favoured by warm and humid conditions.

Early symptoms include circular to oval-shaped lesions which are often accompanied by light green to yellow halo. The lesions erupt from the epidermis of the leaf surface. Heavily infected leaves die at premature stage. Spores are spread by wind and grow when humid conditions are provided (Debnath et al. 2019; Wang et al. 2019a, b). Another type of rust, which is known as common corn rust, is caused by *Puccinia sorghi*. Common rust is common wherever corn is grown. The favourable conditions for *P. sorghi* to cause common rust are similar to those for southern corn rust to occur. The disease causes lesions on the leaves that look like small tan spots on both sides of the leaves. These spots later turn into brick red or cinnamon blisters. One distinctive feature of this disease is that the symptoms appear on leaves only, not on sheaths, ears, or stalks. Infection is favoured by extreme temperatures, high humidity, and temperatures of 16–25 °C. Rust development and spread are favoured by cool temperatures. Pustules develop on corn varieties and hybrids which are susceptible after 7 days of infection (Dey et al. 2012; Debnath et al. 2019). There are many fungi species that cause maize to suffer from diseases, and some of them are mentioned in Table 4.2.

4.2.3 Parasitic Diseases

Plant parasitic nematodes can severely affect corn fields by deteriorating its quality thereby reducing its yield. These parasites attack the root system of the plants to lower the efficiency of the roots to take up the water and nutrients (Topalović et al. 2020). The damage usually appears on roots and above ground parts of the plants. The degree of damage is related to the types and population levels of nematodes present as well as the environmental conditions present in a field. Different species of nematodes include dagger (*Xiphinema*), lance (*Hoplolaimus*), lesion (*Pratylenchus*), needle (*Longidorus*), spiral (*Helicotylenchus*), and stunt nematodes (*Tylenchorhynchus*), but needle (*Longidorus*) and sting (*Belonolaimus*) species are the most dangerous ones. Above-ground symptoms usually include stunted growth, yellowing of leaves, uneven growth of tassels and ears, etc. while browning of roots is also visible (Ismaiel and Papenbrock 2015). Root-knot nematodes (*Meloidogyne* spp.) are present in all over the world, but the majority is found in areas with warm and hot climates and short or mild winters. Root-knot nematodes result in poor growth, decreased quality and yield of the crop, and reduced resistance of other stresses (Ye et al. 2019). Nematode damaged roots do not utilize water and fertilizers

Table 4.2 Fungal diseases their casual agents and favourable conditions for disease development in maize

Disease	Causal organism	Favourable conditions	Reference
Anthracnose leaf blight Anthracnose stalk rot	*Colletotrichum graminicola* *Glomerella graminicola* [teleomorph] *Glomerella tucumanensis* *Glomerella falcatum* [anamorph]	Low fertility and wet and warm weather provide favourable conditions. Spores transmit through rain water	Nicoli et al. (2016)
Black kernel rot	*Lasiodiplodia theobromae* = *Botryodiplodia theobromae*	Moist and humid conditions	Rehman et al. (2021)
Brown spot	*Physoderma maydis*	Abundant rainfall and temperatures ranging between 73 and 90 °F	Subedi (2015)
Brown stripe downy mildew	*Sclerophthora rayssiae*	High moisture conditions	Basandrai and Basandrai (2020)
Crazy top downy mildew	*Sclerophthora macrospora* = *Sclerospora macrospora*	Saturated soil conditions for 24–48 h from planting to about the five-leaf stage of growth. Accumulation of soil and water in the whorl of small plants results in infection	Kim et al. (2020)
Eyespot	*Aureobasidium zeae* = *Kabatiella zeae*	Cool and wet conditions	Wang et al. (2021)
Fusarium ear and stalk rot	*Fusarium subglutinans* = *Fusarium moniliforme*	High nitrogen, low potassium fertility, high moisture in the mid to late season after a dry early season	Gai et al. (2018)
Late wilt	*Cephalosporium maydis*	Moist soils and temperature of 30 °C	Molinero-Ruiz et al. (2010)
Penicillium ear rot Blue eye Blue mold	*Penicillium* spp. *Penicillium chrysogenum* *Penicillium expansum* *Penicillium oxalicum*	Moist and humid conditions	Kieh (2014)
Pythium root rot	*Pythium* spp. *Pythium arrhenomanes* *Pythium graminicola*	Cold temperatures and saturated soils	Binagwa et al. (2016)
Red kernel disease Ear mold, leaf and seed rot	*Epicoccum nigrum*	Warm and humid conditions	Oldenburg and Ellner (2015)
Yellow leaf blight	*Ascochyta ischaemi* *Phyllosticta maydis* *Mycosphaerella zeae-maydis* [teleomorph]	Wet and warm conditions	Rehman et al. (2021)

as effectively, and below-ground symptoms include severe galling, stunting, and chlorosis of crops. Root swellings can be formed in other diseases as well, but the root-knot galls have firm tissues near the fibrous vascular tissues of the roots (Eisenback and Triantaphyllou 2020). The male and female nematodes of root-knot have distinct morphology. Males are worm-like, with 1.2–1.5 mm in length and 30–36 m in diameter. The females are pear-shaped and about 0.40–1.30 mm long and 0.27–0.75 mm wide. The life cycle includes egg, juvenile, and adult stages. A life cycle is completed in 25 days at 27 °C, but it takes longer at lower or higher temperatures. Each female lays approximately 500 eggs in a gelatinous substance produced by the female (Eisenback and Triantaphyllou 2020). Cyst nematodes (*Heterodera zeae*) produce light brown to dark reddish brown and brown cysts that are shaped in an ovate and spheroid form. The symptoms relate to root damage and include stunting of plants in patches, yellowing of leaves, and reduced size of shoot parts. When a female dies, her body is tanned into a brown capsule containing hundreds of eggs. Mature females are found attached to roots with heads embedded in steles (Zheng et al. 2021). The male is wormlike, about 1.3 mm long and 30–40 m in diameter. Fully developed females are lemon-shaped, 0.6–0.8 mm in length and 0.3–0.5 mm in diameter. Approximately 21–30 days are required for the completion of the life cycle of the cyst nematode. 4- to 6-week-old plants can be seen in the root system by 4- to 6-week-old plants (Ibrahim et al. 2017).

The root lesion nematodes (*Pratylenchus* spp.) are economically important Phyto nematodes in maize fields. The plants show chlorosis, stunting, and a loss of vigour which results in wilt (Puerari et al. 2015). Lesion nematodes reduce root development by forming lesions on young roots. The affected roots then rot because of secondary fungi and bacteria attacks. Both male and female nematodes are worm like with 0.4–0.7 mm long 20–25 μm diameter. They are migratory endo-parasitic nematodes. The life cycle of *Pratylenchus* is completed within 45–65 days (El-Nuby 2020). *Rotylenchulus reniformis*, commonly known as reniform nematodes, is the most significant after root-knot. Maize serves as a good host for this nematode. Affected plants show stunted growth and poor yields of maize (Lima et al. 2017). This nematode is characterised as a semi-endoparasite, and it remains attached to roots. Plants infested with nematodes exhibit stunted growth and root discoloration. Loss in germination and crop stand occurs when nematodes damage the crops at the pre- and post-emergence stages of seedlings. Under optimum conditions, the duration of the life cycle of this nematode is about 4–5 weeks. Soil pH, moisture, and temperature have significant roles in penetration, infectivity, and the biology of nematodes. The life cycle is completed in 25 days (Soler et al. 2021).

Hoplolaimus species (lance nematodes) is a very common nematode which can be found on plant roots in all types of soil and climate (Holguin et al. 2016). These parasites are known as sedentary ectoparasites because they often feed at a particular spot for long time, and half of their bodies are embedded in the root system of the plant. Damage may show up as patches of yellowing and dying. These symptoms also can be caused by drought or nutrient deficiency. Lance nematodes multiply slowly in comparison to endoparasitic nematodes, but they inflict significant crop damage at a lower level of infection. The availability of feeder roots and temperature

are important factors for population buildup of this nematode (Singh et al. 2020). Life cycle is completed in 13–38 days. Stem and bub nematodes (*Ditylenchus* spp.) are found worldwide but are particularly present in areas with temperate climates (Sturhan and Brzeski 2020). It is known to be the most destructive pathogens with several hosts. Stem nematodes are responsible of heavy killing of seedlings, dwarfing, causing distorted development of the plants, twisted and swollen stems, as well as foliage. This nematode is barely present in soil and feed on stem and leaves of the host plants. The nematode is 1.0–1.3 mm long and about 30 μm in diameter. The females lay 200–500 eggs, mostly after fertilization by the males. The total duration of life cycle ranges from 19 to 25 days (Sturhan and Brzeski 2020).

4.2.4 Viral Diseases

Viruses are microscopic organisms that are unable to reproduce themselves but need a host to continue their life cycle (Asiimwe et al. 2020). In most cases, viruses are spread by insect vectors, which harm the plant by opening a channel for infections or by dipping into the phloem to feed on the diseases. Once within the plant cells, viruses manage the machinery using the few genes in their little genomes, all the while evading the plant's defences. RNA viruses are the most common types of plant viruses that cause diseases and losses in maize fields (Carino et al. 2020). There are more than 700 species of viruses, many of which cause diseases in a wide range of hosts. These species of viruses are classified into 3 families and 32 groups based on the type of nucleic acid, whether the nucleic acid is single-stranded or double-stranded, and the means of transmission (Roossinck et al. 2015). Barley yellow dwarf virus (BYDV) is a luteovirus that is distributed worldwide and affects major cereal species. Two of the major viral diseases of maize are maize dwarf mosaic (MDM) and maize chlorotic dwarf (MCD). Together, these two diseases can cause losses of up to 90% in fields. These viruses attack all types of corn, but popcorn and sweet corn are the hosts for these pathogens (Wijayasekara and Ali 2021; Bernardo et al. 2021). Symptoms of these diseases resemble and can be influenced by environmental conditions. Maize dwarf mosaic virus produces a faint or pale green streaking pattern in young leaves along the veins. Spots of dark green tissues appear on a light green background (Wijayasekara and Ali 2021). As the infected plants move towards maturity, the discoloration of the leaves increases and becomes yellowish green. Infected plants set a few seeds and produce multiple ear shoots. Early infections cause stalk rots and root rots. Sometimes the plant can experience death due to this infection (Bernardo et al. 2021).

The most destructive of all are maize streak virus (MSV), (Tembo et al. 2020; Monjane et al. 2020; Emeraghi et al. 2021). This virus is transmitted by leafhoppers, persistently. Streak viruses panicum streak virus (PanSV), sugarcane strains Réunion and Egypt, and *Digitaria* strains digitaria streak virus (DSV) are all linked to MSV in terms of genetics (Ma et al. 2015; Shafiq et al. 2020).The virus is transmitted by the insect leafhopper *Cicadulina* spp. commonly found in fields of late-planted maize to varieties that are susceptible to the disease. Disease symptoms

in infected maize plants, streak disease initially manifests as minute, pale, circular spots on the lowest exposed portion of the youngest leaves. The only leaves that develop symptoms are those that formed after infection, with older leaves remaining healthy. Maize-infecting maize rough dwarf virus is one of the viruses found in Fiji (MRDV). Early disease: white streaks and waxy swellings on the veins of the lower side of the leaves indicate early disease. Symptoms begin to manifest 4–5 weeks following the immunization. There are two known maize-infecting tenuiviruses, or tenui-like viruses. It's the maize stripe virus (MSpV) and the maize yellow stripe virus (MYSV) that are causing the problem (Guadie et al. 2018). MSpV and MYSV have been discovered in sorghum, where they cause sorghum stripe disease (SStD) (Sharman et al. 2016). The maize planthopper, *P. maidis*, is the vector of MSpV (Dumón et al. 2018). Although the virus reproduces and stays in the planthopper (Liu and Wang 2018), it may be transferred occasionally. Nymphs are better at passing on MSpV than adults, and they have the ability to spread the virus for a long time (Barandoc-Alviar et al. 2016). The vector's typical latent period is between 10 and 15 days, with transmission by people commencing as early as 4 days and lasting as long as 22 days. Nymphs in their first instar are the most effective transmitters (64%), followed by second to fourth instars (50%) and adults (50%) (33%). MSpV is passed to a tiny percentage of progeny via the egg (Liu and Wang 2018).

4.3 Management of Maize Diseases

Diseases of the maize crop cause several losses to the human world. If these diseases could not be eliminated, there would be a lot of starvation and famine, especially in the zones where maize is used as a staple. In the USA, southern corn leaf blight (casual agents were *Cochliobolus maydis* and *Bipolaris maydis*) caused direct economic losses to the hundreds of corn growers. The annual estimation of economic losses was one billion dollars (Maloy 2005). Annually, various maize diseases cause minute dramatic losses throughout the globe. However, these diseases tend to cause massive losses collectively to the farmers and also reduce the aesthetic appeal of the farmlands (Kupatt et al. 2018). Management of maize diseases usually relies upon the anticipation of the disease occurrence and the prone sites of the disease cycle. These sites provide vulnerable links in the infection chain; therefore, correct diagnosis of the disease cycle is the key to pathogen identification. For effective disease management, a correct analytical knowledge of the disease host, the prevailing environment, including climate, and other factors such as cultural necessities of the host plants are necessary. The tactics of disease management can be grouped under two major principles. However, the difference between these two principles is often vague. The simplest rule of disease management is prevention and therapy (Tripathy et al. 2020).

4.3.1 Prevention

The very first principle of disease management includes the application of techniques before infection and disease attack. In this way, the plant is ready to cope with the upcoming challenges of severe disease attack. An example of this principle is quarantine law enforcement (Ilyas et al. 2021).

4.3.2 Therapy

The second principle includes the curative functions in which any kind of disease-eradicating measure is applied after the initiation of infection. Chemical treatment, in which chemicals are applied to infected or diseased plants and plant parts, is an example of this principle (Tripathy et al. 2020).

4.3.3 Other Principles

Other principles of disease management include:

Exclusion: It includes the principle of avoidance of pathogen introduction into a new area (Maloy 2005; Pal and Gardener 2006).
Eradication: It includes elimination, eradication, and destruction of the inoculum (Maloy 2005; Pal and Gardener 2006).
Protection: It includes the use of any toxicant or some other disinfectant barriers (Maloy 2005; Pal and Gardener 2006).
Immunization: It includes the principle of resistance or tolerance towards infection (Maloy 2005; Pal and Gardener 2006).

4.3.4 Studies on Cultural Control

Cultural practices have a major role in the management of a wide variety of plant diseases. The purpose of cultural practices is to ensure the provision of favourable environmental circumstances for crop development, which results in excellent plant health, and to limit the establishment of phytopathogens, which reduces disease incidence. Modifications to a variety of cultural techniques are critical for disease control in every crop. Several common practises include the following: selection of crop seasons and safe areas, proper tillage to excavate diseased plant debris, cultivation of crops other than disease hosts, selection of disease-tolerant planting materials, proper crop direction to maximize sun and air exposure, proper irrigation, and nutrition management of crop spacing and population, which helps to promote root growth and prevent injury to the plants (Kumar and Gupta 2020).

Some of the cultural control techniques are listed below.

4.3.4.1 Tillage Techniques

Tillage, or turning maize residues, benefits the next crop by decreasing inoculum survivability. Burying infected trash promotes decay and deprives the fungus of a feeding source. The fungus is unable to thrive in the soil on its own. It can only overwinter on dead corn tissue that remains on or above the soil surface (Ghanney 2017). Disking alone is not adequate to bury contaminated material. While mould board ploughing is effective, it may not be recommended in certain areas because of the increased erosion risk. Erosion may be minimized by ploughing in the autumn and sowing a winter cover crop followed by a no-till maize plantation in the spring. On the other hand, burial of infected material, on the other hand, may not be an efficient method of decreasing grey leaf spot inoculum in areas where conservation tillage is widely used, since the pathogen may be carried into a field by wind from neighbouring fields (Munkvold 2003).

4.3.4.2 Agronomic Practices

Grey leaf spot intensity may be eliminating a field from corn cultivation or rotating to a non-host crop for 1 year. The fungus cannot live in contaminated maize waste for more than one season. Maize is the only crop known to be attacked by this fungus. However, the risk of herbicide carryover may limit the crops included in the rotation plan (Afzal et al. 2020). Another way of reducing disease infestation includes growing maize for silage purpose. This can substantially decrease the quantity of inoculum accessible to infect the next crop (Aglave 2018). To begin, silage corn is often harvested before the severe attack of grey leaf spot blight. This strategy limits the quantity of pathogen available to survive the winter months. Second, when maize is harvested for ensilage, just approximately 6 in. of stalk remains in the ground. This approach generates very minute contaminated material for the fungus to overwinter (Aglave 2018). The timing of planting has an effect on disease incidence. Afzal et al. (2020) discovered that maize plant spacing had a substantial impact on turcicum leaf blight infection. Close spacing resulted in an intense degree of disease. This was also true in the case of grey leaf spot and rust. The motive of this increment was that increasing the space between and between rows lowered both the relative humidity and free moisture on the leaves, thus reducing disease infestation (Afzal et al. 2020).

When maize was intercropped with sweet potato, leaf blight and common rust severity were decreased. Additionally, when haricot bean was planted during intercrop cultivation, intercropping maize with haricot bean decreased the amount of infestation of both diseases (intercrop cultivation) (Afzal et al. 2020). Planting maize and sorghum together revealed that a high sorghum population resulted in greater leaf blight intensity and lower corn rust, while a high maize population resulted in low leaf blight severity (Afzal et al. 2020). The optimum levels of fertilization are known to reduce the incidence of disease. Fertilizer application with NPK (0.96, 0.60, and 0.22 kg, respectively, for each pot of 25 cm diameter) levels reduced the damping off disease in maize, reportedly (El-Demerdash et al. 2017). Contrastingly, NPK levels of 0.77, 0.60, 0.22, and 0.77, 0.75, and 0.15 kg per pot reduced damping off post seedling emergence. Another NPK level of 0.58, 0.75, and 0.075 kg minimized the incidence of root rot in maize seedlings (El-Demerdash et al. 2017).

Different fertilizer application levels of biofertilizers such as farm yard manure have been analysed against root rot and damping off in maize cultivars (El-Demerdash et al. 2017). Varietal screening can prove helpful in reducing pathogen incidence in a respective maize field. At Bako and Kelalbero in western Ethiopia, (Afzal et al. 2020) tested 34 maize accessions for resistance to turcicum leaf blight (TLB). Despite the absence of an immune host, some varieties had a reduced incidence of disease, while others showed a greater incidence (Afzal et al. 2020).

4.3.5 Cultural Control of Various Maize Diseases

Common corn smut is produced by the fungus *Ustilago maydis*, which may persist for many years as dormant spores in soil and maize waste. Spores are dispersed by wind or sometimes by water splashing up onto the new plants. Spores may also be transmitted via the dung of animals who have eaten contaminated maize (Kumar and Gupta 2020). It can be controlled well via conducting a soil test based on fertility. Prevention of mechanical damage during spraying and cultivation is another factor that also helps to overcome the disease infestation. Gall removal is important before the smut boils break and teliospores get discharged (Aglave 2018). Southern corn leaf blight is usually caused by a fungal agent named *Bipolaris maydis*. There are only two strains of this pathogen, "Race O" and "Race T". Race O usually hits and just leaves. The lesions produced by this type are tan-coloured, slightly rectangular in form, and have reddish-brown borders. Race T damages leaves, husks, leaf sheaths, and cobs. The most critical point in the removal of all corn blight strains is the disease and inoculum prevalence. As the *B. maydis* overwinters in leaves and sheath debris, it is quite crucial to remove the old plant debris. Efficient tillage practise helps to break up the soil clods and plant debris. Thus, in this way, all remnants of the past infestation are wiped out. In comparison to minimum tillage, which might leave residue on the soil surface, burying residues by ploughing has been found to minimise the incidence of SCLB. Another way to reduce the spread of SCLB is to rotate crops with crops that are not host plants (Aglave 2018).

Grey leaf spot of corn, generally caused by the fungus named *Cercospora zeae-maydis*, is a perennial and economically very harmful disease in the United States. The disease infestation can be controlled via following the late sowing dates and adaptation of minimum tillage systems. One should also know the previous disease history of the corn fields before shifting towards intensive maize cultivation (Aglave 2018). Downy mildew is generally caused by *Peronosclerospora sorghi*. It is a widely distributed disease of maize throughout many regions of the world. To prevent this disease, there is a recommendation of planting corn away from the low, damp areas where the disease is known to develop. Appropriate ground drainage will decrease inoculum spread via flooding and infection thereafter (Aglave 2018). Destroying maize plant detritus, as well as removing and destroying collateral hosts, aids in disease control. Proper nutrition at right rate may help to control disease infestation (Javed et al. 2022b). Removal of such volunteer host is a safest way towards the cultural control of dwarf mosaic virus. Best control is achieved

when all farmers in a community work together to eradicate Johnson grass. Plant the maize crop early so that the aphid population could not grow faster (Aglave 2018).

Anthracnose is a fungal infection that affects the majority of corn tissues during the plant's growth. The fungus, entitled *Colletotrichum graminicola*, displayed symptoms of necrosis with maize leaf samples exhibiting grey, brownish to black, and oval to elongated lesions. Grain yield losses due to anthracnose are estimated to range from 0% to 40%, depending on the hybrid, climate, infection timing, and other stressors (Kumar and Gupta 2020). Tillage can minimise the danger of incorporating the waste into the soil and the effects of a breakdown. At least 1 year of rotation to non-corn crops may reduce anthracnose early in the season, but it does not affect the disease late in the season. Another strategy is crop scouting. Examine maize at regular intervals of 2 weeks until the dough stage is achieved (Aglave 2018). Non-grass crop rotation, for example, the rotation of maize to legumes, can help to lower the spread of disease inoculum. Soil sanitation is another remedy for disease prevention. Weed management, selection of well-drained soils, and high fertility gradients with optimum soil pH also reduce the inoculum spread (Aglave 2018). In maize, younger leaf tissues are more prone to fungal attack then mature leaves. Delayed disease progression in mature crops reduces the risk of productivity loss. Therefore, some farmers prevent disease or limit its impacts in places where rusts are a more constant concern. They lower the disease risks by not sowing late or utilizing short-term hybrids. In doing so, the rust spores couldn't enter the field and occasionally disease may be entirely prevented (Aglave 2018). Maize eyespot (*Kabatiella zeae*) has decreased the maize production up to 9% in the areas where maize is cultivated on a large scale. Aglave (2018) proposed the crop rotation to minimize the infection caused by *K. zeae*, a proper crop rotation and the thorough ploughing and removal of after-harvest leftovers, particularly from severely affected plants. It can also minimize the amount of infectious material. Deep burial of plant wastes reduces sporulation and promotes breakdown of the spores which limits the early spread of diseases (Aglave 2018). Corn cultivated for one growing season followed by tillage operations is recommended to cut back the disease development in the subsequent corn crops. Crop rotation for two growing seasons is considered more accurate to prevent the amount of diseases inoculum (Aglave 2018).

4.3.6 Biological Control

The terms "biological control" and its shortened form "biocontrol" have been used in several fields of biology, most notably entomology and plant pathology (Degani and Dor 2021). It is a phrase used in entomology to describe the method of reducing populations of specific pest insects by the use of live predatory insects, entomopathogenic nematodes, or microbial illnesses. A biological control agent is an organism that inhibits a pest or illness (BCA) (Bressan 2003). In a broader term, the phrase "biological control" refers to the utilization of natural materials extracted or fermented from a variety of sources. These formulations may be relatively basic combinations of natural substances with particular activities, or they may be

complicated combinations having numerous effects on both the host and the target pest or disease. Additionally, although non-living inputs may imitate the actions of live creatures, they are more appropriately referred to as bio-pesticides or bio-fertilizers, depending on the main benefit given to the host plant (Pal and Gardener 2006).

4.3.6.1 Biocontrol of Seed-Borne Fungi Via *Actinomycetes*

Bressan (2003) reported two *Streptomyces* strains that were evaluated for their ability to suppress pathogenic fungus in stored maize grain. Seeds were disinfected and inoculated with *Streptomyces* strains secluded from maize rhizospheres. The *Actinomycete* inoculum was composed of filtered suspensions and complete suspensions of *Streptomyces* spp. strains biomass. *Streptomyces* strains solely inhibited the growth of *Drechslera maydis*, *Curvularia lunata*, and other *Aspergillus* spp. and substantially decreased the exposure of *Cephalosporium acremonium* and *Fusarium subglutinans*. In the development of techniques of inoculations, only non-disinfested seed conjugated with filtered suspension did not constrain the *Penicillium* development. However, inoculation of maize seeds in a complete suspension of strains was the most efficient method of reducing the fungus prevalence. On the other hand, the combination of seed disinfection and inoculation with complete suspension of strains was an excellent approach towards inhibiting the growth of the *Diplodia maydis.* However, the strain DAUFPE 11470 was most efficient in controlling fungi harmful to seeds. The treatment with this strain, however, restricted root and shoot growth (Bressan 2003).

4.3.6.2 Biocontrol of Southern Corn Leaf Blight (SCLB) Via *Trichoderma* Species

Southern corn leaf blight is caused by *Cochliobolus heterostrophus*, and it is among the major global productivity threats to maize crop. Synergic applications of low-toxicity chemical fungicides and bio-control agents may increase the stability and effectiveness of biocontrol agents against plant diseases, thus reducing the need for chemical fungicides (Wang et al. 2015). *Trichoderma* is a popular biocontrol fungus and has been employed successfully to control a variety of foliar diseases. However, few studies on the synergistic use of chemical fungicides and *Trichoderma* have been published. Wang et al. (2019a, b) determined the control impact of combining *Trichoderma harzianum SH2303* and difenoconazole-propiconazole (DP) against SCLB. The results indicated that when DP and *SH2303* were applied synergistically, the leaf spot area was decreased in comparison to the control. The synergistic use of DP + *SH2303* against SCLB was shown to be effective for 15–20 days in pot trials conducted under greenhouse conditions. The treatment increased the production of defence-related enzymes such as phenylalanine ammonia lyase (PAL), catalase (CAT), and superoxide dismutase (SOD) and the expression of *PR1* genes (Wang et al. 2019a, b). Wang et al. (2015) also determined the levels of RNA expression for PAL, SOD, and CAT. It has been reported that the RNA expressions were increased, which correlated to the enzyme activity simultaneously. *Trichoderma SG3403* generated more evident enzyme

activities and related gene expression than pathogen alone, implying that *T. atroviride SG3403* promoted corn defence gene expression against pathogen infection. Thus, an induced resistance mechanism may have been implicated via *T. atroviride SG3403* against SCLB (Wang et al. 2015).

4.3.6.3 *Bacillus* Species as a Biocontrol Agent Against *Fusarium*

Fusarium verticillioides causes rotting of stalk, ear, and root in maize. It has a significant effect on crop output in tropical and subtropical territories. The team of researchers isolated *Firmicutes* and *Proteobacteria* from rhizosphere samples which were collected from infected spots of the maize plants either symptomatic or asymptomatic. The whole collection was tested for potential action against *Fusarium verticillioides*. The researchers used a liquid antagonism assay by preparing a dual culture in solid medium. It resulted in the identification of 42 bacterial species (*Bacillus*, *Pseudomonas*, and *Paenibacillus*) that suppress *Fusarium verticillioides* growth (less than 45%). However, further assays revealed that three *Bacillus* isolates had the maximum antagonistic activity against *Fusarium verticillioides*. It includes *Bacillus megaterium* (*B5*), *Bacillus cereus* sensu *lato* (*B25*), and *Bacillus* sp. (*B35*).

4.3.6.4 Use of Biopesticides Against Maize Disease

Biopesticides are naturally occurring chemicals or agents derived from animals, plants, and microorganisms such as bacteria, cyanobacteria, and algae. These biochemical agents are used to manage agricultural pests and diseases. The US Environmental Protection Agency defines biopesticides as substances originating from natural sources such as animals, plants, microorganisms, and some minerals. These biocontrol agents or products, such as genes and metabolites, may be utilized to avoid crop damage. Biopesticides are much more beneficial than their chemical equivalents, since they are environmentally benign and host specific. Biopesticides can significantly enhance the usage and use of agro-based chemicals in the agricultural industry to protect crops against invasions of infectious pests.

4.3.7 Chemical Control

The utilization of agrochemicals and pesticides, in particular, is common in agriculture to control a variety of pests and diseases of crops. Fungicides and bactericides are chemicals that play a critical role in a wide variety of disease control strategies. Foliar applications usually protect plants from diseases that harm growing plants. These sprays may be used as a preventative or curative measure. These compounds may be either surface protectants or systemic in nature, depending on their mode of action. Protectant pesticides work prophylactically, forming a protective barrier on the seed or plant surface that inhibits pathogen development. Systemic insecticides penetrate the plant tissues and destroy pathogens that have already developed. Fungicides have been used to treat a variety of diseases for over a century, and the process of developing new fungicide formulations is ongoing. The Bordeaux combination was the first extensively used fungicide; it is a copper sulphate fungicide

that is being used in different forms today. The early classes of inorganic fungicides were developed from simple components like sulphur, metallic mercury, or copper complexes. Organic fungicides introduced in the 1950s, such as captan, thiram, and carbamates, are protectant or contact fungicides and very effective against a variety of fungal diseases (Kumar and Gupta 2020).

Usually chemicals are applied in two different ways.

4.3.7.1 Spraying

According to Afzal et al. (2020), a combination treatment of mancozeb and propiconazole at a dosage of 2.0 kg per hectare of maize (two to three times at 10-day intervals) effectively controlled the two diseases, turcicum leaf blight and common rust. It has been reported that, in general, fungicide treatment is not cost efficient for small-scale farmers in Ethiopia. It may, however, be lucrative for hybrid seed producers (Afzal et al. 2020).

4.3.7.2 Chemical Treatment of Seeds

Maize kernel rot infections can cause serious injury to the grain after 3 months of storage during the year's dry and warm season. Research done at the Bako Research Center determined that the use of a chemical named Luxan TMTD contributed to the least amount of kernel rot destruction (9.16%) (Afzal et al. 2020).

4.3.7.3 Chemical Control for Various Maize Diseases

Numerous fungicides authorized for use on maize are efficient in controlling rust diseases when applied properly. However, fungicide applications usually cost between $15 and $20 per acre (including the $5 per acre cost of application), making them often uneconomical. When corn prices are low, fungicide treatments provide economic benefits when applied after infection to a sensitive hybrid with a high yield potential, such as seed, white, or popcorn. Analyse weather predictions prior to application to evaluate whether the weather conditions are friendly, especially high humidity and warm temperatures. Due to the frequency of rust formation, it has been unable to generate accurate treatment threshold data for local circumstances. Other fungicide experiments for controlling corn rust usually show that they work best on seed corn or sweet corn.

Globally, the most destructive fungal systemic disease of maize is common rust, induced by *Puccinia sorghi*. It has been observed that common rust infections may significantly decrease grain yields by up to 40% in many genotypes of maize. Foliar disease management in maize is frequently described as an inappropriate pesticide application or an over-reliance on host-plant resistance. IDM has demonstrated conclusively that when low concentrations of host-plant tolerance are combined with field intervention and low chemical management, expected yields and economic returns are larger than when sensitive genotypes are chemically managed. studied local agronomic practices. Tebuconazole was significantly superior and very successful in decreasing disease rigorousness (19.74%) and increasing yield at 35 and 50 days after sowing at 0.1% foliar treatment. A 0.1% foliar spray of Hexaconazole (35 and 50 days after planting) was the next most effective treatment

(28.23%), followed by a 0.1% foliar spray of Tebuconazole and Neemazole F (5%) at 50 days after sowing.

4.3.7.3.1 Chemical Control for the Downy Mildew of Maize

On maize leaves, downy mildew (*P. sorghi*) is identified by elongated chlorotic streaks with a downy development of conidia and conidiophores. 3–6 days after infection, symptoms manifest as light yellow to white discolorations on the leaf blade. Tassels and ears may be distorted. Metalaxyl (Apron 35SD) seed treatment at a rate of 1.75–2.00 g/kg significantly decreases the disease incidence. Spray the maize crop 3–4 times with Metalaxyl MZ (Ridomil MZ) at 0.2% beginning on the 20th day after planting to suppress the disease (Javed et al. 2022a). Spraying Dithane (M 45) at 0.25% concentration or any other copper fungicide at 0.3% concentration is also effective. The first spraying should be done as soon as the disease symptoms start to show up, followed by two to three more times, depending on how bad the condition is (Javed et al. 2022a).

4.3.7.3.2 Chemical Control of Corn Eyespot

In 2015, corn eyespot caused an annual loss of six million bushels in Ontario and United States (Mueller et al. 2016). Early application of fungicide sprays may have a major effect on disease and production. Fungicides may be financially beneficial, particularly in the seed corn industry. Fungicides should be considered only in fields where maize was damaged by eyespot the previous year and decreased tillage techniques are being utilized. Hybrids that are resistant should be the primary option. Mancozeb, propiconazole, chlorothalonil, and benomyl are all fungicides licensed for use against *K. zeae*. Seed treatments are suggested for efficient protection against *K. zeae*, followed by spraying the crop during the early stages of disease development, when 1% or less of leaf area is affected. Multiple applications may be required when disease circumstances are favourable. Except in seed-producing areas, the use of fungicides against eyespot may be prohibitively costly. Pest management is critical in decreasing the incidence of eyespot, especially reduction of *Aphididae* and *Thysanoptera*, which feed on maize and may promote conidia penetration (Aglave 2018).

4.3.7.3.3 Chemical Control for Grey Leaf Spot of Corn

Grey leaf spot of maize is a foliar disease usually spread by *Cercospora zeae-maydis*. In the 1990s, reported yield losses due to grey leaf spot are as high as 50% in some US maize fields (Liu and Xu 2013). Currently, five fungicides are available to treat maize grey leaf spot: EC Headline (active ingredient, pyraclostrobin), Quilt (azoxystrobin + propiconazole), Proline 480 SC (active ingredient, prothioconazole), Tilt 250 E, and Bumper-418 (active ingredient, propiconazole) (Aglave 2018).

4.3.7.3.4 Chemical Control of Northern Corn Leaf Blight (NCLB)

Setosphaeria turcica is the pathogen that causes this disease. Northern leaf blight lesions may coexist with other pathogenic diseases on the same or distinct leaves of

the plants and is identified by greenish tan lesions on leaves. The disease lowered plant height by 8%, grain yield by 43%, biomass by 43%, and 1000 grain weight by 25% (Subedi 2015). Fungicide treatments are advised exclusively for farms producing fresh market sweet corn and hybrid seed. Spraying should begin as soon as the first lesions develop on the leaves below the maize ear. Numerous fungicides are currently available for NCLB control on maize (Subedi 2015). Fungicides that are effective against NCLB include (strobilurins, such as Quadris and Headline FRAC) (code provided by Fungicide Resistance Action Committee) group 11 and FRAC group 3 (triazoles, e.g. Tilt). Additionally, there are a few items that include both FRAC groups (11 + 3, e.g. Quilt and Stratego) (Aglave 2018). When signs of NCLB are first detected in the field, rotate these FRAC codes and mix with a broad-spectrum protectant against resistance. Because PHIs differ across each product, therefore, it is critical to check labels carefully when a crop reaches harvest maturity. Additionally, depending on the brand, NCLB may be referred to as *Helminthosporium* leaf blight, a term used to refer to both NCLB and Southern corn leaf blight together (Aglave 2018).

4.3.7.3.5 Chemical Control of Stewart Bacterial Wilt

The pathogen that induces this sickness is *Erwinia stewartii. Erwinia stewartii's* overwintering host and vector are corn flea beetles. Based on the cultivar's tolerance or sensitivity, chlorotic or necrotic tissues may stretch the entire expanse of the leaves or may be restricted to a few centimetres. Stewart's wilt might induce the weakened plant to stem rot, resulting in decreased yield. Stewart's wilt affects the output of corn by roughly 0.8% per each 1% of seedlings impacted systematically (Pataky 2003). Control flea beetles using pesticides, especially on vulnerable types during the seedling stage. While this is not as much successful as resistant types, it can mitigate losses in areas where the growers cultivate susceptible hybrids of corn. Gaucho seed treatments suppress corn flea beetles systemically and mitigate the intensity of Stewart's wilt. Numerous pesticides are available under different brand names for use as foliar sprays to combat maize flea beetles. While some of these products last longer than others, the fast development of leaf tissue implies that untreated surfaces are accessible to flea beetles that move into fields prior to treatment. Stewart's wilt can be controlled with flea beetle scouting (two or three times a week) and re-applying pesticides if populations start to grow back (Aglave 2018).

4.3.7.3.6 Chemical Control for Corn Smut

Maize diseases, such as common smut, are caused by *Ustilago zeae*, while other types of smut such as head smut are spread by *Sphacelotheca reiliana*. There are few management options for these pathogenic fungi, but in other countries where maize is grown, seed treatment and fungicides applied during the vegetative stage are employed to prevent the incidence of maize smuts (Korbas 2006). Corn breeders usually avoid utilizing highly disruptive inbred lines and their hybrids or variations. Corn may be protected against insects (e.g. corn earworms and European corn borers) by using pesticides as suggested by entomologists on a timely basis. This

often results in a reduction in the prevalence of common smut in sweet corn (Aglave 2018).

4.3.7.3.7 Chemical Protection Against Maize Late Wilt

Late wilt is a peculiar disease that is wreaking havoc on maize fields across Israel. Its symptom includes the fast withering of maize plants prior to tasseling and until just before maturity. The fungus *Harpophora maydis* is the disease's causative agent. *Harpophora maydis* is a soil and seed-borne pathogen that is presently managed via the use of decreased sensitivity in maize varieties. In previous research, it has been demonstrated that injecting azoxystrobin (AS) into a drip irrigation line allocated to each specific row may effectively inhibit *H. maydis* in the field. Additionally, seed coating with AS can also offer an extra layer of protection. Another more cost-effective protective treatment employing this fungicide is in a combination with Difenoconazole mixture (AS + DC), or Fluazinam, or Fluopyram and Trifloxystrobin mixture, or Prothioconazole and Tebuconazole mixture via seed coatings or drip irrigation (Javed et al. 2022b).

4.3.7.3.8 Chemical Control for Southern Corn Blight (SCLB)

This disease caused by *Helminthosporium maydis* is the most common disease that usually appears at the time of tasseling. Southern corn leaf blight occurs worldwide and is important in regions of warm damp climate of 20–30 °C temperature. Yield reduction of up to 50% was recorded (Subedi 2015). Fungicides applied to the leaves may be utilized. Foliar disease management is essential between 14 and 21 days during tasseling; this is the most vulnerable period for leaf blight damage. Fungicides should be administered to plants infected with SCLB immediately upon the appearance of lesions. Re-applications may be required throughout the growth season, depending on the environmental circumstances. Headline, Quadris, Quilt, PropiMax EC, Stratego, and Tilt are all common fungicides (Aglave 2018).

4.3.7.4 Biotechnological Measures

Biotechnological techniques have been shown to be effective against some crop-disease combinations; they are generally underutilized. Thus, there are just three well-publicized instances of crops utilized on a worldwide scale that show beneficially increased disease resistance. Biotechnological methods include the use of the BT toxin Cry1Ab from the *Bacillus thuringiensis* bacterium to impart insect resistance in maize plants (*Zea mays*). Despite their insect resistance, these transgenic cultivars show some resistance to *Fusarium* spp., particularly *Fusarium verticillioides*. Several more transgenic crops have been created and field-tested. In certain instances, they are also authorized for commercialization; for example, potatoes are resistant to viruses (Collinge 2018).

4.3.7.4.1 Advances in Genetic Engineering Against Maize Diseases

RNA Interference Is Being Used to Combat Maize Pathogens

Plants have developed a complex defensive system against microbial diseases. For instance, pre-existing biotic barriers, as well as their reinforcements, prevent pathogens from getting access to the interior of the cell. Immune receptors in the plasma membrane and intracellular compartment trigger defensive retorts in response to pathogen detection, both directly and physically engaging with pathogen-derived immunogens or indirectly by governing pathogen-induced changes to host targets. Antimicrobial peptides and other chemicals produced by plants may reduce pathogenicity either directly or indirectly by inhibiting the action of virulence factors. In addition, plants use RNA interference (RNAi) to find and destroy viruses that come into their bodies (Rosa et al. 2018).

Pathogen's Counterstrategies Against Plants' Defence Mechanism

Some virulent strains have developed ways to circumvent their plant hosts' defensive mechanisms. For example, many bacterial and fungal diseases generate and release enzymes that degrade cell walls. Additionally, pathogens may transport effectors into the host cytoplasm, some of which inhibit host defence and increase vulnerability. Almost all plant viruses generate viral suppressors of RNAi in order to combat plant RNAi-based defence mechanisms. Additionally, some viruses use the host RNAi system to silence host genes, thus increasing their virulence (Rosa et al. 2018).

Targeting Genes Against Mycotoxins Produced by Fungi

Aflatoxins are poisonous secondary metabolites generated by some *Aspergillus* species and are a global agricultural economic and public health concern. Despite decades of management attempts, aflatoxin contamination results in an annual worldwide agricultural loss of millions of tonnes (Thakare et al. 2017). Some counts of host-microbe interactions offer possibilities for gene targeting against diseases. For example, plants may be genetically modified to express genes that encode proteins capable of digesting mycotoxins or suppressing the activity of cell-wall disintegrating enzymes. Additionally, there are possible genetic modifications in plants that can manufacture and release antimicrobial peptides or other chemicals to prevent microbial colonization directly. By targeting viral RNA, the RNAi machinery may be used to impart strong viral protection (Rosa et al. 2018). Individual or combined immunological receptors that detect several strains of a pathogen may be introduced for strong, broad-spectrum disease tolerance. Essential regulatory genes of the defence hub can be re-altered to fine-tune defensive responses. Susceptible host targets may be transgened in order to avoid pathogen introduction and manipulation. This also applies to host decoy proteins, which act as a "trap" for infectious pathogens. They can be genetically changed to make them more specific for pathogen detection (Dong and Ronald 2019).

Use of Host-Induced Gene Silencing (HIGS) in Maize

Thakare et al. (2017) demonstrated that host-induced gene silencing is a very efficient approach for removing aflatoxins from transgenic maize. The researchers modified maize plants with an RNA interference (RNAi) gene cassette specific for the *aflC* gene. This gene is responsible for encoding an enzyme involved in the aflatoxin manufacturing pathway. Aflatoxins were not found in kernels from these RNAi transgenic maize plants after pathogen infection. Contemporarily, the toxin burdenized millions of non-transgene kernels. This technique in maize involves the double-stranded RNA expressions to silence genes responsible for aflatoxin production. Nowadays, HIGS have also been used to suppress genes specific for hosting nematode feedings and fungi attacks (Thakare et al. 2017).

Use of CRISPR-Cas Against Maize Lethal Necrosis (MLN)

Some genetic engineering techniques may be more beneficial for developing MLN-resistant maize variants. Genetically modified virus resistance conceived by sequential expression of virulence exploits the plant's inherent ability to induce RNAi against viral sequences. The recent discovery of CRISPR-Cas for maize enables the alteration of maize gene alleles to confer viral resistance on lines and hybrids that lack non-maize regions in their genomes. Alternatively, CRISPR-Cas may be used to build viral resistance in maize through RNAi engineering (Redinbaugh and Stewart 2018).

Use of Quantitative Polymerase Chain Reactions (qPCR) to Identify Resistant Genes

Miranda et al. (2017) investigated the global gene expression alterations in maize. Researchers studied the male and female inflorescences after local and systemic fungal infection treatments. To identify genes involved in plant defence against fungi such as *Colletotrichum graminicola*, RNA sequences were combined with qPCR. The sequences revealed that the systemic acquired (SA) resistance in female inflorescences is primarily mediated by the increase of SA-inducible defensive genes (*ZmNAC*, *ZmHSF*, *ZmWRKY*, *ZmbZIP*, and *PR1*) and candidate genes for chromatin modifications. Moreover, transcripts implicated in the jasmonic acid and ethylene signalling were collected, which later on suggested that these may have contributed in further immunization. Additionally, many genes were functionally used to annotate the domain signatures, suggesting new possibilities for testing the techniques of gene deletion and overexpression in maize plants (Miranda et al. 2017) (Fig. 4.1).

Resistance Breeding Against Various Maize Diseases

The apparent resistance varies among different maize variants and hybrids. The greatest method of controlling common smut is to choose the most suited, resistant hybrids and cultivars available. These hybrids are resistant to the corn-smut fungus in general or in the field. The apparent resistance differs in between corn lines is often due to the sheath and husks providing protection. The management of eyespot of corn requires resistance to *K. zeae*, and disease resistant hybrids should be planted. Aglave (2018) reported that Julia, Heros, Agio, and Aura are susceptible hybrids; Kosmo and Elsa are more resistant hybrids. Even a well-known source of

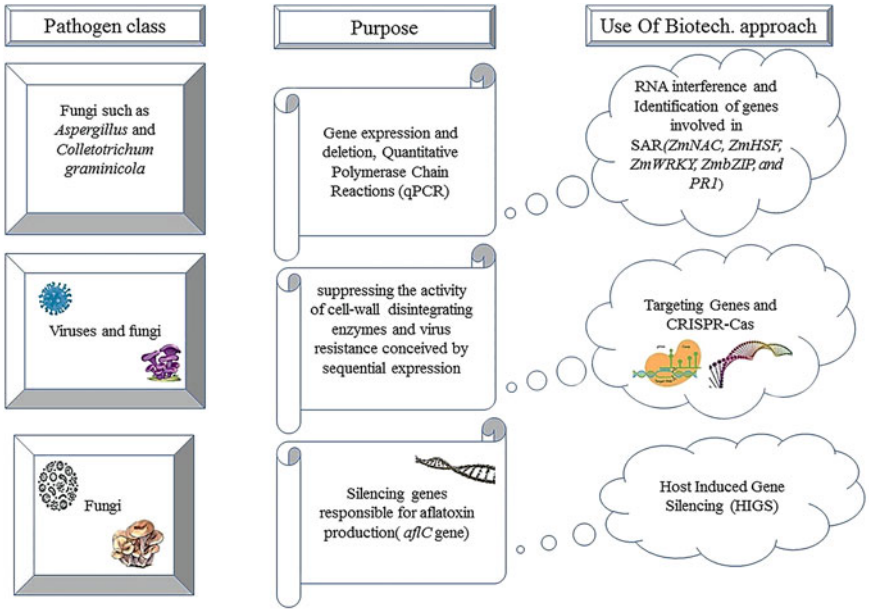

Fig. 4.1 Use of different biological approaches against maize specific pathogens

resistance, such as the Oh43 line, may get infected in the event of epiphytosis (Aglave 2018). The most cost-effective way of reducing production losses due to maize grey leaf spot is the introduction of resistant crop variants. These crops may eventually grow in areas where leaf spot develops while remaining resistant to the diseases although the disease is not eradicated and resistant cultivars exhibit symptoms. The disease is less efficient at decreasing crop output at the end of the growing season. Aglave (2018) reported a corn variety resistant to grey leaf spot entitled SC 407. If the incidence of grey leaf spot is significant, this variety may need fungicide treatment to reach its full potential (Aglave 2018). Host resistance is a useful strategy for controlling NCLB, particularly in sweet corn crops. Through conventional breeding, several kinds of resistance genes have been introduced into sweet corn hybrids (not GMOs). Hybrids may exhibit polygenic or partial resistance, which offers resistance to both pathogen races but is not utterly against either any race. Monogenic resistance hybrids used to impart resistance to just particular pathogen races. These diverse resistance hybrids with various genes will serve to restrict the size, quantity, and amount of sporulation inside each lesion. Due to the presence of resistance genes in a hybrid, the size, form, and colour of lesions may vary. For example, hybrids with one of the monogenic resistance genes *Ht1*, *Ht2*, or *Ht3* would have chlorotic lesions but will have restricted sporulation, preventing the disease from spreading rapidly. Some seed firms employ a numerical rating scale to indicate the degree of resistance, but pay careful attention to these scales. Different

companies use various numbers to represent the amount of resistance. Producers should seek for hybrids with race-specific resistance genes (known as Ht genes) in regions where NCLB is a persistent issue (Aglave 2018). Farmers should grow wilt-resistant sweet corn types that are well-adapted to growing conditions. At the moment, there are only a few early maturing hybrids with high levels of Stewart's wilt resistance. Resistance enhanced hybrids can withstand more infection with less yield loss. Resistance inhibits the bacteria's mobility inside the plant (Aglave 2018).

The most effective method of managing SCLB is to breed for host resistance. Single gene and polygene resistance sources have been identified. Normal cytoplasmic maize is resistant to both Race T and Race C, which explains why Race O is more prevalent. Although flecking may be seen in certain resistant hybrids, it is a response to resistance and will not result in significant economic loss. The disease may be controlled via breeding hybrids that are MDMV-tolerant or MDMV-resistant. In dent corn, there is a high level of tolerance and resistance to strain A, but only a fair level of tolerance and almost no resistance to strain B. There is a lack of resistance to the maize chlorotic dwarf virus and only a moderate level of tolerance (Aglave 2018). There is no significant control method against crazy top that can be recommended. Very little is known about the degree of resistance to this disease in corn hybrids (Aglave 2018). At the moment, the majority of popular sweet maize hybrids are susceptible to rust. However, rust-resistant varieties are available. Commercial breeders of sweet maize use two kinds of resistant varieties: race-specific and partial resistance. If maize farmers want to plant their crops later, they should choose resistant or moderately resistant cultivars because there will be more fungal spores in the air because of the early planting (Aglave 2018).

4.4 Conclusion and Future Prospects

Corn diseases cause costly crop losses every year through problems with germination and establishment of a stand and through damaging effects on the quality and size of the harvest. This chapter is designed to help in the identification of diseases and present management strategies. Several methods, from physical to chemical control of different maize diseases, have been employed to achieve higher productivity with minimal losses. However, each method has some pros and cons. Therefore, integrated pest management (IPM) techniques in combination with modern biotechnological approaches could be helpful for sustainable management of different corn disease-causing pathogens.

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Viral Diseases of Maize

5

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Abstract

Maize is a widely cultivated grain that is a staple food in many countries, including the United States, Africa, and other areas of the world. After wheat and rice, maize is the world's third-largest crop, and it is cultivated in more countries than any other. Maize is consumed as a major food source in many regions of the world, with more maize production than that of wheat and rice. In tropical, subtropical, and temperate climates, maize is farmed on almost 177 million hectares. Global production is at 875 million metric tonnes per year, which is more than wheat or rice. It is grown practically everywhere on the planet, except for Antarctica. The availability of high-quality nutrients provides the health benefits of maize. Virus infections are common in maize-growing locations around the world, and they can result in significant losses for farmers. Maize has been linked to the spread of more than 50 viruses. Maize streak virus (MSV), maize stripe virus, and maize mosaic virus are the three primary tropical maize viruses. Virus infection is usually first identified by signs, including stripes, mosaics, and chlorosis. Plant viruses are responsible for a significant share of agricultural illnesses and economic losses globally, with annual crop losses exceeding USD 60 billion. In 2012, annual maize losses are expected to be 3%, or around USD 8 billion. The global maize crop is suffering from a high prevalence of virus infections, and losses might be significantly larger locally or regionally. This chapter deals with maize virus infections as well as the microorganisms that cause them. The factors that influence disease spread by viruses and their management will be investigated, as well as our present knowledge of the genetics of viral resistance in this essential crop.

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Maize streak virus · Maize dwarf mosaic virus · Maize stripe virus · Maize lethal necrosis · Control · Symptoms

5.1 Introduction

Maize (*Zea mays* L.) is the third most important annual crop worldwide and has been ingrained in modern culture. It is used as a prime meal and animal feed by over 4.5 billion people worldwide (FAO 2016). It is also the most significant grain in sub-Saharan Africa, covering a larger area than other grains (Gent et al. 2011). In a wide range of conditions, it is commonly produced for food and income. Of the entire area planted, 7.85 million tonnes of maize were harvested during 2016/2017. The most cost-effective approach to gaining basic calories is to eat the crop. It accounts for 17% of total calorie consumption per capita. Maize also has the advantage of being a less expensive protein source (Rashid 2011). With all its economic and nutritional importance, in Pakistan its annual average is only 3.6 t ha^{-1} which is too less than global average yield of 5.6 t ha^{-1} (FAO 2015).

Plant viruses cause diseases and huge economic losses around the globe (Gómez et al. 2009; Jahn et al. 2005), with annual crop losses exceeding $60 billion USD. Annual losses in maize were estimated by Oerke and Dehne (2004) to be 3%, or over $8 billion USD in 2012. Because virus infections affect the worldwide maize crop in sporadic fashion, losses can be significantly higher locally or regionally. Several viruses have been discovered to infect maize, and their consequences can be damaging. There have been more than 32 viruses found in maize across the world (Damsteegt 1981). Maize streak virus (MSV; genus *Mastrevirus* in the family Geminiviridae), maize dwarf mosaic virus (MDMV), all belonging to the genus *Potyvirus* in the family *Potyviridae*, and maize stripe virus (MSV) were previously identified in the country (Mesfin et al. 1991; Lencho et al. 1997). Maize has been shown to be infected with more than 50 viruses (Lapierre and Signoret 2004). Sorghum mosaic virus (SrMV) (Shukla et al. 1998), Johnsongrass mosaic virus (JGMV) (Seifers et al. 2000), Maize chlorotic mottle virus (MCMV), and Maize chlorotic dwarf virus (MCDV) are a few examples. Maize dwarf mosaic virus (MDMV) is the most important disease-causing agent in crops worldwide, with maize dwarf mosaic disease (MDM) mostly occurring in Africa, the United States, Asia, and Europe (Table 5.1).

The prevalence and spread of the four maize viral illnesses described above (Table 5.2), as well as virus identification methodologies, were discussed in this chapter to offer a foundation for reliable virus identification and diagnosis in corn production.

Table 5.1 Describe the common symptoms, host range, and mode of transmission of viral diseases of maize

Diseases	Host range	Symptoms	Transmission
Maize streak virus	MSV is generally recognized as being endemic throughout sub-Saharan Africa (Rossel and Thottappilly 1985)	Chlorotic streaks in veins of leaf are common symptoms of this disease (Dhau et al. 2018)	Transmitted and spread by leafhopper (Shepherd et al. 2010)
Maize dwarf mosaic virus	Dwarf mosaic virus of maize is found in sugar cane, sorghum, maize, *Eleusine* spp., *Panicum* spp., and *Setaria* spp. (Redinbaugh and Stewart 2018)	Chlorotic mosaics, mottles, or streaks on the green tissues of infected plants In open-pollinated maize populations, the severity of disease symptoms can vary greatly (Jones 2021)	*Schizaphis graminum* and *Aphis craccivora* are among the aphid genera that disseminate maize dwarf mosaic virus in a non-unrelenting manner (Ghosh et al. 2017)
Maize stripe virus	Its host and geographical distributions are effectively described by vector	At start the symptoms are fine chlorotic lesions between leaf veins, followed by chlorotic stripes of varying severity and width (Falk and Tsai 1998)	Maize stripe virus is disseminated in a persistent-propagative way by the maize delphacid Peregrinus maidis
Maize lethal necrosis	The *Poaceae* family is the only experimental host, with maize being the most prevalent natural host. *Zea mays* var. *parviglumis* and *Zea luxurians* were also infected by Kansas serotype 1 (Gordon and Thottappilly 1978)	Causes a wide spectrum of symptoms in maize. Stunting, leaf necrosis, premature plant mortality, shortened male inflorescences with few spikes, and/or shortened, deformed, partially filled ears are among the symptoms	A broader panel of possible carriers was investigated in the United States, and it was discovered that some beetles can spread MLN at both larval and adult stages, but some aphid and leafhopper species, as well as a mite, are virus-free

Table 5.2 Maize important viruses which transmitted with different vectors and cause the yield losses

Virus	Host	Vector
Maize Streak Virus	Maize	Leafhopper
Maize dwarf mosaic virus	Maize	Aphid
Maize stripe virus	Maize	*Peregrinus maidis*
Maize lethal necrosis	Maize	Aphid and leafhopper

5.2 Maize Streak Virus (MSV)

A 100 years ago, maize streak was known as the most crucial viral disease of maize worldwide. MSV is a dangerous virus and is one of the most widespread viruses. Maize yield is declining, putting food security at risk (Mbong et al. 2021). This

disease was discovered in 1901, and its symptoms were known as "mealie variegation" before being called "maize streak" (Fuller 1901).

The maize streak virus (MSV) belongs to the family *Geminiviridae*, which is a disease-causing agent and a well-known member of the genus *Mastrevirus*, which is the major cause of the maize streak virus sickness (MSVD). With a single component of a circular, single-stranded DNA genome of around 2700–2800 nucleotides contained in germination particles, the virus is continually transmitted by migratory leafhoppers of the genus *Cicadulina* (Family Cicadellidae, Order Hemiptera) (Shepherd et al. 2010).

5.2.1 Transmission

Its transmission and spread by virus were revealed for the first time by a renowned scientist, H.H. Storey, and transmitted by leafhopper (Shepherd et al. 2010). The transmission cycle, latent period, and sensitivity of different host plants to the same viruses were all interpreted by Storey and his colleagues (Dhau et al. 2018). For more than 10 years (Mesfin et al. 1992; Bosque-Pérez 2000), MSV has been intensively examined as the most important virus affecting corn yield in sub-Saharan Africa (Mesfin et al. 1992) MSV's geographic distribution (Alegbejo et al. 2002), diversity, genomics and strain levels, also its host plant range, virus/vector ecology and epidemiology in key African regions (Bosque-Pérez 2000; Magenya et al. 2009; Martin and Shepherd 2009), the biology of its vector, which causes diseases and its connection with MSV, and efforts at virus resistance breeding (Welz et al. 1998).

5.2.2 Symptoms

- Chlorotic streaks in veins of leaf are common symptoms of this disease (Dhau et al. 2018).
- These disease symptoms appear on infected maize plants as small, pale, circular dots on the youngest leaves' lowest clear region. Only young infected leaves show symptoms, and older leaves are uninfected.
- As the disease proceeds, fresher leaves acquire streaks in the leaf veins that can be a few centimeters long, with tertiary veins being more affected than principal veins.
- Streaks or lesions move latterly, forming narrow, broken chlorotic stripes that can stretch the length of badly damaged leaves.
- Few viral strains cause red pigmentation on maize leaves, while some others cause white to yellow lesions (Jacquat et al. 2020).

5.2.3 Control

It can be controlled by preventing maize plants from being close to the oldest grains and using crop cycles that reduce leafhopper invading (Gichuru 2014). The vector can be suppressed using systemic pesticides sprayed on the planting furrow during maize planting. On the other hand, chemical seed treatments might be harmful if not used correctly because they provide only minimal protection during intense stress. The most efficient and cost-effective approach to preventing streak outbreaks is the creation and application of streak-resistant cultivars (Emeraghi et al. 2021). According to recent investigations, the strain of MSV (MSV-A), which is tolerated by maize and causes more MSD, is causing diseases more quickly across Africa than those less adapted variants, which are the cause of diseases in grasses (Varsani et al. 2008). Although the enhanced mobility might be due to the maize-adapted strain's wider host range than its grass-adapted siblings, as MSD is only transmitted through humans, that's why humans' migration with infected material from one place to the other is blamed. African governments are looking to control the flow of maize leaf material and insects across various nations in the coming future.

Whatever more ways are adopted to control MSD, it is likely that the disease will continue to spread and be the reason for losses until a major portion of African farmers have easy access to commercially acceptable MSD immune maize varieties. Now we have no such types, but it is more likely that we will have them in the future by adopting modern technologies. As with many other problems faced by the world's poorest people, it appears that the more financial assistance, scientific efforts, and state leadership required to apparently address the MSD problem are all in short supply. We won't know how harshly to judge our inability to control MSD until we know the true costs of the illness.

5.3 Dwarf Mosaic Virus of Maize (MDMV)

MDMV is a *Potyvirus* viral with a single-trapped, positive-sense RNA genome. Maize, sorghum, and Johnson grass are among the plants infected with this virus (Kannan et al. 2018). MDMV is a significant maize pathogen that has been spreading over the world and is one of the most frequent viral infections for monocotyledonous plants, causing up to a 70% loss in corn output since 1960. MDMV belongs to the *Potyvirus* (*Potyviridae*) family and was initially discovered in maize and Johnsongrass in Illinois in 1964. MDMV is a common single-stranded RNA virus that is spread by numerous aphid species in a common way. MDMV is one of the most common viral infections in maize across the world.

5.3.1 Host Range

MDMV is an intermediate host of maize and Johnson grass (*Sorghum halepense*) (for specific strains). SCMV is found in sugar cane, sorghum, maize, *Eleusine* spp., *Panicum* spp., and *Setaria* spp. (Redinbaugh and Stewart 2018).

5.3.2 Transmission

Rhopalosiphum maidis, *Rhopalosiphum padi*, *Myzus persicae*, *Schizaphis graminum*, and *Aphis craccivora* are among the aphid genera that disseminate maize dwarf mosaic virus in a non-unrelenting manner (Ghosh et al. 2017). The virus spread quickly through the sap, and seed transmission was also observed, though at a modest rate (Kyallo et al. 2017).

5.3.3 Symptoms

There are various symptoms caused by this strain based on the of development of the host at the start of infection, with early infections generating more severe effects than late infection. Symptoms are:

- Chlorotic mosaics, mottles, or streaks on the green tissues of infected plants (Fig. 5.1).
- Slowed growth and a halt in ear development.
- In open-pollinated maize populations, the severity of disease symptoms can vary greatly (Jones 2021).

Fig. 5.1 Symptoms of MDMV in maize

5.3.4 Control

There is no cure for this disease, and affected plants should be eliminated from the crop immediately. The principal approach to the disease's management is the use of resistant crop species (Dusfour et al. 2019). Interrupting vector-maize contact by lowering vector virus populations in sensitive maize is one of the most popular techniques for controlling the spread of viral infections in corn. Chemical pesticides or aphicides can be used to do this (Ferro et al. 1980). However, this strategy simply slows the virus's internal spread inside a site, which has a negative impact on soil fertility (Ismail et al. 1996). Furthermore, Toler (1985) has established that pesticides have no effect on MDM illness. Breaking pathogen-vector and pathogen-maize interrelationships by reducing viral sources is another typical way to reduce maize dwarf mosaic. MDMV is mostly transmitted by Johnson grass (*Sorghum halepense*) (Knoke et al. 1983).

5.4 Maize Stripe Virus

Maize stripe is a virus's disorder that affects maize. Maize stripe virus infects maize and sorghum in subtropical and tropical areas across the world (Bolus et al. 2021). That can be found in the southern United States, Central America, Africa, Australia, and a few Pacific islands and is likely to be found in most tropical maize-growing areas. The disease first appeared in the continental United States in Florida in 1975 (Gordon and Thottappilly 2013).

Plant viruses can infect important crop plants and reduce their commercial production, posing a danger to world food security and agricultural economies. Stippling symptoms in maize caused by stripe virus, leaf veins finally converted into chlorotic stripes. And more, young plant infection ultimately results in stunting and striking "hoja blanca" or symptoms like turning leaf into white (Falk and Tsai 1998).

5.4.1 Host Range

Its host and geographical distributions are effectively described by vector, the maize planthopper *Peregrinus maidis* (Ashmead), which spreads maize stripe virus by a circulative-propagative mechanism (Bolus et al. 2021).

Maize stripe virus (MStpV) was initially detected in Hawaii, Cuba, Trinidad, Mauritius, and East Africa, according to scientific sources (Storey 1936). MStpV isolates from Florida, Venezuela, Peru, Australia, India, Mauritius, Réunion, Thailand, and Taiwan were all found to be linked by serological testing (Gingery et al. 1979; Greber 1981; Peterschmitt et al. 1987, 1991; De Doyle et al. 1992; Chen et al. 1993; Sdoodee et al. 1997).

5.4.2 Transmission

Maize stripe virus is disseminated in a persistent-propagative way by the maize delphacid *Peregrinus maidis* (Ashmead) (Homoptera: Delphacidae) (Tsai and Zitter 1982).

MStpV's host specificity and geographic dispersion are primarily explained by the vector, the maize planthopper *Peregrinus maidis* (Ashmead), which transmits MStpV by a circulative-propagative mechanism (Tsai and Zitter 1982; Nault and Gordon 1988; Falk and Tsai 1998; Singh and Seetharama 2008). MSpV can also be transmitted transovarially by corn planthoppers (Tsai and Zitter 1982).

5.4.3 Symptoms

- At the start the symptoms are fine chlorotic lesions between leaf veins, followed by chlorotic stripes of varying severity and width (Falk and Tsai 1998).
- The new leaf shows complete chlorosis when young plants are infected at the four- to five-leaf stage, whereas the central leaf is curled mostly (Falk and Tsai 1998).

5.4.4 Control

Traditional virus disease control measures are recommended, including vector control, early removal of sick plants, eradication of itch grass near plantings, and insect vector or virus resistance breeding. In Reunion, I RAT is working on developing MStpV and other maize virus-resistant cultivars (Marchant and Hainzelin 1986). MStpV resistance has been derived from the variety Revolution.

5.5 Maize Lethal Necrosis (MLN)

The virus was first detected in Kenya (Wangai et al. 2012) and then in Rwanda (Adams et al. 2014), the Democratic Republic of Congo (Lukanda et al. 2014), and Uganda's border territories (Lukanda et al. 2014; Adams et al. 2014). According to Grabherr et al. (2011), Ethiopian maize plants with severe symptoms were detected sick in July 2014, proving the occurrence of MLN disease in the nation (Mahuku et al. 2015). The fast spread of the MLN disease in east Africa has posed a serious threat to maize output and impacted the region's agricultural produce significantly. The sickness has the potential to cause significant production losses, grain quality deterioration, and food supply problems.

From seedling through maturity, MLN can impact maize plants at any stage of growth. MLND is diagnosed by chlorotic mottling of leaves, necrosis from the leaf border to the midrib, and a dead heart; later-stage infection might result in sterile pollen, undersized cobs with poor seed set, or plant mortality. New and possibly highly virulent MCMV and SCMV strains, a favourable environment for the

survival and spread of the two viruses' insect vectors (Cabanas et al. 2013), a favourable environment for the proliferation of the two viruses' insect vectors, and continuous maize cropping in certain regions leading to virus inoculum build-up are all possible factors that contributed to the devastating effect of MLND in eastern Africa.

5.5.1 Host Range

The *Poaceae* family is the only experimental host, with maize being the most prevalent natural host (Gordon et al. 1984). Mechanical inoculation has been used to infect *Bromus* spp., *Digitaria sanguinalis*, *Sorghum* spp., and *Triticum aestivum* (Castillo and Hebert 1974; Niblett and Claflin 1978; Zhao et al. 2004), as well as *Zea mays* (Castillo and Hebert 1974; Gordon and Thottappilly 2013). *Zea mays* var. *parviglumis* and *Zea luxurians* were also infected by Kansas serotype 1 (Nault et al. 1978).

5.5.2 Transmission

A broader panel of possible carriers was investigated in the United States, and it was discovered that some beetles can spread MLN at both larval and adult stages, but some aphid and leafhopper species, as well as a mite, are virus-free. The transmission of maize chlorotic necrosis by beetles is undeniable (Awata et al. 2019).

5.5.3 Symptoms

Depending on genotype, infection age, and climate changes, this virus (MCMV) causes a wide spectrum of symptoms in plants. Stunting, leaf necrosis, early plant mortality, truncated male inflorescences with few spikes, or diminished, deformed ears partially filled are some of the symptoms (Castillo and Hebert 1974; Uyemoto et al. 1981).

5.5.4 Control

Crop rotation is a good technique to keep this virus away from crops (Claflin et al. 1981). Two seasons, crop rotation at least with other crops such as potatoes, root crops, cassava, legumes, column bulbs, green onions, vegetables, and garlic is recommended. To diversify farm enterprises, new crops are planted each season. Plant life may be enhanced by compost and basal/top dressing fertilizers.

To reduce alternate hosts for probable vectors, good field cleaning techniques, including weed management methods, are required (Wangai et al. 2012). To minimize pathogen and vector populations, infected foliar plant material should be

removed and discarded from the field. Although this material is safe for cattle, it is not safe for humans or animals to eat decaying grain or cobs. The best approach to get rid of them is to burn them. Farmers should avoid reusing seed and should only plant seed that has been approved. Plant corn just before start of the major rainy season rather than during the short rainy season to provide a buffer between maize planting seasons. As a result, the number of vectors will decrease. MCMV expanded to other Hawaiian Islands, but it was kept under control for a long time on Kaua'i (Nelson).

5.6 Maize Virus Diseases: Genome Tools

Maize is the most important food and feed source for meeting food demands of the world's population. Its yield and production must be increased to meet the rapidly increasing world's food demand. In so many ways, maize research is looking for a breakthrough, applaude to researchers as maize B73 genome sequence which is nearly completed (Schnäble et al. 2009), there are few other sequences which are not far enough from completion, the 5000-line nested association mapping (McMullen et al. 2009). However, by applying genome sequence and relating DNA sequence to function often happen research methods that have been not present in maize, where generating transgenic plants is particularly difficult.

The present BMV-VIGS system works by generating capped in vitro transcripts. Adapting the existing approach to *Agrobacterium* binary-based vectors for viral inoculation would be a first step toward creating a system that allows for cost-effective, high-throughput investigation of several candidate genes. Alternative viral inoculation strategies in maize might potentially be investigated. For example, to differentiate different maize lines for MSV resistance, direct inoculation of Agrobacteria bearing T-DNA constructs that begin infection of maize streak virus (MSV) into the coleoptilar node is regularly utilized (Grimsley and Bisaro 1987; Martin et al. 1999). In addition, a vascular puncture inoculation approach has been correctly used to inoculate several distinct maize viruses that are resistant to typical inoculation processes such leaf rubbing (Redinbaugh et al. 2001).

The creation of effective VIGS tools for maize is a huge step forward in maize research. Together with all of the tremendous resources available for maize genetics, the availability of functional genomics methods for researching maize geneticists' preferred candidate genes promises rapid findings (Benavente and Scofield 2011).

5.7 Conclusion

With a solid understanding of the consistency of virus incidence of disease and virus trajectories, as well as their relationship to crop and climate, an effort will be made to begin reducing plant virus losses by eradicating the source of infection, practising proper field plant and soil management, cutting off virus transmission paths,

effectively adjusting cultivating time, and improving the management and farming of powerful seedlings, as well as eradicating fungus.

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Barley Diseases: Introduction, Etiology, Epidemiology, and Their Management

6

Heba S. Abbas

Abstract

Barley is regarded as the globe's fourth major cereal crop. A variety of airborne, seedborne, and soilborne infective agents attack barley, causing a variety of barley diseases and substantial losses in agricultural output. Brown and yellow rusts, smut, net blotches, spot blotches, barley yellow dwarf, and molya disease are among the most serious diseases. In general, employing integrated disease management approaches is the best way to handle barley diseases. Growing resistant or tolerant varieties with the fewest foliar fungicides is the most effective approach for barley disease treatments. However, managing soilborne pathogens in barley plants is problematic due to a deficiency in distinguishing symptoms for diagnosis and the absence of fungicides or nematicides that are effective for these pathogens. Recently, nanotechnology has driven the advancement of creative concepts and agricultural productivity with a broad scope for managing plant infections and pests. The antimicrobial properties of metallic and metal oxide nanoparticulates such as silver, selenium, titanium dioxide, zinc oxide, and iron oxide have been extensively researched. In this chapter, we go over barley disease and the role of nanomaterials in reducing the incidence of disease and diagnosis, as well as barley seed germination, physiology, and nutritional quality of barley grain.

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Leaf rust disease · Net Blotch disease · Powdery mildew · Barley yellow dwarf · Barley smut · Spot blotch · Fungicides · Nanoparticulate

6.1 Introduction

Nanotechnology causes the progress of innovative concepts and agricultural yield with a vast perspective to manage plant pathogens and pests. Nanotechnology has considerably developed in the field of pharmacological medicine, but has gained moderately less awareness for agronomic purposes (Balaure et al. 2017; Sinha et al. 2017). The application of agricultural nanobiotechnology is presently being discovered in the germination of seeds and the delivery of phytohormones, water managing, target genes transference, nano-barcoding, agro-nanosensors, and restricted discharge of agrichemicals.

Nowadays, researchers have designed nanoparticles (NPs) with desired features, to offer new pesticides and other actives for controlling plant disease and protect plants through two diverse approaches: (a) nanoparticles for plant protection, or (b) nanocarriers for the offered pesticides or other actives, including ds- RNA, and can be practiced by spray purposes or onto waterlogged seeds, leaves, or roots. Nanocarriers can offer some advantages, similar to (1) a better shelf life, (2) transferred the weakly water-soluble pesticides into soluble substances, (3) decreased toxicity, and enhanced the uptake, efficiency, and constancy of the nano-pesticides under unfavorable circumstances (Hayles et al. 2017; Khandelwal et al. 2016).

Metallic and metallic oxide nanoparticulates including silver, copper, iron oxide, zinc oxide, and titanium dioxide have been widely investigated for their antimicrobial properties (Gogos et al. 2012; Kah and Hofmann 2014; Kim et al. 2018). Recently, silver nanoparticulates have revealed inhibition of the fungal growth, such as *Alternaria alternata, Macrophomina phaseolina, Sclerotinia sclerotiorum, Curvularia lunata, Botrytis cinerea,* and *Rhizoctonia solani* (Krishnaraj et al. 2012a, b). Also, low concentrations of copper nanoparticulates increase the resistance of seedlings to the harmful fungi, which cause root decaying in sprouts (Maslobrod et al. 2014). Furthermore, NPs have a main effect on the plant's morphology and genome. A trivial number of nanoparticles can enhance crop productions, but a large amount of nanoparticulates' exposure can cause disorder in plants' physiology and oxidative damage. Furthermore, NPs can decrease the efficiency of the oxidative enzymes that cause genotoxicity and toxicity (Ali et al. 2016; Rizwan et al. 2017).

One of the most crucial cereal plants is barley (*Hordeum vulgare* L.), which is commonly used not only in agriculture but also in food manufacturing. Barley is affected by different diseases, frequently caused by pathogens (Aubert et al. 2018; Giraldo et al. 2019; Gozukirmizi and Karlik 2017; Kumar et al. 2012). The demand for barley grains is rising because of their different uses and high nutritive significance. Therefore, extensive production will be required over the next few years.

Several biotic and abiotic factors should be controlled to enhance the yield of barley. Barley diseases significantly affect net blotch, rusts, spot blotch, stripe disease, molya, powdery mildew, and barley yellow dwarf disease which are the main biotic factors in improving the barley grain yield. Other diseases are vital for manufacturing because they spoil the value of malt and beer.

Understanding the pathogens associated with the disease and modulating the reacted variables are the most effective ways to manage it. Resistant variants are the simplest and most efficient way to treat serious diseases. It is critical to employ integrated disease management strategies that focus on variables for successful disease management. Adoption of resistant barley cultivars provides the most long-term pathogen control (for instance, cultivars with diverse MLO genes). Using resistant cultivars for pathogens enhances output in their cultivated areas automatically (Gangwar et al. 2018). Moreover, fungicide seed dressings or fungicides sprayed in-furrow with fertilizer can protect barley from diseases or reduce early seedling infection. The target diseases should guide the choice of fungicide. Foliar fungicide treatment in the crop is intended to prevent disease growth and keep the greening of leaves. It lessens the effect of diseases on productivity and grain quality. The economic effectiveness of foliar fungicide treatments is determined by disease severity, variety susceptibility, crop production potential, grain quality prognosis, and the environment. For example, triazole fungicides have been effective at a rate of 0.1% against barley rust diseases (Bhardwaj et al. 2017).

Moreover, the disease still faces a critical challenge. Therefore, there is an urgent need to achieve progress in the growing and productivity of barley crops as well as develop an alternative control approach against barley diseases. However, the absence of nanomaterials in the early stage of the plant indicates their unharmful effects and the safety of their use. For example, manganese ferrite NPs, magnetite NPs, and Fe/SiO2 enhance the growing factors of barley and can be planned for future barley breeding applications (Disfani et al. 2017; Tombuloglu et al. 2018). Also, iron oxide or magnetite nanoparticulates endorsed gene expression and proficient photosynthetic activity of barley (Tombuloglu et al. 2019a) and stabilized selenium NPs enhanced barley seed germination (Siddiqui et al. 2021). Barley diseases and the impact of nanomaterials on controlling such diseases, germination of seeds, physiology, and nutritional quality of barley grains were all explored in depth in this chapter.

6.2 Barley Diseases and Their Managements

Barley is a major cereal crop that has been farmed for thousands of years, dating back to early times, and is used in animal feed, malt products, and food production. With around 150 million tons of grain production, it ranks fourth in the world (Arabi and Jawhar 2004). In all places where barley is grown, barley leaf diseases produce major output reductions while also lowering quality. Barley, like other cereals, is susceptible to a variety of plant infections and illnesses, resulting in a considerable

drop in output and poor grain quality. In his "Compendium of Barley Diseases," Mathre (1997) listed around 80 diseases caused by pathogens, including net blotch, yellow and brown rusts, powdery mildew, smut, spot blotch, speckled leaf blotch, barley stripe, barley yellow dwarf, and molya disease, which are cautiously significant in several countries. The routine of fungicides or disease-resistant varieties is efficient in disease control, although pathogens have the potential to overcome plant resistance genes and neutralize fungicide treatments (Ellwood et al. 2019; Hawkins et al. 2014; Mohd-Assaad et al. 2016). The ability of diseases to evolve is useful in the development of control approaches (Palumbi 2001; McDonald and Linde 2002a, b).

6.2.1 Leaf Rust Disease

Leaf rust is the most common rust disease in the *Hordeum vulgare* crop, and it may be found almost everywhere the crop is planted. It doesn't happen very often, but it can be very important in some places where barley is grown.

It has been stated as potentially harmful in North America (Reinhold and Sharp 1982; Mathre 1982) and Kenya (Reinhold and Sharp 1982; Mathre 1982). Actual losses in field crops are hard to come by. However, in New Zealand (Arnst et al. 1979) and England, losses of 10–20% have been reported, at least in part due to leaf rust (Jenkins et al. 1972). Infections caused by *Puccinia hordei* uredial grow on the barley as little (up to 0.5 mm) orange-brownish pustules that blacken with time. The pustules spread mostly on the superior and inferior leaf surfaces and sheaths and are generally accompanied by chlorotic halos. Some stem, glume, and awn infections can happen late in the season with severe infections, and there is often broad tissue chlorosis and final necrosis accompanied by this severe pathogen. Blackish-brown telia appear late in the season. They usually appear as stripes, especially on leaf sheaths, and they can also be seen on stems, heads, and leaf edges. The host's consequences vary depending on the length and strictness of the infection, but biotrophy generally has an unfavorable influence on photosynthesis, respiration, nutrient passage, and water interactions, resulting in overall debilitation. Spring barley is predominantly vulnerable, particularly if planted late, since it is susceptible when the infection is vigorously growing. Primary, severe infections can cause restricted growth and a lessening in the number of fertile tillers and grains per year (Lim and Gaunt 1981; Udeogalanya and Clifford 1982). A lot of people have problems with grain size and quality because epidemics don't start for a long time (Lim and Gaunt 1981; Udeogalanya and Clifford 1982).

Up until roughly 1970, leaf rust was thought to be not nearly as serious as other Hordeum vulgare diseases. However, the disease's recent spread, mainly in northern and western Europe and portions of the US, has prompted an increase in both basic investigation and the progress of disease management strategies that depend on both plant resistance and fungicides. Despite the success of these efforts, more study is needed to uncover new bases of resistance and novel fungicides to control any damage to the outputs of current trials due to variations in the pathogen population.

To that end, research into pathogen evolution and the relationship between type II plant resistance and current systemic fungicides should be pursued. There seems to be a requirement for extra data on the resistant plant in order to make predictions about its long-term viability (Clifford 1985). Until 2015, 21 seedling resistance genes were known. It is expected that achieving long-term resistance to leaf rust in *Hordeum vulgare* will necessitate the introduction of both seedling resistance genes and adult-resistant plant (APR) genes (Park et al. 2015).

6.2.2 Net Blotch Disease

The ascomycete *Pyrenophora teres* causes net blotch, which has become one of the most serious diseases of *Hordeum vulgare*. Net blotch is easily identified by brown reticular bands on the susceptible barley leaf. It reduces production by up to 40% and lowers seed quality. The pathogen's life cycle, mechanism of spread, and expansion allow for rapid infection of the host. Agricultural wastes, seeds, and grasses are the pathogen's origins. The relationship between the Hordeum vulgare plant and the fungi is complicated, involving physiological fluctuations such as the appearance of signs on the barley plant as well as genetic alterations such as the modification of many genes involved in defensive pathways.

Net blotch resistance genes have been found, and their locations on 7 barley chromosomes have been determined. Because of the disease's importance, numerous management measures have been used to combat net blotch. For instance, the use of plant growth promoting rhizobacteria, which are helpful bacteria that colonize the rhizosphere. The preventive role of these bacteria and their bioactive compounds against possible pathogens has been described in several investigations. (Backes et al. 2021). Small bacteriocins and fungal defensins are among the antimicrobial peptides produced by bacteria (Waghu and Idicula-Thomas 2020). Microbes can synthesize secondary products via non-ribosomal pathways (Montesinos et al. 2012). Useful bacteria also create antifungals known as cyclic lipopeptides, which permit them to function as antagonists against pathogenic fungi. These compounds are harmful to the progress of further species and have a low molecular weight (Beneduzi et al. 2012). Due to their amphiphilic properties, lipopeptides, which are synthesized non-ribosomally, have antibacterial and surfactant capabilities that have piqued the interest of researchers (Cazorla et al. 2007). For instance, *Bacillus* sp. and *Burkholderia* yield the majority of these antibiotics (Ongena et al. 2007; Pérez-García et al. 2011; Esmaeel et al. 2016, 2018).

6.2.3 Powdery Mildew

Powdery mildew (caused by the fungus *Erysiphe graminis* D.C.) is the most serious disease afflicting barley around the globe. On the leaves, it is simply recognized by its conidial phase, which usually appears in distinct lesions. However, it will occasionally cover the entire leaf in a weft of spore-bearing mycelium. The fungi

demonstrate a high level of physiological specialization (Marchal 1902). It's been fascinating to see how the discovery of a successful systemic fungicide has affected the amount of mildew research being done around the globe. Researchers now have an active tool for estimating disease-related costs, and results from 25 nations show that mildew is causing larger losses than previously thought. Because mildew stagnates mostly in the winter season, the harvest is regarded as extremely risky in places where spring barley is also cultivated. To avoid the initial formation of reproductive structures in the spring barley, it is critical to evaluate the efficiency of pesticides in reducing mildew over the winter. In the autumn of 1968, trials were put up in the UK to investigate this issue. Due to the extremely rainy autumn, mildew did not quickly expand into the developing crop. Ethirimol provided almost perfect treatment of mildew attacks in the autumn. The next spring, there was no disease in the treated plants, whereas the untreated plants showed a modest but unchanging infection. Moreover, some control was maintained in the treated plants until June, resulting in a significant reduction in crop spore production (Brooks 1970). Breeding the broad-based *mlo* gene in barley is a good source of long-lasting resistance. It's possible to stack a lot of different types of resistance genes on top of each other or use introgressions from bulbous barley (Dreiseitl 2020).

6.2.4 Barley Yellow Dwarf

The most common viral disease of cereals is barley yellow dwarf (BYD), which is caused by the barley yellow dwarf virus (BYDV). The virus is delivered to phloem cells by aphids feeding on the leaf phloem. When viruses enter the plants, they proceed to multiply and build new virions. This mechanism, which causes the symptoms of this disease, necessitates a considerable metabolic input from the plant. Symptoms begin about 14 days after the viral infection. Susceptible plants exhibit yellowish or reddish leaves, an erect posture with thicker, stiffer leaves, decreased root growth, and a reduced harvest. Because of saprotrophic fungus colonization, the heads of infected plants persist erect and turn black and discolored throughout maturation. Young plants are especially vulnerable. When the aphid feeds, the viruses are propagated via the phloem. When an aphid eats, the virus's coat protein is detected by the epithelium of the aphid's hindgut, and the virus particle is permitted to enter the hemolymph of the insect and persist forever. However, the virus is unable to multiply within this insect. The virus is energetically carried into the attachment salivary gland, where it is discharged into the salivary canals. In the aphid's next feeding, the virus is expelled in its saliva (Gray and Gildow 2003). Insecticide management of the aphid insect is one method of preventing BYDV contamination. However, the use of insecticides is aggressively discouraged because of environmental conditions and the potential for resistance to progress. As a result, developing virus-resistant varieties is the most effective way to mitigate the harmful effects of viral infection on farming. Exposure to viruses indicates that they can proliferate and propagate within the plant, resulting in severe disease signs. Because viral management is not achievable, resistant barley genes are

regarded as the best strategy to avoid the loss of products. Though multiple genes and numerical trait loci for viral tolerance are recognized and employed in barley breeding, little is known about the molecular and physiological basis of this characteristic (Paulmann Maria et al. 2018). The higher productivity of the resistant variety, which harbors the Ryd2 gene, was shown to be related to small degrees of hormone signaling, offering innovative indicators for resistance and a novel framework for researching the origin of viral resistance in barley (Ordon et al. 2009).

6.2.5 Barley Smut

Smut of barley is caused by the fungus *Ustilago hordei*. The disease is present all over the world and is more widely transmitted than loose smut. Infected kernels are substituted by masses of dark brown smut spores. Smutted heads are compact and hard. Plants that have been infected may become stunted. Smut sori can also emerge as lengthy streaks on leaf edges on rare occasions. To control covered smut disease, resistant cultivars and seed treatments are applied (Mathre 1997). On the other hand, *Ustilago nuda* generates loose barley smut. It is a disease that has the potential to wipe out a large section of barley yield. Loose smut substitutes grain heads with spores that invade open blossoms on plants and produce seed without causing visible signs. The seeds seem to be in good health, and it is only after they mature the next time of year that it is obvious that they were diseased.

The real-time PCR results showed that loose smut infection occurs at the secondary leaf phase and that it is therefore appropriate for practice in different barley cultivars (Wunderle et al. 2012). Systemic fungicides are the primary technique of controlling loose smut disease (Thomas 1984a, b). For covered smult, five barley cultivars, including HBL 391, HBL 316, HBL 113, DWRUB 123, and DWRUB 92, were extremely resistant, although BL 1656 and BL 1562 germplasm lines displayed a resistant response to *Ustilago horde* (Singh et al. 2020).

6.2.6 Spot Blotch

The causal agent of the spot blotch disease is *Cochliobolus sativus*. The disease can be found anywhere barley is planted, but it only causes major output losses in warm, humid areas (Mathre 1997; Martens et al. 1984). Infections manifest in the form of dark, chocolate-colored spots. The spots meld together, leaving uneven necrotic areas on the leaves. A zone of yellow leaf tissue of varied width may edge leaf spots. During kernel filling, infections on the standard leaf are the most dangerous, with heavily diseased leaves entirely drying up. Resistant cultivars, rotation by non-cereal crops, seed treatments, and foliar fungicides are used to fight the disease. (Martens et al. 1984). An eco-friendly foliar spray for control of this disease, *Trichoderma harzianum*, neem, and tulsi extracts as biological control agents, and SAR chemical (SA) can be applied (Kaur et al. 2021).

6.2.7 Molya Disease

The *Heterodera avenae* nematode is responsible for "Molya disease" in wheat and barley. The second juvenile (J2) swells and becomes a lemon-shaped, creamish-white adult female as she grows. When this white female reaches maturity, she will transform into a brown female known as a "Cyst" (dead female), with 400 eggs inside her body acting as a protective cover against the harsh environment. When the second stage, J2, detects humidity and a host plant, it raptures the cyst and emerges from the birth hole to attack the crop the following season. Dissimilar to other pathogens, nematode signs are not diagnostic since they are similar to water or nutritional deprivation or any other physiological problem. There are two types of nematode symptoms, and normally, above ground signs are not distinguishable and can be readily confused with any other infection. However, in blown ground signs, roots frequently become bushy, with mild swelling at the site of infection. The brown cyst matures, it detaches from the roots and remains in the mud until the following crop is grown, behaving as a source of infection for future years, and J2 hatches out upon identifying the host crop, precise temperature, and humidity conditions. There are no other options for managing the nematode in standing crops. To avoid additional output losses, it is recommended that certain agronomic treatments (seed treatments, resistant cultivars, etc.) be implemented to regulate the nematode population (Priyanka 2018).

6.2.8 Barley Diseases Control Using Fungicides

Fungicides are commonly employed to shield crops because they can offer extremely high rates of disease avoidance. Foliar fungicides are applied to the majority of *Hordeum vulgare* diseases in Europe. Nevertheless, unselective fungicide usage, combined with disease adaptation, can significantly impair fungicide efficiency. If administered before severe symptoms progress, metrafenone, proquinazid, and cyflufenamid fungicides can provide excellent defense against powdery mildew. It is very hard to control the disease when it has established itself in the plant. Morpholines can eliminate powdery mildew and give effective short-term elimination and protectant action. However, disease resistance renders strobilurin fungicides ineffective against powdery mildew (HGCA 2011).

In net blotch disease, seed should be examined to determine if the treatment is mandatory or not. In susceptible plants, SDHI fungicides and prothioconazole can provide good protection. Furthermore, in order to eradicate brown rust disease, SDHIs, as well as the majority of triazoles and strobilurins, are good controls. However, the disease can be treated by combining morpholine with one or more other fungicides. The optimum control for leaf spot disease is obtained by combining a triazole with, for instance, boscalid or chlorothalonil.

Suitable fungicide choice is thus required to reduce yield losses. Before applying fungicides, the counsellor or planter should assess the grade of fungicide resistance. The Fungicide Resistance Action Group (FRAG) is the primary foundation of these

data in the United Kingdom. The majority of fungicides have extremely exacting approaches to their target fungus. This uniqueness can frequently lead to fast fungal development. The fungicide's target place is a critical factor driving pathogen progress because fungicides with only one target site frequently generate quick resistance against fungicides, as seen with methyl benzimidazole carbamate fungicides. As a result, to reduce the losses of active fungicides, an integrated management system for the control of barley diseases must be adopted. The most effective ways now being used are: delivering the suitable dose at the suitable time and combining multiple compounds with distinct mechanisms of activity in conjunction with the adoption of resistant varieties (Walters et al. 2012).

6.3 Nano Diagnostics for Barley Infections

Rapid detection solutions for plant pathogens with elevated sensitivity and selectivity are required to avoid disease propagation and limit losses to ensure maximum production and food security. Microscopy and culturing are time-consuming, labor-intensive methods that require complicated sample management. Immunological and molecular approaches have evolved, although there are still significant challenges with speed, signal strength, and equipment. The combination of molecular and immunologic diagnosis with nano-approaches yields a solution in which all detection processes can be housed on a portable tiny instrument for quick and precise diagnosis of plant infections (Kashyap et al. 2017).

Nanotechnology, nanoparticles, and quantum dots (QDs) have developed as critical instruments for the rapid and precise detection of a specific biological signature. Using biosensors, QDs, nano platforms, nanopore DNA sequencing technologies, and nanoimaging can help improve disease diagnosis and crop protection. These technologies can also help with high-throughput analysis and crop protection.

6.3.1 Nano Diagnostic Kits for Barley Mycotoxins

The term "nano diagnostic kit," also known as "lab in a packet," refers to the practice of packing a laboratory's instruments, reagents, power supply, and other components into a package no larger or heavier than a briefcase (Khiyami et al. 2014). This allows for the simple and rapid identification of plant diseases in fields, permitting specialists to assist agronomists in disease epidemic inhibition (Pimentel 2009; Nezhad 2014). A mycosensor is a dipstick-based antibody-based test for the real-time diagnosis of Zearalenone, Trichothecene, Deoxynivalenol, and Fumonisin B1/Fumonisin B2 mycotoxins in barley samples (Lattanzio et al. 2012).

Nano diagnostics using immunoassay kits and nucleic acid-based tests are quick, inexpensive, and simple to use, making them ideal for on-site testing. However, there are several hurdles, such as the detection and choice of efficient antigens, antibodies, nucleotide targets, nanomaterials, and their manufacture as kits, which

need more research work to make them practicable at the ground level on a wide scale (Lattanzio et al. 2012). Furthermore, the transportable diagnostic device, nanoparticle-based, bio-barcoded DNA sensor, and QD might all be used to identify plant diseases and toxogenic fungus. Transportable diagnostic tests have been established to identify plant diseases quickly and may be applied to avert outbreaks. These nano-based kits are rapid for pathogen identification and also improve diagnostic precision. Furthermore, the grouping of nanotechnology and microfluidic devices has been successfully used in molecular studies of plant pathology and may be customized to identify definite infections and poisons. For instance, the micro-PCR, which can execute 40 cycles of PCR in a short time. In the near future, nano-instruments with unique features might be employed to create smart agricultural systems in the near future. These nanodevices, for example, may be applied to detect plant health concerns before they become observable to the planter. Such devices may be able to respond to unusual events, identifying the problem and initiating disease management intervention. Nano-smart instruments will therefore serve as both a defensive and an initial alarm system. Nanodevices that can do thousands of measurements quickly and affordably will become available during the next few years. The downsizing of biochip technology to the nanoscale level will continue to improve future possibilities in plant disease diagnostics. Nano-phytopathology can be used to better understand plant-pathogen interactions, perhaps leading to novel crop protection measures. Specific nano-instruments and DNA nano-instruments might provide precise tracking, diagnosis, and monitoring of the pathogens in the first stage of plant infection (Khiyami et al. 2014).

6.4 Effect of Metallic Oxide Nanoparticulates on the Barley Varieties

Plants require iron as an essential micronutrient for their growth, whereas copper is a microelement that aids in plant metabolism. Fertilizers containing iron oxide and copper oxide nanoparticulates are applied in trace amounts to improve the necessary metal content of the soil, thus enhancing crop development. These NPs are employed in large dosages as antifungals to protect plants from diseases caused by fungal pathogens (Anderson et al. 2018; Devi et al. 2019; Elmer et al. 2018). Also, zinc oxide nanoparticles are found in a variety of commercial items, including sunscreens, cosmetics, and paints (Hussain et al. 2018; Vance et al. 2015). Furthermore, ZnO NPs have been recommended as a fertilizer to provide Zn to plants.

Metal oxide nanoparticulates have a significant effect on the morphology of the plant. Wheat, tomato, and lettuce roots can be lengthened with Fe_3O_4 nanoparticles. Different concentrations of CuO nanoparticulates can lower the length of roots and shoots in chickpea plants. CuO NPs stress decreased the germination of cucumber, lettuce, rice, and radish seeds (Konate et al. 2018; Kumar et al. 2019). Also, the levels of microRNA expression in plants can be influenced by metal oxide nanoparticles. It is known that microRNAs can defend plants against biotic stress, such as infections that cause powdery mildew.

6.4.1 Barley Morphology and Seedlings Germination

Petrova et al. (2021) investigated the morphology, genotoxicity, and miRNA156a of *Hordeum vulgare* L. cultivars Marthe and KWS Olof when they were grown in different concentrations of iron oxide and copper oxide nanoparticles. The impact of diverse doses of iron oxide and copper oxide nanoparticulates on shoot length on Marthe and KWS Olof barley cultivars was compared; the 17 mg/L dose of iron oxide nanoparticulates generated a substantial increase in the Marthe and KWS Olof varieties. Only the Marthe variety's shoot length was greatly boosted by treatment with 35 mg/L of iron oxide nanoparticulates. Copper oxide nanoparticulates at 35 mg/L enhanced shoot length exclusively in the KWS Olof cultivar. The shoot length of the Marthe cultivar control group was 16.15 cm, whereas the shoot length of the groups treated with 17, 35, and 70 mg/L iron oxide nanoparticulates was 16.04, 18.96, and 17.23 cm, respectively (Fig. 6.1). However, when they were treated with copper oxide nanoparticulates, the shoot length of the groups was 16.08, 15.58, and 15.18 cm at 17, 35, and 70 mg/L, respectively. On the KWS Olof cultivar, the shoot length of the control group was 15.78 cm, whereas the shoot length of the groups treated with iron oxide nanoparticulates at 17, 35, and 70 mg/L was 18.53, 18.13, and 17.35 cm, respectively. The shoot length of copper oxide nanoparticulates-treated KWS Olof variety attained 15.06, 17.36, 16.95 cm at

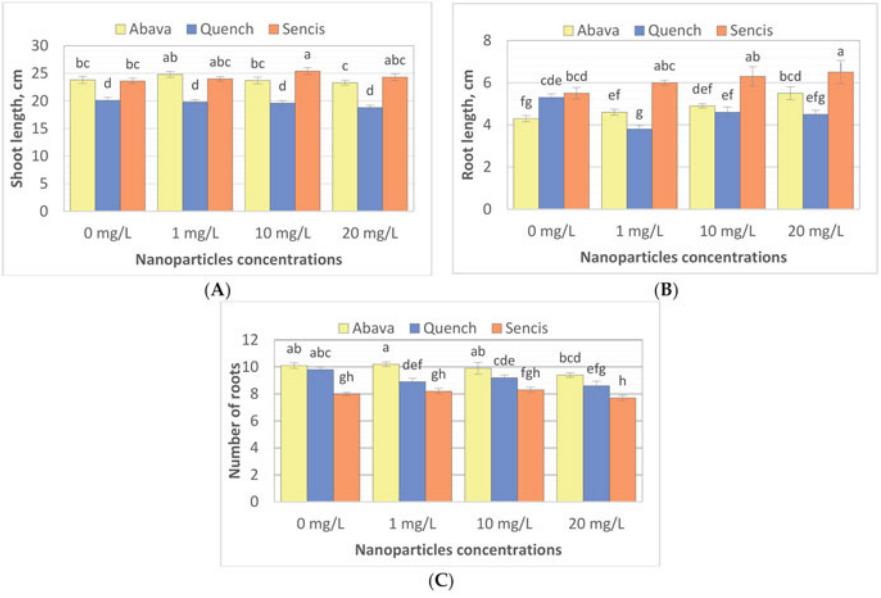

Fig. 6.1 Growth parameters expressed as the % of control; in barley cultivars, seedlings have grown 8 days with different doses of iron oxide nanoparticulate. Diverse letters show significant differences at $p < 0.05$. However, the similar letters show no significant difference (Kokina et al. 2021)

17, 35, and 70 mg/L, respectively. All other iron oxide nanoparticulates treatments improved the shoot length of both cultivars of barley.

Copper oxide nanoparticulates at all treatments reduce the shoot length of Marthe cultivar, but in the KWS Olof cultivar, all doses of CuO NPs in this cultivar enlarged shoot length except in case of using 17 mg/L concentration of copper oxide nanoparticulates (Petrova et al. 2021).

The root length of the Marthe and KWS Olof cultivars was unaffected by different treatments of iron oxide nanoparticulates. All treatments of copper oxide nanoparticulates lowered Marthe and KWS Olof roots lengths substantially. The root length for the control group of Marthe cultivar was 7.58 cm, whereas the root length of the group with iron oxide nanoparticulates at 17 and 35 mg/L concentrations was 7.17 and 6.33 cm, respectively. However, at the 70 mg/L concentration, the root measured 9.86 cm long. The Marthe set with copper oxide nanoparticulates at 17, 35, 70 mg/L concentrations had a height of 3.08, 5.31, 5.76 cm, respectively. All Fe_3O_4 NPs concentrations had a beneficial effect on the fresh biomass of the Marthe and KWS Olof cultivars, with biomass increasing. However, iron oxide and copper oxide NPs at 17, 70, and 35 mg/L did not influence the fresh biomasses of seedlings.

On the contrary, recent research by Kokina et al. (2021) showed the increase in root length and shoot length in both Sencis and Abava varieties when they were treated with iron oxide nanoparticulate. Abava seedlings grew to 1 cm in shoot length and 0.1 cm in root number when given a 1 mg/L dose. However, insignificant root development of Abava was observed when given a 20 mg/L dose. Moreover, the reduction of growth parameters was observed only in the Quench variety (Fig. 6.1).

Also, Petrova et al. (2021) showed that ZnO NPs improve barley seed growing, shoot/root extension, and stress level of hydrogen peroxide and reduce the viability of root cell, the stability of genomic template, and up/downregulated miRNAs in the seeds. The seeds grown with the supplements 4 mg/L of ZnO NPs had the highest germination rate (66%), while the control seedlings had a much lesser germination percentage (42%). Germination rates at 2 mg/L and 1 mg/L were 57 and 63%, respectively. ZnO NPs had a substantial influence on the regular length of shoots. There was no noteworthy statistical variation between the length of the seedling root and the number of seminal roots. The maximum dose (4 mg/L) of ZnO NPs had the greatest impact on barley germination and shoot and root length. In another study, Tombulogu et al. (2019b), cultivated Barley for 3 weeks in a hydroponic solution enriched with different concentrations of NiFe2O4 NPs and the results in rising in iron and nickel levels of leaves that were 5.5 and 8 times larger than the control, respectively. Furthermore, the NPs treatment boosted the leaf's calcium, potassium, manganese, sodium, and magnesium constituent (Tombuloglu et al. 2019b).

Also, Rico et al. (2015) proved that cerium oxide NPs ($nCeO_2$) improved biomasses, plant height, and chlorophyll composition while decreasing spike formation in *Hordeum vulgare L.* Ce buildup by 294%, which was associated with increased nutrient storage including phosphorous, potassium, magnesium, calcium, iron, copper, sulfur, and zinc in grains. Similarly, $nCeO_2$-amended soil (250 μg/kg DW) improved the levels of amino acids including methionine, aspartic acid,

Table 6.1 Amino acid and fatty acid compositions in barley grains harvested from $nCeO_2$-amended soil (Rico et al. 2015)

N CeO$_2$ Concentrations (mg kg^{-1})			
0		125	250
Amino acids (μg g^{-1} dry wt)			
Alanine	61.10 + 539	67.62 + 1 24	88.72 + 25 M
Amide-NH_3	78.37 + 5_12	84.36 + 236	99.52 + 24.45
Arginine	13.08 + 232c	3 7.19 + 3 25b	62.53 + 2.10a
Aspartic *acid*	126.56 + 83,713	123.05 + 292b	160.84 + 18.95a
Cysteine	6.57 + 1 36	832 + 0.70	6.25 + 0.77
Glutamic acid	500.49 + 52.65	47,327 + 14.55	573.74 + 189.40
Glycine	75.02 + 5.91	77.02 + O31	82.79 + 32.99
Isoleucine	41.30 + 5.48	5030 + 230	47.51 + 24.69
Leucine	67.48 + 830	7626 + 737	130.75 + 56.94
Lysine	42.16 + 3.19	4430+ 1.78	7 1.24 + 22.36
Methionine	4.36 + 0.433	5.80+ 1.98b	3 1.24 + 0.58a
Phenylalanine	35.56 + 2.78	3829 + 3.61	61.42 + 20.57
Proline	373.96 + 31.15	345.97+ 10.78	395.94 + 25.49
Serine	26.70 + 239	3 1.97 + 2.41	45.86 + 16.55
Threonine	65.86 + 5.5 8b	74.82 + 2.65ab	103.79 + 18.44a
Tyrosine	36.64 + 4.3 lb	6048 + 5.1 5ab	8 &35 + 25.95a
Valine	82.62 + 899	101.92 + 1 26	125.94 + 36.55
Total	1637.84 + 12,138	1702.14 + 36.82	1816.96 + 448.64
Fatty acids (relative % abundance)			
Linoleic acid	55.17 + 0.1 2b	54.76 + 04913	56.11 + 0.28a
Linolenic acid	6.62 + 0.11	6.8 1 + 0.1 1	7.10 + 0.29
Oleic acid	15.11 + 031	14.99 + 030	14.86 + 0.20
Palmitic acid	2 1.72 + 0.12a	2130 + 0.13b	21.54 + 0 1 2ab
Stearic acid	0.84 + 0.0313	1.00 + 0.05a	0.89 + 0.07ab

tyrosine, threonine, linolenic acid, and arginine in grains by up to 617, 31, 141, 58, 2.47, and 378%, respectively (Table 6.1) (Rico et al. 2015).

In that concern, $nCeO_2$ and $nTiO_2$ exhibited differential effects on the content and nutritional value of *H. vulgare* kernels. Both MNPs did not affect β-glucans, but lowered amylose concentration by around 21%. The majority of amino acids and crude protein levels rose. Lysine, followed by proline, showed the greatest growth among amino acids (51% and 37%, respectively) (Pošćić et al. 2016).

The oxidative stress in the leaves was not always caused by the $nCeO_2$ treatment; nonetheless, yield was reduced at the maximum $nCeO_2$ concentration (500 mg/kg). Further, the plant couldn't form grain at this high concentration (Rico et al. 2015).

6.4.2 Barley Genotoxicity

CuO NPs had a greater impact on the barley genome than Fe_3O_4 NPs which decreased genome constancy to 72% in the Marthe cultivar and 76.34% in the KWS Olof cultivar, whereas CuO NPs raised genome stability from 53.33 to 78.66%, in the Marthe cultivar and reduced genome constancy to 68.81% in the KWS Olof cultivar. After Fe_3O_4 NPs treatments, levels of miRNA expression were not altered in the Marthe cultivar, but rose in the KWS Olof cultivar. The treatment by CuO NPs raised the expression levels of miRNA in the Marthe cultivar, but it decreased in the KWS Olof cultivar. The results imply that the examined NPs may be useful because they may alter the expression of miRNA, which affects plant resistance (Petrova et al. 2021). Forthcoming research is required to examine the impact of NPs stress on expressions of miR156 and other miRNA in mlo and non-mlo barley seedlings, as well as the prospect of using NPs to boost the disease resistance.

6.5 Effect of Metallic Nanoparticles on the Barley Diseases, Seed Germination, Root, and Shoot System

Seed nanoparticles are beneficial to seed growth and sowing quality. Plants grow more resistant to harsh situations such as diseases and pests as a result of their effects. In studies, nanoparticles have been shown to dramatically enhance seedling germination during the early phases of growth (Barabanov et al. 2018; El-Ramady et al. 2014; Krishnaraj et al. 2012a, b). The impact of nanoparticulates on plant development can vary depending on the dose. It has been demonstrated, for example, that increasing the absorption of silver nanoparticulates can delay seedling growth compared to the control (Gubbins et al. 2011; Lee et al. 2012; Mirzajani et al. 2013). Furthermore, the toxicity of nanoparticles may be affected by their size (Jiang et al. 2014). For instance, small silver nanoparticles with a diameter of 6 nm, for example, have been shown experimentally to be more hazardous than the big ones (20–1000 nm) (Musante and White 2012).

6.5.1 Selenium Nanoparticles (SeNPs)

The impacts of critical trace elements such as selenium are being studied in depth. This component is required for the plant organism to function properly. The influence of SeNPs on diverse plant species differs substantially depending on the development of plant growth, the extent of SeNPs exposure, as well as the nanoparticle's morphology, chemical structure, absorption, surface construction, solubility, and aggregation (Romero et al. 2019). The effect of SeNPs on the germination features of *Hordeum vulgare* L. seeds was examined by Siddiqui et al. (2021). SeNPs were found to have a favorable influence on the shoot and root length and the percentage of germination. The treated sample with the

Fig. 6.2 Photos of Barley seeds: (**a**) barley seeds were treated with Selenium nanoparticulate in a Petri dish; (**b**) only one germinated *Hordeum* seed (Siddiqui et al. 2021)

formulation of SeNPs at a dose of 4.65 g/mL had the highest percentage of seed germination (Siddiqui et al. 2021) (Fig. 6.2).

6.5.2 Silver Nanoparticles (AgNPs)

The dispersion of AgNPs in the shoot and root tissues and seedlings of *Hordeum vulgare* was examined by Linares et al. (2020). The strong, linear responses of barley seedlings to soil AgNP doses over a 14-day exposure time validate barley's usefulness as a detective examination for silver bioavailability in AgNP biosolid-amended soils. The growth of root and shoot was reduced linearly by the increased concentration of AgNPs. Furthermore, Elamawi and Al-Harbi (2014) reported that the lower doses of AgNPs enhanced the percentage of barley seed germination and lessened the prevalence of barley seed rot disease produced by *Fusarium oxysporum*. However, the higher doses of AgNPs reduced the germination of barley grain and showed a robust lessening in the length of roots. The chlorosis of leaves was caused by a loss in chlorophyll pigments and disorganization of chloroplast thylakoids in positive silver ions and the treated barley groups with AgNPs. As a result, increased monoaldehyde content in response to the influence of positive silver ions and AgNPs gave an indication of oxidative stress intensification. Silver toxicity caused the death of mitochondria, chloroplasts, and the nucleus, which showed that these were the main goals of silver poisoning (Fayez et al. 2017).

6.5.3 Gold Nanoparticles (AuNPs)

Feichtmeier et al. (2015) investigated the influence of 2–19 nm spherical AuNPs on barley seedling germination. There was no noteworthy influence on germination, but there was wilting of leaves, blackening of roots, and reduced biomass, which

worsened as the concentration of AuNPs increased. However, a relatively modest concentration of AuNPs in the nutritional media (1 g/mL) stimulated growth. It is supposed that low concentrations trigger hormone roles (Barrena et al. 2009), whereas higher concentrations and larger AuNPs have a negative outcome on barley growth and biomass yield. Adsorption of AuNPs onto the primary root may have reduced pore size, hindering water passage capacity and thus lessening barley growth and related features. Previously, researchers explained this as well (Feichtmeier et al. 2015; Asli and Neumann 2009).

6.6 Conclusion

One of the most vital cereal plants is barley (Hordeum vulgare L.), which is widely employed not only in agronomy but also in nutrient production. Barley is susceptible to a variety of diseases, the majority of which are caused by plant pathogens. Fast diagnosis methods for crop pathogens are needed to avoid disease spread and limit losses in order to maximize output and food security. For instance, a mycosensor is a dipstick-based antibody-based test that detects mycotoxins in barley samples in real time. Barley diseases: nanotechnology propels a broad range of options for managing barley diseases. Silver, selenium, copper, iron oxide, zinc oxide, and titanium dioxide nanoparticles have received a lot of attention. These nanomaterials have a role in reducing disease incidence, as well as barley seed germination, physiology, and nutritional quality of barley grain. Future studies are needed to investigate the role of miR156 and other miRNA expressions in NP-stressed barley seedlings, as well as to evaluate the feasibility of applying NPs to boost barley resistance to diseases.

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Part II

Plant Breeding and Diseases Management

Identification of a New Susceptibility Gene and Its Role in Plant Immunity

7

Zohaib Asad, Maria Siddique, Muhammad Ashfaq, and Zulqurnain Khan

Abstract

Host–pathogen interaction and crosstalk are very crucial to study disease susceptibility and resistance. Various susceptibility genes (S-genes) have been reported and studied for understanding the mechanism of disease development in plants. Developing disease resistance using modern techniques is dependent on a comprehensive understanding of the role of susceptibility genes in disease development. By disrupting susceptibility genes in the host, resistance has been developed in rice, tomato, pepper, and many other plants. More precisely, in the case of bacterial blight, effector-binding elements (EBE) in the promoter region of the S-gene are important targets to restrict bacterial transcription factor proteins from S-gene activation. Identification of S-genes along with R-genes is very important for building the foundation of third-generation disease resistance in plants.

Keywords

Alleles · Antibiotic · Cell wall · Genes · Jasmonate · Salicylic acid · Stomata · Susceptibility

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7.1 Introduction

A genetic mutation increases a person's susceptibility to a disease or ailment. Symptoms are far more likely to arise when a variation (or mutation) is hereditary, albeit not always known as a predisposing mutation, harmful mutation, disease-causing mutation, pathogenic variation, or pathogenic variant (McCarthy 2004). Plants often use dominant resistance genes to impart resistance to diseases and pests. Because susceptibility is predicated on the identification of a particular microbe's molecular pattern, these narrow-range genes are usually easy to overcome. Infection develops based on compatibility between both the plant and the pathogen. As a result, changing a plant gene that is vital for compatibility may provide an extra broad-spectrum as well as long-lasting form of resistance. This section focuses solely on factors that lead to loss of compatibility for certain susceptibility (S) genes. We found three distinct groups of susceptibility genes that act at various phases of disease: early pathogen development, host defense modulation, and microbe sustenance. Susceptible genetic traits have the ability to be used in resistance breeding, as evidenced by the numerous examples presented here. Because S genes have a function other than microbial compatibility, the negative consequences of their mutation need a one-by-one assessment of their applicability.

With the widespread planting of "Victoria-type" oats, which possess the Pc-2 gene for rust susceptibility to *Puccinia coronate*, a rust fungal disease, a disorder epidemic in oats appeared in the nineteenth century. Victoria blight, caused by the fungus *Cochliobolus victoriae*, was found to be universally susceptible in oats containing Pc-2. The pathogenicity of *C. elegant victoriae* is mainly reliant on the production of a toxic chemical known as victorin, and the prominent (Vb) gene imparts toxin susceptibility as well as high susceptibility to Victoria blight disease in oats. Despite considerable attempts, rust resistance (Pc-2) and Victoria blight susceptibility (Vb) have yet to be genetically differentiated and are thought to have the same identity, suggesting an unanticipated connection among plant disease resistance and susceptibility (Eckardt 2002).

The main objectives of these chapters are the identification of susceptible genes and highlighting their roles in plant defense mechanisms. Plant *S genes* may interfere with host–pathogen compatibility and provide long-term resistance to plant disease development.

7.1.1 Difference

Inherited tractability is thought to be the cause of the information gap between the natures of resistance and susceptibility. Gene-for-gene type resistance is usually initiated by a dominant, pathogen-derived avirulence (Avr) gene product activating a genetically dominant resistance gene product. In the absence of their R protein partners, Avr proteins frequently serve as virulence determinants, showing that their primary function is pathogenicity and that recognition by R genes developed from this role. Nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins are

the most common R proteins. These proteins' only known function in plants is to condition disease resistance. In mammals, structurally similar proteins mediate the innate immune response. Salicylic acid (SA), Jasmonic acid (JA), and/or ethylene are required for R gene-mediated signaling cascades, which frequently involve activation of hypersensitive cell death (HR) (Kwiatkowski 2000).

The genes of vulnerability are less apparent in the bulk of plant diseases. Microbes often have numerous viral proteins (called effectors), each adding progressively to the disease phenotype, and host susceptibility is usually characterized in terms of gains or losses of resistance. A noteworthy exception is Os8N3, a sexually prominent rice gene which is upregulated by a bacterium type-III effector protein and imparts disease susceptibility gene-for-gene. Also, susceptibility to Victoria blight and other diseases caused by pathogens that kill cells during the process of infection is determined by a single dominant region and a single pathogen-derived host-selective toxin (HST) (Eckardt 2002). These diseases are caused by necrotrophic pathogens, which are pathogens that kill cells during the process of infection.

Viruses are cellular parasitic molecular parasites that consume cellular resources throughout their reproduction cycle. Plant viruses also utilize virus-encoded moving proteins as well as cellular components to travel from cell to cell (local) in infected leaves and large distances via the vascular system (systemic movement). In most cases, an insect vector delivers plant viruses into the cell, and infection begins in a single cell. Viral proteins must be translated in order for viral replication, virion assembly, and virus migration to adjacent cells to take place. The cycle is repeated for every newly infected cell. Viruses travel vast distances after entering the circulatory system. Some viruses are only found in the blood vessels. Most viruses, on the other hand, escape the vascular system and infect roots and young leaves far from the original infection site. So, when a virus gets into a plant, it keeps multiplying at the cell level and moving from one cell to another.

Plants defend themselves against viruses via a variety of mechanisms that target viral nucleic acids or proteins. While gene silencing targets viral RNA and DNA, autophagy and R-mediated innate immunity detect viral proteins. Antiviral defense, with or without a hypersensitive reaction, inhibits viral RNA translation, replication, movement, or virion assembly, resulting in virus buildup and/or movement delays (Garcia-Ruiz 2018).

7.1.2 Virus Susceptibility Is Determined by Host Factors

At the cellular level, hosts possess components that are needed for all aspects of viral reproduction. In plants, factors needed for local and systemic viral transport are found. In the absence of necessary host components, this model predicts that viral accumulation is decreased at the cellular and/or organism levels owing to ineffective virus replication, mobility, or a combination of these factors. The final outcome is a viral-resistant phenotype with decreased virus accumulation and mild symptoms in comparison to susceptible plants, or no infection, comparable to a nonhost

phenotype. As a result, the existence of host components needed for viral infection or transmission is a genetic determinant of virus susceptibility (McGee and Nichols 2016).

7.1.3 Alleles Associated with Host Susceptibility

The majority of plant diseases change the expression patterns of host genes in order to benefit the pathogen directly. Disease susceptibility genes are reprogrammed genes that help pathogens survive and reproduce. Disease susceptibility genes are recessive resistance genes. Powdery mildew resistance was conferred through a mutation in an Arabidopsis gene that produces pectate lyase (an enzyme involved in cell wall breakdown). For example, *Golovinomyces cichoracearum*, the Barley MLO gene, as well as its spontaneously changed pea and tomato MLO orthologs, gives resistance to powdery mildew. In wheat, the *Lr34* gene confers moderate resistance to leaf and yellow rusts, as well as powdery mildew. Adenosine triphosphate (ATP)-binding cassette (ABC) transporter is encoded by *Lr34*. The disease-resistant dominant allele was recently discovered in cultivated wheat (not wild strains) and provides broad-spectrum resistance in barley, similar to MLO.

The eif4e and eif4g host translation elongation initiation factors have natural alleles that provide virus resistance. Potyviruses have been used to manage barley, rice, tomato, pepper, pea, lettuce, and melon. Following the finding, a successful mutant screen for chemically induced eif4e alleles in tomato was carried out. The development of recessive disease resistance alleles may be aided by natural promoter variation. The rice recessive resistance gene xa13, for example, is an allele of Os-8N3. *Xanthomonas oryzae* pv. oryzae strains expressing the TAL effector *PthXo1* activate *Os-8N3* transcriptionally. The promoter of the *xa13* gene contains a mutant effector-binding region that prevents *PthXo1*-binding, making these lines resistant to *PthXo1*-dependent strains. This discovery also proved that *Os-8N3* is necessary for susceptibility.

Pollen formation requires *Xa13/Os-8N3*, indicating that disease susceptibility mutant alleles may be troublesome if their role in other processes is altered. Fusing TAL effectors to nucleases, on the other hand, was used to make changes in the *Os11N3 (OsSWEET14)* TAL effector-binding element (TALENs). Rice plants with changed Os11N3-binding sites were resistant to *Xanthomonas oryzae* pv. Oryzae, but nevertheless functioned normally throughout development (Garcia-Ruiz 2018).

7.1.4 Susceptibility Genes Have Many Different Types

A Warm Welcome to S Genes That Allow Basic Compatibility
- Structure of the cuticle or cell wall
- Stomata serve as entrance points
- Immune Suppressor-Producing S Genes
- Maintaining a healthy amount of salicylic acid
- Sustenance for the Guests: Susceptible Genes Ensuring Sustained Compatibility

7.1.5 A Warm Welcome to S Genes That Allow Basic Compatibility

Bacterial pathogens penetrate into apoplast through stomata via wounds, where they often establish type III and type IV secretion systems for effector injections. Fungus and oomycetes produce spores, which germinate and produce runner's hyphae which also enter the recipient via natural apertures or force entrance through cell walls utilizing appressoria. After that, a haustorium may be built for nutrition and effector transfer. Plant genes determine if a compatible relationship can be formed in the new infection phases, from the synthesis of attractants through the formation of structural components to produce a feeding site (van Schie and Takken 2014).

7.1.6 Structure of the Cuticle or Cell Wall

The cuticle, a sticky layer covering the leaf surface, is made up of cutin, waxes, polysaccharides, and lesser chemicals like flavonoids. Glossy11, a corn mutant, reduced very long-chain aldehyde content in leaf cuticles, leading to poor PM spore germination. Decreased differentiation of fungus rust and anthracnose parasites (*Puccinia emaculata, Colletotrichum trifolii,* and *Phakopsora pachyrhizi*) was observed in a Medicago mutant, irg1, with lower levels of primary alcohols in the surface wax. Because of decreased sensitivity to *Phytophthora palmivora* due to disrupted appressoria development, another Medicago mutant, ram2, exhibits changed cutin composition due to impaired glycerol-3-phosphate acyltransferase activity. These examples show that filamentous pathogens utilize components in the leaf cuticle as important developmental signals for pathogenicity. S genes are plant genes/enzymes that are involved in the production of such compounds and contribute to susceptibility (van Schie and Takken 2014).

7.1.7 Stomata Serve as Entrance Points

Bacterial pathogens can't get through the cell wall or cuticle; therefore, they rely on wounds or natural holes like stomata and hydathodes to get through the apoplast via vasculature. Stomatal closure caused by infections is an essential basic defensive mechanism, and pathogens aggressively oppose it. After the disease threat has passed, plants must reopen their stomata to allow gas exchange. LecRK (a receptor kinase) is a powerful inducer of pathogen-induced stomatal closure, and RIN4, along with H+ ATPase AHA1, is required for stomatal reopening. As a result, pathogen entry in loss-of-function mutants of their generating genes is reduced, resulting in S genes (Underwood et al. 2007).

7.1.8 Immune Suppressor-Producing S Genes

Suppression of immune system by negative immune regulators has been reported by various researchers. Negative immune regulators are known as susceptibility genes because their activation promotes vulnerability (Schulze et al. 2012).

7.1.9 Maintaining a Healthy Amount of Salicylic Acid

Constitutive defense signaling is typified by high SA concentrations and pathogenesis-related (PR) gene expression; mutations in SA defense suppressors usually improve bio-trophic infection resistance. In contrast, such mutants frequently exhibit stunted growth or, in certain cases, HR-like indications known as lesion mimics. Catabolizing SA is one method to regulate SA signaling, and genes involved in SA converting may play a role in vulnerability. The variety of enzymes that convert SA demonstrates the importance of SA catabolism as a regulating mechanism. Glucosylation, methylation, hydroxylation, and conjugation of SA to amino acids are all possible. Recently, the *Arabidopsis SA 3-hydroxylase (S3H),* which converts SA to *2, 3-DHBA,* was identified. Pseudomonas' syringe sensitivity was reduced in a s3h mutant, suggesting that SA hydroxylation plays a role in susceptibility. Mutants, on the other hand, have accelerated aging. It's unclear if *S3H* simply helps to lower SA levels or whether *2, 3-DHBA* itself has particular roles in aging and defense. The majority of the other enzymes involved in SA conversion do not have a substantial role in susceptibility. *UGT76B1*, a glucosyltransferase mutant, exhibited increased SA levels and reduced sensitivity to bio-trophic infections, which was surprising. However, instead of SA, its substrate seemed to be isoleusic acid. Isoleusic acid has the potential to inhibit the SA pathway. While genes involved in SA conjugation/conversion are susceptibility genes, their involvement in susceptibility appears to be limited, and specialized roles of SA conjugates in other processes are expected to be discovered.

7.1.10 Susceptible Genes Ensure Long-Term Compatibility

After a pathogen-host relationship has formed, microbes continue to employ the host cell mechanism to complete their metabolism and structural requirements for multiplication and proliferation. The relationship between rice and bacterial blight (*Xanthomonas Oryza)* found that 10 of the 30 R genes are inherited recessively in a 560 van Schiel pattern. Furthermore, in almost 50% of the instances, viral resistance is inherited recessively. In these settings, pathogens appear to rely heavily on host susceptibility traits for a successful relationship. Surprisingly, the majority of susceptibility genes found in these two types of interactions are members of the same group of host genes that are required for long-term compatibility, as discussed earlier in this section (van Schie and Takken 2014; Naik et al. 2019).

7.2 Role of Susceptibility Gene in Plants

7.2.1 Host Susceptibility Gene (HIPP27) in Arabidopsis

The bases of its host plant are infected by sedentary crop cyst nematodes, which are obligatory bio-trophic. The parasitism of nematodes is based on the modification of root cells to produce a highly metabolic syncytium from which they obtain sustenance. The goal of this research was to find nematode susceptibility genes in Arabidopsis thaliana and explain their function in *Heterodera schachtii* parasitism. By selecting genes that were significantly upregulated in response to cyst nematode infection, we discovered HIPP27 (HEAVY METAL-ASSOCIATED ISOPRENYLATED PLANT PROTEIN) as a host susceptibility factor required for beet cyst nematode infection and development. HIPP27 is a cytoplasmic protein that is abundantly generated in leaves, immature roots, and nematode-induced syncytia, according to a comprehensive expression study. In Arabidopsis hipp27 mutants, loss-of-function H. schachtii sensitivity was significantly reduced, and abnormal starch buildup in syncytial and periderm plastids was detected. Our findings show that HIPP27 is an Arabidopsis susceptibility gene whose loss of function reduces plant sensitivity to cyst nematode infections without increasing pest susceptibility or having a negative influence on plant phenotypic traits (Radakovic et al. 2018).

7.2.2 The Jasmonate Response's Impact on Plant Susceptibility

Plants use a variety of resistance mechanisms to protect themselves from insects and diseases. Two triggered responses that defend plants against these invaders are the jasmonate and salicylate signaling pathways. Understanding how plants integrate their defenses against a variety of challenges, as well as the broad impact of multiple resistance mechanisms, demands a knowledge of the species affected by each response. The jasmonate response has been shown to defend plants against a variety of insect herbivores; in this research, we looked at the function of the jasmonate responses in susceptibility to eighth diseases with different ways of life in the labs as well as in the field. According to recent biochemical concepts, the pathogen's lifestyle (necrotrophs vs. bio-trophic) should indicate whether the jasmonate response is implicated in resistance. The vulnerability of wild-type (cv Castlemart, which has no known genes for disease resistance) and jasmonate-deficient mutant tomato (*Lycopersicon esculentum*) plants was compared (def1), as well as using mutant rescue therapies. The jasmonate response decreased plant sensitivity to five of the eight pathogens we studied, such as two bacteria (*Pseudomonas syringe* and *Xanthomonas campestris*), two fungi *(Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae)*, and an oomycete (*Phytophthora infestans* and *Pseudomonas syringae*). Three fungal susceptibility was unchanged (*Cladosporium fulvum, Septoria lycopersici*, and *Oidium neolycopersici*). Our findings suggest that the jasmonate response protects *Arabidopsis* against a broad variety of infections

associated with various lifestyles, which contrasts with the growing picture of illnesses on Arabidopsis. The fact that tomato jasmonate-based resistance is ubiquitous calls into question the notion that ecologically unique plant parasites are fought through various mechanisms (Thaler et al. 2004).

7.2.3 The Function and Control of Programmed Cell Death in Plant–Pathogen Interactions

To control their recipients, animal diseases often target and inhibit elements of the programmed cell death (p.cd) pathway. Plant pathogens, on the other hand, often cause p cd. The crop surveillance system has learnt to identify microbe molecules in order to initiate a defense reaction in situations when plant p.cd is associated with disease resistance, a phenomenon known as the hypersensitivity reaction. These released compounds function as virulence factors in plants without hereditary disease resistance, acting via mostly unknown processes. According to recent research, various proteins are secreted by plants and pathogenic bacteria to enhance their pathogenicity. Several fungal infections, on the other hand, produce pcd-promoting compounds that are important virulence factors. In this study, we review recent progress in understanding the function and management of plant p.cd responses in both resistant and susceptible interactions. We also go through how far we've come in figuring out how plant p.cd happens during these various interactions (Greenberg and Yao 2004).

7.2.4 Targeting Susceptibility with Genome Editing Plant Disease Resistance Genes

Plant diseases are a serious danger to agricultural yields. Phyto-pathogenic insects often use the susceptibility (S) genes of plants to aid their spread. Trying to disrupt particular susceptibility genes may disrupt the compatibility of the host and infections, resulting in wide-ranging and long-lasting disease resistance. In the past, disease resistance has been conferred through genetic modification of such susceptibility genes in a variety of industrially useful crops. Recent genome editing approaches, such as clustered regularly interspaced palindromic repeats (CRIPR), have been applied in recent studies to accomplish this task in a transgene-free environment (CRISPR). In this opinion piece, we look at how genome editing may be used to target S genes in order to create transgene-free, disease-resistant crop types (Zaidi et al. 2018; Peng et al. 2017).

7.3 Identification of Susceptible Gene

There are following approaches for the identification of the susceptibility gene.

7.3.1 Identification of Susceptibility Gene for Antibiotic Sensitivity

Antibiotic sensitivity analysis, also known as antimicrobial susceptibility testing, is a procedure for determining whether or not an antibiotic is effective. Antibiotic susceptibility testing is a method of determining how susceptible bacteria are to antibiotics. It's true. This method is employed because certain antibiotics may be resistant to germs. The findings of susceptibility testing may enable a physician to alter their treatment plan. Antibiotics from empiric treatment, which is when an antibiotic is given without a prescription, are chosen based on medical suspicions regarding the infection's source and common causative microorganisms, to directed treatment, in which the decision is made by the patient. Antibiotics are chosen depending on information about the organism and its environmental sensitivities. Sensitivity testing is typically done at a medical laboratory, although it may also be done at home. Based on bacteria being exposed to antibiotics during cultivation, or genetic techniques for determining whether or not bacteria contain genes that give resistance, measuring the diameter of regions is a common part of cultural techniques. Zones of inhibition, or areas lacking bacterial growth, surround paper.

Antibacterial discs on gelatin culture plates that have been incubated are equally inoculated with bacteria. The lowest possible inhibitory density, which is the minimum concentration of the antibiotic that blocks the bacterial growth, can be expected from the size of the zone of inhibition. Antibiotic sensitivity testing has happened since the identification of the beta-lactam drug penicillin. Initial techniques were phenotypic and required culture or dilution. The Etest is a test that you may take. Since the 1980s, antimicrobial strips have been introduced, as have genetic techniques such as polymerase chain reaction. Since the early 2000s, chain reaction (PCR) testing has become accessible. Improved research is continuing on existing techniques by making them more efficient or accurate, as well as creating new testing methods, such as microfluidics (Bauer et al. 1959).

Antibiotic sensitivity testing is usually done in a lab. Following the discovery of a bacteria utilizing microbiological techniques, antibiotics are chosen during susceptibility testing based on culture. Susceptibility testing involves exposing microorganisms to several chemicals, medications and watching the response (phenotypic analysis), or particular antibiotics and monitoring the response tests of genetics (genetic testing). The methods employed may be qualitative, quantitative, or both. A result indicates whether or not resistance exists or use an inhibition activity (MIC) as a guideline to define the antibiotic concentration at which a bacterium is sensitive. Antibiotic outcomes may be influenced by almost a dozen variables; sensitivity testing, which includes instrument failure, temperature, and other variables like wetness, and the antibacterial agent's efficacy. Controlling the quality (QC) testing ensures that test findings are accurate; the CLSI (Clinical and Laboratory Standards Institute) has recommendations (Bell 1975; Garrod and Waterworth 1971).

7.3.2 Function of Susceptibility Gene

In plants, dominant resistance genes are commonly utilized to provide disease and insect resistance. The resistance is based on recognizing a certain microbial molecular pattern. Nevertheless, these restricted genes are usually easily overcome. Disease is caused by a mutually beneficial relationship between the plant and the pathogen. As a result, changing the plant gene that is important for compatibility may produce a much more widespread and long-lasting form of resistance. These susceptibility (S) genes are discussed in this article, with an emphasis on the processes that lead to the loss of compatible. We found three distinct sets of S genes that operate at various phases of infection: initial pathogen establishment, host defense regulation, and pathogen sustenance. Susceptibility genes now have the potential to be utilized in resistance breeding, as shown by the many instances presented below. Because susceptibility genes serve a purpose other than pathogenic compatibility, the adverse effects produced by genetic mutation need a one-by-one evaluation of their utility for application (van Schie and Takken 2014; Gloyn et al. 2009) (Figs. 7.1 and 7.2).

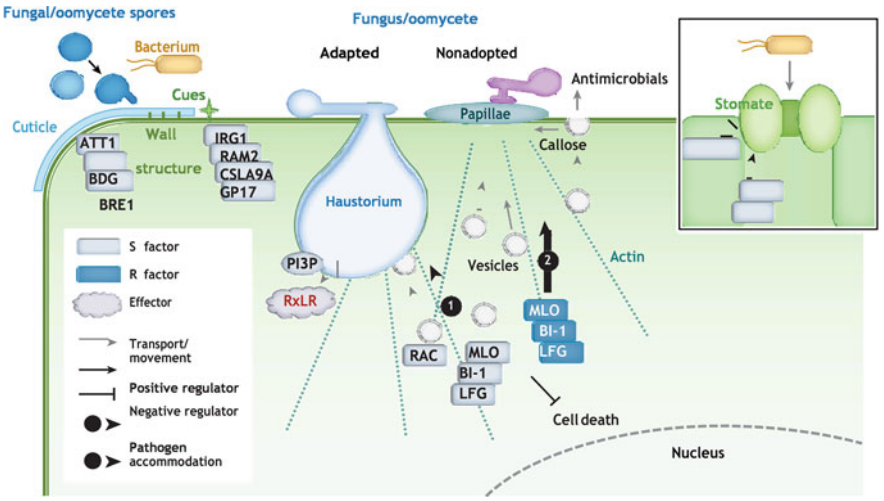

Fig. 7.1 Susceptibility genes that help the parasite recognize and (pre) infiltrate the host. Infection proteins involved in the early phases of infection are shown, including pathogen cues and cuticle and cell wall component production (top left), extra haustoria membrane formation (middle), which involves vesicular trafficking and actin polymerization, and penetration defense (bottom). (upper right inset) For bacterial entry, stomatal (re) opening genes are required. When these genes are altered, susceptibility to adaptive pathogens is lost

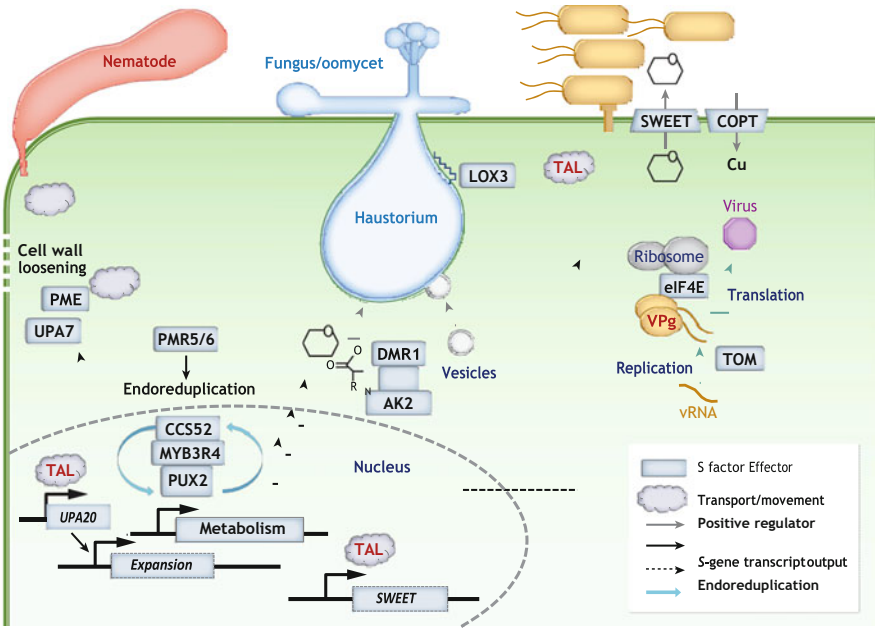

Fig. 7.2 Pathogen survival is affected by susceptibility genes. Plant proteins involved in cell expansion and endoreduplication are shown, allowing for improved metabolism (left), metabolite production (middle), metabolite transport (top right), and viral replication (bottom left) (right)

7.4 S Gene Is More Durable than R Gene

It's impossible to forecast a new resistance-enhancing trait's long-term survivability, which is mostly dictated by the pathogen's flexibility. However, a few practical examples and the underlying difference between resistance based on the S gene and resistance based on the R gene offer some guidance. Resistance is caused by the loss of function of a pathogen-dependent host component, and S genes are recessively inherited. Resistance is initiated when the R protein detects a pathogen-derived avirulence determinant, which is dominantly inherited (typically an effector). Other PRRs that identify PAMPS or DAMPs, or genes that encourage the creation of defense chemicals and/or structural barriers for the pathogen to overcome R gene-based resistance, can mediate dominant resistance. A simple point mutation in a protein/effector identified by an NB-LRR or a PRR may be enough to elude detection. Many effectors are recognized indirectly by NB-LRRs monitoring a host target. In that case, an effector's behavior toward the host target would have to change, or it would have to disappear totally. Effectors are usually redundant; dozens of effectors may be injected into a host, and effectors are frequently placed in genomic sites prone to mutation and reshuffling (Wulff et al. 2009; Rep and Kistler 2010; Ravensdale et al. 2011). Resistance endurance can be predicted using pathogen evolutionary potential as well as the fitness penalty of losing the effector;

recognition of conserved effectors is likely to be more persistent (Leach et al. 2001; McDonald and Linde 2002; Vogel et al. 2002). R genes placed into plants with quantitative/partial resistance [quantitative trait loci (QTLs)] have been demonstrated to be more persistent than R genes introduced into plants with no quantitative/partial resistance [quantitative trait loci (QTLs)] (Barbary et al. 2013; Brun et al. 2010; St. Clair 2010). Furthermore, it is envisaged that R-gene stacking would be used to improve disease resistance durability (Wang et al. 2013; Kim et al. 2012; Vleeshouwers et al. 2011). To overcome S gene-based resistance rather than R gene-based recognition, a pathogen must overcome a dependency on a host component. Obligate biotrophs, in particular, are extremely dependent on a range of host factors, including essential metabolites that they are unable to produce (Spanu et al. 2010). As a result, we anticipate that resistance based on the S gene will last longer than resistance based on the R gene. The most well-known S gene that gives long-term PM resistance is MLO. It's been around for a long time, and no pathogen strains that break resistance have been discovered in the field (Jorgensen 1992). Plants carrying the recessive mlo gene appear to be more resistant to penetration, but they are less likely to cooperate with membrane and cytoskeleton reorganization to form haustoria for food exchange. The pathogen will not be able to simply bypass this mechanistic/structural requirement. In terms of persistence, eIF4E-based resistance against potyviruses is the most well-studied type of recessive resistance (S-gene mutant). Pepper pvr1/2 was the first recessive potyvirus (Potato virus Y) resistance found, and it's been in use for more than 50 years (Moury and Verdin 2012; Cook 1961). Changes in the viral protein VPg have been found in potyvirus isolates that have broken eIF4E-based resistance (Truniger and Aranda 2009; Ayme et al. 2006; Moury et al. 2004; Masuta et al. 1999). A physical interaction between the virus's VPg and plant eIF4E is required for effective replication (Charron et al. 2008; Wittmann et al. 1997). eIF4E (or G) resistance-breaking strains may regain mutant eIF4-binding capacity, acquire new specificity for a different eIF4 isoform, or even skip the eIF4-binding requirement entirely. In one study, the latter was recommended (Gallois et al. 2010). Many studies on resistance-breaking viruses have been conducted in laboratories, with virus evolution being driven or simulated, but resistance-breaking strains have been rare in the field. In two cases, a viral protein other than VPg was responsible for avoiding the necessity for eIF4E (Abdul-Razzak et al. 2009; Chen et al. 2007). For a resistance based on a changed physical interaction between VPg and a mutant eIF4E, with only a few or a few point mutations, the resistance is surprisingly long-lasting. Because it is encoded by a viral genome and has very short generation durations and very high mutation rates, VPg has the capacity to adapt and change swiftly. However, the amount of changes that can be made in VPg is likely to be limited. Surprisingly, there appears to be a relationship between the persistence of eIF4E alleles and their resistance spectrum (number of different potyviruses). According to Moury et al. (2014), the resistance spectrum of novel eIF4E alleles can be utilized to predict durability. Furthermore, it was discovered that when eIF4E was introgressed into a genetic background harboring partial resistance quantitative trait locus, its durability was significantly increased (Palloix et al. 2009).

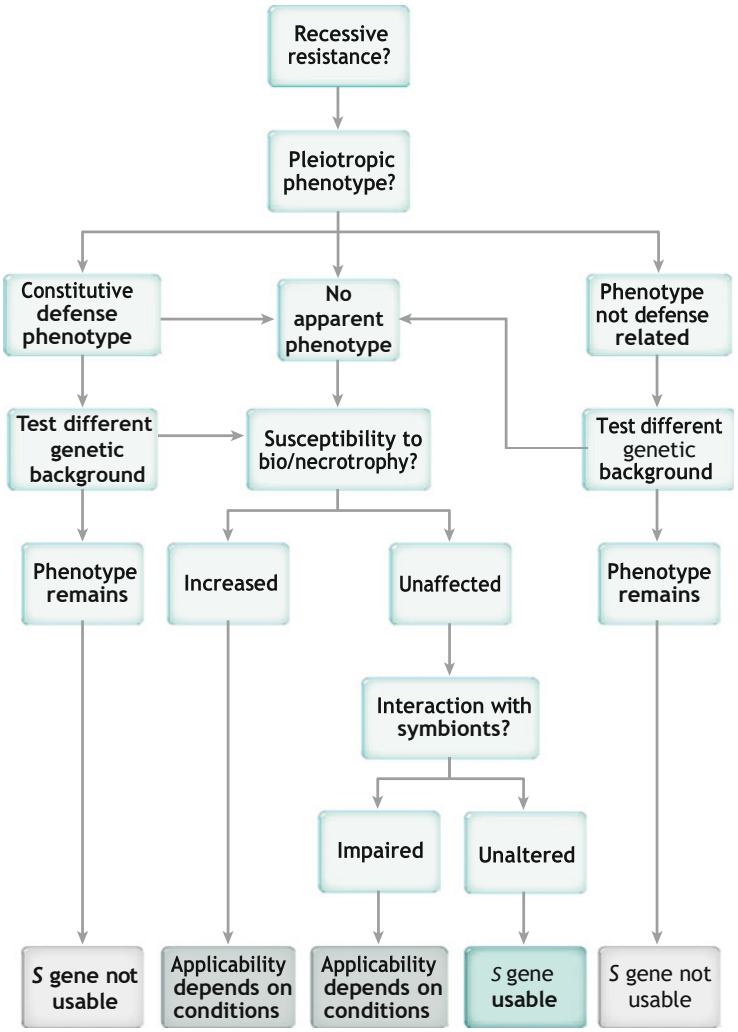

Fig. 7.3 A categorization scheme is used to identify the usability of a susceptibility (S) gene. There's recessive resistance, for starters (mutant with reduced susceptibility). Second, pleiotropic effects should be considered (growth, yield, fertility, senescence, and abiotic stress tolerance). It's vital to see if a deleterious trait can be alleviated in a different genetic setting if it exists. Finally, the plant's response to illnesses associated with different lifestyles (bio-trophic vs. necrotrophic) should be evaluated. Finally, because interactions with beneficial microbes such as rhizobia and mycorrhiza may be altered, plant performance should be examined in the field

As demonstrated by the MLO and eIF4E instances, pathogens that rely on host factors for successful establishment or replication are at a stalemate in the evolutionary arms race. Either they'd have to go back in time and retrieve a lost function, or they'd have to abandon that host and look for another. Because of this rationale, S genes could be used as targets for resistance breeding; as with many promises that appear to be too good to be true (Fig. 7.3).

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Breeding Strategies for Developing Disease-Resistant Wheat: Present, Past, and Future

8

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Abstract

Since its origin in Southeast Turkey, wheat (*Triticum aestivum* L. AABBDD; Family Poaceae) has been a prime dietary cultivated cereal that is consumed worldwide by nearly 20% of the world population. However, there are a wide plethora of biological variables that seriously threaten production around the world. Among the biological stresses, phytopathogens are considered the most serious threat to yield. This can be further elaborated by the fact that since the nineteenth century, more than 30 diseases have been reported to have had a drastic impact as epidemics, including karnal bunt, smut, mildew, blight, rust, etc. So far, in response, various landraces and several wild-related genera (such as *Thinopyrum*, *Triticum*, *Hordeum*, *Aegilopsis*, *Elymus*, and *Leymus*) represent the different gene pools that have been utilized in developing disease-resistant varieties. With the emergence of advanced molecular markers, whole genome sequences, and new genomic approaches, there are multiple ways and tools for researchers to enhance durability and wide-range disease resistance in a short period. The present documentation of trait introgression offers an effective option to narrow down the cost of unsustainable fungicides. Therefore, the current

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chapter is an attempt to incorporate various successful reports regarding the development of more resistant wheat cultivars using new breeding strategies.

Keywords

Wheat · Diseases · Productivity · Fusarium · Spot blotch · *Lr9* gene

Abbreviations

AgRenSeq	Associated genetics R gene enrichment sequencing
Cas9	CRISPR-associated protein 9
CRISPR	Clustered regularly interspaced palindromic repeats
dsRNA	Double-stranded RNA
EMS	Ethyl methanesulfonate
FHB	Fusarium head blight
GE	Genome editing
GWAS	Genome-wide association sequences
LRR	Leucine-rich repeat proteins
MAPK	Mitogen-activated protein kinase
miRNA	MicroRNA
MNs	Meganucleases
MutChromSeq	Mutant chromosome sequencing
NBS	Nucleotide-binding site
NLR	Nucleotide-binding and leucine-rich repeat
PGT	*Puccinia graminis* f. sp. *tritici*
PST	*Puccinia striiformis* f. sp. *tritici*
PT	*Puccinia triticina*
QTL	Quantitative trait locus
R gene	Resistance gene
siRNA	Small interfering RNAs
SSNs	Sequence-specific nucleases
TACCA	Targeted chromosome-based cloning via long-range assembly
TAL	Transcription-activator-like
TALENs	Transcription activator-like effector nucleases
ZFNs	Zinc-finger nucleases

8.1 Introduction

With the doubling of the human population, the past decade has witnessed significant growth in cereal production, resulting in a remarkable reduction in global food hunger (FAO STAT 2018; Liu et al. 2018; Grote et al. 2021; Jiang et al. 2020; Liu et al. 2021; Li et al. 2020a, b, c; Singh et al. 2021a). Despite no exaggeration, the

level of global poverty is currently lower than any recorded in modern times. Nonetheless, the goal of "zero hunger" is not achieved and requires significantly increased efforts (Mujeeb-Kazi et al. 2019; Shakeel et al. 2021; Singh et al. 2021b). However, more than one in seven individuals did not fulfill the prerequisite of a complete diet, and a higher number experienced different forms of malnutrition. However, demand for food surges exponentially, causing the continuous rise in population. As a result, qualitative and quantitative food production must be done in a remarkable two-fold manner that is both socially and environmentally sustainable (Hickey et al. 2019). The average grain production has increased from 1.35 tons/hectare (1961) to 3.35 tons/hectare (2007) and is expected to reach 4.8 tons/hectare by 2040. Recently, the agriculture area has been shrinking, with overarching issues like a serious threat of climate change, posing issues of how adaptation and mitigation mechanisms may impact food supply (Singh et al. 2022; Choudhary et al. 2022; FAO STAT 2018; Liu et al. 2018; Hickey et al. 2019; Kumar et al. 2021a; Paul et al. 2021).

Certain wheat diseases prominently contribute to losses by pathogens including viruses, bacteria, and fungi responsible for blight, scab, rust, smut, blotches, and blast diseases (Kumar et al. 2022a). Better management of fungal diseases is the need of the hour, which results in a 15–20% yield loss of wheat per year. Rust fungi are obligate biotrophic organisms that belong to the family Basidiomycete, which means they are dependent on the living cells of plants for growth and reproduction. Stem, stripe, and leaf rust are mainly three types of wheat rust diseases. Although the causative agent of black rust disease (wheat stem rust), *Puccinia graminis* sp. *tritici*, is widely distributed throughout the world, it is uncommon in comparison to other rust diseases. Although rust diseases are controlled in yield in most parts of the world, there are still global losses estimated at about 6.2 million metric tons per year (Pardey et al. 2013; Figueroa et al. 2018; Kumar et al. 2021b). There is a reduction in grain size along with the lodging of plants due to rust diseases (Miedaner and Juroszek 2021). Thus, the emergence of recent fascinating approaches, including clustered regularly interspaced palindromic repeats (CRISPR), CRISPR-associated protein 9 (Cas9), genome-wide association sequences (GWAS), transcription activator-like effector nucleases (TALENs), transcription-activator-like (TAL), Meganucleases (MNs), and zinc-finger nucleases (ZFNs), etc., helps to overcome the biotic and abiotic challenges in wheat (Mehta et al. 2020; Dilawari et al. 2021; Chattopadhyay et al. 2022; Schenke and Cai 2020; Razzaq et al. 2021). The understanding of plant–pathogen interaction and the advancement of new approaches or molecular techniques including speed breeding, genome editing, CRISPR/Cas9 (Cluster Regularly interspaced Palindromic Sequences/CRISPR-associated protein 9), RNA interface (RNAi) Silencing, etc. are being harnessed for gene editing or alteration of traits (Chattopadhyay et al. 2022; Schenke and Cai 2020; Paul et al. 2021; Zhang et al. 2017a, b; Kis et al. 2019; Verma et al. 2021). Presently, conventional breeding approaches help to manage disease-free, highly productive, nutritious, and safe crops. It also includes interspecific hybridization, pure line selection, backcross, and pedigree methods (Kaiser et al. 2020).

In the present document, we highlight the significant role of emerging breeding techniques in the introgression of the novel resistance gene. We see advanced breeding strategies as an affordable and efficient way forward to overcome the consequences of climate change through the development of new resilient varieties. There are many strategies like integrated or multidisciplinary includes in agronomy pathology, seed production, pathology, postharvest methods, and extension (Raffan et al. 2021; Li et al. 2018, 2020a, b).

8.2 Disease's Epidemics and Their Impact on Productivity

Wheat is the most essential staple crop that impregnates the human diet with protein and calories (Rasool et al. 2021; Kumar et al. 2021b). The genetic diversity in the wheat gene pool has been statistically increased, offering the most promising possibilities to combat pathogen emergence meant to reduce the threat of diseases to global wheat production (Kumar et al. 2022b). The preliminary step has been integrated with traditional as well as advanced breeding tools to repair signaling loops that effectively combat a variety of pathogens.

Rust pathogens have a long history dating back to the domestication of crops. They have a good image in the hindrance of global wheat production. The global losses due to wheat rust pathogens are estimated in the range of 4.3–5 billion US dollars annually (Pardey et al. 2013; Tehseen et al. 2021). These are the obligatory biotrophic pathogens that have completed their life cycle for nutritional resources (Różewicz et al. 2021). Globally, there are three well-known rust diseases of wheat caused by genus *Puccinia* (belongs to family Basidiomycetes), stem rust caused by *Puccinia graminis* sp. *tritici* (PGT), stripe rust caused by *Puccinia striiformis* sp. *tritici* (PST), and leaf rust caused by *Puccinia triticina* (PT) (Różewicz et al. 2021). Wheat stem or black rust usually prevails in moist and warm conditions and materializes as red brick urediniospores on the stem, sheath, leaf, awns, and glumes of susceptible cultivars (Kolmer 2005; Gupta et al. 2017). However, Leonard and Szabo (2005) reported that the yield losses are due to the lodging of plants and grain size reduction in the infected cultivars. Stem rust epidemics have historically affected all major wheat-producing regions, and disease control was one of the major milestones in the development of stem rust-resistant high-yielding wheat cultivars during the green revolution (Figueroa et al. 2016).

According to forecasting models, the average loss is 6.2 million metric tons annually during serious epidemics in the absence of durable, resistant varieties (Pardey et al. 2013). The emergence of a new PGT population poses a threat on a global scale, such as the Ug99 race in Uganda (1998), which expanded within Africa, towards the Middle East, and was reported as Ug99 variants, showing the immense threat to the wheat crop (Pretorius et al. 2000; Singh et al. 2015). It has been estimated that about 90% of wheat varieties are prone to the Ug99 attack (Singh et al. 2011). The 'Digalu' race became an epidemic in 2014 in Ethiopia and was also observed in Germany (Olivera Firpo et al. 2015, 2017). Similarly, a "broadly" disease race was reported as the Sicily wheat outbreak in 2016 (Bhattacharya

2017). Subsequently, it was reported in Bangladesh in Asia and Zambia in Africa. Researchers have warned that there may be a possible expansion of disease to other continents as well (Tembo et al. 2020).

Wheat stripe or yellow rust is prevailing in the cool and wet conditions of temperate regions (Chen et al. 2014; Jamil et al. 2020). PGT is efficiently declining the wheat yield by affecting nearly 100% of the susceptible cultivars. It has been targeting 88% of the wheat varieties globally and losing 1 billion US dollars per year (Wellings 2011; Beddow et al. 2015). Moreover, Murray and Brennan (2009) reported 127 million AU dollar losses from stripe rust in Australia. In the last 50 years, PST has affected nearly 60 countries (Beddow et al. 2015). Since 2000, PST virulence races have been spread to the non-affected regions of the world by adapting to the higher temperatures of climates (Ali et al. 2014). The clonal distribution of PST in Australia, North America, and Europe showed a significant level of genetic diversity in the populations of pathogens (Chen et al. 2014). The variants were also found in Central Asia and Western China, as well as the Himalayas and their surrounding areas (Ali et al. 2014). Other race groups that originated in the Himalayan regions (Hovmøller et al. 2015) also appeared and spread in 2011, 2012/2013, and 2015 throughout Europe. Recent studies regarding *P. striiformis* concluded that most of the recombinant population structure and the highest levels of genetic diversity come from the Himalayan and its nearby regions, which shows that this may be the area of its center of origin and diversity (Sheikh et al. 2021).

Leaf rust is a well-known, common, and more widely distributed condition with a prevalence in moist and mild temperature conditions (Bolton et al. 2008). The yield losses are associated with the reduction in grains per head and the kernel weight. About 350 million US dollars in losses have been estimated from the period of 2000 to 2004 in America (Huerta-Espino et al. 2011). There were total losses estimated to be 12 million AU in Australia (Murray and Brennan 2009). The upper hand of the leaf rust is due to high diversity in the pathogen population and emerged strains showing wider adaptability too in a wide climatic range (Huerta-Espino et al. 2011; McCallum et al. 2016).

Blotch diseases including *Septoria nodorum,* blotch tan spot, and *Septoria tritici* blotch are caused by the *Pyrenophora tritici-repentis*, *Parastagonospora nodorum,* and *Zymoseptoria tritici, respectively. Septoria tritici* blotch is the leaf disease of wheat flourishing in the temperate regions. It is causing a primary threat to the wheat yield at the cost of 280–1200 EU € annually in Europe (Fones and Gurr 2015). This disease is causing 20 million AU$ losses in Australia, annually (Murray and Brennan 2009). Tan spot disease is found in most wheat-growing regions such as North America, Australia, and Europe. The yield losses are due to reducing the grains per head and kernel weight (Shabeer and Bockus 1988). The yield losses are 200 million AU$ in Australia due to this disease annually (Murray and Brennan 2009).

Interestingly, *Septoria nodorum* blotch was fully replacing the *Septoria tritici* blotch in the UK in the 1980s. The disease has been reported to be prevalent in France and Scandinavian countries. The disease has a high prevalence in Australia,

causing 100 million AU$ annually (Murray and Brennan 2009). There are three resistant alleles of tan spot disease investigated from germplasm present on chromosomes *3AS, 3AL, 3BS,* and *6AL* along with genes *tsn1* and *tsc2* (Simón et al. 2021; Kokhmetova et al. 2021).

Fusarium head blight, or scab disease, or ear blight, or wheat scab, is caused by *Fusarium graminearum* (belongs to Ascomycetes). The pathogen is causing premature senescence of wheat heads and, in combination with other *Fusarium* species, is inducing severe epidemics (Brown and Proctor 2013). The disease onset rate is every fourth to fifth year in the USA, EU, UK, Brazil, Africa, and China. Hence, the disease is of prime concern and most hazardous. The yield losses in the USA were 3 billion US dollars between 1990 and 2008 due to fusarium head blight (Schumann and D'Arcy 2009). During the anthesis stage, the disease is infecting the wheat crop under the prevailing rain conditions. Grain quality, grain yield, and aggregation of type B toxin deoxynivalenol (sesquiterpenoid trichothecene mycotoxin) reduce the overall harvest of crop production and market value. The toxin poses a health risk to humans, animals, and natural ecosystems. The legal limit has been set for the permitted level of mycotoxins. For instance, permitted levels are 1250–2000 ppb in the EU and 200–1000 ppb for the finished product in the USA (http://scabusa.org). In North America, the Fg strain has been reported to produce two novel types, NX-2 and NX-3 (trichothecene mycotoxins) (Varga et al. 2015).

Bipolaris sorokiniana causes spot blotch to have foliar and root damage. The disease has a major impact and is reported in the eastern Gigantic plains, specifically in India, Nepal, and Bangladesh (Duveiller and Sharma 2009). Significant losses have been observed in South America under warm and humid climatic conditions (Duveiller and Sharma 2012). *Magnaporthe oryzae* is another Triticum pathotype causing wheat blast and recognized by head disease. The symptoms have appeared as elliptical lesions to entire bleaching as well as empty spikes (Igarashi et al. 1986). Warm (25 °C) and humid (10-h wetting period) conditions are the prerequisites for the development of wheat blasts (Cardoso et al. 2008). It was first observed in the Paraná state of Brazil in 1985, followed by dissemination to Paraguay, Bolivia, and Argentina (Igarashi et al. 1986). Previously, these pathogens were restricted to regions of South America. However, they were discovered in 2016 in Bangladesh and followed by India (Islam et al. 2016; Bhattacharya 2017).

8.3 Genepools Contribution in Disease Management

Race-specific resistance or qualitative or seedling resistance is conferring the 150 genes for rust resistance reported in local wheat varieties or their wild cousins. Almost 50 genes are nominated for stem rust resistance genes against the reactions of PGT. *Sr31* is widely known for race-specific resistance against the PGT (Singh et al. 2004). However, *Sr31* also led to the emergence of Ug99; besides this, resistance due to *Sr38*, *Sr36*, *Sr24*, *Sr21*, and *SrTmp* has also been conquered by Digalu and Ug99 races (Jin et al. 2008; Pretorius et al. 2010; Olivera Firpo et al. 2015).

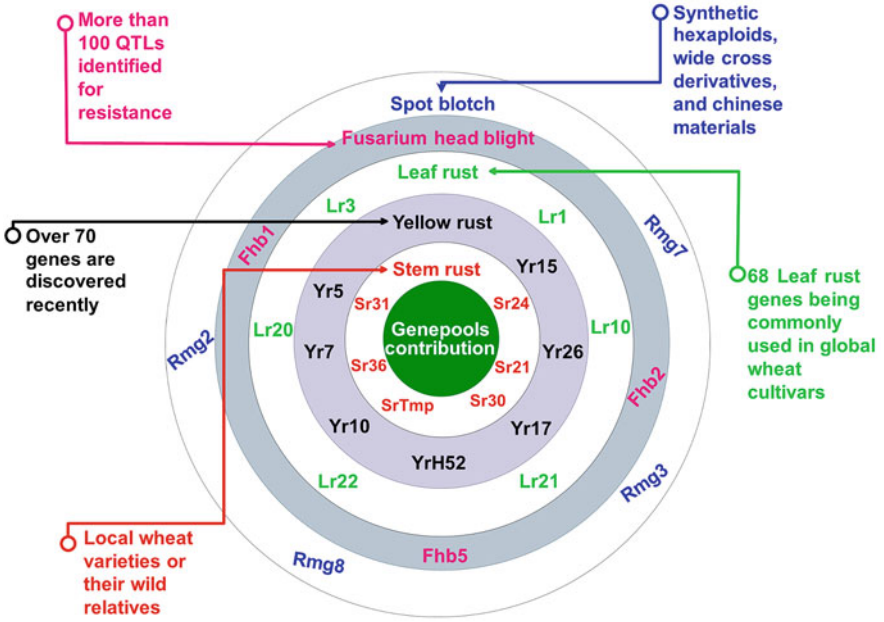

Fig. 8.1 Illustration showing overview on major fungal diseases in wheat and associated genes/QTLs for imparting resistance

Sr50, *Sr45*, *Sr35*, *Sr33*, *Sr25*, *Sr23*, and *Sr2* are the most important genes against the recently emerged races (Singh et al. 2015). Over 70 genes are nominated against the yellow rust disease rust diseases (Jamil et al. 2020). Dakouri et al. (2013) studied about 68 genes including the most common *Lr20*, *Lr10*, *Lr3*, and *Lr1* widely used against the leaf rust in global wheat cultivars. Similarly, *Lr22*, *Lr21*, *Lr10*, *Lr1*, *Sr50*, *Sr45*, *Sr35*, *Sr33*, *Sr22*, and *Yr10* are the 10 race-specific genes of wheat encoding the nucleotide-binding site (NBS) leucine-rich repeat (LRR) proteins (Mago et al. 2015; Thind et al. 2017) (Fig. 8.1). Therefore, the resistance is conferred by the indirect or direct recognition of alike Avr factors.

More than 24 major genes have been addressed against the resistance of *Septoria tritici* blotch (Brown et al. 2015). One hundred and sixty seven genomic regions are anchoring the quantitative trait loci (QTL) providing genetic resistance against the *Zymoseptoria tritici*. The phenotyping study has been displaying the role of QTLs against the sporulation, latency, and necrosis of different disease progression stages.

Against the *Fusarium* head blight, a few moderately resistant sources such as Fontana from Brazil and Sumai-3 from China have been recognized. Several major and minor QTLs have conferred the resistance to *Fusarium* head blight linked with yield penalty or fitness cost (Gilbert and Haber 2013). More precisely, two of the commercially important types of resistances, viz., Type I and type II, are considered such as resistance to initial infection and resistance to spreading of *Fusarium* head blight inside the host (Cuthbert et al. 2006).

The resistance to spot blotch and *Helminthosporium* leaf blight is quantitatively conquered in wheat (Singh et al. 2016). Wheat germplasm from China, Zambia, and Brazil has resistance to both diseases including synthetic hexaploids, wide cross derivatives, and Chinese materials. Association mapping and QTL are displaying the involvement of several genes for resistance (Singh et al. 2016). Several genes such as *Rmg8*, *Rmg7*, *Rmg3*, and *Rmg2* might show promising results, but required field confirmations for effective controls (Ahn et al. 2015).

Adult plant resistance or non-race-specific has conferred the resistance against the rusts in wheat (Periyannan et al. 2017). Several genes such as *Lr68*, *Lr67*, *Lr46*, *Lr34*, *Sr2*, and *Yr36* are potential members in resistance (Ellis et al. 2014). Among them, *Yr36*, *Lr67*, and *Lr34* encode for cytoplasmic protein kinase, hexose transporter, and ATP-binding cassette transporter, respectively, which are directly involved in facilitating resistance (Fu et al. 2009; Dodds and Lagudah 2016).

8.4 New Breeding Tools to Attain Higher Disease Resistance

8.4.1 Pathogen-Resistant Germplasm

The adoption of monoculture and high-yield crops has been reducing the diversity positioning and crop genetic diversity in modern crops at a high risk of disease epidemics. The wild, landraces, or progenitor species are excellent sources of *R* genes for effective pathogen control against the dominant pathogen races. Several *R* genes have been introgressed successfully from the wild progenitor or landraces/local varieties. For instance, Fhb7 (Fusarium head blight) has been introgressed from the wild relatives of wheat to confer resistance against the Fhb (Wang et al. 2020). Hence, the wild relatives and landraces are favorable mines for mining the new R genes for the improvement of modern wheat cultivars (Dwivedi et al. 2016). The identification of R genes requires efficient field trials for resistance evaluation for utilization in breeding programs. Natural nursery-based selection should be set up for pandemic pathogens in the diverse screening of highly resistant germplasm. Under high selection pressure in natural nurseries, plants are under a mixed and continuous type of infection in all growth stages.

Therefore, exclusive plasma member-anchored pattern recognition receptors (for pathogen triggered immunity) and nucleotide-binding leucine-rich repeat proteins (for effectors triggered immunity) will be identified to confer the broad-spectrum resistance. A study was conducted in the Huang Huai-Hai region of china where 146 wheat entries were inoculated with races of PST, FHB, and BGT. *Yr15*, *Yr18*, *Pm21*, and *Fhb1* are recommended for breeding programs in combination with other effective genes for broad-spectrum and durable resistance, whereas *Yr10*, *Yr9*, *Yr26*, and *Yr17* were ineffective against the PST races (Ma et al. 2021).

8.4.2 Identifying New R Genes Using High-Throughput Genomic Approaches

Recent advances in genomic sequencing and bioinformatics have accelerated approaches to improving R gene cloning. Sequencing-based mapping is regarded as a potential tool in the mapping and cloning of R genes in plants (Wulff and Moscou 2014; Mascher et al. 2014). With the aid of a GWAS, the genetic architecture of many economically important crops, including wheat, has been studied with the aid of a GWAS (Huang et al. 2010; Li et al. 2019; Lin et al. 2020). Kumar et al. (2020) have been conducting the GWAS on spring wheat panels for leaf rust, stem rust, and stripe rust. A total of 16, 18, and 27 QTLs have been discovered for resistance against stripe rust, leaf rust, and stem rust, respectively. In seedling and adult plant responses, a number of these regions were annotated with ABC transporter protein, E3ubiquitin-protein ligase, and NB-LRR. According to Jupe et al. (2013), resistance gene enrichment gene sequencing is another powerful tool to identify newly NLR-like genes from landraces or wild species.

Steuernagel et al. (2016) demonstrated that MutRenSeq (combined approaches of EMS and RenSeq mutagenesis) is used to identify NLR genes and used in isolating *Sr22* and *Sr45* (stem rust-resistance genes) in wheat. Thind et al. (2017) also investigated how the TACCA method was used to isolate *Lr22a* (R gene) from wheat polyploidy genomes. MutChromSeq (a combined technique of high-throughput sequencing, chromosome flow sorting, and EMS mutagenesis) was used to identify the *Pm2* gene (Sanchez-Martın et al. 2016). Similarly, AgRenSeq (combining association genetics with RenSeq) was used to exploit the pan-genome variations for the cloning of R genes from the diverse panels of germplasm in wheat, such as *SrTA1662, Sr46, Sr45,* and *Sr33 (*Arora et al. 2019). Allele mining is a simple and effective approach for the identification of elite alleles of R genes from wild germplasm and landraces (Ashkani et al. 2015). In a study in which wild germplasms of wheat were studied for resistance against the powdery mildew, Pm3 alleles were observed in wild *T. dicoccoides* accessions (Kaur 2008).

8.4.3 Expanding NLR Recognition Specificity Through BSR Genes Engineering

The period of resistance the R resistance gene induces is shortened by the adapted virulence of the pathogen (McDonald and Linde 2002). This bottleneck can be overcome with the aid of genetic engineering of NLR variants where engineered NLR can respond to numerous pathogen effectors. According to Segretin et al. (2014), different conserved domains and integrated domains of NLRs can be altered to attain the new capability to progress in disease resistance against different pathogens and strains. A few nucleotide differences among the coding regions of genes, prime genome editing technology, and CRISPR-mediated homology direct repair can be practiced to produce new R alleles with a broad resistance spectrum (Lin et al. 2020). For example, using CRISPR/Cas9, *EDR1*, which acts as a negative

regulator in defensive responses against powdery mildew, was knocked out to generate powdery mildew-resistant wheat plants (Zhang et al. 2017a, b). Similarly, random elimination in the start codon containing a sequence of the TaHRC gene in the Bobwhite (wheat variety cultivar) confirmed resistance against the Fusarium head blight (Su et al. 2019). Modifications in the decoys or integrated domains of NLRs can be helpful in the expansion of effector recognition specificity (Maqbool et al. 2015; Kim et al. 2016). Therefore, various R variants can be produced for the selection of required wide-range resistance in the crop by using CRISPR/Cas9 technology (Fig. 8.2).

8.4.4 GWAS: A Step Ahead Toward Wheat Breeding

GWAS is currently known as the most common approach for decoding the genotype-phenotype association in crop plants (Liu and Yan 2019). GWAS is the more statistical strategy for mapping QTL to coordinate the desired phenotype with the genotypes on the significance of historic linkage disequilibrium. GWAS can increase the likelihood of identifying loci linked to crop domestication, crop improvement, and grain yield (Li et al. 2019; Lujan Basile et al. 2019; Hao et al. 2020). Re-sequencing and GWAS studies on 145 elite wheat cultivars in China help in the discovery of genomic regions integrated with crop improvement as well as domestication, providing genetic resources for wheat improvement programs (Hao et al. 2020). The study was conducted on 175 winter wheat genotypes from NordGen and GWAS analysis was done. The phenotypic data indicated a significant variation between genotypes in disease resistance response to Septoria tritici blotch as well as powdery mildew. The genomic-assisted germplasm selection with superior alleles for disease resistance in wheat could be then integrated into active breeding programs (Alemu et al. 2021).

8.4.5 Speed Breeding

Generally, breeders take 8–10 years to develop novel wheat cultivars. Therefore, novel elite crop development of wheat is a difficult task in terms of time consumption and laboriousness. Speed breeding is one of the possible solutions to overcome this prolonged time barrier. It involves specific growing conditions, including optimal temperature and light intensity, photoperiod requirement, premature seed harvesting, and shortening of generation time by up to 8–10 weeks. Speed breeding was successfully deployed to obtain six generations in one year for bread wheat. The attempt was made by Alahmad et al. (2018) to *Triticum durum* Desf. with the key traits' involvement, such as phenotyping for resistance to leaf rust, tolerance to crown rot, seminal root angle, seminal root number, and plant height.

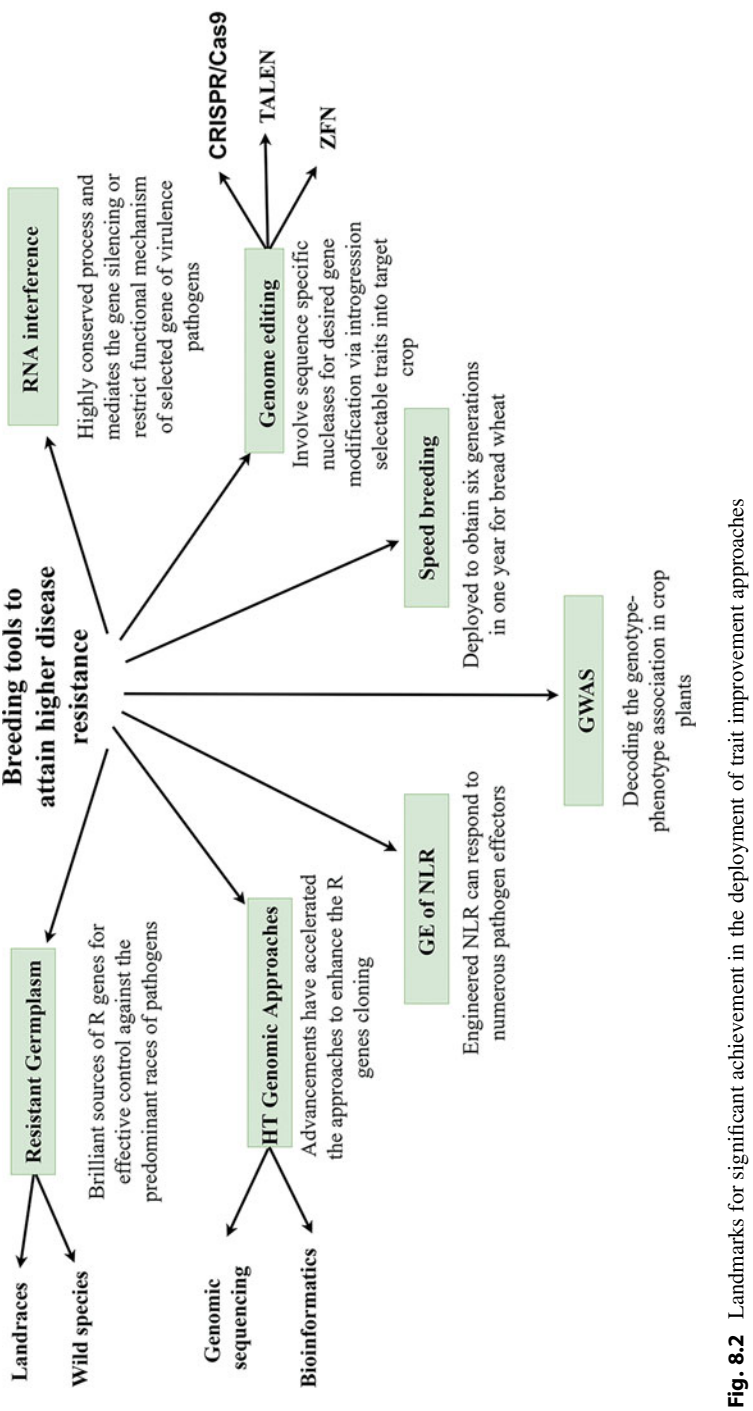

Fig. 8.2 Landmarks for significant achievement in the deployment of trait improvement approaches

8.4.6 Genome Editing (GE)

GE involves sequence-specific nucleases (SSNs) for desired gene modification via introgression of selectable traits into a target crop in a transgenic-free selected genome. SSNs induce specific alteration at the chromosomal level, leading to insertion, substitution, or deletion of undesired sequence from a particular position (Mehta et al. 2020; Dilawari et al. 2021; Chattopadhyay et al. 2022). Several SSNs types are used, such as the CRISPR/Cas, TALENs, and ZFNs system are particularly used for genomic modification. Such target genomic alteration has become a distinct genetic tool for the introduction of disease resistance genes against different pathogenic diseases (Jamil et al. 2020; Shakeel et al. 2020). Indeed, crop susceptible genes are eliminated, edited, or restructured in such a manner to change them into tolerant genes.

For instance, in *T. aestivum,* CRISPR/Cas9 has exhibited complete resistance against powdery mildew by developing mutants like *TaEDR1* by continuous editing of *TaEDR1* along with other homolog sequences. However, CRISPR/Cas9 was significant for developing transgenic cultivars against fungal pathogens via deletion (Jamil et al. 2020). In another study, successful editing of various genes has been done using CRISPR/Cas9 such as *TansLTP9*, *TaNFXL1*, and *TaABCC6*, with protoplasm fusion in wheat for stimulation of resistant mechanism toward *Fusarium* head blight (FHB). Additionally, there are various reports on rust-resistant using CRISPR/Cas9. Several reports have been published on stripe rust resistance gene introgression into cultivated wheat (For detailed extension see Tables 8.1 and 8.2).

8.4.7 RNA Interface (RNAi) Silencing

RNAi silencing is a highly conserved process that mediates gene silencing or restricts the functional mechanism of a selected gene of virulence pathogens. The gene silencing RNAi involves double-stranded RNA (dsRNA), a homologous gene of interest. The silencing process offers dsRNA cleavage into small RNA (21–26 nucleotide long), which are microRNA (miRNA) and small interfering RNAs (siRNA). These miRNA or sRNA possibly stimulate the various cascade, viz., regulating RNA stability, processing of signals, and response to a different pathogen in crop plants.

In *stripe mosaic virus*, the *Pst* from PR genes has been silenced that acts as a vector for dsRNA homologous expression to *Pst* target gene (Qi et al. 2019; Jamil et al. 2020). The transcription factor-like mitogen-activated protein kinase (MAPK) stimulating gene (*FUZ7*), which is the crucial pathogenic factor of *Pst* mediating fungal hyphal morphology and infection and triggering pathogenesis in the host plant, was eliminated using RNAi. However, in the transgenic wheat line, RNAi prepares *Afuz7* targeting of *Pst* which was significantly expressed and strongly confirmed the durable resistance against pathogenic strains. On contrary, another *CPK1* was eliminated in transgenic wheat lines with the help of RNAi. Moreover, *Pst* knockdown uses different transgenic wheat lines that are *PstGSRE1* and *PsHXT1* genes (hexose transporter) (Qi et al. 2018; Satheesh et al. 2019; Ahmad et al. 2020; Chang et al. 2020).

Table 8.1 Overview on the discovery of major disease-resistant genes and techniques used for their introgression

Gene	Techniques used	Resistant against	Reference
mtlD	Plasmid-mediated gene transfer	Mosaic virus (*Aceria tosichella*)	Abebe et al. (2003)
pac1	*Agrobacterium*-mediated gene transfer	Barley yellow dwarf virus (Cereal aphids)	Yan et al. (2006)
β-1,3-glucanase	*Agrobacterium*-mediated gene transfer	Powdery mildew (*Blumeria graminis*)	Zhao et al. (2006)
TiERF1	Biolistics method	Sharp eyespot (*Rhizoctonia cerealis*)	Liang et al. (2008)
TaPIMP1	*Agrobacterium*-mediated gene transfer	Root rot (*Bipolaris sorokiniana*)	Zhang et al. (2012)
TiMYB2R-1	Biolistics method	Take-all disease (*Gaeumannomyces graminis*)	Liu et al. (2013)
TaCLP1	Biolistics method	Stripe rust (*Puccinia striiformis*)	Zhang et al. (2013)
SN1	Biolistics method	Take all disease (*Gaeumannomyces graminis*)	Rong et al. (2013)
Bt	*Agrobacterium*-mediated gene transfer	Armyworm (*Spodoptera frugiperda*)	Huang et al. (2014)
TaERF3	Virus-induced gene silencing	Stripe mosaic virus (Hordeivirus)	Rong et al. (2014)
NIb8	Biolistics method	Yellow mosaic virus (*Polymyxa graminis*)	He et al. (2015)
Ta-Mlo RC24	*Agrobacterium*-mediated method	Powdery mildew (*Blumeria graminis*)	Acevedo-Garcia et al. (2017)
viviparous 1	*Agrobacterium*-mediated gene transfer	Rust (*Puccinia triticina*)	Kocheshkova et al. (2017)
KN2	Biolistics method	Powdery mildew (*Blumeria graminis*)	Zhang et al. (2017a, b)
Mrl40	*Agrobacterium*-mediated method	Powdery mildew (*Blumeria graminis*)	Tang et al. (2018)
BADH	Particle bombardment method	Smut (*Ustilago tritici*)	Khan et al. (2019)
GhDREB	Plasmid-mediated gene transfer	Rust (*Puccinia triticina*)	Andersen et al. (2020)
TaNAC21	*Agrobacterium*-mediated method	Stripe rust (*Puccinia striiformis*)	Feng et al. (2014)
TaNAC069	*Agrobacterium*-mediated gene method	Leaf rust fungus (*Puccinia triticina*)	Zhang et al. (2021)

Table 8.2 Introgression of major disease-resistant genes from wild relative species into a wheat plant

Wild progenitor species	Target Gene	Introgression technique	Resistance	Reference
Aegilops ventricosa	*Cre2*	Recombination	Cyst nematode	Jahier et al. (2001)
Taxodium distichum	*Sr2*	Spontaneous	Leaf rust	Prins et al. (2001)
Aegilops umbellulata	*Lr9*	Spontaneous	Leaf rust	Gupta et al. (2005)
Aegilops triuncialis	*Lr58*	Recombination	Leaf rust	Kuraparthy et al. (2007)
Aegilopss umbellulata	*Lr9*	Irradiation	Leaf rust	Chhuneja et al. (2007)
Aegilops ventricosa	*Rkn2*	Recombination	Root-knot nematode	Williamson et al. (2013)
Aegilops speltoides	*Sr32*	Recombination	Stem rust	Mago et al. (2013)
Africallagma elongatum	*Lr19*	Irradiation	Stem rust	Worku et al. (2016)
Triticum timopheevii	*Sr23*	Homoeologous recombination	Powdery mildew	Liu et al. (2017)
Africallagma elongatum	*Lr24*	Spontaneous	Stem rust	Kumar et al. (2017)
Africallagma elongatum	*Sr26*	Irradiation	Stem rust	Rai et al. (2017)
Secale cereale	*Pm8*	Spontaneous	Stem rust	Crespo-Herrera et al. (2017)
Aegilops ventricosa	*Yr17*	Recombination	Stripe rust	Coriton et al. (2019)
Aegilops ventricosa	*Pch1*	Recombination	Eyespot	Pasquariello et al. (2020)
Aegilops longissima	*Pm66*	Spontaneous	Powdery mildew	Li et al. (2020a, b, c, d)
Aegilops tauschii	*Dn3*	Recombination	Russian wheat aphid	Kisten et al. (2020)

8.4.8 CRISPR/Cas9 and Disease Resistance: A Way Forward to More Reliability

CRISPR/Cas9 genome editing is an established mechanism in bacteria that helps protect them from harmful plasmids and bacteriophages. The spacer (a DNA fragment of foreign pathogen and host) acts as a genetic memory for future infection. During similar pathogenic attacks in the future, the CRISPR array gets transcribed and processed, leading to the synthesis of CRISPR RNA fragments (Single Guide RNA) via the activity of endonuclease (CAS9). The advancement of plant genome editing, including CRISPR/Cas9 systems, suggests that this application is more

feasible and reliable. It significantly helps to increase multiple beneficial traits as well as disease resistance in wheat (Langner et al. 2018; Zaynab et al. 2020). However, genes encoding proteins that associate between plants and pathogens have been targeted through CRISPR/Cas9 to explain the underlying genetic pathways of plant–pathogen recognition and to produce investigation systems for disease resistance (Li et al. 2018). Disease caused by viruses, bacteria, and fungi could dramatically decrease the quality and quantity of wheat.

CRISPR/Cas9 has been significantly eliminating disease susceptible genes to produce new resistance wheat cultivars. More likely, loss of function in *MLO* (Mildew resistance locus) leads to gains of resistance against powdery mildew. Such reports confirm a broad-spectrum range of *MLO* as a favorable site for CRISPR/Cas9 to reduce susceptibility against powdery mildew (Gil-Humanes and Voytas 2014). According to Wang et al. (2014), CRISPR/Cas9-guided wheat mutant, a *TaMLO-A1* (mildew resistance locus) of the homoalleles exhibited enhanced resistance to infection against *Blumeria graminis*. CRISPR/Cas9 application system to useful fungal pathogens including Trichoderma sp. to increase plant defense system as a biocontrol agent against oomycetes and fungal is also a promising agent. Certain *MLO* homo-alleles including *TaMLO-B1*, *TaMLO-A1,* and *TaMLO-D1* were edited using CRISPR/Cas9 and showed that *TaMLO-A1*-mutagenized wheat plants have confirmed resistance against Blumeria graminis (Tyagi et al. 2021). Such techniques like CRISPR/Cas9-dependent plant–pathogen genome editing will draw much attention as well-adapted to increased pathogenic resistance and transgenic-free plants and will be required for global food demand (Paul et al. 2021).

In another study, the fusarium head blight, induced by *Fusarium* spp., was managed in CRISPR/CAS9-silenced mutants. Studies demonstrated that RNA interference on trehalose 6-mutant (Δtri 6) of *Fusarium* spp. confirmed lowered disease indices that lie from 40 to 80% in durum wheat (Muñoz et al. 2019). However, two mutants (*Δtri1* and *Δtri6*) of *Fusarium* spp. were incapable of pathogenic response to the inflorescence and also elicited plant defense response. Moreover, *Δmap 1* mutants of *Fusarium* spp. demonstrated two times reduction in the production of mycotoxins, but was unable to colonize pathogens in other plant parts except for grains. Such competition for nutrients and space between non-virulent and virulent strains could decrease the disease severity, and the field liberation of non-virulent CRISPR/Cas9-mutant strains of *Fusarium* spp. might help to overcome the emerging issues (Zaidi et al. 2020; Zaynab et al. 2020; Zhang et al. 2017a, b; Wang et al. 2020; Verma et al. 2021; Liu et al. 2021).

According to Wang et al. (2014), using CRISPR/Cas9 to mutate wheat cultivars exhibits improved resistance toward powdery mildew resistance, caused by *Blumeria graminis*. Additionally, CRISPR/Cas9 mediated site-specific mutagenesis in springer and wheat varieties (Hahn et al. 2021). Similarly, mutagenization of 3 genes such as *AUR1* (a visual marker), *Tri5* (toxin production and infection), and *MGV1* (required for infection and reproduction) was done by CRISPR/Cas9. More likely, silencing of *Tri5* and *MGV1* could suppress the fungal ability to suppress infection in crops, whereas *AUR1* silencing acts as an effective visual marker during

mutagenesis (Sack 2020). Recently, in wheat a serious fungal pathogen like powdery mildews caused by *Podosphaera xanthii* was enhanced via editing *Mildew Locus O* (*MLO*) gene through CRISPR/Cas9 technique. Knockdown of susceptibility loci becomes highly complicated in wheat like targeting *MLO* homologous genes and *Enhanced disease resistance 1* (*EDR1*) using CRISPR/Cas9 editing (Wang et al. 2020; Verma et al. 2021; Raffan et al. 2021; Li et al. 2018, 2020a, b).

However, various target locus in the *wheat dwarf virus* (WDA) genome was screened using CRISPR/Cas 9 direct sequences enclosing the PAMs motif. Several target positions were designated that demonstrated no specific effect and were efficient in attacking different viral DNA sequences. The single-guided RNA WDA1 (sgRNA WDA1) displays the complementary overlapping in certain coding sites; sgWDA2 targets the Rep/Rep (Shahriar et al. 2021). Moreover, crop engineering using CRISPR/Cas9 like techniques has helped to develop disease-resistant varieties that are more resilient to climate change (Zaidi et al. 2020). Application of CRISPR/Cas9 ultimately helps to improve disease resistance in wheat and other crops and is well-documented (Schenke and Cai 2020; Paul et al. 2021; Zhang et al. 2017a, b; Kis et al. 2019; Verma et al. 2021; Gil-Humanes and Voytas 2014; Tyagi et al. 2021; Duy et al. 2021).

8.5 Concluding Remarks

The conventional breeding approaches for disease-free wheat in modern agriculture are transgenic, mutation breeding, and cross-breeding. These are laborious, time-consuming, and unfocused crop improvement programs that are unable to meet food demand. To cope with these challenges and to increase crop selection ability, transgenic and marker-assisted breeding has been developed, harnessing target traits via introgression into elite wheat varieties. Such advancements in plant breeding are an excellent tool that maintains rapid mutation and can recognize the significant genetic approaches for disease resistance. No doubt, crop breeding strategies are propelled by next-generation breeding methods. New breeding resistant varieties should remain the key focus. Some alternate approaches, viz., shifting plantation date, integrating fungicide, and eradicating volunteer plants, should also be considered. Precise modification of existing allelic diversity via advanced genomic editing is an efficient alternative for accelerating wheat improvement and sustainably increasing wheat production. Although it is convenient to attain precise allele/gene targeting or replacement in different cereal plant species, impressive work has been published in past years in wheat molecular breeding, which eradicates the constraints of the pathogen during crop improvement programs. In precise genome editing, replacement, deletion, site-directed artificial evolution, knockdown module, and insertion of allele/gene will be significantly facilitated by functional genomics. The advancement of the different genome modifications provides ease to gene pyramiding of novel resistance genes in the desired cultivar in a user-derived way immediately, efficiently, and cost-effectively without any linkage drag from undesired genes. More recently, CRISPR/Cas9 like techniques help in the transformation

of agriculture via the deletion or addition of alleles. No doubt, such techniques are cost-effective but eco-friendly, thus becoming a reliable trend. However, approaches like GWAS, RNAi silencing, genome edition, speed breeding, etc. will offer a huge amount of genetic information and enhance disease resistance via genomic editing. Genome editing approaches have various advantages over conventional breeding techniques, given their high efficiency, simplicity, amenability to multiplexing, and high specificity. Breeders strongly believe that combining molecular approaches with numerous breeding strategies will underpin an attempt to create super wheat cultivars for sustainable agriculture and ensure food security in an eco-friendly way.

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9 Potential Breeding Strategies for Developing Disease-Resistant Barley: Progress, Challenges, and Applications

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Abstract

There is a pressing call for enhancing world food production by at least 60% by 2050 using the same acreage. Barley (*Hordeum vulgare* L.), considered to be a risk-avoidance crop, is the fourth-most important grain crop in the world in terms of production after maize, wheat, and rice. The major barley producing countries are the Russian Federation, Germany, Canada, France, Spain, and Ukraine. Cultivated barley is an annual self-pollinating, true diploid ($2n = 2x = 14$) cereal, primarily grown for its grain and mainly used as feed for livestock. The rest of the barley grain is used as malted barley, as well as for human food and health food. Barley also yields valuable forage that can be grazed; cut for green forage, hay or silage while still green; cut for dual purpose (green forage and grain); or cut for straw after grain harvest. Cultivated barley is adapted to stress-prone environments, marginal and waste lands. Its wider adaptability, however, exposes the barley crop to different biotic stresses such as insects, phytopathogens, and weeds. Among them, plant pathogens are the most important constraints for the quality production of barley. Although more than 250 different plant pathogens infect barley, only a few of them cause considerable economic yield loss. In commercial barley production, disease management relies heavily on fungicide applications around the globe, which leads to higher production costs. Further, the heavy doses of fungicides create residue problems in fodder and grain and also lead to the development of resistant races or pathotypes. Hence, the best approach for managing barley diseases is by developing disease-resistant varieties. Earlier, the classical breeding approaches were followed to develop resistant varieties, but this approach provides only short-term relief, and the

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breakdown of resistance occurs very fast. To overcome these challenges, researchers changed their aim to advance breeding strategies with new molecular approaches like marker-assisted selection (MAS); marker-assisted backcrossing (MABC); targeting induced local lesions in genomes (TILLING); RNA interference (RNAi); virus-induced gene silencing (VIGS); genome editing; and RNA-dependent DNA methylation (RdDM) to breed disease-resistant barley varieties.

Keywords

Disease resistance · Molecular breeding · Powdery mildew · Rusts · Spot blotch · Stripe disease · Smut disease

9.1 Introduction

Cultivated barley, botanically known as *Hordeum vulgare* (L.), is the earliest domesticated coarse cereal (Zohary and Hopf 2000; Harwood 2019) in the Poaceae family, grown during the winter season. It is the fourth most important grain crop grown in the world after maize, rice, and wheat, with a share of 7% of global cereal production (Gangwar et al. 2018; Reddy et al. 2014). Barley is primarily grown for its grain, which is mainly used as animal feed. The second use of barley grain is as malted barley for alcoholic beverages, particularly beer. Barley grain is used as human food as well as healthy food. The main type of fiber found in whole grains is beta-glucan. It is also commonly used in the preparation of bread, soups, cakes, and other healthy products. Almost 70% of total barley production is used for cattle and poultry feed, 25% for malt and malt extract, and 5% for human consumption (Gangwar et al. 2018; Singh et al. 2016). Barley produces valuable forage in addition to grain, which can be grazed, cut for green forage, hay, or silage while still green in the field, cut for dual purpose (first for green forage at vegetative stage and then regenerated for grain), or cut for straw after grain harvest. Barley straw is used as fodder for ruminants and as bedding material. Cultivated barley is a self-pollinating, diploid ($2n = 2x = 14$), annual temperate grass capable of growing in various stress conditions like salinity, drought, higher altitude, and low fertilization. Hence, this characteristic makes barley grow in marginal and waste lands, so it is also known as the poor man's crop (Verma et al. 2012).

On the basis of spike morphology, barley is grouped into two types: two row and six row, while on the basis of growth habit into three types: winter, spring, and facultative (Poehlman 1994). It is also classified into hulled and hulless barley on the basis of grain type. The lemma and palea are fused to the pericarp in hulled barley, whereas chaff is easily separated from the grain in hulless type. Hulless barley is used for human consumption due to its higher nutritive value. Barley grain consists of 20% of dietary fiber and 3–7% of β-glucan (Oscarsson et al. 1996). The β-glucan of barley has significant blood cholesterol-lowering effects (Martinez et al. 1991). Moreover, Barley-glucan and non-starch polysaccharide increase the viscosity of

food material in the intestine which decreases its rate of digestion and absorption (Anderson et al. 1990; Newton et al. 2011), thus useful to people with diabetes (Gosain 1996). Because of its multifarious utilities, nutritive value, and increased industrial demand, sustainable yield gains will be needed over future decades. However, biotic stresses are the most serious constraints to barley production in which phytopathogens cause total crop loss to the tune of about 20–45% (Bellard et al. 2012; Savary et al. 2012). Barley is infected by more than 80 different plant pathogens which cause diseases like yellow and brown rust, covered smut, powdery mildew, net blotch, spot blotch, barley stripe, barley yellow dwarf, and molya diseases which are economically important in a global context (Mathre 1997). Disease resistance has been the second highest priority after grain yield in barley breeding. Here, we are trying to highlight the major diseases of barley along with their major symptoms and disease developmental conditions. We are also including various molecular techniques that have been utilized in the discovery and classification of disease-resistant genes in barley.

9.2 Major Diseases of Barley

The wider adaptability exposes the barley crop to different biotic stresses such as insects, phytopathogens, and weeds. Among them, diseases are the most important constraint for the production of quality barley (Pessarakli 2016). Phytopathogens include fungi, bacteria, plant parasitic nematodes, and viruses that cause infection in cultivated barley. The most important diseases responsible for considerable losses are mentioned below.

9.2.1 Powdery Mildew

It's a common disease of cultivated barley, caused by fungal pathogen *Blumeria graminis* f. sp. *hordei*. Early infection can cause yield loss to the tune of 25%, while infection at later stages affects yield loss by 10%. The disease incidence is more during the early crop growth stage, but symptoms are first noticed at tillering stage (Fig. 9.1). Both winter and spring barley varieties are susceptible to powdery mildew disease. *Blumeria graminis* f. sp. *hordei* is a biotrophic pathogen and disease is favored by cool (15–25 °C) and humid weather, but can also occur in warmer and semiarid environments. The important symptoms of the disease are whitish, fuzzy fungal mycelium seen on the surface of leaves. Later, powdery or fluffy white pustules of conidial chain are noticed on the leaves. The entire spikes of plants can be infected with powdery mildew in addition to the leaves and leaf sheaths.

Fig. 9.1 Powdery mildew in barely

9.2.2 Rusts

Rusts are the most devastating diseases of barley (Duplessis et al. 2011), and these pathogens have evolved further into many distinct physiological races or pathotypes. Barley is infected by four different rusts, i.e., stem, leaf, yellow, and crown rust, all caused by members of the genus Puccinia, family Pucciniaceae, order Pucciniales, class Pucciniomycetes, subphylum Pucciniomycotina, Phylum Basidiomycota, kingdom Fungi, and domain Eukarya (Bauer et al. 2006).

9.2.2.1 Black Stem Rust

Black stem rust of barley caused by *Puccinia graminis f.* sp. *tritici* is the most important disease. It infects the crop late in the season; therefore, the losses are minimal. The symptoms that develop predominantly occur on the leaf, blade, sheath, and stem. Severe infections with many stem lesions may weaken plant stems and result in the breaking of stems at the point of infection. Initially, rust pustules are reddish-brown and later turn into black telia containing teliospores (Bhardwaj et al. 2017). Favorable conditions for infection require a temperature range of 15–28 °C with 6–8 h of free moisture on the leaf surface. Secondary infection occurs if wet weather persists and the temperature remains in the range of 26–30 °C. Several cycles of uredospore production occur during the growing season.

9.2.2.2 Crown Rust

Crown rust of barley is caused by *Puccinia coronate* f. sp. *hordei*. Outbreak of crown rust disease on barley was seen during 1991 in south central Nebraska, U.S.A. (Jin and Steffenson 1999). Pathogen infects leaf blades, leaf sheaths, peduncles, and awns. Symptoms starts on leaf blades; uredial pustules are linear, oblong with orange to yellow color, followed by chlorosis.

9.2.2.3 Yellow (Stripe) Rust

Yellow rust is an important foliar disease of barley caused by *Puccinia striiformis* f. sp. hordei. Early infection of yellow rust causes severe yield loss and also prevents spike emergence or grain formation/development (Prakash and Verma 2009). In cooler climates (2–15 °C), the disease is more severe, followed by prolonged leaf wetness (8–10 h). Uredial pustules are seen on leaves as narrow stripes that are orange to yellow in color, and as disease progresses, the yellow stripes continue to enlarge because of the partial systemic nature of pathogens. Black telia readily develops from uredia as infected barley plants approach maturity. The uredial and telial spore stages of *P. striiformis* f. sp. *hordei* occur on barley and various *Hordeum* spp. (Marshall and Sutton 1995).

9.2.2.4 Leaf (Brown) Rust

Leaf rust, or brown rust, is a sporadic and most common disease of barley, caused by the basidiomycota fungi Puccinia hordei. Small orange or brown uredial pustules are mainly scattered on the upper surface of the leaf. Infection is also seen on the leaf sheath. Uredial pustules are surrounded by chlorotic halos, or green islands. Secondary spread occurs by urediospore, which is formed within 7 to 10 days after infection. A temperature ranging from 20 to 25 °C and prolonged wet weather are prerequisites for the faster spread of the disease.

9.2.3 Spot Blotch

Spot blotch, caused by the fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*), is a major foliar disease of barley (Arabi and Jawhar 2004). It occurs in the warmer and more humid regions of the world. Yield losses in susceptible varieties range from 10% to 30%. Spot blotch disease development is favorable when temperatures are 15–22 °C and relative humidity is greater than 90%. Hence, the spot blotch disease of barley is considered to be one of the major threats to barley production under climate change (Singh et al. 2014a, b). Infection is characterized by small, dark brown lesions. As disease progresses, lesions are restricted in width by leaf veins and turn dark brown with a chlorotic margin. Heavily infected leaves dry out and die prematurely. If inoculum is available and the environmental conditions are conducive to infection, the kernel blight phase (black point) of this disease may develop.

9.2.4 Stripe Disease

Stripe disease of barley is caused by *Drechslera gramineae*, and the fungal pathogen causes systemic infection only in barley. Symptoms start as small lesions on seedlings and the most characteristic symptoms are long, narrow, and straw-colored streaks or stripes that appear on the leaves. Later, parallel stripes may extend the entire length of the leaf blade. The light straw-colored streaks soon turn to brown,

which leads to the drying out and splitting of the leaf blade. Severely infected plants shrivel and die prematurely. Infected plants are severely stunted with few tillers and the spikes fail to emerge. The ears that do emerge are greyish brown, withered, twisted, erect, and often barren. The fungal pathogen remains alive for 3 years.

9.2.5 Net Blotch

The fungal pathogen fungi *Pyrenophora teres* causes barley net blotch, an important and destructive disease of barley. Under favorable environmental conditions, the disease can be prevented (Murray and Brennan 2010). Disease has the potential to cause yield losses of 10–44% in susceptible cultivars. Small dark brown lesions are seen on leaves, sheaths, and glumes, which later develop into short brown stripes or irregular blotches. Lesions may be surrounded by a yellow area. The ear can also be infected, but lesions do not usually appear. The infection is more severe in humid periods lasting for 10 or more hours at an optimum temperature of 15–20 °C.

9.2.6 Smut Diseases

9.2.6.1 Loose Smut

Loose smut, an internally seed-borne disease of barley, is caused by *Ustilago tritici*. When an infected seed germinates, the dormant mycelium inside the seed begins to grow and causes systemic infection. The smut pathogen shows host specialization, i.e., isolates that attack wheat do not attack barley and vice versa. The most obvious symptoms occur only after the emergence of spikes. Infected ear heads emerge earlier than normal, and grains are replaced with a mass of dark brown to black teliospores. Disease spread is by wind-blown teliospores from smutted ears to adjacent healthy flowering ears of barley. The teliospores grow and invade the female parts of barley flowers. They then spread to the developing embryo.

9.2.6.2 Covered Smut

Covered smut of barley is one of the most common diseases caused by *Ustilago hordei.* Smutted ear heads emerge at the same time or slightly later than healthy plants. All the grains in the diseased spike and the entire spikes in the diseased plants are infected. All the infected grains in the diseased spike are transformed into masses of teliospores and these teliospores are held by tough greyish white membrane. The membrane is the glume that usually remains intact until harvest or threshing.

9.2.7 Barley Yellow Dwarf Disease

It is caused by the *Barley Yellow Dwarf Virus* (BYDV), a member of the Luteovirus group. The virus causes a 100% yield loss if infection occurs at an early stage of growth (Mathre 1997). Initial symptoms are seen in plants randomly scattered in the

field. The most common noticeable symptoms are yellowing of leaves and a reduction in the growth of plants, which appear either singly or in small patches. Discoloration in shades of yellow, red, or purple is observed in the leaves of infected plants, which typically starts at the tip or margin and moves towards the downside or midrib, respectively. Leaves stand upright and rigid with rough leaf margins along with less tillering, flowering, and sterile florets, which results in fewer filled and smaller kernels with corresponding yield losses.

9.3 Sources of Disease-Resistant Genes

In the absence of genetic resistance, crop production is highly dependent on chemical control of pathogens. Barley disease management depends on repeated application of chemical fungicides, but use of resistant varieties offers both an economical and an environmentally sound method of management. The development of resistant varieties is complicated and needs time, besides being broken by different pathotypes of the pathogen. Bovill et al. (2010) attempted to identify the source of resistance against spot blotch disease of barley caused by *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*). Australian barley cultivars are highly susceptible to spot blotch disease, and hence, resistance sources have been identified in North American two-row barley lines. In adult plants, spot blotch-resistant QTL were found on chromosomes 3HS and 7HS, but seedling resistance is controlled by a locus on chromosome 7HS. A total of 124 accessions of two-row barley were screened for spot blotch resistance for 3 years under natural epiphytotic conditions (Singh et al. 2014a, b). Accessions, viz. BCU422, BCU1204, and BCU5092, are identified as resistant sources against the spot blotch pathogen, while BCU711, K603, and RD2506 are noted as the most susceptible fungal pathogens to *Bipolaris sorokiniana*. Several resistance genes (Mla1-Mla31 except Mla4, and Mlmr) are identified against powdery mildew disease in barley and many more specific resistances have been detected in cultivars, landraces, and wild barley. Dreiseitl (2011) described three specific powdery mildew-resistant genes (Ml (Ro), MlaLv, and Ml (Ve)); they are widely used in commercial cultivars. In 20 barley accessions, 39 powdery mildew-resistant genes are identified (Mastebroek et al. 1995). Dreiseitl and Bockelman (2003) screened 1383 accessions collected from United States Department of Agriculture (National Small Grains Collection). Among 1383 accessions, 123 accessions were resistant to 22 isolates.

9.4 Breeding Approaches for Disease Resistance

Durable resistance offers great prospective for global food security and sustainability. Developing high-yielding barley varieties with enhanced resistance to biotic and abiotic stresses and improved quality for feed, malt, food, and fodder is imperative. Presently, researchers are trying to bring two or more desirable traits together, like, for example, higher yield with enhanced resistance towards different

Table 9.1 Various approaches for disease resistance breeding in barley

S. No.	Approaches
1	Conventional breeding • Introduction of exotic lines • Selection • Hybridization • Backcrossing • Mutagenesis using chemicals and radiations
2	Marker-assisted breeding • Marker-assisted selection • Marker-assisted backcrossing • Genome-wide association mapping • Genomic selection
3	Targeting induced local lesions in genome • Eco-TILLING • DEco-TILLING
4	Transgenics • Agrobacterium-mediated transformation and regeneration protocols • RNA interference • Virus-induced gene silencing • Genome editing tools • Overexpression of genes • Tissue or developmental stage-specific expression of genes • Constitutive expression of genes • Promoter trap • Enhancer trap

biotic and abiotic factors and improved dietary value of grain and fodder. Classical, genetic, molecular, and new breeding approaches/technologies against diseases in barley are mentioned in Table 9.1.

The modern high-yielding barley varieties and breeding lines developed worldwide are found to have a restricted genetic base in contrast to their natural ancestors as most of the breeding objectives were mainly restricted to fewer traits (Caldwell et al. 2005). Due to narrow genetic diversity, cultivated barley gene pool is vulnerable to various diseases. The gene pool of cultivated barley was defined and the wild progenitor, *H. vulgare* subsp. *Spontaneum,* is classified in the primary, *H. bulbosum* in the secondary, while all other species in the tertiary gene pool. Crop wild relatives are bestowed with desirable agronomic and stress-(biotic and abiotic) resistant traits which could be useful for plant breeding initiatives. Due to limited variability of resistant genetic resources in the cultivated gene pool of barley, significant attempts have been made to introduce promising alleles from natural ancestors and landraces into current breeding populations (Schmalenbach et al. 2008; Friedt et al. 2011).

Globally, around 4,66,531 accessions of barley gene pool are conserved, mainly by Canada and USA (FAO 2010). In order to increase the utilization of conserved barley germplasm for breeding programme, Knüpffer and van Hintum (2003) formed two core collections of wild barley (one with 70 accessions and another 144 accessions), while Steffenson et al. (2007) established Wild Barley Diversity

Collection (WBDC) with 318 accessions. These core subsets are presently preserved at the International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. Fu and Horbach (2012) developed a core subset of 269 accessions representing 16 countries from the collection of 3782 accessions. Neupane et al. (2015) assessed 2062 accessions and identified 15 of them to have effective resistance against four diverse isolates of *Pyrenophora teres maculata* collected worldwide. Cope et al. (2021) analyzed 131 heritage cultivars and landrace lines of barley against four diverse isolates of Barley 'Scald' and three lines with new source of resistance were identified. The disease resistance against leaf stripe (*Drechslera graminea*) was reported in wild barley (*Hordeum spontaneum*) and barley landraces (Oğuz 2019). Fetch et al. (2003) reported high frequency of resistance for septoria speckled leaf blotch, leaf rust, net blotch, powdery mildew; intermediate for spot blotch; and low for stem rust in *Hordeum spontaneum*. They also reported two *H. spontaneum* accessions (Shechem 12–32 and Damon 11–11) having resistance for all the six diseases as mentioned. *Hordeum bulbosum* L. ($2n = 4x = 28$) belongs to the secondary gene pool of cultivated barley and has long been searched for novel disease-resistant alleles (Pickering et al. 2006; Fetch et al. 2009). The quantitative barley leaf rust resistance gene, Rph26, was fine-mapped within a *H. bulbosum* introgression on barley chromosome 1HL for pyramiding with other resistance genes (Xiaohui et al. 2018).

In barley, chromosome substitution lines (CSL) (Matus et al. 2003; Inostroza et al. 2008), nested association mapping (NAM) panels (Schnaithmann et al. 2014), advanced backcross lines (Pillen et al. 2003; Nice et al. 2016), and multi-parent segregating populations (MAGIC) (Sannemann et al. 2015) are being utilized for the identification of QTLs/genes responsible for disease resistance. Leng et al. (2018) identified, fine-mapped, and physically anchored a dominant spot blotch susceptibility gene Scs6 to a 125 kb genomic region containing the Mla locus on barley chromosome 1H against pathotype 2 isolate (ND90Pr) of *C. sativus* in barley cultivar Bowman. Leng et al. (2020) also mapped genetic loci controlling spot blotch and powdery mildew diseases of barley using 138 recombinant inbred lines (RILs). They recognized two QTLs, QSbs-1H-P1 and QSbs-7H-P1, responsible for spot blotch on chromosomes 1H and 7H, respectively. Hickey et al. (2017) applied a novel modified backcross strategy for rapid trait introgression to the European two-rowed barley cultivar, Scarlett. Hautsalo et al. (2021) used four Multi-parent Advanced Generation Inter-Cross (MAGIC) populations in Genome-Wide Association Studies (GWAS) and identified nine areas on chromosomes 1H, 3H, 4H, 5H, 6H, and 7H associated with resistance, in which three of these regions are putatively novel resistance sources. Pogoda et al. (2020) assessed the severity of powdery mildew infection on detached seedling leaves of 267 barley accessions using two poly-virulent isolates and identified four candidate genes against powdery mildew attack. Therése et al. (2017) performed a genome-wide association study in a Nordic spring barley panel consisting of 169 genotypes and identified a total of four QTLs, one located on chromosome 4H and three on chromosome 6H.

Amouzoune et al. (2021) compared the Generation Challenge Program Reference set (GCP) with 188 accessions against the Focused Identification of Germplasm

Strategy (FIGS) with 86 accessions for identifying new sources of resistance against leaf rust of barley, and they reported FIGS as a better approach than GCP in yielding higher percentages of resistant accessions at adult plant-resistant stage. Bilgic et al. (2005) identified a gene (Rcs5) on chromosome 7H conferring seedling resistance to pathotype I (ND85F) whereas in another study, Bilgic et al. (2006) used a doubled haploid (DH) mapping population to identify a gene (designated as Rcs6) on chromosome 1H conferring resistance to pathotype 2 (ND90Pr) of Bipolaris sorokiniana.

9.5 Molecular Breeding Approaches for Disease Resistance

Barley production is harshly affected by a range of biotic stresses. Usually, breeding for disease-resistant genotypes involves manual inoculation of the pathogen into the host at the right stage along with the desirable conditions for disease development, but this technique is very cumbersome and can also lead to false negatives (Figs. 9.2 and 9.3). Therefore, use of advanced breeding approaches like Marker-Assisted Selection (MAS), Genome-Wide Association Studies (GWAS), QTL mapping, and high throughput molecular techniques like sequencing and genomics has been utilized in accelerating breeding programs for various qualitative and quantitative traits (Figs. 9.1 and 9.2). Long-lasting resistance requires combinations of several resistance genes and QTLs in a genome.

Host-based resistance is one of the most feasible and eco-friendly approaches for controlling disease-related losses in crop plants, and a diverse genetic base is one of

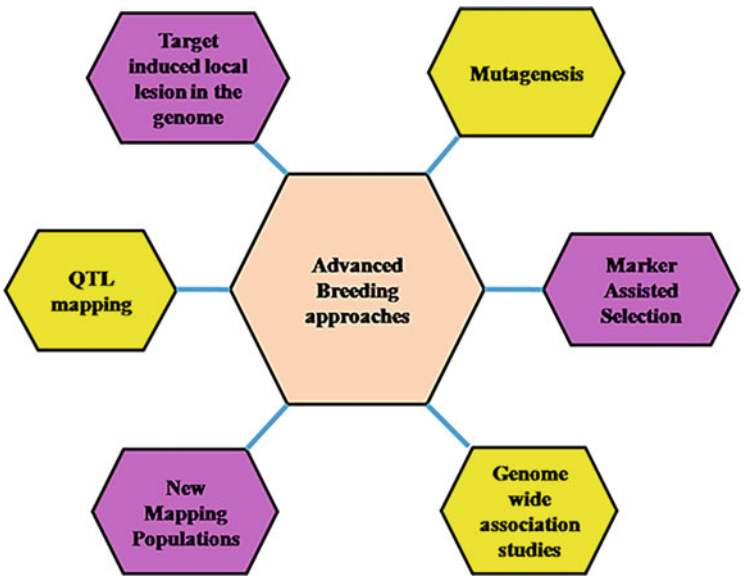

Fig. 9.2 Advanced breeding approaches for disease resistance

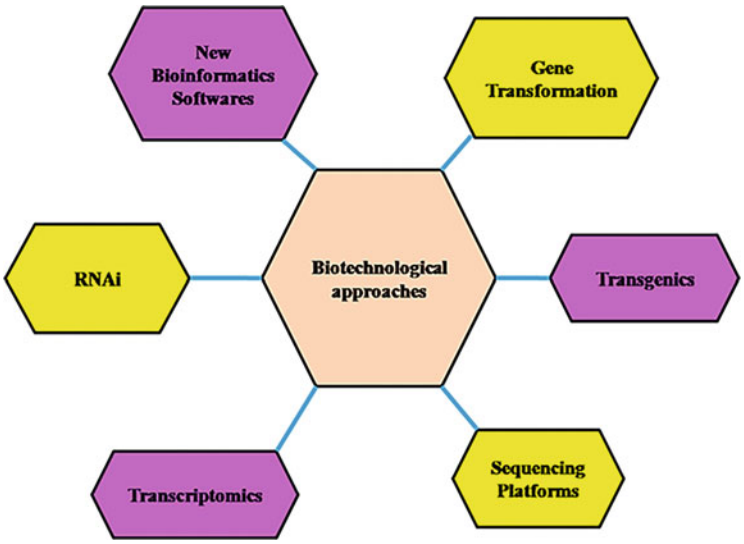

Fig. 9.3 Biotechnological approaches for disease resistance

the primary requisites for it. Barley has one of the oldest cultivated crops and has a rich genetic base, having geographically diverse wild accessions, landraces, and cultivars. The genome of barley has already been mapped, and there are many genomic resources available in public databases. These include expressed sequence tags, full length cDNA (FL-cDNA) sequences, genome sequences, and pan genomic data for 20 varieties of barley that include landraces, cultivars, and wild races (Zhang et al. 2004; Sato et al. 2009; Mayer et al. 2012; Jayakodi et al. 2020). Barley has a haploid genome size of nearly 5.1 Gigabases (gb) with nearly 26,000 highly confident genes as supported by transcript and homology data. The International Barley Genome Sequencing Consortium attempted sequencing of barley cv. Morex in 2012 using a hierarchical shotgun sequencing approach (Mayer et al. 2012). Molecular markers have served extensively in barley breeding programs for tracking useful genes and in their isolation (Stein and Graner 2005; Perovic et al. 2018). Single nucleotide polymorphism (SNP) markers are currently the most chosen markers due to their high throughput detection employing NGS (Ganal et al. 2018). With the advent of the 9 K Illumina iSelect chip and the 50 K Illumina Infinium array, the number of existing SNP markers has improved to 44,040 SNPs (Stein et al. 2007; Close et al. 2009; Bayer et al. 2017). Zang et al. (2015) tried to fine map the candidate gene responsible for loose smut resistance in barley by utilizing dense linkage map saturated with various useful markers like RFLP, microsatellite, and SNPs. They were able to enrich the genomic region associated with loose smut resistance. Sayed and Baum (2018) screened two groups (homozygous-resistant and susceptible), each comprising of 10 plants for barley scald disease caused by *Rhynchosporium commune* from the recombinant inbred line (RIL) population at F_7 generation with the help of 25 markers which lie close to scald-resistant genes.

Out of the 25 markers, only 5 markers showed clear discrimination between resistant and susceptible plants. They reported that most of these markers reside near to the centromeric region of the long arm of 3H chromosome. They anticipated that presence of polymorphic markers will be extremely helpful in discriminating breeding material in barley. Brueggeman et al. (2002) cloned *Rpg1* (Resistant to *Puccinia graminis* 1) gene against stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) through high-resolution map-based cloning. The *Rpg1* gene encodes a receptor kinase-like protein with two tandem protein kinase domains. In Northern America, *Rpg1*gene offered strong resistance to barley varieties for nearly 40 years, but with the appearance of a new race of *Puccinia graminis* f. sp. *tritici* (*Pgt*) TTKSK, an alternative to *Rpg1*-resistant gene was needed. Jin et al. (1994) identified, cloned, and characterized the *rpg4*, a recessive gene against the same. The *rpg4* gene was later found on the chromosome 5H in barley (Borovkova et al. 1995). Fusarium head blight (FHB) is another devastating malady of barley which results in the reduction of grain yield and accumulation of deoxynivalenol (DON) mycotoxin in grains. It has been reported that the morphological and developmental characters of the host plant, e.g., earliness and plant height, are linked with pathogen infection and its severity. Ogrodowicz et al. (2020) investigated 100 recombinant inbred lines (RILs) by employing a barley Ilumina 9 K iSelect platform and found a set of 70 quantitative trait loci (QTLs). They suggested tight association of yield-related traits with FHB-associated QTLs should be followed while designing a barley breeding programme for FHB resistance. Powdery mildew of barley is caused by *Blumeria graminis* f. sp. *hordei*. Recessive allelic form *(mlo)* of the barley *Mildew resistance locus o* (*Mlo*) locus provides broad spectrum resistance to the fungal pathogen, *Erysiphe graminis* f. sp. hordei. Büschges et al. (1997) utilized RFLP and AFLP marker systems for the purpose of gene isolation. Later, Hoseinzadeh et al. (2019) did high-resolution genetic and physical mapping of major powdery mildew-resistant locus in barley through GBS approach. Vatter et al. (2018) followed SNP-based nested association mapping (NAM) to map stripe rust and leaf rust resistance QTL in barley. They reported 8 new QTLs for stripe rust and 2 new QTLS for leaf rust. Hu et al. (2019) identified new major QTL on chromosome 5H along with two minor QTLs on chromosome 7H providing tolerance against barley yellow dwarf infection in barley. Visioni et al. (2020) employed High Input Association Mapping (HI-AM) panel comprising 261 spring barley genotypes (including released varieties, breeding material from ICARDA, and germplasm from GenBank) to map spot blotch-resistant QTLs at seedling and adult plant stages in barley by utilizing genome-wide association mapping (GWAM) approach. It was reported that expression of wheat Lr34 gene in transgenic barley lines imparted resistance against multiple fungal pathogens (Risk et al. 2013; Chauhan et al. 2015). Its constitutive expression in transgenic barley lines caused upregulation of senescence and pathogenesis-related genes, resulting in leaf tip necrosis in general and reduced height and total gain weight in extreme cases which can be overcome through regulated expression.

Host-delivered RNA interference (HD-RNAi) approach has been effectively used in various crop species to impart resistance, especially against viral diseases and

insect-pests damage (Tiwari et al. 2017; Joshi et al. 2020). In barley, there are few reports where researchers have used RNAi against phytopathogens, e.g., Kis et al. (2016) designed and expressed a polycistronic cassette of artificial microRNAs in barley against wheat dwarf virus and found higher level of resistance at low temperature conditions which are highly favorable for the insect vector to survive and spread disease. In contrast to HD-RNAi approach, RNA-spray-mediated approach has been also attempted, similar to pesticide application. Koch et al. (2016) sprayed 791 nt long noncoding dsRNA molecule (*CYP3*-dsRNA) targeting three essential genes of ergosterol biosynthesis pathway of *Fusarium graminearum*. Their study revealed increased level of resistance in the sprayed as well as non-sprayed portion of the detached leaves of barley. Acquired resistance observed in the non-sprayed areas of the leaves indicated systemic movement of interfering RNA from applied to adjacent non-applied areas through plant conducting tissues. Moreover, their research also enlightened the role of fungal RNAi machinery like fungal DICER-LIKE 1 (*FgDCL-1*) in spray-based RNAi approach. As per their study, mutant form of *FgDCL-1* was found to be nonfunctional against same insecticidal dsRNA. Recently, Werner et al. (2020) also used spray-based RNAi approach for silencing *ARGONAUTE* and *DICER* genes of *Fusarium graminearum* (*Fg*). They also observed enhanced resistance in barley leaves. Genome editing technologies have been deployed in various crop plants for imparting disease resistance. But, in barley very few attempts have been made due to lack of enough knowledge on techniques like gene transformation and tissue culture. Moreover, success of transformation is highly genotype-dependent. CRISPR-Cas9 technology has been employed in deciphering role of orthologous disease-related genes in barley by using model organisms in which protocols of genetic transformation are well-standardized (Low et al. 2020). Golden Promise is one such cultivar of barley which is highly amenable for genetic transformation and shows higher regenerability. Its genome has been recently sequenced and assembled through illumina-based next-generation sequencing platform (NGS), which could be definitely useful for entire barley research groups especially through CRISPR-Cas 9 platform (Schreiber et al. 2020). Kis et al. (2016) utilized CRISPR platform to enhance viral resistance in Golden Promise cv. of barley against *wheat dwarf virus*. Due to its successful transformation and regeneration ability, Golden Promise cv. was extensively used for transgenic research. Recently, Han et al. (2021) developed a highly efficient and genotype-independent gene editing technique based on anther culture. They found that their platform can generate a greater number of transgenic plants within a similar time frame along with high editing efficiency as compared to immature embryo protocols. This technology may play a crucial role in imparting disease resistance trait in commercial cultivars of barley as well as in functional validation of disease-related genes.

9.6 Conclusion

Barley, one of the oldest crops primarily grown for its grain, has the largest single use in feeding livestock throughout the world. Despite the overall decline in barley acreage, total production has increased due to the continuous improvement in barley productivity (yield per hectare). But no breeding program can develop varieties with acceptable levels of resistance to all diseases under all conditions. Moreover, the climate is constantly changing owing to various anthropogenic activities, which may further affect host and pathogen relationships. Therefore, our primary focus after higher grain yield is to impart broad spectrum resistance to the crop species with long-lasting impact. Traditional breeding methods (introduction of exotic lines, selection, hybridization, backcrossing, gene pyramiding) and modern breeding methods have been used to bring and improve resistance to biotic stresses in barley. Modern breeding approaches overcome the problems of traditional breeding strategies like more effort, more labor, transfer of non-desirable genes along with resistant genes, short-term relief, limited resistance sources, breakdown of existing resistance due to continuous evolution of new pathogen races, and being time-consuming. Advanced breeding and biotechnological methods like QTL mapping, gene mapping, MAS, MABC, TILLING, transgenics, RNA interference (RNAi)-mediated gene silencing, gene and genome editing using CRISPR-Cas9, along with bioinformatics and high throughput computational technologies, can enable us to engineer durable resistance in cultivated barley. The ability of the CRISPR platform to provide a transgene-free crop with desirable attributes is getting sincere applause among the scientific community. The availability of various bioinformatics tools will help us in the identification of pathogen-inducible promoters, key transcription factors, and noncoding RNAs pertaining to pathogen attack and disease development. They also allow us to decipher the actual biochemical roles of various disease-related genes in the disease signaling pathways, as gene annotation has become a major challenge in understanding their role. Global expression profiling techniques like suppression subtractive hybridization, microarray, serial analysis of gene expression (SAGE), and RNA seq. will allow us to capture the expression status of several genes in resistant and susceptible genotypes, which will definitely help us to focus on key or vital disease-related genes that could be used in the future. Whatever the approach (conventional or molecular breeding), our main concern is to increase productivity and minimize yield loss due to phytopathogens. It is very certain that advancements made in the science of molecular biology will become important pillars towards successful breeding methods in barley.

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Economic and Eco-friendly Alternatives for the Efficient and Safe Management of Wheat Diseases

10

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Abstract

The achievement of high cereal production while considering environmental and health safety standards is an essential goal for all countries to meet their own food needs and feed the rapidly growing population around the world. In this regard, wheat (*Triticum aestivum* L.) is a strategic crop of great importance to global food security, especially in developing countries. It is even more important for the consumers of all sectors and regions where people rely on wheat as a significant element in their diets. However, several biotic and abiotic stress factors bring about the limiting and declining of local wheat production in return for the increasing needs of the growing population. To deal with such challenges, procedures allow for the use of agrochemicals as a means of achieving a high wheat yield. However, the unrestricted use of such chemicals causes serious damage to the agricultural ecosystem, particularly in those ecosystems that lack organic soil content and a high level of biodiversity, which help to restore its natural vigor after extensive use of agrochemicals. As a result, these demands to look for other eco-friendly alternatives will help us make satisfactory progress in

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controlling wheat disease and successfully restore and sustain our agricultural ecosystem. In this chapter, we're going to talk about natural ways to boost the production of wheat cultivars by making them more able to fight off or at least tolerate wheat diseases.

Keywords

Wheat · Biostimulants · Wheat diseases · Biocontrol · Biotic stress

10.1 Introduction

Triticum aestivum L. is one of the world's most important staple cereals and a major source of 20% of calories and plant-based proteins in human meals (Almoneafy 2006; Mehta 2014b; Tilman et al. 2011; Vidal et al. 2020). As per the latest statistical report of the Food and Agriculture Organization, global wheat production in 2019–20 was about 765,769,635 tons (FAO 2020). Moreover, the current supply of wheat is adequate for world demand, according to the FAO's most recent wheat production valuation (http://www.fao.org/worldfoodsituation/csdb/en/). In the future, as the world's population grows, the wheat supply needs to be expanded further, which is expected to reach nine billion by 2050. Wheat growth and production will be boosted largely by an increase in yield due to high competition for arable lands with limited production (http://www.fao.org/state-of-food-security-nutrition/en/). Integrated disease and pest management, tolerance to warmer climates with increased frequency of abiotic challenges, and reduced water and other resource use can all help to improve this situation (Mehta 2014a). Wheat, like many other crops, is vulnerable to a variety of diseases that, if left unattended, can result in alterations in the chemical properties and quality of wheat grain as well as a significant decrease in yield (Matzen et al. 2019). Many strategies have been used to eliminate or mitigate the negative impact of wheat diseases, such as the use of various agronomic practices, the introduction of resistant varieties, and the use of microbiocidal synthetic compounds or chemical control. The latter is widely used as the most effective and common method of controlling wheat phytopathogens. The haphazard use of synthetic microbiocidals, on the other hand, has several serious drawbacks and threats, including pathogen resistance to these chemicals, eradication of beneficial microorganisms in the surrounding environment, reduction of soil organic content, and a decrease in biodiversity within plant-associated niches. Furthermore, because pathogen sensitivity to these compounds decreases, the applied dose of them must be increased. Due to this dosage increase, the resulting control costs, the environmental threats posed by these compounds, and their negative side effects on human health will significantly rise (Gao et al. 2020). The harmful effects of these synthetic chemicals are exacerbated in arid and semiarid lands due to their lower organic matter content and, as a result, lower biodiversity, which do not allow them to mitigate any deleterious changes caused by uncontrolled application of these chemicals (Santos et al. 2011). Therefore, in this chapter, we highlight and discuss

the beneficial effects of some inexpensive, safe, available, and efficient alternatives to managing wheat diseases in order to reduce agrochemical inputs in the agricultural sector and sustain our environmental resources.

10.2 The Well-Reported Eco-friendly Approaches Used in Wheat Disease Management

Several approaches have been evaluated by researchers as safe alternatives to synthetic pesticides for controlling wheat phytopathogens. The search for and evaluation of the biocidal activity of various biological/nonbiological procedures or biologically based means against wheat phytopathogens is still ongoing. In this section, we will look at some well-known methods for controlling wheat diseases.

10.2.1 Applying of Biogenic Nanoparticles

Nanotechnology has been suggested as a potential new technology for satisfying worldwide demands for sustainable agriculture and crop loss deterrence (Rai-Kalal et al. 2021). Within this context, numerous researchers have focused their efforts on developing nano-formulations that are low-cost, biodegradable, environmentally friendly, and exhibit biocontrol activities towards phytopathogens (He et al. 2019; Partila 2019). To minimize hazardous waste produced by the nanomaterials industry, "green" synthetic processes should be used to supplement the increasing demand for these materials. Biogenic NP synthesis is a very appealing, greener, and more eco-friendly production option due to the use of lower toxicity compounds, pressures, and surrounding temperatures during the synthesis (Chhipa 2019). As a safe and eco-friendly technology, a variety of prokaryotic and eukaryotic organisms are utilized in the biosynthesis of metallic nanoparticles such as platinum, gold, silver, zirconium, iron, cadmium, and palladium, as well as metal oxides like titanium oxide and zinc oxide (Luo et al. 2018). Around 75% of the potential application of nanoparticles in agriculture was directed toward the primary goal of controlling plant diseases (He et al. 2019; Luo et al. 2018). Of their physicochemical properties and nano size, they can easily penetrate the cellular envelops and membranes of microbial phytopathogens' internal compartments and induce a fatal effect on them (Khan and Rizvi 2014). Due to the obvious differences in charges between nanoparticles and microorganism macromolecules, they can act as electromagnetic absorbers and attach to the cell surface, causing oxidation of microbe surface molecules, and eventually cell death (Lin and Xing 2007). Many researchers have used biogenic nanoparticles as an efficient, low-cost, and safe method of managing wheat diseases. Most of the time, these materials were able to help wheat plants fight off phytopathogens, and this was usually accompanied by a boost in plant growth. For instance, Satti et al. (2021) biosynthesized and applied 40 mg/L titanium dioxide nanoparticles from *Moringa oleifera* Lam. aqueous leaf extract, which resulted in biocontrol of *Bipolaris sorokiniana* that causes spot blotch

in wheat. Additionally, the biosynthesized silver nanoparticles (AgNPs) have been demonstrated to inhibit fungal growth and reduce mycotoxin production of *Fusarium graminearum*, which cause Fusarium head blight in wheat. According to electron microscopy images, nanoparticle treatment caused hypha deformation and collapsing, which resulted in the leaking of genetic materials and proteins outside of the fungal hypha (Ibrahim et al. 2020). In a different research, researchers revealed that applying biogenic AgNPs significantly reduced *Bipolaris sorokiniana* infection in wheat plants in vivo. AgNPs caused lignin deposition in the host plant's vascular bundles, according to further histochemical analysis (Mishra et al. 2014). Table 10.1 shows some remarkable outcomes.

10.2.2 Harnessing of Beneficial Microorganisms (Biological Control)

Using beneficial microbes to improve plant growth and agricultural sustainability is a promising strategy. Beneficial microorganisms associated with plants can alleviate the harmful effects of pathogenic/environmental stresses in plants, boost plant growth, improve the cycle of biologically active compounds (e.g., enzymes, hormones, and vitamin), and decompose organic matter residues in agricultural soil (Saberi-Riseh et al. 2021; Smolińska and Kowalska 2018). These microorganisms can also effectively colonize the plant phytosphere, i.e., rhizosphere, phyllosphere and anthosphere. As a result of their capability to promote plant growth, improve plant health, and control plant diseases, beneficial microorganisms are a promising strategy for long-term plant disease management (Almoneafy et al. 2021). They can directly benefit plants by exerting an antagonistic effect on plant pathogens via colonization of infection site, competition for nutrient uptake, and occupation of niche (Köhl et al. 2019). The indirect mechanisms include interaction with plants that involves the induction of plant resistance to phytopathogens and the promotion of plant growth by facilitating nutrients uptake and phytohormones' production (Vos et al. 2015; Wang et al. 2021). Furthermore, their positive interaction with the concerned plant causes changes in the plant's secondary metabolite status (Etalo Desalegn et al. 2018). This method is useful and promising when applied to all arable lands, and its benefit increases in arid and semiarid lands because its soil organic content is increased, which improves its ability to retain water for a longer period. Moreover, it strengthens the environment's ability to recover from the detrimental consequences of randomized agrochemical application (Kaushal and Wani 2016). Biocontrol of phytopathogens through microbial agents or their metabolites is a cheap and environment-friendly component of a successful wheat disease management program (Sood and Kaushal 2021; Xu et al. 2021). In order to combat these pathogens, the quest for biocontrol agents for wheat diseases and the importance of various beneficial microorganism–pathogen interactions have been highlighted in numerous reports. In this regard, one of the most notable examples of antagonistic bacteria protecting plant roots can be found in soils that suppress wheat take-all disease. Take-all decline (TAD) is well-known to result from the accumulation of populations of 2,4-diacetylphloroglucinol

Table 10.1 Some effective instances of nonbio/bio-derived agents used to safely manage wheat diseases

Type of controlling approach	Pathogen	Disease's name	Resulted effect	Reference
Biogenic nanoparticles (titanium nanoparticles)	*Bipolaris sorokiniana*	Spot blotch disease	Significant reduction of disease severity	Satti et al. (2021)
Biogenic nanoparticles (silver nanoparticles)	*Fusarium graminearum*	Fusarium head blight	Inhibition of fungal growth and reduction of mycotoxin in wheat grains	Ibrahim et al. (2020)
Biogenic nanoparticles (silver nanoparticles)	*Bipolaris sorokiniana*	Spot blotch disease	AgNPs deposited lignin in the vascular bundles of the host plant	Mishra et al. (2014)
Plant extracts (extracts of 5 plants)	*Puccinia triticina*	Wheat leaf rusts	Treatment resulted in a significant decrease in the coefficient of infection of wheat leaf rust as well as an increase in wheat yield	Draz et al. (2019)
Plant extracts (*Curcuma zedoaria* rhizomes or its substance)	*Puccinia triticina*	Wheat leaf rusts	In vivo, the treatment significantly suppressed wheat leaf rust	Han et al. (2018)
Plant extracts (extracts of neem, clove, and garden quinine)	*Puccinia triticina*	Wheat leaf rusts	Treatment completely prevented the development of leaf rust in treated plants	Shabana et al. (2017)
Plant extracts (*Agapanthus africanus* extracts)	*Puccinia triticina*	Wheat leaf rusts	The treatment increased the activities of -1,3-glucanase, chitinase, and peroxidase in both susceptible and resistant wheat cultivars	Cawood et al. (2010)
Plant extracts (aqueous leaf extracts of *Jacaranda mimosifolia*)	*Puccinia triticina*	Wheat leaf rusts	Plant extract treatment alone or in combination with 0.05% Amistar Xtra increased PR protein expression in treated plants	Naz et al. (2014)
Plant resistance inducers (*N*-hydroxypipecolic acid)	*Fusarium graminearum*	Fusarium head blight	Treatment boosts immune response, enabling wheat plants to defend themselves against pathogens	Zhang et al. (2021)
Plant resistance inducers (saccharin)	*Zymoseptoria tritici*	Septoria tritici blotch	Treatment reduced disease severity by 77% by eliciting and priming	Mejri et al. (2020)

(continued)

Table 10.1 (continued)

Type of controlling approach	Pathogen	Disease's name	Resulted effect	Reference
			lipoxygenase and PR gene-related defense pathways	
Plant resistance inducers (several chemical inducers)	*Mycosphaerella graminicola*	Septoria leaf blotch	The treatment had a suppressive effect on Septoria leaf blotch, as well as an increase in wheat grain yield	El-Gamal et al. (2021)
Plant resistance inducers (salicylic acid)	*Zymoseptoria tritici*	Septoria tritici blotch	Treatment resulted in the induction of pathogen resistance in wheat-treated plants by increasing the expression of both the PAL and PR2 genes	Mahmoudi et al. (2021)

(2,4-DAPG)-producing fluorescent *Pseudomonas* spp. during wheat monoculture. This is due to the unique fungicidal activity of 2,4-DAPG against the causal agents of this disease, *Gaeumannomyces graminis* var. *tritici* (Durán and de la Luz Mora 2021; Kwak et al. 2012; Kwak and Weller 2013). Similarly, Yang et al. (2014) discovered that the in vitro growth of *Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia solani* AG-8 was inhibited by cyclic lipopeptide (CLP) produced by *Pseudomonas fluorescens* HC1–07. In another study, *Bacillus velezensis* CC09 was shown to demonstrate 66.67% disease-control efficacy (DCE) of take-all and 21.64% DCE of spot blotch by efficiently colonizing the wheat leaves, roots, and stem and leaves, respectively (Kang et al. 2018). Furthermore, wheat powdery mildew was significantly suppressed by *B. subtilis* (4×10^5 CFU ml^{-1}) during in vitro via inhibition of conidial germination and normal appressorium development, or in vivo via induction of disease resistance in wheat (Xie et al. 2021). Additionally, pathogenicity-related genes of *Gaeumannomyces graminis* var. *tritici* were downregulated in pathogen-inoculated roots of wheat treated with the biocontrol agent *Bacillus velezensis* CC09 (Kang et al. 2019). Similarly, *Bacillus amyloliquefaciens* subsp. *plantarum* XH—9 demonstrated a high capacity to colonize wheat roots and significantly reduced *Fusarium oxysporum* in roots of the treated plants as revealed by qRT PCR analysis (Wang et al. 2018). Despite the fact that antagonistic fungi have been shown to have biocontrol capacity against various cereal pathogens, chemical fungicides are not quietly replaced by commercial fungal biocontrol. So far, research on bio-management of wheat pathogens by antagonistic fungi has primarily focused on using *Trichoderma*. *Trichoderma harzianum,* for example, outperformed *T. viride* as a bioagent, inhibiting the growth of spot blotch disease by 60.82% in vitro (Kaur et al. 2021). Arbuscular mycorrhizal fungi (AMF), which share symbiotic relationship with nearly all plants, are

important fungi that live in the rhizospheric soil and could be used to control wheat diseases. AMF has been shown in this symbiosis to improve growth and crop yield, as well as to provide tolerance against different stress factors, including protection against many phytopathogenic fungi and heavy metal toxicity (Eke et al. 2016; Spagnoletti et al. 2017, 2018). In this context, inoculation with AMF *Rhizophagus intraradices* significantly reduced the population density of *Fusarium pseudograminearum* by 75.7% and 39% disease severity in wheat grown in greenhouse (N.C. Schenck and G.S. Sm.), via a mechanism of redox balance and competition for root colonization compared to the untreated control (Spagnoletti et al. 2021). Plant endophytic microorganisms may be better adapted than epiphytic microorganisms to enter, colonize, and secrete secondary metabolites within the plant (Busby et al. 2016; Ulloa-Ogaz et al. 2015). In this respect, pre-colonization of wheat with endophytic fungi *Sarocladium zeae*, followed by *F. graminearum* inoculation, resulted in a significant reduction of fusarium head blight symptoms (57.9%) and a 61.2% reduction in mycotoxin content in harvested wheat heads (Kemp et al. 2020). Remarkable results are listed in Table 10.2.

10.2.3 Applying of Plant Extracts

Plant-derived natural products have grown into one of the leading resources for discovering novel compounds with distinct biological functions, resulting in remarkable number of novel phytopathogen-controlling agrochemicals (Agarwal et al. 2020; Lorsbach et al. 2019; Umetsu and Shirai 2020). Several natural plant products have been shown to reduce foliar pathogen populations and limit disease development, implying that these plant extracts could be used as eco-friendly alternatives and components in integrated pest management approaches (Draz et al. 2019). Several plant species have been found to contain natural substances that are either toxic to several wheat pathogens or can induce plant systemic resistance against them (Draz et al. 2019; Han et al. 2018). In this context, numerous related experiments have been performed to investigate the effectiveness of plant extracts in controlling leaf rust in wheat caused by *Puccinia triticina*. For example, pre-application of five plant extracts significantly reduced the coefficient of infection of wheat leaf rust, and the yield was significantly increased (Draz et al. 2019). Likewise, spraying *Curcuma zedoaria* rhizomes or its isolated substance sesquiterpene ketolactone showed significant activity against wheat leaf rust in vivo (Han et al. 2018). In another study, spraying four-day post-inoculated wheat plants with clove, neem, and garden quinine extracts resulted in complete prevention of leaf rust development in treated plants (Shabana et al. 2017). More intriguing results were observed during foliar application of *Agapanthus africanus* extracts, which unanimously improved the in vitro activities of three pathogenesis-related proteins (PR); i.e., chitinase, -1,3-glucanase, and peroxidase; in susceptible and resistant wheat cultivars, regardless of whether they were infected or uninfected with leaf rust (Cawood et al. 2010). Furthermore, it has been reported that spraying wheat leaves as a pretreatment with bioformulations consisting of aqueous leaf extracts from

Table 10.2 Some successful examples of environmentally friendly biological agents/products used to control wheat diseases

Type of controlling approach	Pathogen	Disease's name	Resulted effect	Reference
Biological control (*Pseudomonas fluorescens*)	*Gaeumannomyces graminis* var. *tritici*	Take-all disease	Take-all declination in wheat plants caused by the toxic effect of 2,4-DAPG on the pathogen	Durán and de la Luz Mora (2021)
Biological control (*Pseudomonas fluorescens* HC1–07)	*Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia solani AG-8*	–	In vitro inhibition of fungal growth	Yang et al. (2014)
Biological control (*Bacillus velezensis* CC09)	*Gaeumannomyces graminis* var. *tritici* and *Bipolaris sorokiniana*	Take-all disease + spot blotch disease	66.67% and 21.64% disease-control efficacy of take-all and spot blotch, respectively	Kang et al. (2018)
Biological control (*B. subtilis*)	*Blumeria graminis* f. sp. *Tritici*	Wheat powdery mildew	Treatment inhibited conidial germination and normal appressorium development in vitro and induced disease resistance in wheat in vivo	Xie et al. (2021)
Biological control (*Bacillus velezensis* CC09)	*Gaeumannomyces graminis* var. *tritici*	Take-all disease	Pathogen pathogenicity-related genes are downregulated as a result of bioagent treatment	Kang et al. (2019)
Cultivar mixtures	*Zymoseptoria tritici*	Septoria tritici blotch	AUDPC of susceptible plants was reduced by 68% in the heterogeneous mixture and by 32% and 34% in the homogeneous mixtures with 75% and 25% of resistant plants, respectively	Vidal et al. (2017)
Cultivar mixtures	*Puccinia striiformis* f. sp. *tritici*	Wheat yellow rust	In comparison to pure stands, heterogeneous mixtures reduced the variability of disease severity and yield	Vidal et al. (2020)
Cultivar mixtures	*Zymoseptoria tritici*		Adding 25% of a resistant cultivar to a	Ben M'Barek et al. (2020)

(continued)

Table 10.2 (continued)

Type of controlling approach	Pathogen	Disease's name	Resulted effect	Reference
		Septoria tritici blotch	pure stand of a susceptible cultivar reduces disease severity by nearly 50%	
Biofumigation (*Brassica carinata* as a break crop)	*Bipolaris sorokiniana* and *Fusarium culmorum*	Common root rot and fusarium foot rot	Treatment reduced the incidence and severity of fusarium foot rot by 40.6% and 56.3%, respectively, and completely eliminated common root rot on wheat	Campanella et al. (2020)
Biofumigation (white mustard meal)	*Fusarium culmorum* Sacc	Common root rot	In a greenhouse trial, treatment reduced pathogen infection by 38% and improved wheat growth and grain quality parameters in a field trial	Kowalska et al. (2021)
Biofumigation (mulch layer and botanical extracts of three plants)	*Fusarium graminearum*	Fusarium head blight	Treatment resulted in consistent suppression of fusarium head blight and a significant reduction of mycotoxins in wheat grains	Drakopoulos et al. (2020)
Biofumigation (isothiocyanates compounds)	*Fusarium graminearum*	Fusarium head blight	In vitro inhibition of conidial germination and mycelium radial growth is enhanced by isothiocyanates, allyl, and methyl isothiocyanates	Ashiq et al. (2021)

Jacaranda mimosifolia only and/or combined with 0.05% Amistar Xtra improves leaf rust resistance due to the extract's ability to increase PR protein expression in treated plants (Naz et al. 2014). Plant extracts, on the other hand, were used to counter other diseases that afflicted the wheat plant and demonstrated remarkable efficacy in controlling those diseases. For instance, several studies have reported that plant extracts have remarkable antimicrobial activities against *Bipolaris sorokiniana* and other wheat fungal pathogens (Bahadar et al. 2016; Magar et al. 2020; Naz et al. 2018; Perelló et al. 2013). Findings related to the controlling effect of plant extracts are listed within Table 10.1.

10.2.4 Cultivar Mixtures for Wheat Disease Management

Cultivar mixtures/multiline cultivars can be an efficient technique for managing crop diseases, especially those caused by airborne pathogens. Though some seedlings can be affected by certain elements of the pathogen population, the host mixture overall elicits considerable resistance, owing primarily to host diversification (Brooker et al. 2021; Mundt 2002; Zhang et al. 2018). Several mechanisms can be used to demonstrate disease suppression among combinations of susceptible and resistant plant cultivars. In this regard, de Vallavieille-Pope (2004), Finckh et al. (2000), and Mundt (2002) demonstrated several potential mechanisms for elucidating the reductive effect of cultivar mixtures on plant diseases. Such mechanisms include pathogen spread restriction because of the resistant plants present among susceptible plants, induction of host resistance as a result of infection by avirulent strains, which can decrease subsequent infection by virulent strains, and competition among pathogen populations for available host tissues (Fig. 10.1). Vidal et al. (2017) recently demonstrated four mechanisms by which cultivar mixtures reduce disease. The first is the density effect, which involves distancing the susceptible cultivars within the mixture, minimizing the amount of inoculum reaching them. Second, there is the barrier effect, which is represented by the interception of pathogen spores in the mixtures by resistant plants. Third, induced resistance occurs when an avirulent pathogen race infects an incompatible host. Finally, microclimate modification occurs as a result of the varied characteristics of mixed cultivars, such as plant height, canopy structure, and so on, which alter the microenvironment, making it unsuitable for the development of plant diseases. Cultivar mixtures are not used to get rid of phytopathogens. Instead, they are used to reduce disease development in

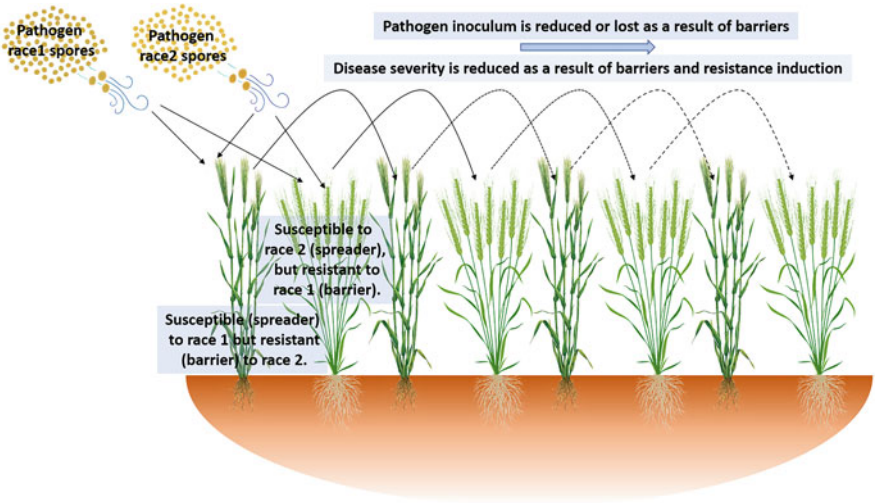

Fig. 10.1 Some of the known mechanisms by which cultivar mixtures can suppress foliar diseases in wheat

the mixture by lowering the amount of inoculum needed for a severe infection (Kumar et al. 2021; Vidal et al. 2017, 2020). Besides, it has also been demonstrated that cultivating mixtures of cultivars with varying disease resistance levels and agronomic traits in the same field at the same time produces higher yields than pure cultivars (Fang et al. 2014; Župunski et al. 2021). Similarly, Kristoffersen et al. (2020) revealed that cultivar mixtures reduced (by 10.6%) the severity of *Septoria tritici* blotch caused by *Zymoseptoria tritici* and improved yields by 1.4% across all tests in a meta-analysis of 406 trials conducted over 19 years. However, in another study, cultivar mixtures reduced wheat stripe rust to a lesser extent than susceptible pure stands (Huang et al. 2011). Recently, it was stated that increasing the number of cultivars with regard to diversification in agronomical characteristics such as plant height and earliness did not have a negative impact on the performance of the mixtures, but rather contributed to stabilizing the reduction of pathogen spread within the mixtures population and improving their yield when compared to their corresponding pure stands (Vidal et al. 2020). Another meta-analysis study found that disease reduction of wheat stripe rust provided by cultivar mixtures may be more effective during intensive disease infection and moderate wheat sowing density (Huang et al. 2012). Due to the fact that farmers prefer susceptible components within cultivar mixtures for their agronomical traits over resistant components, researchers are increasingly investigating the performance of mixtures with a considerable ratio of the resistant cultivar which offer a comparable level of disease reduction as pure resistant components. In this regard, Ben M'Barek et al. (2020) revealed that mixing 25% resistant cultivar with pure stand of susceptible cultivar leads to a considerable reduction (nearly 50%) in *Septoria tritici* blotch disease severity when compared to the pure susceptible component. Canopy architecture of cultivar mixture components must be considered in addition to disease resistance in order to improve cultivar mixture performance in disease reduction (Vidal et al. 2017). Furthermore, wheat cultivar mixtures had no beneficial effect on soil collembola as beneficial insects contribute to the regulation of the activities of decomposing microorganisms, consuming fungal phytopathogens in soil, and regulating the activity of mycorrhizal fungi (Salmon et al. 2021). Additionally, cultivar mixtures have proven to have a higher potential for yield increase when compared to pure components, particularly in low pesticide input cropping systems (Borg et al. 2018). The presentative findings related to the role of cultivar mixtures in the reduction of wheat diseases are summarized in Table 10.2.

10.2.5 Estimation of Plant Resistance Inducers' Mitigating Effect Against Wheat Phytopathogens

Agents that improve plant resistance to pathogens by stimulating the plant's particular defense mechanisms, or its own induced resistance, are called Plant resistance inducers (Alexandersson et al. 2016). To deal with biotic stresses or pathogens, plants typically have an advanced immune system. Plants physically defend themselves by barriers like dense cuticles, waxes, and unique trichomes that inhibit

pathogens and insects from staying on plants. Additionally, chemical complexes are produced by plants as defense against pathogens and herbivores (Moustafa-Farag et al. 2020). However, in order to properly deal with such a challenge, the plant must first identify a biotic stress casual factor or pathogen as an unfriendly component that must be dealt with. Pathogens can be recognized by plants via two pathways that activate defense responses. First, pathogen-associated molecular patterns (PAMPs) including peptidoglycans, fungal chitin, bacterial lipopolysaccharides, and quorum sensing are recognized by the pattern recognition receptors. PAMP-triggered immunity is the most basic form of defense (PTI) (Monaghan and Zipfel 2012). The second pathway of the immune system (ETI) comprises secretion of plant resistance proteins (R), which through effector-triggered immunity process detect pests'/pathogens' specific effectors (Avr proteins) and activate the plant defense response. As a result, hypersensitive responses (HR) are triggered, which involve programmed cell death in affected cells and their adjacent regions (Spoel and Dong 2012). Generally, phytohormones such as ethylene (ET), salicylic acid (SA), and jasmonic acid (JA) act as signaling molecules for two types of efficient plant pathogen resistance. The first type is called systemic acquired resistance (SAR) that occurs when necrotizing pathogens infect the cells and are associated with large amount of SA and pathogenesis-related proteins (Grant and Lamb 2006). The second type of plant resistance is induced systemic resistance (ISR), which is activated by the application of plant resistance inducers, which can be either biotic, such as non-pathogenic root-colonizing microorganisms or any other non-virulent pathogen, or nonbiotic, such as chemical agents or plant extracts. Such resistance necessitates the use of signaling compounds such as JA and ET (Alexandersson et al. 2016). However, as many studies have shown, many chemical inducers can also activate the first type of resistance in plants (Lee et al. 2014, 2015; Zhao et al. 2019). Plant resistance inducers can have a systemic effect, as described above, or a local effect, such as changes in composition of the cell wall, hypersensitive response (HR), and producing antimicrobial protein and phytoalexins (Alexandersson et al. 2016). Many chemical inducers, such as SA, benzothiadiazole (BTH), 2,6-dichloroisonicotinic acid (INA), acetylsalicylic acid, β-aminobutyric acid (BABA), and trehalose.... etc., have been widely used to control wheat disease. For instance, in a three-year field trial, wheat plants treated with chemical inducers or some plant extracts exhibited long-term-induced resistance and a reduction in powdery mildew disease severity ranging from 2% to 53% (Vechet et al. 2009). In another study, although pretreatment of wheat plantlets with *N*-hydroxypipecolic acid moderately increases resistance to *Fusarium graminearum*, it improves immune response, allowing wheat plants to defend themselves against pathogens (Zhang et al. 2021). Furthermore, under greenhouse conditions, foliar treatments of wheat seedlings with saccharin, a metabolite derived from probenazole, caused a 77% decline in Septoria tritici blotch disease onset, and the protective effect of saccharin was attributed to induction and priming of lipoxygenase and PR gene-related defense pathways (Mejri et al. 2020).

Meanwhile, spray application of several chemical inducers under field conditions suppressed Septoria leaf blotch, particularly treatment with potassium silicate and sodium silicate; this positive effect was accompanied by an increase in grain yield in

sprayed wheat plants that were sprayed (El-Gamal et al. 2021). Also, pretreatment of wheat seedlings with SA resulted in the induction of resistance against *Septoria tritici* blotch in wheat plants by significantly upregulating the phenylalanine ammonia-lyase (*PAL*) and β-1,3-glucanase (*PR2*) genes in wheat-treated plants compared to untreated ones (Mahmoudi et al. 2021). Table 10.1 summarizes the preliminary findings concerning the function of plant resistance inducers in the reduction of wheat diseases.

10.2.6 Biofumigation for the Safe Management of Wheat Diseases

Biofumigation is the suppression of soilborne pathogens through the decomposition of organic material, such as agricultural by-products or manure, which releases volatile chemicals that have the ability to reduce different types of phytopathogens including bacteria, fungi, and nematodes (Baysal-Gurel et al. 2020; Madhavi Gopireddy et al. 2019; Matthiessen and Kirkegaard 2006). Biofumigation can be accomplished by including green manure, seed meals, or dried plant matter that has been treated to retain isothiocyanate activity into the soil (Lu et al. 2010; Matthiessen and Kirkegaard 2006). Plants in the *Brassicaceae* family are more appropriate for biofumigation because their tissues contain a high concentration of glucosinolates and other sulfur-containing compounds (Campanella et al. 2020). As a result of the hydrolysis of glucosinolates by the action of the myrosinase enzyme, these plants emit toxic substances such as nitriles, thiocyanates, isothiocyanates, oxazolidine, methanethiol, and dimethyl sulfide (Fahey et al. 2001).

The hydrolyzation process happens when plant tissues are injured or chopped. Consequently, fresh Brassicaceae plants or seed meals are chopped and mixed into the soil to perform biofumigation (Ziedan 2022). Because of their exposure to the slowly released toxic substances, many harmful soilborne phytopathogens, weeds, and insects are effectively suppressed (Madhavi Gopireddy et al. 2019). Furthermore, these substances can boost the activity of beneficial soil microorganisms and increase their competitiveness against non-beneficial microorganisms (Galletti et al. 2008; Gimsing and Kirkegaard 2009). Additionally, this process can help improve soil fertility by increasing available nutrients, enhancing soil properties, and enriching soil organic matter (Galletti et al. 2008; Gimsing and Kirkegaard 2009; Matthiessen and Kirkegaard 2006). Biofumigation with brassica plant materials has proven to be an operative method for controlling wheat soilborne pathogens, either alone or in combination with other methods. In this regard, using *Brassica carinata* as a break crop with durum wheat reduced the occurrence and intensity of Fusarium foot rot by 40.6% and 56.3%, respectively, as well as no symptoms of common root rot on wheat plants cultivated after *B. carinata* break crop. These positive results were accompanied by a significant boost in wheat yield when compared to wheat monoculture (Campanella et al. 2020). Moreover, using white mustard meal as a wheat seed wet dressing reduced *Fusarium culmorum* Sacc. infection by 38 to 44% in a greenhouse trial and improved wheat growth and grain quality parameters in a field trial (Kowalska et al. 2021). Similarly, mulch layer and botanical extract

treatments of *Sinapis alba*, *Brassica juncea*, or *Trifolium alexandrinum* on top of the maize remains infected with *Fusarium graminearum* after wheat planting demonstrated consistent suppression of fusarium head blight and remarkable reduction of mycotoxins contents in wheat grains over 2 years of field experiments (Drakopoulos et al. 2020). Additionally, Ashiq et al. (2021) found that isothiocyanates, allyl, and methyl isothiocyanates have greater inhibition abilities against conidial germination and mycelium radial growth than the other isothiocyanates compounds during in vitro evaluation of antifungal activity against *Fusarium graminearum*. Table 10.2 illustrates the initial findings regarding the role of biofumigation as an effective means of suppressing soilborne wheat diseases.

10.3 Conclusions and Prospects for the Future

The uncontrolled use of agrochemicals has caused significant damage to resource-poor ecosystems with low biodiversity. The severity of these damages has reached an unprecedented level in recent years, particularly in developing countries. For example, the accumulation of high concentrations of agrochemicals in agricultural soils has negatively impacted the positive role of their beneficial microorganisms and significantly reduced their organic matter content, significantly reducing their ability to retain moisture content and negatively affecting their other physical properties. Such that if these irresponsible behaviors of random and excessive use of these chemicals are not remedied, this may result in irreversible damage to these poor ecosystems' vitality, components, and natural resources. This is in addition to the significant health consequences for humans and animals. As a result, it is critical that we use safe environmental alternatives to achieve environmentally friendly pest management of agricultural pests in order to restore and repair damaged ecosystems. The continued use of these alternatives will allow us to sustain our ecosystems' limited natural resources. This is accomplished practically by incorporating these alternatives into integrated pest management programs for cereal crops, particularly wheat, either individually or in combination with the other available alternatives. Furthermore, such incorporation should be carried out in a way that does not negatively impact the performance of currently used methods, as well as the components and resources of existing ecosystems, and is also compatible with the limited material capabilities of low-income farmers. This will contribute to the gradual disappearance of pollution challenges caused by the accumulation of high levels of agrochemical concentrations in soil and other ecosystem components. Figure 10.2 depicts a number of anticipated benefits from the use of environmentally friendly approaches in the control of wheat diseases.

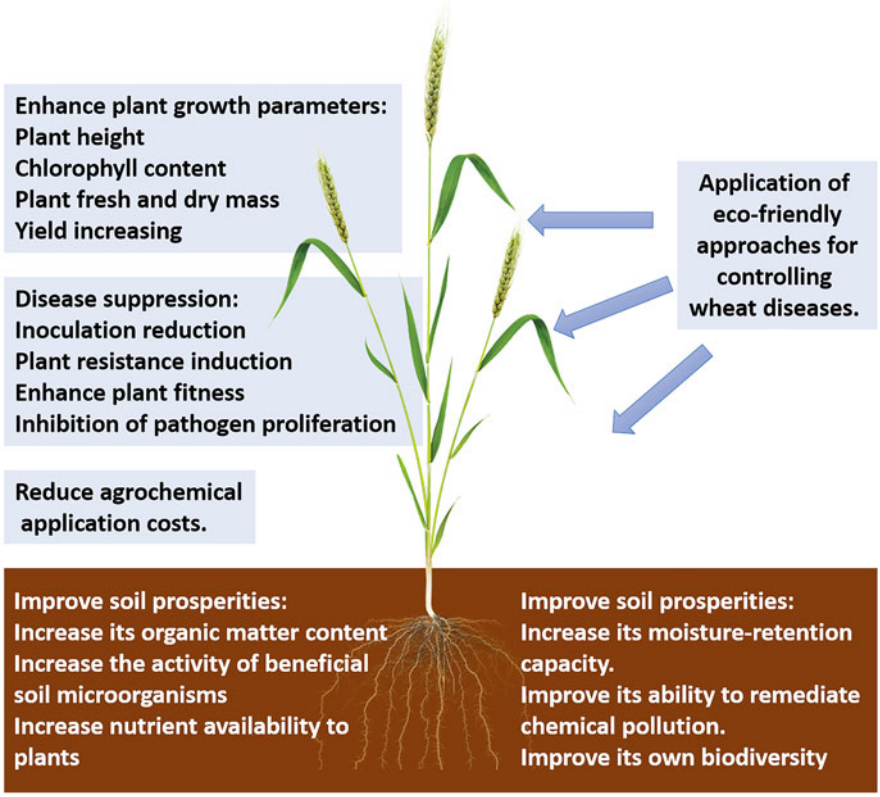

Fig. 10.2 The anticipated advantages of using eco-friendly alternatives for the safe management of wheat diseases are many

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Part III

Genome Editing

Resistance Gene Identification, Cloning, and Characterization in Plants

11

Muhammad Abu Bakar Saddique, Saad Zafar, Zulkifl Ashraf, Muhammad Atif Muneer, Babar Farid, and Shehla Shabeer

Abstract

Various plant diseases and diverse microbial communities, including bacteria, fungi, oomycetes, viruses, and nematodes, drastically deteriorate crop quality and yield worldwide. Plant-pathogen interaction mechanisms have been extensively studied, which involve the activation of signaling events that lead to the suppression of pathogen attacks. Several R genes have been found in plants containing conserved functional domains and nucleotide-binding sites with leucine-rich repeats (*NBS-LRR*). So far, different experimental approaches have been used to identify resistant genes in a variety of plant species. For example, PCR-based cloning has been employed to identify putative NB-containing R genes that help to identify potential resistance gene homologs (RGHs). Besides, multiple or complicated features connected to a single or several stress responses can be studied using genome-wide association studies (GWAS). In recent years, for the cloning and mapping of resistance gene analogues (RGAs), a sequence-homology-based approach has been extensively used. In this chapter, the identification of resistant genes, their resistance, cloning types, and the identification

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and characterization of RGA have been discussed. Simultaneously, the mechanisms of the different resistant genes and their functions in different crops have been reviewed. Furthermore, the *RGAs* that have been cloned in many different crops have been suggested as a source of genetic material for cultivars that are resistant to disease for a long time in crop-breeding programs.

Keywords

Plant pathogens · Resistance · Plant disease · Plant breeding · Biotechnology · Cloning · Food security

11.1 Introduction

Climate change is the major threat to mankind. Globally, it causes 0.4 million deaths per year due to an increase in temperature and greenhouse gas (GHG) concentrations. These changes cause drastic effects on crop growth, development, and cultivation of crops on the globe. Simultaneously, these changes cause disturbances in the reproduction and severity of many plant pathogens (Gautam et al. 2013). Plant diseases are the result of the interaction of these plant pathogens like bacteria, fungi, viruses, nematodes, and insects with susceptible hosts and the environment. It causes a huge reduction in crop production globally. According to FAO, about 20–40% of global crop yield is reduced each year due to biotic factors (pests and diseases). Climate change has made it easier for many plant pathogens to move around, which has resulted in the emergence of new diseases that could spread in uncontrollable rashes and endanger food security (Piquerez et al. 2014). A lot of research work has been done by plant scientists to deal with these issues by finding disease resistance genes and their mechanisms in plants for better crop productivity and developing resistant cultivars.

To cope with this biotic stress, plants develop several tolerance mechanisms through the activation of specific genes. Interaction of a plant with a pathogen is one of the well-understood mechanisms. It involves the activation of signals, which often leads to a quick defense response against a variety of diseases. This response supports the host plant's defense against that disease infestation. Belkhadir et al. (2004) describe the specialized genes that induce defense signaling and recognition of pathogen effectors by plants (Belkhadir et al. 2004). Resistance genes (R genes) have a significant role in crop protection against diseases. In the last 30 years, researchers have identified 300 functional R genes in different crops (Kourelis and Van Der Hoorn 2018). Numerous identified plant-specific R genes are currently being employed in crop improvement programs. Plant R genes are used to generate disease-resistant varieties as an alternative to conventional means of disease control, such as using pesticides or other chemical control approaches. Introduction of R genes into susceptible plants will result in an efficient reduction of pathogen growth, low host plant damage, and zero pesticide application by farmers. The conventional breeding methods for developing disease resistance are time-consuming and

laborious because, for this purpose, repeated backcrossing has to be performed until complete transfer of resistant genes into susceptible genotypes is achieved. Currently, a large number of nonconventional techniques have been developed to develop tolerant genotypes against disease. Plant resistance genes have undergone extensive functional research, cloning, characterization, and genetic transformation. That is expected to fuel researchers in resolving these issues (Gururani et al. 2012). R genes provide host resistance, which is a cost-effective and eco-friendly biotechnological technique. It reduces agricultural diseases and increases crop yield by producing disease-resistant crops (Kumar et al. 2017). This review accentuates plant diseases caused by climate change, the identification and characterization of disease-resistant genes, and tolerance mechanisms in crop plants.

11.2 Identification of Resistant Genes for Plant Diseases

There are increasing strains of plant pathogens producing harmful diseases. The spread of plant pathogens and the severity of these diseases cause huge yield reductions. To minimize this effect, many resistant genes have been identified in crop plants. Many other advanced techniques have been developed as well. For the identification of resistant genes, in silico analysis was performed by scientists with their expressional profiling. Over the last two decades, new DNA sequencing technologies have proliferated. Mainly, next-generation sequencing (NGS) and third-generation sequencing (TGS) have improved the reliability and speed of sequencing, although the entire sequencing still takes hours or days to complete (Van Dijk et al. 2018). For medical and research purposes, sequencing is essential in biological classification, cell biology, forensic investigation, gene identification, and gene manipulation. Experimental approaches such as PCR cloning have been used to find probable NB-containing R genes in a range of plant species, including those that also identify RGHs in different plant species such as Arabidopsis (Meyers et al. 1999), rice (Bai et al. 2002), and cotton (He et al. 2004). Homology-based bioinformatics techniques have found thousands of possible NB-containing R genes in crops like rice (Monosi et al. 2004), potato (Lozano et al. 2012), and soybean (Nepal and Benson 2015). These genes are thought to be R genes.

Genome-wide association studies (GWAS) are an effective method for examining multiple or complex traits linked to a single or many stress responses. Novel candidate genes or quantitative trait loci (QTLs) responsible for abiotic and biotic stress have been identified by GWAS in many crop plants. Traditional gene mapping has several limitations, which are overcome by the genome-wide association technique (GWAS) (Brachi et al. 2011). Since the introduction of high-density single-nucleotide polymorphisms (SNPs), whole-genome scans have been able to uncover discrete haplotype blocks that are strongly linked to quantitative trait variation. Resistance gene analogues (RGAs) have been shown to be an important method for identifying disease resistance genes in a variety of crops. A large number of disease resistance genes have been found and successfully cloned in a few crops until today. RGAs have opened new paths in research on genetic organization and

evolutionary aspects of distinct classes of resistance genes among diverse plant species (Zhang et al. 2016). The Plant Resistance Genes database (PRGdb) has been updated with a new user interface, sections, tools, and data for genetic improvement. Plant scientists and plant breeders use these resources to develop high-yielding and disease-resistant genotypes.

11.3 Mechanism of Resistance Gene

Plant resistance genes encode special proteins that can detect pathogens. For decades, R genes have been used in plant breeding approaches for developing resistant genotypes with varying success rates. In many agricultural plants, genetic resistance has been the most successful disease management approach. The two main types of host resistance are major gene resistance and quantitative resistance. Major gene resistance is predominantly controlled by a single gene and also engaged in ETI, whereas quantitative resistance is under the influence of many genes that involve plant defenses induced by PTI. It also helps in understanding the significant connections between the processes triggered by ETI and PTI. In agronomic terms, significant gene resistance results in a "clean" crop, but its durability is unknown. On the other hand, quantitative resistance allows for some disease infection while maintaining stability. Plants have the ability to identify pathogens and their modes of reproduction and growth (Leach et al. 2014). Plants have a complicated immune system based on their ability to recognize phytopathogens. The presence of pathogen recognition genes (PRGs), which encode specific receptors, is activated. Pathogen-associated molecular patterns-triggered immunity (PTI) and effector-triggered immunity (ETI) are two tiers of defensive mechanisms in the plant immune system. Hundreds of immunological receptors activate these pathways when pathogen-derived signals are detected (Xue et al. 2020). Death of infected cells occurs due to the activation of resistant R proteins in the ETI mechanism. NBS-LRR/NLR (nucleotide-binding domain and leucine-rich repeats) is the class of intracellular receptors that contains many R proteins (DeYoung and Innes 2006; Jacob et al. 2013). PAMPs (Pathogen-associated molecular patterns) receptors are classified as R genes because they provide partial or even complete resistance to pathogens (Lacombe et al. 2010). Recent molecular techniques are influencing the use of R genes due to their signaling mechanisms for disease response (Fig. 11.1).

11.3.1 Different Identified R Genes and Their Resistance Mechanisms

In different plant species, different numbers of R genes are present, and when a pathogen attacks, those genes are activated against that disease and develop tolerance mechanisms. A single R gene can produce complete resistance against one or more strains of harmful pathogens when transferred to the susceptible plant of the same species (McDowell and Woffenden 2003). That's why they are used in

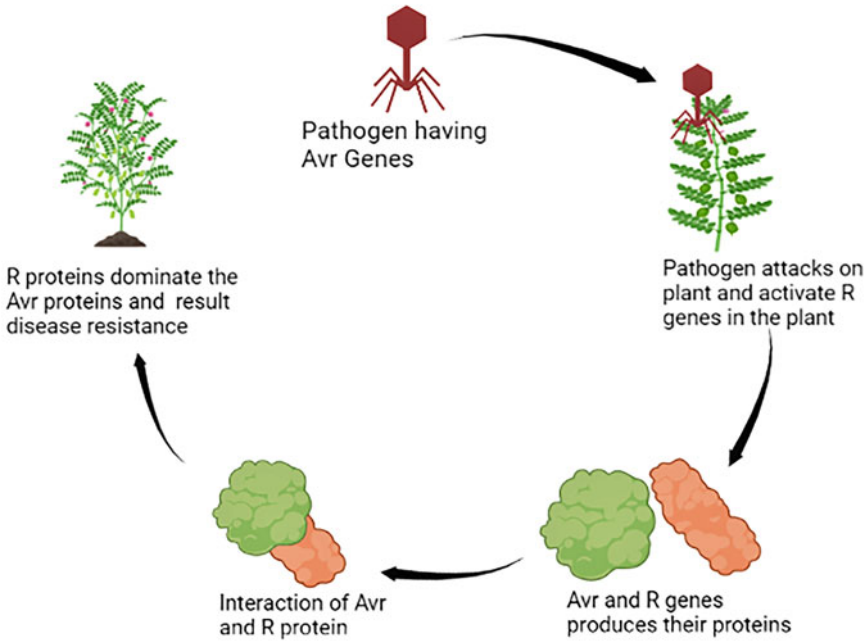

Fig. 11.1 Interaction between plant R proteins and pathogen Avr proteins occurs during the resistance mechanism when a pathogen attacks a host plant having an *Avr* gene, resulting in the activation of R genes to provoke the disease infection. Their genes produce corresponding proteins, which results in their strong interaction, which causes disease resistance. Plants missing R genes suffer from disease attack and yield reduction

different breeding programs for developing resistant genotypes. Further, the variations in plant phenotype and response to pathogen attack urge scientists to work on their cloning and investigations into their molecular modes of action (Table 11.1).

11.4 Genetics of Resistance

For sustainable agriculture, disease-resistant plants are one of the prerequisites. Basically, genetic resistance is classified into two categories.

11.4.1 Race-Specific or Vertical Disease Resistance

As the name indicates, resistance to a specific race of pathogen. These genes are effective against a limited number of pathogens, but not all. They generally follow the gene-for-gene model. This type of resistance is controlled by a single gene known as monogenic resistance, but it is not durable or long-lasting because

Table 11.1 R genes and disease tolerance mechanisms

Mechanism	Description	R Genes	References
RLP/RLK, direct	A pathogen-derived effector interacts directly with a cell surface RLK/RLP receptor to trigger the recognition of disease-resistant genes	*RLP23, EFR*, *RBGP1*, *LORE*, *LYM1/LYM3, LYK3*, *FLS2, LYK4*, *LYK5*, *LYM2*, (Arabidopsis), *CEBiP*, *LYP4/LYP6*, *OsFLS2* (rice), *FLS3*, *SlFLS2, LeEIX2*, *CORE*, (tomato), *VvFLS2* (grapevine)	Hind et al. (2016), Katsuragi et al. (2015), Zhang et al. (2013)
RLP/RLK, indirect	The activation of resistant genes occurred by the binding of effector to the host plant or by binding of effector-mediated modification of host triggered by an RLP/RLK	Cf-*2* (tomato)	Seear and Dixon (2003)
NLR, direct	Recognition triggered by direct interaction of a pathogen-derived component and an NLR	*HRT1, SUMM2*, *RPS2*, *RPM1, RPS5*, *ZAR1* (Arabidopsis), *Gpa2*, *R2-like*, *Rpi-abpt*, *Rx1*, *Rx2, Rpi-blb3*, *R2*, (potato), *Rpg1r, Rpg1-b* (soybean)	Kourelis and Van Der Hoorn (2018), Krasileva et al. (2010)
NLR, indirect	The activation of resistant genes occurred by the binding of effector to the host plant or by binding of effector-mediated modification of host triggered by an NLR	*RanGAP2*, *BSL1* (soybean), *RIN4*, *TIP*, *PBS1*, *CRCK3*, *RKS1*, *PBL2, ZED1*, *ZRK3*, (Arabidopsis)	Lewis et al. (2010), Russell et al. (2015), Seto et al. (2017)
NLR-ID	In this mechanism, the recognition of resistant genes is triggered by the binding of effector with a domain or by effector-mediated modified domain that is integrated in the host NLR	*R1* (potato), *RRS1-R*, *RRS1B*, *RRS1-S* (Arabidopsis), *RGA5-A*, *Xa1* (rice)	Cesari et al. (2013), Saucet et al. (2015)
Executor	The pathogen effector TAL activates the executer gene which helps in recognition of resistant gene	*Bs3*, *Bs4C-R, Bs3-E* (pepper), *Xa10*, *Xa7, Xa23*, (rice)	de Lange et al. (2013), Gu et al. (2005)
Other, active	This mechanism helps in reducing the susceptibility by directly affecting pathogen by	*Hm1*, *qMdr9.02*, *Hm2,* (maize), *At1*, *At2* (melon), *STV11-R* (rice), *Ty-1*, *Tm-1*, *Ty-3* (tomato)	Butterbach et al. (2014), Johal and Briggs (1992), Sindhu et al. (2008)

(continued)

Table 11.1 (continued)

Mechanism	Description	R Genes	References
	disturbing its pathogenicity process		
Other, passive	This mechanism helps in losing the susceptibility of host plant by mutation that leads to the failure to manipulate the host	*rwm1*, *lov1* (Arabidopsis), *Mo-1* (lettuce), *Rym-4*, *Rym-5* (barley), *sbm1* (pea), *retr01* (cabbage), *bc-3* (French bean), *Pvr2* [1], *pvr6, Pvr2* [2] (pepper), *xa13*, *xa25, xa5* (rice), *Eva1* (potato), *Asc-1*, *Ty-5*, *Pot-1* (tomato)	Huang et al. (2016), Kang et al. (2005), Reddy et al. (2009)
Reprogramming	A deregulated host causes loss of susceptibility	*Lr34*, *Lr67*, *YrL693*, *Yr36* (wheat), *GH3-8*, *GH3-2* (rice)	Ellis et al. (2014), Moore et al. (2015)

pathogens continuously evolve with the passage of time for their survival. Mendelian-resistant genes are found in higher plants and are genes for race-specific resistance. The resistance in plants is based on a genetic interaction between pathogen avirulence (Avr) genes and host-resistance (R) genes (Periyannan et al. 2017). The response of these genes varies from specie to specie. The Avr genes in flax rust (*Melampsora lini*) code for tiny, secreted proteins are produced in the haustoria and recognized by the host cell. The host plant cell contains specialized sense organs that easily identified the pathogen avirulence genes and show response to that type of genes. The recognition event that leads to resistance occurs on at least two occasions. One is that the flax NLR protein directly interacts with the pathogen Avr protein and develops resistance (Anderson et al. 2016). The second mechanism is that, following the entry of Avr genes, the host plant activates the transfer of signals to effectors that act to minimize the effect of the pathogen Avr genes. In this mechanism (Ravensdale et al. 2011), the host plant activates the transfer of signals to the effectors that act to minimize the effect of the pathogen Avr genes. Nucleotide-binding domain is connected to ATP rather than adenosine diphosphate (ADP) in this mechanism. Some genes that make wheat and other cereals more resistant to different races have been genetically found and cloned (Steuernagel et al. 2016). The present advancement of NLR gene capture techniques promises to boost the number of cloned rust resistance genes significantly. In wheat, nine genes *Lr1, Lr10, Lr21* and *Sr33, Sr22, Sr45, Sr50, Sr35,* and *Yr10* conferring to leaf, stem, and stipe rust pathogens, respectively, have been identified, cloned, and characterized (Ellis et al. 2014; Krattinger and Keller 2016; Steuernagel et al. 2016). R genes are the first class of resistant genes that have been genetically defined, and they produce high phenotypic variations from single genes. These genes can be easily identified in glasshouses at seedling stages.

11.4.2 Non-race-Specific or Horizontal Disease Resistance

Non-race-specific resistance refers to a host plant's resistance to all races of a disease pathogen, and it can be effective against multiple infections at the same time. This type of resistance is usually quantitative and controlled by many genes. It is also known as polygenic resistance and is also known as durable resistance. Here, in this type of resistance, there is no visible immune response, but plants show partial resistance, which inhibits pathogen growth (Ellis et al. 2014). In wheat, this type of resistance occurs at later stages of development, which is why it is referred to as adult plant resistance (APR). APR genes are highly durable as compared to NLR-encoding R genes (Periyannan et al. 2017). The cloning of numerous wheat APR genes recently revealed new information on the mechanisms of non-race-specific resistance (Gou et al. 2015). The most important stem rust-resistant APR gene in wheat is *Sr2* and *Lr34*, a gene that produces resistance to leaf rust, stripe rust, and powdery mildew. Similar to these, scientists are trying to identify and clone new genes that produce resistance (Ellis et al. 2014).

11.5 Gene Cloning

Gene cloning is a molecular technique that involves copying a gene of interest into numerous identical copies. Plant cloning (e.g., roots, shoots, or axillary buds) seems to be the most important modern technique of plant cloning (e.g., roots, shoots, or axillary buds) (Renneberg et al. 2017). Plants employ a variety of tactics to defend themselves against the wide range of diseases that can be found in their environments. For example, the Hm1, the first resistance gene (R gene) to be cloned in maize (*Zea mays*), was reported more than 25 years ago; countless more R genes have since been discovered and isolated (Kourelis and Van Der Hoorn 2018). The presence of the R gene may detect the presence of a specific pathogen by identifying the ligands transcribed by *Avr genes* (a-virulence genes) and is recognized as the most important and effective strategy to combat the pathogen attack (Peng et al. 2014). PCR cloning is a quick approach to cloning genes, and it's commonly used for applications that require higher throughput than other cloning methods can provide. Scientists can also clone DNA fragments that aren't easy to get in large amounts.

MutRenSeq is a technique for the cloning of disease resistance genes (R genes) in plants. It combines the R genes of structural class and the mutational genes. It also identifies the single gene mutations by comparing the wild-type parental sequence to numerous independently produced (Peng et al. 2014; Steuernagel et al. 2016). The MutRenSeq process necessitates the genetic isolation of a single R gene in an otherwise susceptible background. Random mutations will, with a certain frequency, knock out the R gene and cause the loss of resistance conferred by that gene. A mutation directly in the R gene causes loss-of-function mutants (Steuernagel et al. 2017). A single gene must govern disease resistance in the mutagenesis line in order to get susceptible mutants. The majority of EMS-induced (ethyl methanesulfonate)

mutations are point mutations, while some are deletion mutations (Periyannan et al. 2013).

AgRenSeq, or speed cloning, is a technique developed by John Innes Centre researchers and collaborators in the United States and Australia to help speed up the fight against diseases that endanger food crops around the world.

11.5.1 MutMap Technique

The era of genomics starts with the discovery of DNA sequencing techniques developed by Sanger. With the advancement and improvement in the sequencing techniques, the time and cost of sequencing have significantly reduced that resulted in high quality genomic data. Along with improved sequencing technologies, new bioinformatic tools have also contributed for generating quality data. MutMap technique depends on the cross of mutant specie with its wild type itself. It identifies SNPs generated through mutation that causes phenotypic variation. It also involves the chemical mutagen to create a mutant population for choosing lines with highly desirable traits in M_2 or subsequent generations (Takagi et al. 2013). MutMap is a technique for mapping the traits controlled by single recessive genes and was demonstrated in rice (Abe et al. 2012). In a similar way to QTL-sequence, whole-genome sequencing is performed to phenotype bulks of F2 individuals generated by crossing the selected mutant with the appropriate wild-type parent and categorized according to the intended mutant phenotype (Zhang et al. 2019). MutMap-Gap is a slight modification of MutMap that helps to find causal SNPs in genomic regions where the standard genome sequence is missing. The causative gene of the lmm24 lesion mimic mutant was cloned using MutMap in rice (Sánchez-Martín et al. 2016), which boosted the fungus *Magnaporthe oryzae* resistance and upregulated the defense responsive genes (Fig. 11.2).

11.6 Resistance Gene Analogs (RGA) Identification and Characterization Through In Silico Analysis

In the past 20 years, genome sequencing has grown rapidly, which has resulted in an increase in the quality and quantity of the available genomic resources. In 2000, the genome assembly of the first land plant, Arabidopsis, was published (Initiative 2000). With this, the genomes of many other crop plants have also been sequenced and assembled. These genomes are also available on different databases and gene banks. According to an estimate, nearly 0.16% of the 350,000 plant species have been sequenced, assembled, and are available on different databases. Of these genomes, some crop plants like Arabidopsis, rice, and maize have been resequenced by using modern sequencing techniques that increase the quality of the genomes (Marks et al. 2021). Publically accessible databases such as NCBI, TAIR, Phytozome, and Ensemble Plants FTP sites, among many others, store the published whole-genome sequences. As a result of advancements in next-generation

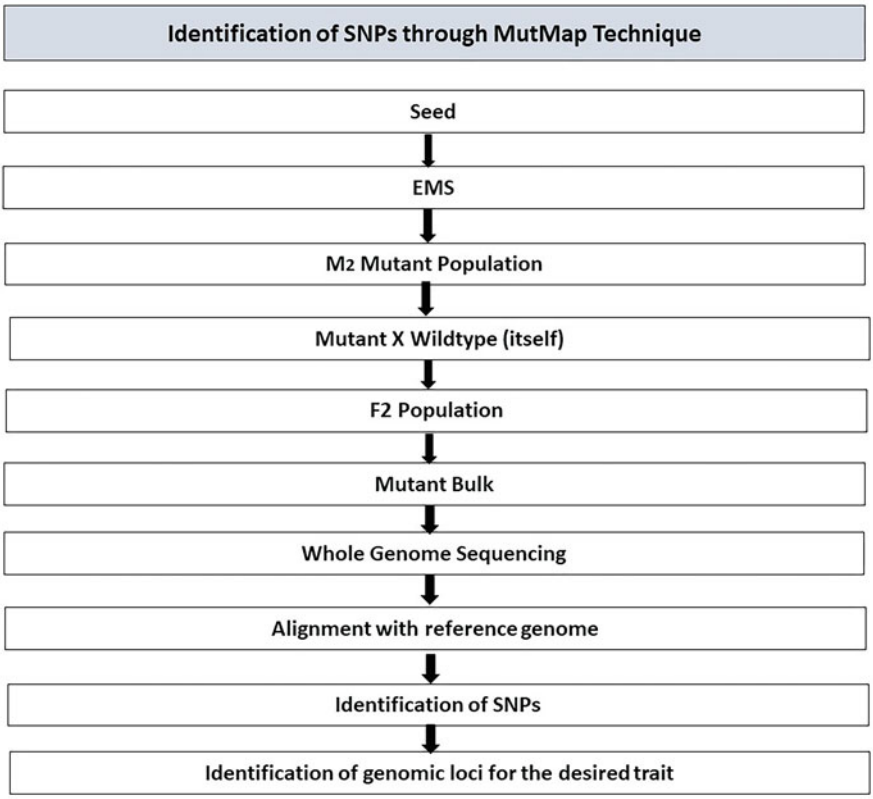

Fig. 11.2 MutMap technique for identifying SNPs

sequencing technology, whole-genome sequencing has become one of the most significant tools in current biological studies. The absence of functional annotations for the enormous number of macromolecules is one of the most significant obstacles to genome sequencing. On the other hand, experiments to assign protein functions are costly and time-consuming. Hence, the computational strategies for functional prediction are extremely interesting for resolving this difficult problem (Peng et al. 2014). Therefore, computational approaches can be used to find and analyze the genome-wide plant RGAs owing to their major structural characteristics and conserved domains.

First, an RGA database was developed that contained all reported RGA genes and their protein sequences. GenBank and PRGdb are two significant repositories of well-organized RGA sequences (Pruitt et al. 2009). Second, to find putative RGA candidate genes, the BLAST search tool was used against the RGA database with an E-value ranging from 1e − 5 to 1, depending on genome size. Third, numerous software programs are utilized to detect and align numerous conserved domains and motifs utilizing the RGA candidates as input. For example, Sanger's pfam scan.pl

and InterproScan programs can be executed concurrently (Sanseverino et al. 2010). A script is also needed to classify RGA candidates by their domain and motif structures, or a combination of both, so that they can be put into groups.

11.6.1 Characterization of RGAs

RGAs have been identified, mapped, and characterized across the plant genome using whole-genome sequencing. RGAs containing NBS-LRR have been extensively investigated in numerous plants, e.g., rice, maize, sorghum, barely, apple, medicago, and Arabidopsis (Sekhwal et al. 2015). The most important class of R genes implicated in disease resistance in plants are NBS-LRR genes (Porter et al. 2009), as they are highly duplicated, clustered, and are evolutionarily diverse (Radwan et al. 2008). For genome-wide characterization and identification, several bioinformatic tools are used for analysis. Their functions are described in Table 11.2.

A lot of NBS-LRR genes have been identified and cloned from different crop plants. These genes are grouped into different superfamilies. The genes that are mostly cloned belong to the eLRR, LRR-Kinase superfamily or NB-LRR superfamilies. These families were initially identified in Arabidopsis, tobacco, and tomato by map-based cloning. The cloning techniques require specialized infrastructure that was mainly not available for many crops which can be the main sources of disease-resistant genes. Angiosperms have NBS-LRR-encoding genes, whereas grass genomes and other monocot genomes lack TNL-encoding genes (Guo et al. 2011).

Through the genome-wide identification and also the characterization, many resistant genes have been identified that show their response to specific plant diseases. The majority of monocots have more NBS-LRR and CNL-encoding genes than dicots (Table 11.3).

11.7 Conclusion

To feed the increasing population, there is need to develop disease-resistant and high-yielding genotypes. Due to change in climate patterns, a large number of new strains of pathogens have developed and cause harmful diseases. Plant diseases reduce the plant growth, development, and ultimately reduce yield. To minimize this yield reduction, different molecular and breeding approaches have been used. The main thing during this pathogen attack is host plant–pathogen interactions which have gained much attention by scientists. Several other factors have driven the research, including disease challenges linked with modern agricultural methods and climate change into a new era. The main purpose of that research is to develop disease-resistant and long-lasting pathogen-resistant crops. For this purpose, disease-resistant genes (*R genes*) have been identified and cloned. Various RGAs have been cloned and characterized from various plant species which have developed DNA markers as well as disease resistance genes. The cloning and

Table 11.2 Bioinformatic analysis used for genome-wide characterization and their functions

Analysis	Function	References
Phylogenetic analysis	A powerful approach used for identification of evolutionary history of current day species. It also explains the similarities and differences in the species. It is also performed to identify the spread of harmful diseases	Munjal et al. (2018)
Conserved domain analysis	Domains are the proteins conserved units. This analysis is used to classify proteins and to identify the function of specialized protein	Fong and Marchler-Bauer (2008)
Subcellular localization analysis	This analysis helps to identify the location of proteins either present in cytoplasm, mitochondria, Golgi apparatus, or any other organelle. These locations help to identify the function of proteins	Pan et al. (2021)
Sequence logo analysis	The graphical representation of multiple aligned sequences used to identify and visualize short, conserved patterns in the RNA, DNA and proteins sequences	Dey et al. (2018)
Multiple sequence alignment analysis	This analysis helps in identifying the similarities and differences of sequences of different species	Pirovano and Heringa (2008)
Ka/ks calculation analysis	This analysis helps to identify the divergence of gene after duplication	Nekrutenko et al. (2002)
Promoter analysis	This analysis helps to predict and identify regulatory elements which perform specific functions	Mariño-Ramírez et al. (2009)
Synteny analysis	This analysis is performed to compare different genomes and to identify the genomic evolution of the related species	Cheng et al. (2012)
Protein and protein interaction analysis	These interactions can handle a variety of biological processes. This analysis helps in predicting the function of specific protein	Rao et al. (2014)
Gene expression analysis	This analysis helps to identify the expression of gene in different plant tissues either leaf, fruit, roots, flowers, seeds, etc. through qRT-PCR	Segundo-Val and Sanz-Lozano (2016)

characterization of *R genes* and RGAs help in identification of resistant genes that can be further transferred into the susceptible plants to develop resistance. Race-specific R genes are more likely to breakdown because of changes in pathogen avirulence (*Avr*) genes, as well as the resistance of non-race-specific genes, which make them less likely to work. In short, due to climate change, the pathogens adapt to the changing environmental conditions that result in increase of new strains of pathogens which affect crop plants. To minimize pathogen virulence and increase resistance duration, several genes have been identified and yet have to develop strategies involving both race-specific and non-race-specific genes and adopt new molecular approaches.

Table 11.3 List of resistance genes identified in different crops

Gene name	Location on chromosome	Class	Disease	Identification in crops	References
RPP13	3	NBS	Downy mildew	Arabidopsis	Bittner-Eddy et al. (2000)
RCY1	5	NBS	Mosaic type	Arabidopsis	Takahashi et al. (2002)
RPP1	3	NBS	Downy mildew	Arabidopsis	Botella et al. (1998)
RPP4	4	NBS	Downy mildew	Arabidopsis	Van Der Biezen et al. (2002)
RPS4	4	NBS	Powdery mildew	Arabidopsis	Gassmann et al. (1999)
RPP5	4	NBS	Downy mildew	Arabidopsis	Noël et al. (1999)
RPS5	1	NBS	Downy mildew	Arabidopsis	Warren et al. (1998)
RRS1	5	NBS	Bacterial wilt	Arabidopsis	Deslandes et al. (2002)
RPP27	1	NBS	Downy mildew	Arabidopsis	Tör et al. (2004)
Hs1pro-1	1	RLP	Beet cyst	Sugar beet	Cai et al. (1997)
Rp1-D	10	NBS	Rust	Maize	Collins et al. (1999)
Hm1	1		Corn leaf blight	Maize	Johal and Briggs (1992)
Pi9	6	NBS	Blast	Rice	Liu et al. (2002)
Rpr1	11	NBS	Blast	Rice	Sakamoto et al. (1999)
Pid3	6	NBS	Blast	Rice	Shang et al. (2009)
Xa21	11	RLK	Bacterial blight	Rice	Song et al. (1995)
Xa3/Xa26	11	RLK	Bacterial blight	Rice	Sun et al. (2006)
Xa10	11	Oth-R	Bacterial blight	Rice	Tian et al. (2014)
Xa25	12	Oth-R	Bacterial blight	Rice	Liu et al. (2011)
Xa27	6	RLP	Bacterial blight	Rice	Bimolata et al. (2013)
Pi-d2	6	RLK	Blast	Rice	Chen et al. (2006)
Xa1	4	NBS	Bacterial blight	Rice	Yoshimura et al. (1998)
Pib	2	NBS	Blast	Rice	Wang et al. (1999)
Pi-ta	12	NBS	Blast	Rice	Bryan et al. (2000)
Pi-36	8	NBS	Blast	Rice	Lin et al. (2007)
Pia	11	NBS	Blast	Rice	Okuyama et al. (2011)
Pi37	1	NBS	Blast	Rice	Lin et al. (2007)
Xa5	5	NBs	Bacterial blight	Rice	Iyer and McCouch (2004)

(continued)

Table 11.3 (continued)

Gene name	Location on chromosome	Class	Disease	Identification in crops	References
Xa13	8	Oth-R	Bacterial blight	Rice	Chu et al. (2006)
Rx	12	NBS	PVX	Potato	Bendahmane et al. (1999)
RB	8	NBS	Late blight	Potato	Song et al. (2003)
Rx2	5	NBS	PVX	Potato	Bendahmane et al. (2000)
Prf	5	NBS	Bacterial speck	Tomato	Salmeron et al. (1996)
Mi	6	NBS	Root knot	Tomato	Milligan et al. (1998)
I2	11	NBS	Fusarium wilt	Tomato	Ori et al. (1997)
Ph-3	9	NBS	Late blight	Tomato	Zhang et al. (2014)
Sw-5	9	NBS	Tomato spotted wilt	Tomato	Brommonschenkel and Tanksley (1997)
Tm-2	9	NBS	Tobacco mosaic	Tomato	Lanfermeijer et al. (2003)
Bs4	5	NBS	Bacterial spot	Tomato	Schornack et al. (2004)
Hero	4	NBS	Potato cyst	Tomato	Ernst et al. (2002)
Cf-*2*	6	RLP	Leaf mold	Tomato	Dixon et al. (1996)
Cf-*4*	1	RLP	Leaf mold	Tomato	Parniske et al. (1997)
Cf-*5*	6	RLP	Leaf mold	Tomato	Dixon et al. (1998)
Cf-*9*	1	RLP	Leaf mold	Tomato	Jones et al. (1994)
Vel1,2	9	RLP	Verticillium wilt	Tomato	Kawchuk et al. (2001)
Fen	5	Oth-R	Bacterial speck	Tomato	Martin et al. (1994)

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The Role of Genetic, Genomic, and Breeding Approaches in the Fight Against Fungal Diseases in Wheat

12

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Abstract

Wheat is a major, widely cultivated staple cereal food resource, representing almost 200 million ha of agricultural areas. It is regarded as the second most important cultivated crop, equally consumed in different forms. In recent years, enormous progress in genomic advancement in fungal disease resistance has partially solved the problem throughout the globe. The landraces, conventional varieties, and wild species (primary, secondary, and tertiary gene pools) have been explored in search of resistant genes. Wheat's cosmopolitan distribution and changes in global climatic conditions exposed the crop to various strains of fungal pathogens. Conventional and advanced breeding techniques provide a platform for identification and introgression of potential genes that help to combat the fungal disease exploits. Furthermore, the use of new genomic techniques such as marker-assisted breeding, RNAi editing, genome editing, speed breeding tilling, and so on empowers the harnessing of new rust-resistant genes. The chapter highlights the importance of potential donors of fungal resistance alleles in breeding strategies and new emerging techniques. Moreover, translational

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approaches are also essential to achieve long-term durable resistance along with the variable resistance nature of fungal pathogens.

Keywords

Wheat · Productivity · Fungal diseases · Markers gene *Lr78*

Abbreviations

AFLPs	Amplified fragment length polymorphisms
BAC libraries	Bacterial artificial chromosome libraries
CAPS	Cleaved amplified-polymorphic sequence
CASS	Chromosome arm-specific sequencing
DArT markers	Diversity arrays markers
DHs	Doubled haploids production
dsRNA	Double-stranded RNA
FST	Flow sorting technology
GM crops	Genetically modified crops
GS	Genomic determination
GWAS	Genome-wide association studies
HIGS	Host-induced gene silencing
MABS	Marker-assisted backcrossing
MAS	Marker-assisted selection
MNs	Meganucleases
NGS	Next-generation sequencing technologies
PM resistance genes	Powdery mildew resistance genes
PtCNB	Calcineurin B
PtCYC1	Cyclophilin
PtMAPK1	Mitogen-activated protein kinase 1
QTL	Quantitative trait locus
R gene	Resistance genes
RFLPs	Restriction fragment length polymorphism
RNAi	RNA interference
S genes	Susceptibility genes
SIGS	Spray-induced gene silencing
SNPs	Single nucleotide polymorphism
SSD	Single-seed-descent
SSRs	Simple sequence repeats
STS	Sequence targeted site
TILLING	Targeting induced local lesions in genomes
WGSA	Whole-genome shotgun approach
ZFNs	Zinc-finger nucleases

12.1 Introduction

In 2050, agriculture will need to produce more than double the food because of the exponentially growing world population and increased reliance on cereal crops (Choudhary et al. 2022; Singh et al. 2022; Zhang et al. 2021). Considering the future projections, the production of major cereal wheat should need to be increased to provide 20% of the protein and calories for human nutrition worldwide (Mehta et al. 2019; Singh et al. 2019; Rahman et al. 2019). The wheat crop is mainly threatened by strong abiotic and biotic undesirable components and is strongly threatened by crop productivity from seed germination to crop harvesting. Increasing food demands will need to be fulfilled via sustainable disease-free plants combining management of pest and pathogen adaptation to changing climate conditions and fluctuating abiotic challenges with adapting to low water conditions (FAOSTAT 2020; Savary et al. 2019; Ahmad et al. 2020; Mbinda and Masaki 2021; Kumar et al. 2022a; Kumar et al. 2022b) (Fig. 12.1). Overall, biotic strains cause 21.1% yield loss managed only by pests and diseases, whereas almost 31 crop pathogens are reported in wheat, such as fusarium head blight, leaf rust, stripe rust, Septoria leaf blotch, tan spot blotch, and powdery mildew which cause severe losses. These diseases' abrupt plant physiological and biochemical processes lead to alteration in qualitative and quantitative crop loss (Corredor-Moreno and Saunders 2020; Fones et al. 2020; Simón et al. 2021; Fernando et al. 2021; Bishnoi et al. 2021). Wheat diseases significantly affect the growth as well as productivity of crops. For example, rust

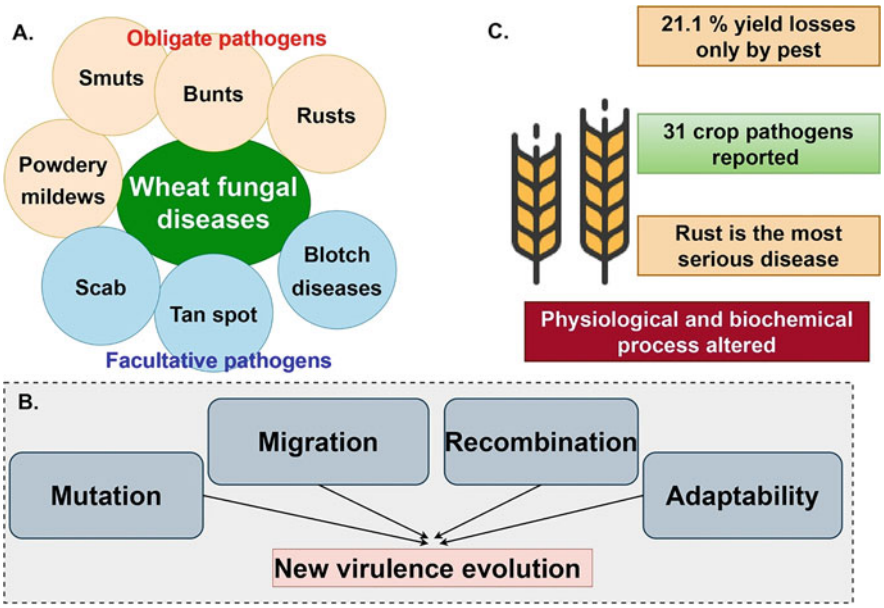

Fig. 12.1 An illustration showing the available genetic techniques used in wheat breeding to improve resistance (**a**) Wheat fungal diseases caused via obligate and facultative pathogens; (**b**) various methods of virulence evolution; (**c**) effects on physiological and biochemical process

is the most serious disease both quantitatively and qualitatively (Fig. 12.1). Wheat germplasm contains a lot of genetic variation when it comes to disease resistance, and several race-specific and long-lasting resistance genes have been identified. The chapter highlights the importance of potential donors of fungal resistance alleles in breeding strategies and new emerging techniques. Moreover, translational approaches are also essential to achieve long-term durable resistance along with the variable resistance nature of fungal pathogens.

By methodically transferring several resistance genes from diverse species and genera linked to wheat by cytogenetic treatments, the diversity has been further enhanced. Resistance is normally conferred from the seedling development stage through physiological maturity by race-specific or main genes. However, resistance expression can begin at later growth stages in some circumstances. Furthermore, the level of resistance provided by these genes varies greatly, ranging from complete immunity to minor decreases in disease symptoms. Although matching virulences in the pathogen population were able to overcome numerous known race-specific rust and PM resistance genes, there is potential to increase their lifetime by pyramiding several undefeated genes through marker-assisted selection (MAS) (Ahmad et al. 2020; Mbinda and Masaki 2021).

Another method, genomic determination (GS), is utilized to foresee breeding qualities, empowering the choice of competitors before phenotyping, and is a better approach than MAS for progeny traits. New genetic methods such as molecular marker technologies offer a viable option for improving wheat-resistant cultivars. In the previous decades, RFLPs, SSRs, AFLPs, SNPs, and DArT markers have played a crucial role in developing resistant cultivars against fungal infections in wheat. For increasing grain production, genomics methods like TILLING RNAi and epigenetics are required. Disease resistance through mutagenesis and bioinformatics is becoming an established scientific method for analyzing wheat genome structure and function.

Single nucleotide polymorphism (SNP) genotyping is employed for gene sequencing processes in huge populations quickly with a large number of markers and a variety of genotyping systems. SNP data are commonly utilized to determine marker-trait relationships in (QTL) mapping investigations and genome-wide association studies (GWAS). The use of high-density SNP arrays has proved effective in genetic research of a variety of commercially significant crops. For GWAS of several rice accessions, a 44K SNP genotyping chip was used and several alleles important for controlling morphological and agronomic features were found (Hane et al. 2007; Islam et al. 2020; Santillán Martínez et al. 2020; Tyagi et al. 2021). The literature used for gene-based tolerance for rust-smut disease and the techniques used for their introgression are summarized in Table 12.1.

Table 12.1 List of genes used for tolerance in rust-smut disease and techniques used for their introgression

Plant species	Genes	DNA marker	Marker systems	Resistance	References
Aegilops umbellulata	*Lr9*	*cMWG684*	Sequence-tagged site (STS)	Leaf rust	Ganeva et al. (2000)
Agropyron elongatum	*Lr19*	*STSLr19*130	Randomly amplified polymorphic DNA	Leaf rust	Prins et al. (2001)
Triticum tauschii	*Lr39*	*Xcmwg682*	Microsatellites or simple sequence repeats	Leaf rust	Singh et al. (2004)
Triticum tauschii	*Lr41*	*GMM210*	Amplified fragment length polymorphism	Leaf rust	Singh et al. (2004)
Triticum ventricosum	*Lr3*	$M39_{175}$	Amplified fragment length polymorphisms	Leaf rust	Diéguez et al. (2006)
Triticum aestivum	*Lr68*	*Psy1-1*	Single nucleotide polymorphism	Leaf rust	Herrera-Foessel et al. (2012)
Triticum aestivum	*Pm38*	*Swm10*	Diversity arrays technology	Leaf rust, stem rust, yellow rust, and yellow dwarf virus, and powdery mildew	Ellis et al. (2014)
Puccinia graminis	*Sr2*	*Xgwm533*	Restriction fragment length polymorphism	Stem rust	Mago et al. (2014)
Cantalupensis Charentais	*Pm1*	*M75/ H35_155*	Sequence-tagged site	Powdery mildew	Elkot et al. (2015)
Aegilops umbellulata	*Yr40*	*gwm190*	Restriction fragment length polymorphism	Stripe rust	Bansal et al. (2016)
Triticum aestivum	*Lr75*	*swm271*	Simple-sequence repeats	Leaf rust	Singla et al. (2017)
Thinopyrum bessarabicum	*Lr24*	*SCS5550*	Restriction fragment length polymorphism	Leaf rust	Singh et al. (2017)

(continued)

Table 12.1 (continued)

Plant species	Genes	DNA marker	Marker systems	Resistance	References
Triticum aestivum	*Lr78*	*IWA6289*	Diversity arrays technology	Leaf rust	Kolmer et al. (2018)
Triticum dicoccoides	*Yr15*	*uhw250*	Randomly amplified polymorphic DNA	Stripe rust	Klymiuk et al. (2018)
Triticum aestivum	*Lr77*	*IWB10344*	Single-nucleotide polymorphism	Leaf rust	Kolmer et al. (2018)
Secale cereale	*Lr26*	*P6M12*	Amplified fragment length polymorphism	Stem rust and powdery mildew	Tomkowiak et al. (2019)
Triticum ventricosum	*Lr37*	*Xcmwg682*	Sequence-tagged site	Leaf rust	Randhawa et al. (2019)
Triticum turgidum	*Yr30*	*csSr2-SNP*	Restriction fragment length polymorphism	Yellow stem and leaf rust	Rani et al. (2019)
Triticum aestivum	*Lr74*	*cfb5006*	Simple-sequence repeats	Leaf rust	Kthiri et al. (2019)
Triticum aestivum	*Ltn2*	*csLV46*	Cleaved amplified polymorphic sequences	Leaf rust and stripe rust	Babu et al. (2020)
Triticum aestivum	*Yr46*	*gwm165*	Amplified fragment length polymorphism	Stripe rust and leaf rust	Huerta-Espino et al. (2020)
Triticum speltoides	*Lr35*	*BCD260*	Restriction fragment length polymorphism	Leaf rust	Gultyaeva et al. (2021)

12.2 Conventional Breeding and Factors Affecting Disease Resistance

Conventional breeding methods for qualitative resistance usually begin with the screening of a large number of lines in which vulnerable plants are removed by producing epiphytic conditions. After that, the resistant lines or plants are chosen and developed in breeding programs, i.e., mass selection or pure selection. Another method is hybridization and selection, which includes intervarietal methods like the

Pedigree Method Bulk Method and Single Seed Descent Method. The Modified Bulk Method includes multiline varieties polyploidy breeding and population approaches mutation breeding. The next method is backcrossing, which entails crossing a resistant parent with an excellent but susceptible parent, then backcrossing progenies with the susceptible parent until the required level of susceptible parent genome is regained. Homozygous-resistant lines may be established in a relatively small number of breeding cycles in qualitative resistance breeding, and no additional resistance allele selection is necessary. However, a variety of highly heritable traits may be chosen for quantitative resistance (Bisht et al. 2019; Zatybekov et al. 2022; Zhang et al. 2022). Molecular markers, csLV34, Gb, Sr24#12, and wmc44 (SSR) markers, helped in the identification of leaf rust and stem rust resistance genes that might be used as important donors in wheat breeding for achieving durable rust resistance. The abundance of Sr24/Lr24 using a specific Sr24 # 12 marker was identified in HS545 that exhibited monogenic regulation of leaf rust resistance toward pathotype. Specific rust resistance of genotypes G19 and G12 had significant grain production superior to HS490, which might be an ideal option for their use in developing rust resistance cultivars. These marker validations also confirmed the abundance of Sr24/Lr24 genes in more than half of the wheat lines, whereas almost 22.2% of the lines are enriched with Lr24/Sr24 and Sr57/Lr34/Pm38/Yr18 gene combinations. Such genetic labels used in this study may aid in the transfer or pyramiding of resistance genes for agronomically important disease susceptible wheat genotypes (Pal et al. 2022).

In wheat, the fungal disease is presently caused by obligate pathogens or parasites (smuts, bunts, rusts, powdery mildews) and facultative parasites (scab, tan spot, spot blotch, Septoria nodorum blotch, Septoria tritici blotch). Such highly specialized obligate parasites show significant variation in the fungal population for pathogenicity towards resistance race-specific genes (Santillán Martínez et al. 2020; Tyagi et al. 2021; Yin and Qiu 2019). The advent of new virulence evolution achieved by mutation migration and preexisting virulence recombination and their adaptability are highly common in powdery mildew and rust fungi (Fig. 12.1). The technologies that can help to combat fungal diseases are genome sequencing, GWAS analysis, CRISPR/Cas-regulated multiplex system, gene stacking by synthetic biology, development of diverse genotypic-independent approaches, and speed breeding (Bisht et al. 2019; Li et al. 2021a, b). However, breeding for disease resistance has always been challenging and specific. In this context, smut and bunts are more specifically known for strains or physiological races, whereas the selection and evolution of new strains are less frequent (Corredor-Moreno and Saunders 2020; Esse et al. 2020; Li et al. 2021a, b; Pandurangan et al. 2021; Rasheed et al. 2021; Upadhyaya et al. 2021).

12.3 Role of Genomics in Wheat Breeding to Combat Fungal Threats

Among the biotic stresses which pose a threat to wheat production, fungal pathogens like rust are mainly considered as major threats with high impact. It causes severe loss in the crop if the epidemic is well-favored by environmental conditions like high humidity, excessive rainfall, etc. (Fabre et al. 2020). In recent years, the emergence of gene-specific DNA markers has made it reliable and efficient to expedite resistance gene pyramiding in new cultivars. Complexity, enormous genome size, and identical sequences in the homologous genome repetitive sequences have created a challenge in creating robust DNA markers and enlisting wheat genome sequence. Several markers-based approaches, such as CAPS (Cleaved Amplified-Polymorphic Sequence), STS (Sequence Targeted Site), and SSR (Simple Sequence Repeats), have been extensively investigated for disease resistance over the last decade. Wheat genomics has been the witness to the advent of NGS (Next-generation Sequencing Technologies) from (CASS) Chromosome Arm Specific Sequencing Flow sorting technology, WGSA (Whole-Genome Shotgun Approach), de-novo whole-genome assembly, and long-range sequencing through the WGSA approach (Babu et al. 2020; Pramanik et al. 2021; Huerta-Espino et al. 2020). The accelerating pace of genomics-based molecular disease characterization and developing resistance wheat is propelled by an enhanced understanding of the pathogen and its molecular components related to resistance interaction. Depending upon the information on the genomic size of pathogens, the genomic sequence also varies in terms of quality and contiguity of segments; more likely the complete genome of *Puccinia graminis f. sp. tritci* (Wheat stem rust), *P. triticina* (wheat leaf rust), and *Stagonospora nodorum Blumeria graminis f. sp. Tritici* (Powdery mildew) has been determined (Duplessis et al. 2011; Hane et al. 2007; Islam et al. 2020; Parlange et al. 2011).

Such molecular sequences have been positively complemented for fungal pathogens by various genomics approaches, including physical mapping and BAC libraries. Furthermore, the host is very concerned about molecular mining for revealing the interaction between wheat and pathogens. The recognition of pathogens depends upon molecular factors identified by the receptors engaged during the resistance system. More likely in powdery mildew, two virulence genes have been discovered using combined methods associated with GWAS or map-oriented cloning techniques. The AvrPm2 (powdery mildew2) gene in wheat shows homology with the rye mildew gene and is confirmed by pm2 immunoreceptors. The finding confirms the successful introgression of wheat-rye against powdery mildew, whereas an identical *Avr* subset also confirms resistance against mildew on other crops. Similarly, two stem rust genes, *AvrSr35* and *AvrSr50*, were isolated based on whole-genome analysis and are considered the first genes of stem rust reported in wheat. On-field pathogenomics was performed on diseased wheat plants in the United Kingdom (Mago 2021). In wheat cultivars, durable resistance is a very effective and well-known approach to managing stripe rust disease. Such durable type resistance can be attained by incorporating multiple APR genes into the specific target so that each introgressed gene is relatively specific

but small and possesses an individual role. In the case of Fengdecun 12 and Ruihua 520, two Chinese wheat cultivars exhibit APR to stripe rust in different environments. More than 170 recombinant wheat inbred lines from crossing the RH520 FDC12 were utilized to evaluate the molecular basis of resistance and recognize genomic regions related to resistance to stripe rust.

On a global scale, the *Lr34* ensures multiple resistance against rust pathogens. For instance, powdery mildew and leaf rust pathogens emerging on partial resistance identified through transcriptomics *Lr34*- carrying barley or wheat host were associated with fungal pathogens growing isogenic host or other cultivars of wheat lack *Lr34*. No doubt, pathogens do not act toward the negative effect of *Lr34* and it is tempering to consider the predictable role of *Lr34* as in durable resistance. In some cases, overexpressing of AtLTP4.4 like nonspecific lipid-transfer gene in transgenic wheat (nsLTP) gene, lower accumulation of (DON) deoxynivalenol, inhibiting oxidative stress and causing accumulation of hydrogen peroxide accumulation showed a reduction in Fusarium head blight caused by *Fusarium graminearum* (McLaughlin et al. 2021; Collinge and Sarrocco 2022). Another, *Trichoderma gamsii* A5MH is an endophytic strain of wheat that suppressed the *F. pseudograminearum* abundance that causes crown-rot disease and increased wheat durum growth in natural Chromosol and Kandosol cropping soils (Stummer et al. 2022). Use of *Trichoderma gamsii* T6085 reduced growth of F. *graminearum* in wheat straw. This interaction with the plant also increased defense-related genes PAL1 and PR1 significantly. The ability to compete, resistance to host, and endophytic behavior can be achieved with the use of T6085 in the soil as well as on crops (Sarrocco et al. 2020).

12.4 Role of Genetics in Fungal Disease Management

Most fungal pathogens affecting wheat possess genetic diversity and its control through using race-specific-resistant genes holds the great capability to generate more permanent resistance and increase worldwide wheat output (Xu et al. 2019; Wang et al. 2018a; Miedaner and Juroszek 2021). Resistance genes (R) are classified as major (seedling resistance vertical and all stage resistance genes) and minor genes (APR). R genes identify pathogen proteins, encode them, and initiate an immune response. Major genes control qualitative resistance and minor genes control quantitative resistance. Among major genes, 21 have been identified that are gene-specific and not likely to be durable (Wang et al. 2018b). These genes usually give resistance from seedling to maturity and sometimes at later growth stages. Minor genes have weaker specificity and are more durable. Analysis using RNA-based disease control strategy, the design that involves the use of RNA silencing, is widely applicable for diverse pathogens. Using the *Fusarium graminearum* pathosystem, the spray of *CYP3*-dsRNA or noncoding dsRNA 791 suppresses the growth of three fungal cytochrome P450 *lanosterol C-14α-demethylases*, which inhibited fungus populations. The effective spray that controls infections rate and severity directly

indulged in the movement of *CYP3*-dsRNA after pathogen uptake and its movement through vascular tissue (Koch et al. 2013, 2016).

The presence of major and minor genes can provide resistance to diseases caused by biotrophic and necrotrophic fungi. Biotrophic fungi attack only the living plants and are obligate in nature. Examples include the Stem rust resistance gene is *Sr26*, leaf rust resistance gene is Lr68, rusts resistance gene is *Lr34-Yr18-Sr57*, powdery mildew resistance gene is *Pm18*, and *Yr5* against stripe rust. Necrotrophic fungi feed on the dead and decaying organisms and they are facultative in nature (Prins et al. 2001; Duplessis et al. 2011; Parlange et al. 2011; Huerta-Espino et al. 2020). The presence of *Rmg8* and *Rmg7* genes provides resistance to the wheat blast fungus and *Fhb1 Fhb2* and *Fhb5* are among the most important that confer resistance against Fusarium head blight (Mandalà et al. 2019; Milne et al. 2019). Quantitative trait locus (QTL) mapping can also be used to identify the chromosomal position of genes or genetic variations that influence a particular trait. Over the past years, 500 QTLs conferred small to moderate effects for the different resistance (Buerstmayr et al. 2002; Wang et al. 2020; Li et al. 2020). Similarly, 79 *Lr*-genes and more than 200 QTLs and 82 *Yr*-genes and 140 QTLs have been reported for seedling and adult plant LR and SR resistance. *Stb6* QTL is determined at or near loci of qualitative genes that provide several kinds of resistance. Plants also have susceptibility (S) genes that encourage and assist the spread of any disease or pathogen infection in contrast to R genes. If these genes are thrown away, resistance to certain infections can be improved (Milne et al. 2019; Zhang et al. 2017; Su et al. 2019; Corredor-Moreno and Saunders 2020). The gene *Lr34* is resistant to stripe rust leaf rust and powdery mildew and generally appears on flag leaf and functions in an adult plant. It appears similar to ATP transporter *PEN3* that causes movement of metabolites to provide resistance. Powdery mildew, a damaging disease caused by *Blumeria graminis*, may drastically impair wheat harvests. At present, 78 powdery mildew resistance alleles and 50 powdery mildew loci have been found and given names. However, only a few genes have been defined molecularly and functionally, for example CC-NBS-LRR protein is encoded by *Pm21* that provides wider powdery mildew resistance. *Zymoseptoria tritici* causes STB that might result in global economic losses. Wheat contains 21 *Stb* resistance genes. Only one gene *Stb6* has been cloned and studied thus far. These are genes for a conserved wall-associated receptor-like kinase that impart pathogen resistance without causing hypersensitivity (Li et al. 2020; Corredor-Moreno and Saunders 2020; Esse et al. 2020). Despite these, the stable overexpression of *TaWRKY19* in wheat or repression of *TaNOX10* enhanced susceptibility toward PST, avirulent race, whereas mutations in different copies of *TaWRKY19* suggested good resistance to PST by alleviating ROS accumulation in host plant species. Studies demonstrate that a transcriptional repressor like *TaWRKY19* binds to the promoter of *TaNOX10* at the W-box element. *TNOX10* encodes for NADPH oxidase and significantly stimulates the production of ROS, confirming host resistance to PST reported by Wang et al. (2022). The detailed case studies on major rust resistance genes and their isolation from different sources have been explained in Table 12.2.

Table 12.2 List of major rust resistance genes and their isolation from different sources

Plant source	Gene	DNA marker	Chromosomal region	Resistance	References
Triticum ventricosum	*Lr37*	*Xcmwg682*	2AS	Leaf rust	Helguera et al. (2002)
Aegilops tauschii	*Lr10*	*KSUD14*	1AS	Leaf rust	Feuillet et al. (2003)
Triticum tauschii	*Lr39*	*BARC124*	1DS	Leaf rust	Singh et al. (2004)
Triticum tauschii	*Lr41*	*GMM210*	1D	Leaf rust	Singh et al. (2004)
Triticum aestivum	*Lr1*	*RGA567-5*	5DL	Leaf rust	Cloutier et al. (2007)
Aegilops tauschii	*Lr22a*	*GWM296*	2DS	Leaf rust	Hiebert et al. (2007)
Triticum spelta	*Lr34*	*cssfr6*	7DS	Stripe rust	Lagudah et al. (2009)
Triticum aestivum	*Lr67*	*csSNP856*	4DL	Powdery mildew	Forrest et al. (2014)
Agropyron elongatum	*Lr19*	*STSLr19*130	7DL	Leaf rust	Wessels and Botes (2014)
Triticum aestivum	*Ltn3*	*gwm 165*	4DL	Stripe rust	Ren et al. (2015)
Triticum aestivum	*Pm38*	*csLV34*	7DS	Powdery mildew	Reddy et al. (2016)
Triticum turgidum	*Yr30*	*csSr2-SNP*	3BS	Yellow and stem rust	Rani et al. (2019)
Triticum aestivum	*Pm46*	*cfb5006*	7BL	Leaf rust	Chandra et al. (2020)
Triticum aestivum	*Ltn2*	*csLV46*	1BL	Stripe rust	Gebrewahid et al. (2020)
Aegilops umbellulata	*Lr9*	*J13*	6BL	Leaf rust	Narang et al. (2020)

12.4.1 Speed Breeding

For the objective of reducing harvest time in wheat, a method known as "Speed Breeding" was developed by researchers at the University of Queensland. Speed breeding is suited for a wide range of germplasm and does not require specialized in vitro culturing technology. The premise of speed breeding is to accelerate the rate of photosynthesis by controlling light intensity, temperature, and daytime duration, which directly encourage early blooming (Ahmar et al. 2020). This is combined with yearly seed harvesting to minimize the generation period. The protocol involved artificial treatment of 22 h daylight and 2 h night light. The light intensity was adjusted to be 360–380 mol/m^2 at the ground level and 450–500 mol/m^2 at the top level. A 22 °C temperature is applied in the light period and a 17 °C temperature in the dark period. The humidity level is kept between 60% and 70% generations and

can be grown in a year and harvesting can be done within 60 days. The shorter breeding time will be beneficial in genetic research tissue culture studies, mapping populations, seed reproduction, marker-based selection characterization, and developing varieties resistant to biotic and abiotic stresses (Ahmar et al. 2020; Watson et al. 2018). The speed breeding method is an inexpensive technique compared to other methods used in classical breeding as it requires less labor (Singh et al. 2016; Qi et al. 2019). In the Single Seed Descent method, one seed per plant is required to develop each generation. It facilitates the production of homozygous lines. Integrating speed breeding and SSD techniques can effectively accelerate the generation of inbred lines for research and plant breeding programs in less time (Bariana et al. 2013; Zhou et al. 2011; Goutam et al. 2015; Li et al. 2020). In addition, the wheat *Stagonospora* leaf and glume blotch are caused by fungus *Parastagonospora nodorum*. *Stagonospora nodorum* interaction of Snn–SnTox3, Snn1–SnTox1, and Tsn1–SnToxA is considered as an extensively studied association because of its involvement in the suppression of ROS production and regulation. The mechanism regulating the concentration of cytokinin during the infection stage might be effectively utilized for disease control and its management strategies (Katoch et al. 2022).

12.4.2 MAS

MAS (marker-assisted selection) has been utilized for protection from certain infections in wheat. MAS is favored over traditional approaches to provide adequate heritability reducing dominant behavior and destructive phenotyping. MAS is used to study the interaction between genotype and phenotypes. With the use of molecular biology, a particular trait from a donor can be selected and can be transformed by crossing over, but only desired trait should be selected (Wang et al. 2014; Nalam et al. 2015; Gupta et al. 2010; Li et al. 2020; Singh et al. 2016). It involves backcrossing up to 3–4 generations and complete recovery is possible. MAS is beneficial in QTL and associated mapping. The efficiency of marker-assisted selection declines when the number of plants reflecting the desired research in a population grows exponentially and the number of QTLs grows.

Different types of molecular markers are used (RFLP: Restriction fragment length polymorphism), where a mutant allele is identified through a restricted digestion pattern; VNTR that shows repeated clusters aligned in the same direction with varied lengths, SSR are the satellite repeats of dinucleotide and trinucleotide; dCAPS in which dominant and recessive are known by PCR and restriction digestion; CAPS that identify recessive alleles and amplify them; RAPD that does not need information about genomic sequence; FLP is used for insertion-deletion; and SNP that gives the highest resolution in the map. The pyramiding of genes through MAB also prevents certain fungal diseases. Many strategies have proved beneficial for MAS in wheat. It entails combining desired traits into a single genotype or transferring them from donor to recipient as a single allele. It can be achieved through either backcrossing, forward breeding, or double haploids (Gupta et al. 2010). Marker-

assisted backcrossing (MABC) is utilized to create parent lines with specific features that will transfer these genes into germplasm. Specific features are transferred into different breeding lines, resulting in the creation of parental lines for the transfer of important genes taken from different locations into the finest germplasm. This selection is made for recurrent parents, and the selection of the targeted locus is based on phenotype. It can delete non-desirable genes. Calculations are done theoretically through simulations of the wheat genome structure on computers, and eventually, the experience of the Yr15 gene transfer was used to suggest a four-stage MABS technique. Forward breeding MAS is the technique in which a locus in the heterozygous state is targeted, then selected plants are self-pollinated till the progeny becomes homozygous. This method is superior to MABC because superior lines are created using genes from both parents, and there is introgression of genes that are targeted. Doubled haploid production (DHs), in which plants derived from single pollen grains are doubled artificially to form homozygous diploids (Gupta et al. 2010; Jamil et al. 2020; Li et al. 2020). The most expensive part of MAS is DNA isolation, but once that's done, markers associated with a variety of attributes may be genotyped to choose a complete genotypic bundle. To properly select a suitable number of genotypes harboring positive alleles for numerous trait loci, larger population sizes are necessary. Several breeding strategies were used to determine the size of the population for MAS with various numbers of loci. In the case of doubled haploid/recombinant inbred lines and single backcross procedures, a significantly smaller number is required (Randhawa et al. 2019). Yr molecular markers may be used to map the detection of stripe rust resistance genes in host plants. One of the most significant and urgently required research fields on rusts, notably stripe rust, is the marker-assisted selection of specific genotypes for the Yr gene. Stripe rust resistance can also be achieved if there is stacking of multiple APR Pst resistance genes through MAB. With the significant expense in the genotyping absence of authentic markers and perfection in phenotypic determination, most reproducing programs rely on phenotypic selection (Randhawa et al. 2019; Prasad et al. 2019; Jamil et al. 2020).

Recently, reports confirmed that Powdery mildew resistance locus-associated trait *TraesCS3B02G014800* was considered as a most promising candidate gene for *QPm. caas-3BS* (*a stagnant* quantitative trait locus (QTL) for offering adult plant resistance (APR) against powdery mildew in the popular population of recombinant inbred line ex: Zhou8425B/Chinese Spring by phenotyping across four environments. In addition, *TraesCS3B02G016400* and *TraesCS3B02G016300* were not much reliable candidates depending on sequence variation and gene annotations between the parents. The above findings result not only provide high-throughput KASP markers for enhancement of resistance against powdery mildew, but also direct the way the map depends upon resistance gene cloning (Dong et al. 2022).

12.4.3 RNAi (RNA Interference)

RNAi is a self-defense mechanism to protect against fungal diseases. Cellular enzymes can cause the degradation of viral mRNA through cellular enzymes.

Plant protection techniques based on RNA interference (RNAi) were developed based on the understanding that exogenous or plant-derived double-stranded RNA (dsRNA) molecules may silence critical or virulence genes in microbial diseases and pests. Due to the extremely specialized, ecologically friendly and changeable character of dsRNAs, these tactics are advantageous. Furthermore, RNAi is successful in suppressing plant-pathogens when further administered by transgenic host-induced gene silencing (HIGS) or environmental absorption (ex. Spray-Induced Gene Silencing; SIGS). A bidirectional method called "cross-kingdom communication" silences virulence genes by exchanging RNA duplexes between plant and microbial cells. Fungal pathogens such as Pst carry out the uptake of nutrients and secretion of effectors. From the transgenic host plants, siRNAs, along with other nutrients, are transported to the obligate fungus, *B. graminis*.

The transgenic host plants that contained RNAi reduced the growth of the biotrophic B. graminis. Thus, RNAi-based crop protection strategy could be deployed against fungal pathogens. The powdery mildew of wheat caused by *Blumeria graminis* f. sp. tritici is a serious disease and dsRNA targeting the avirulence gene *AVRa10* which is recognized by the resistance gene *Mla10* significantly reduced fungal development in the absence of *Mla10* and the silencing of 13-b-glucanosyltransferases (*BgGTF1* and *BgGTF2*) reduced the early development of the pathogen. BSMV-HIGS (Barley stripe mosaic virus) provides a way to analyze the function and to screen RNAi targets for the control of rust diseases. Silencing of the genes through *A. tumefaciens* to genes encoding mitogen-activated protein kinase 1 (*PtMAPK1*) cyclophilin (*PtCYC1*) and calcineurin B (*PtCNB*) from *Puccinia triticina* controlled disease symptoms and decreased sporulation. Further silencing of *PtMAPK1*, *PtCYC1*, and *PtCNB* also reduces the impact of rust fungi. The hairpin RNAi creations of the homologous gene of MAP kinase (*PtMAPK1*) and the cyclophilin (*PtCYC1*) of leaf rust hindered fungal growth and drastically decreased fungal biomass in transgenic wheat (Qi et al. 2019). RNA interference was first used on gene vernalization gene *TaVRN2*, which delays flowering time. A study concluded that with the reduction of *VRN2* by RNAi, flowering time in winter-wheat plants is accelerated by more than a month. Wheat was targeted with MAPK kinase gene *PsFUZ7* which plays a crucial part in developing *Pst* virulence to stripe rust by regulating hyphal morphology and development as the target for RNAi (Zhu et al. 2017).

The expression RNAi construct in transgenic wheat plants imparts significant and long-lasting resistance to *Pst* as well as a severe limitation of *Pst* development. This effective disease inhibition suggests that HIGS is a powerful strategy for engineering transgenic wheat resistant to the obligate biotrophic pathogen Pst and that it could be used instead of conventional breeding or chemical treatment to develop environmentally friendly and long-lasting resistance in wheat and other food crops. Another host-induced gene silencing the *FgCh3b* enhanced resistance against *Fusarium graminearum* (Koch et al. 2013) *PtCNB PtCYC1* and *PtMAPK1* exhibits resistance from *P. striiformis* and *P. graminis* and *P. triticina* (Panwar et al. 2013a) and *FcGls* from *Fusarium culmorum* (Panwar et al. 2013b). The *TaCSN5* like overexpression in wheat lines considerably reduces the accumulation of salicylic acid and enhances

susceptibility to *P. striiformis* (Pst). Similarly, TaCSN5-RNAi wheat lines are confined opposite feedback mechanistic cascade. Also, *TaCSN5* negatively stimulated *TaG3NPR1* genes indulged in the SA-signaling pathway. Additionally, *TaCSN5*-RNAi lines exhibited enhanced multiple races-specific resistance to Pst. The combining results confirm that *TaCSN5* involves in negatively regulating the expression into PST resistance in an SA-based manner (Bai et al. 2021).

12.4.4 Genome Editing

Just like the rice, recognizing and editing the genetic makeup of wheat would help in the development of high-yielding varieties (Mehta et al. 2020; Dilawari et al. 2021; Chattopadhyay et al. 2022). The most current and commonly utilized genome-modifying tools are the meganucleases (MNs), zinc-finger nucleases (ZFNs), TALENs, and the CRISPR/Cas9 system. CRISPR/Cas9 and its variations hold enormous promise for developing novel wheat types with increased yield potential as they are simple and extremely efficient (Fig. 12.2). Using the CRISPR/Cas9

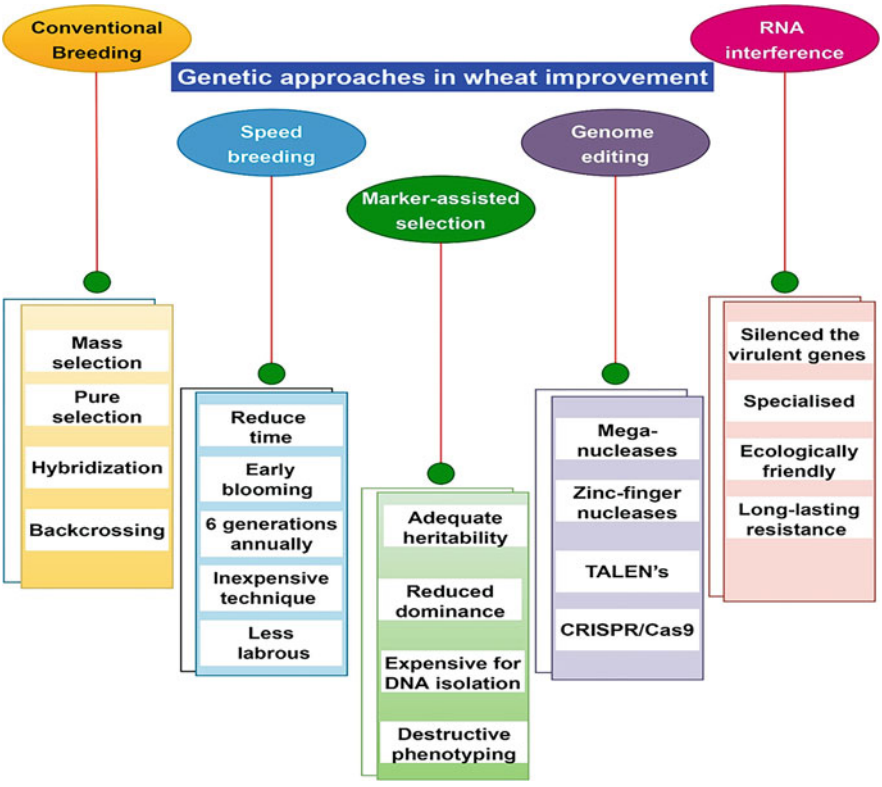

Fig. 12.2 Overview of fungal wheat diseases and other factors that affect the virulence of new pathotypes

system in wheat, a targeted genome of the TaMLO-A1 allele was modified, which resulted in the complete removal of powdery mildew (Yin and Qiu 2019; Ahmad et al. 2020).

MLO loci decrease the defense mechanism against powdery mildew and when MLO was modified, resistance to the fungal pathogens against *Blumeria graminis* f. sp. tritici (Bgt) is increased. The three *MLO* homoeologs in bread wheat (*TaMLO-A1, TaMLO-B1,* and *TaMLOD1*) look identical, and if they are modified, varieties resistant to *Bgt* can be developed (Wang et al. 2014). If genes like *LOX1* or *LOX5* are modified, it can also enhance disease resistance to *F. graminearum* in the corresponding mutant plants (Nalam et al. 2015). In some cases, powdery mildew, caused by the pathogenic fungus *Blumeria graminis,* has restricted wheat production in different major wheat cultivation regions throughout the globe. Introgression of *Pm60* like tolerance genes that were initially identified in *Triticum urartu* (diploid wild wheat) into wheat cultivar can harbor the genetic diversity for various disease resistance breeding. 'Bridge' approach or durum like intermediate species was used to introgress *Pm60b* and *Pm60* into common /hexaploid wheat that was noted by genetic markers and confirms the powdery mildew resistance (Zhang et al. 2022). Using the GWAS technique in cultivated wheat, 11 quantitative trait loci (QTLs) for 5 out of 6 specific strains or races of *Pt* and *Pgt* were reported. Out of 11 QTLs, nine were demonstrated as leaf- and stem-resistant during the growth stages of wheat and these can be used at all growth stages for improving resistance to wheat (Zatybekov et al. 2022). Except these, genotyping was conceded out using 8 QTL and 55 K SNP array and was identified on different chromosome arms 4BL, 3BS, 2DS, 2AL, 5BL, and 7BL through inclusive composite-interval mapping. The *QYr.nwafu-4BL.2* from FDC12 and *QYr.nwafu-3BS* from RH520 were uniform across the four different testing regimes. Results depicted that *QYr.nwafu-3BS* is behaving similar to the *Sr2/Yr30*, a pleiotropic resistance gene (Liu et al. 2022).

12.5 Concluding Remarks

Crop cultivars have been improved using traditional plant breeding techniques. Several existing cases involve outstanding efforts by breeders that resulted in the development and release of disease-resistant crop types. The use of DNA markers has further aided breeders in reducing the breeding cycle, hence improving the efficiency and precision of traditional plant breeding. To some extent, mutation breeding has been effective in generating unique genetic variants. However, because it is a random process, evaluating and identifying acceptable mutants is a time-consuming and arduous procedure. Furthermore, after suitable mutants have been found, additional breeding processes are needed to establish homozygosity and eliminate unwanted mutations. DNA technology offers a viable alternative to traditional breeding since it allows for the transmission of beneficial genes across genus boundaries.

So far, GM crops developed through DNA technology make the plants resistant to viral infections. In addition, plant breeders and pathologists have a fantastic

platform to produce resistant cultivars because of the development of genome editing RNAi silencing technologies like MAS, etc. Although the major focus should remain on breeding resistant cultivars, several other approaches such as integrating fungicides, moving planting dates, modifying crop feeding patterns, eliminating volunteer plants, cultivar mixing, and intercropping may be used as a temporary measure to prevent diseases. These things continue to develop and distribute resistant wheat cultivars side by side to give a cost-effective and environmentally sustainable alternative.

Marker-assisted breeding CRISPR and even bioinformatics allow researchers to investigate and make modifications in hitherto unexplored areas of plant science as well as bring together the genetic underpinnings of a wide range of agricultural plant activities. These instruments are a sign that the plant crop and other plant species will be used properly and in a useful way in the future. These tools are now being studied in many labs across the world. In the future, sequencing tools with low-cost procedures will undoubtedly aid in the identification of novel genes in a short period of time in many crop species in a cost-effective manner.

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Disease Resistance Genes' Identification, Cloning, and Characterization in Plants

13

Siddra Ijaz, Imran Ul Haq, Maria Babar, and Bukhtawer Nasir

Abstract

In plant-pathogen interactions, signal activation and transduction confer resistance in plants against various pathogens. Communication between host and pathogen is the prime step for a pathogen to cause infection. The molecular basis of pathogen response in plants depends on the pathogen types. Hypersensitive reactions usually result from Avr-R interactions that restrict pathogens' development through cell death. These avr genes can be recognized directly and indirectly by the resistance (R) gene. The NBS-LRR family is an important resistance gene (R gene) family in plants, which is divided into subclasses. Resistant gene analogues (RGAs) are candidates for R genes that have a significant role in defense response against disease-causing pathogens and are classified into two classes. The first class is based on the immediate recognition of a pathogen called resistance genes (*R* genes), while the second class is based on the defense response generated by recognition events. Hence, this chapter attempts to delineate a comprehensive overview of resistance genes, their classes, identification, and characterization in plants.

Keywords

Disease resistance · *R* genes · Resistance genes analogs · Plant-pathogen interaction

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13.1 Introduction

Microbes interact with plants in two ways: they are in a symbiotic relationship with each other, or they invade to cause an infection that results in disease occurrence. Generally, invaded pathogens alter plants' various metabolic pathways, developmental stages, and reproduction cycles. Bacterial, fungal, viral, and nematode diseases reduce crop health and productivity, resulting in significant financial losses for agricultural landowners. Among different devastating pathogens, major plant infections are caused by fungi, and approximately 80% of the causal agents of various plant diseases are fungi (El Hussein et al. 2014). Plants have evolved several defense systems to protect themselves against disease attacks (Seherm and Coakley 2003; Fujita et al. 2006).

Plants do not possess the adaptive immunity, usually exhibited by vertebrates, to fight against pathogens. For efficient detection and prevention against pathogens, plants rely on their genetic attributes (Chisholm et al. 2006). There are mainly two mechanisms through which plants respond to pathogen invasion. The first one is the basal defense, which primarily acts through the basal immune system. It was described for the first time over 30 years ago (Albersheim and Anderson-Prouty 1975). The elicitors (mainly elongation factors), which generally indicate the pathogen's presence and help initiate the mechanism, include chitin, lipopolysaccharides, hepta glucosides, or bacterial flagellins (Jones and Takemoto 2004). The second one has induced plant defense immunity, including pathogen-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is activated by PAMP (pathogen-associated molecular patterns) and plays a crucial role in the activation of primary defense responses like stomata closure, ROS (reactive oxygen species) generation, and the initiation of transcription processes of particular genes involved in the defense mechanism. ETI is stimulated by plant resistance genes (R genes) by recognizing pathogenic factors.

Moreover, ETI is linked with plants' HR (hypersensitive response) (Mandadi and Scholthof 2013; Yuan et al. 2021). *R* genes of plants are complementary to Avirulence (Avr) genes products coded by pathogens. These avr genes can be recognized, directly as well as indirectly, by the resistance gene products. The guard and decoy hypothesis perturbs the adaptive immune system components due to the Avr gene products, separate from the R proteins. The resistance genes (*R* genes) are activated by perturbation, which acts as a trigger. The avirulence gene of *Pseudomonas syringae* (AvrPphB) is an example of protease involved in the host protein kinase's cleavage. Cognate R protein (RPS5) plays its role in detecting this cleavage, resulting in its activation (Ade et al. 2007; Dodds and Rathjen 2010; Yuan et al. 2021). The MAMP (microbe-associated molecular patterns) receptors are relatively heritable as well as stable, so the diversification and selection are employed on the adaptive immune system's components in individuals' somatic cells, giving rise to a constant coevolution of plants and pathogens (Bent and Mackey 2007). The chapter covers the basics of resistance (*R*) genes, their classifications, and their role in disease resistance. It also emphasizes the

identification, characterization, and cloning of numerous *R* genes and RGAs in several crop species to combat harmful infectious diseases.

13.2 Resistance Genes

Resistance (*R*) genes are categorized into various classes. Nucleotide-binding leucine-rich repeats (NBS LRR/NLR) are the most significant type of *R* gene (Van Ooijen et al. 2007). Phylogenetic analysis of the NBS gene was reassembled by an extensive study of resistance gene analogs (RGAs) available in various genomes and NBS domains (Pan et al. 2000). Depending on the presence or absence of multiple domains at the N-terminal (amino-terminal) region, NL proteins are classified into two subclasses; the Toll/ interleukin receptor-nucleotide-binding site-leucine-rich repeats (TIR-NBS-LRR/TNL) and coiled-coil nucleotide-binding site-leucine-rich repeats (CNL). In a phylogenetic analysis, TNL and CNL formed discrete clades (McHale et al. 2006).

The first group of NBS domain of *R* genes contains TIR sequence in the N-terminal region (TNL) that is extensively distributed in dicot species of plants. In contrast, the second group is always associated with the CC domain in their amino terminal region (CNL) and is widely distributed in angiosperms. CNL and TNL are based on the N-terminal region, and their distribution in plant species is not in the same ratio. The absence of TNL among monocotyledonous species is the most prominent example. The genomes of *A. lyrate*, soybean, and *A. thaliana* contain two to three folds more TNL than CNL genes. However, potato and *Medicago truncatula* have a more significant number of CNL genes in their genome (Kang et al. 2012). Both TNL and CNL classes are comprised of the members who undergo the mechanism of alternative splicing. In animals, alternative splicing of TIR receptors is commonly accruing. For instance, the splicing of mouse TIR receptor, i.e., TLR4 variants, is the part of the regulatory mechanism that inhibits the excessive responses to bacterial lipopolysaccharide (Jordan et al. 2002).

13.3 NBS-LRR Class of *R* Genes in Plants

NBS-LRR are categorized into TNL and CNL (Hammond-Kosack and Jones 1997). TNLs are solely found in dicotyledonous plants, while CNLs are found in both monocotyledonous and dicotyledonous plants (Jacob et al. 2013). These two classes of NBS-LRR are distinct due to N terminal domains. LRR domain plays a vital role in protein-protein interaction (Martin et al. 2003) and recognizing various pathogenic avr proteins. Like LRR, NBS domains are very conserved and have the potential of binding with GTP or ATP. The P-loop sub-domain plays a vital role in binding nucleotides with protein. The operating range of NLRs increases when it gets translocated to some unlinked locus (Wu et al. 2017). NLRs get activated when their interaction occurs with pathogen effectors, which makes them quite helpful in detecting and controlling pathogens in various crops and various plant species.

Proteins containing the NBS-LRR (NLR) domain have a variable N-terminal domain (~200 amino acids), NBS domain (~300 amino acids), and LRR domain (10–40 short leucine-rich repeats) (Young 2000; Kang et al. 2005). The accessibility to plant genome sequences has encouraged scientists to identify and characterize NL encoding genes and RGAs in various plants and crops genomes to protect them against pathogens. Several hundred NL encoding genes have been investigated in alfalfa (*Medicago sativa*), *Arabidopsis thaliana*, grapevine (*Vitis vinifera*), rice (*Oryza sativa*), Medicago species, soybean (*Glycine max*), and chickpea (*Cicer arietinum*) (Zhou et al. 2004; Tan et al. 2007; Moroldo et al. 2008; Porter et al. 2009) using molecular biology and computational biology techniques..

NLR is the largest class of *R* genes, having conserved domains (Yue et al. 2012). Out of 150 cloned RGAs, 80% encodes these conserved domains (Guo et al. 2016). Resistance genes (*R* genes) are categorized into four structurally discrete classes. Serine-threonine kinase protein is the first class among these classes, which phosphorylate the ser/thr residues and control signaling networks (Ellis et al. 2000). The second class of *R* genes comprises putative transmembrane receptors along with the extracellular LRR domain (Chakraborty et al. 2019). The third class of *R* genes encodes receptors like kinase protein possessing the first- and second-class properties.

Moreover, the fourth class belongs to the NLR associated either with the CC domain or the TIR domain at N terminus, which exhibits significant plant disease resistance against pathogen. LRR domain involves recognizing pathogen specificity through ligand-binding and protein-protein interaction (Ellis et al. 2000). The interaction between distinct R proteins and other proteins acting downstream in the cascade is altered by nucleotide triphosphate-binding. LRRs play a vital role in protein-protein interactions, which are highly adaptable structural domains, and are involved in recognizing multiple pathogenic avr proteins (Bent 1996; Jones and Dangl 2006).

NBS-LRR class is categorized into TNL and non-TNL types on the basis of amino terminal region. TNL type of NBS-LRR class possesses domain homologs to the interleukin-1 receptor and toll receptors; on the contrary, non-TNL types of NBS-LRR class have coiled-coil protein commonly known as CC-NBS-LRR (Meyers et al. 2003). The interleukin-1 receptor domain is involved in pathogen detection, while the coiled-coil domain is associated with protein-protein interaction. The nucleotide-binding site domain consists of kinases (2a and 3a), P-loop, and hydrophobic GLPL motif. LRR domain interacts with pathogens directly or indirectly. These conserved *R* gene domains have been used to design the primer to identify and screen resistant gene analogs (RGAs) within or related crops (Kanazin et al. 1996).

The conserved domain of resistance genes (*R* genes) is part of the superfamily known as STAND with ATPase activity. To maintain the close conformation, the conserved domain of NB-ARC interacts with both its N and C-terminal. To get activated, the leucine-rich repeat must separate from the nucleotide-binding site domain. After separation, the conserved domain of NB-ARC modifies its state from ADP nucleotides to an ATP nucleotide to allow the rotation within the

NB-ARC domain that leads to an open conformation. It allows the amino terminal region to get exposed (Leipe et al. 2004; Takken and Goverse 2012). The effector recognition specificity depends on the leucine-rich domain (LRR), which ensures the coevolution with the pathogen effector under diversifying selection. Some other conserved motifs have also been recognized and characterized in the available NL conserved domains, which form the R proteins, nucleotide-binding adaptor shared by APAF-1 (apoptotic protease activating factor 1), and CED-4 (*C. elegans* Death-4) in the NB-ARC domain. The presence of ARC1 and ARC2 subunits was revealed during functional analysis of the NB-ARC domain (Qi et al. 2012).

The conserved nucleotide-binding site domains are associated with signaling, and they contain several conserved domains as well as motifs, including P-loop (also known as a kinase-1a motif or Walker A motif), kinase-2 domain (also called Walker B), and GLPL motifs (Tan and Wu 2012). Among these essential motifs of the NBS region, the ATP/GTP-binding loop (P-loop)/Walker A/Kinase-1a motif has a pivotal role. This motif is consisted of a glycine (Gly)-rich, flexible loop and also has lysine (Lys) residues that allow binding to the phosphate group of nucleotides (Saraste et al. 1990). The ATP hydrolysis is governed by the coordination of threonine or serine (Thr/Ser) residues with the magnesium ion ATP (Mg^{+2} ATP) and coordination of positively charged primary amine (ε-amino group) of Lys residue with beta and gamma phosphates (β– and γ– phosphates) of ATP (Cremo et al. 1989). Therefore, the classical signature sequence of the Walker A motif is (G/A)(4X)GK(T/S), where "X" is for any amino acid. The P-loop motif is essential in plant defense signaling (Hishida et al. 1999). A mutation study at the P-loop motif predicted that any substitution in Lys-residues reduces or even loses ATP-binding and ATP hydrolysis (Tameling et al. 2002). Moreover, ATP-binding (or ATPase activity) is the general recognition feature of the NBS site. LRRs are involved in protein interactions, which are highly adaptable structural domains, and they can evolve different binding specificities. At the level of predicted solvent-exposed residues, the leucine-rich repeats are under differentiating selection. In this region, these conserved domains lack conservation property and are more diverse than random genetic drift. (Ellis et al. 2000; Jones and Dangl 2006). It proposes that the emergence of new pathogen specificities is promoted by selective pressures to recognize different pathogenic Avr proteins.

The NL class of *R* genes confers a hypersensitive response in viral diseases, showing its resistance potential against them (Hull 2002). In extreme resistance, the multiplication of viral pathogen is constrained to a single cell level where necrotic lesions do not appear at the site of primary infection. The extreme resistance feature is showed in *Solanum tuberosum*, where Rx genes (CNL) resist Potato Virus X (Sekine et al. 2008). In *A. thaliana*, HRT gene-mediated resistance against TCV (Turnip CrinkleVirus) is an example of HR resistance. The type of resistance determines the *R* genes' expression level. In *A. thaliana*, the overexpression of RCY1, which confers resistance against CMV (Y), is an allelic form of hypersensitive response to the TCV extreme-resistance phenotype (Sekine et al. 2008).

For NLR proteins, the induction of alternative splicing of variants upon pathogen recognition has also been observed in plants. This observation suggested that the

variant's alternative splicing may involve the defense mechanism's regulatory process (Jordan et al. 2002). Several transcripts have been identified for TNL encoding proteins. The TNL encoding genes identified in various plants species include RPP5, RAC1, and RPS4 in Arabidopsis (Parker et al. 1997), L6 gene in flax (*Linum usitatissimum*) (Ayliffe et al. 1999), N gene against Tobacco Mosaic Virus in tobacco (*Nicotiana tabacum*) (Marathe et al. 2002), and Y-1 and Bs4 genes in potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*), respectively (Vidal et al. 2002; Schornack et al. 2004).

Regardless of the specific nature of interactions among pathogens' avirulence gene and *R* genes of plants, no distinct specificities are present in reactions of NL proteins against any specific pathogen. The allelic forms residing at the same NLR locus provide resistance to various disease-causing microbial strains, even though the strains belong to different classes. Three proteins of *A. thaliana*, i.e., RPP8, RCY1, and HRT, are encoded by multiple alleles residing at a single locus. RPP8 protein confers resistance against oomycetes, while RCY1 and HRT resist viral pathogen. Similarly, there are two highly alike proteins, the Rx protein of potato and Gpa2 (Globodera pallida 2) protein, but the potato Rx protein recognizes a virus, and Gpa2 protein recognizes a nematode (Van der Vossen et al. 2003).

In NBS conserved domain, the P-loop motif is a prerequisite for nucleotide-binding. Moreover, any mutation or alteration in the P-loop motif leads to loss of NBS-LRR protein functionality (Williams et al. 2011). Mutations determine the auto-activation of NBS-LRR protein in the MHD (Met-His-Asp) motif present in the ARC2 subunit, which involves nucleotide-dependent conformational changes (Van Ooijen et al. 2008). The coiled-coil domain exclusively triggers the cell-death response in CNL protein. In Arabidopsis, three proteins (RPS5, RPM1, RPS2) resist *P. syringae* Pv Maculicola 1 attack and activate the ADR1 (disease resistance 1) gene. The locus A10 gene resists barley mildew disease in barley, and in *Nicotiana benthamiana*, NRG1 genes resist TMV infection (Collier et al. 2011).

13.4 Resistance Gene Analogs (RGAs)

Resistance gene analogs (RGAs) are candidates of *R* genes having conserved motifs and conserved domains. RGAs can be categorized into NBS-LRR and TM-LRR (transmembrane leucine-rich repeat). NBS-LRR are complex *R* gene families which alter their state from ADP to ATP on occurrence with pathogen effector. NLR is present in the cytoplasm; however, LRR is positioned on the C-terminal, recognizing different effectors. A homology region is present between these domains, ARC, i.e., APAF1, resistance (R) proteins, and CED4 (*C. elegans* Death-4) domain. The functional characterization revealed the presence of ARC1 and ARC2 subunits. Structurally, ARC1 is a four-helix bundle, while ARC2 forms a winged-shaped helix bundle. ADP-binding occurs through four water-mediated and eight direct interactions between ARC1 and ARC2 subunits (Riedl et al. 2005). Conserved motifs including P-loop, kinase-2 motif, and kinase-3a motif are present in NB site of P-loop NTPase (Walker et al. 1982; Traut 1994). The lysine residue in the Walker

A motif helps in the coordination of β and γ phosphate inbound NTP. In comparison, the first Asp residue of Walker B helps in Mg^{2+} ion coordination at the catalytic site, while the second residue plays the role of the catalytic base in ATP hydrolysis.

13.5 Resistance Genes in Cereals

A large group of microbes, including bacteria, fungi, viruses, and nematodes, are responsible for causing infectious diseases in cereal crops like wheat, sorghum, barley, and maize. Every year, farmers face huge economic losses due to the pathogen attack. Numerous *R* genes have been identified and characterized for long-term disease management from wheat, maize, barley, and rice. NLR not only recognizes and provides resistance against bacteria and fungi, but also against nematodes and viruses. In maize, Rp1-D and Rp3 genes encode NL and confer resistance against leaf rust disease caused by *Puccinia sorghi*, while the Hm1 gene encodes HC toxin reductase, which resists Southern corn leaf blight disease caused by *Cochliobolus carbonum*. In barley, Mla1 and Mla6 encode NL, which resists *Blumeria graminis*, which is responsible for powdery mildew disease.

Additionally, the Rpg1 gene encodes protein kinase, which helps provide resistance against *Puccinia graminis* (causal agent of stem rust disease in barley). Lr21, Lr10, and Pm3 genes have been identified in wheat which encodes NL. The former two genes confer resistance against *Puccinia triticina* (causal agent of leaf rust disease), while the latter one helps resist *Blumeria graminis*. In rice, Xa21 and Xa26 encode receptor kinases that play an imperative role in the defense mechanism against *Xanthomonas oryzae*, responsible for bacterial blight. However, Pi-b and Pi-ta encode NL genes which help in conferring resistance against *Magnaporthe grisea*, a fungal pathogen responsible for rice blast (Ayliffe and Lagudah 2004).

13.6 Resistance Genes' Identification, Cloning, and Characterization

Disease-resistant genes are identified by their degree of expression in the defense response and their ability to boost the defense mechanism (Wang et al. 2010). The proteins of disease-resistant genes encode structural proteins (which assimilated into the extracellular space that contribute to the confinement of pathogen), secondary mechanism enzymes, regulatory genes (that control the expression level of defense response genes), as well as the catalases, chitinase, or peroxidase, etc. enzymes that are directly involved in defense mechanisms (Dixon and Harrison 1990). According to structural and functional similarities and the presence of conserved regions, sequencing of identified *R*-genes has revealed signal transduction and protein-protein interaction in specific conserved domains (Gassmann et al. 1999). NBS-LRR conserved domains are present in most of the genes. For example, CLASS-1 includes the Prf gene in tomato (*Solanum lycopersicum*), N in tobacco (*Nicotiana tabacum*), RPS2 and RPMI of Arabidopsis, and L6 gene of flax (*Linum*

usitatissimum) which encode cytoplasmic receptor containing an LRR and NBS domain. Class-II includes CF2, CF4, CF5, and CF9 of *S. lycopersicum* resistant to different *Cladosporium fulvum*. Class-III includes Xa21 of *O. sativa* resistant to bacterial pathogens and *Xanthomonas oryzae* pv *oryzae* with an extracellular LRR domain and an intracellular serine kinase domain. Class-IV includes the Pto gene of *Solanum lycopersicum* resistant to *Cochliobolus fuluvm*, and the Hml gene in maize (*Zea mays*) encodes functional HC toxin reductase domain. Cloning and characterization of RGAs are based upon different strategies like map-based positional cloning, homology-based degenerate primers, and transposon tagging, which have exposed the amino acid domains with extensive sequence homology (Madsen et al. 2003).

In various crops and plant species, multiple RGAs have been identified against different pathogens. A study conducted by Liang et al. (2005) revealed the identification and characterization of RGAs in peach (*Prunus persica* L.) against peach scab. The identified and isolated resistant gene analogs in peach provide the basis for studying the genes involved in conferring resistance in peach and other closely and distantly related species (Liang et al. 2005). Wan et al. (2012) proposed a research study to isolate potential NBS type *R* gene in sweet pepper. Degenerate primers identified the resistant gene analogs (RGAs) from conserved GLPL, P-loop, and NBS regions. Of 78 RGAs, 51 RGAs encoded conserved kinase-2a, P-loop, GLPL motifs, and some previously identified resistant (*R*) genes from tomato and Arabidopsis. The phylogenetic tree grouped the identified pepper RGAs into TIR and non-TIR clusters, and their findings fully support that both these types are widely present in dicot plants. Analysis of qRT-PCR revealed that abscisic and salicylic acids change the expression of identified RGAs, ultimately suggesting their involvement in plant defense response by activating signaling cascades.

Subcellular localization is imperative for R protein functionality, and various R proteins are localized in the nucleus and the cytoplasm. In barley, Bai et al. (2012) showed that the activity of MLA10 in a nucleus is suppressed to cell-death signalings, while in a cytoplasmic location, it is observed to be enhanced. Sufficient MLA10 is present in the cytoplasm; the enforced retaining in the cytoplasm has strengthened MLA10 function in cell-death signaling. Particular intramolecular and intermolecular interactions are linked to the role of MLA10 residing in the nucleus. The latest study has also disclosed that a rod-shaped homodimer is formed through the CC domain of MLA in solution. The same study also revealed that a minimal functional unit is mainly defined by MLA dimers, ultimately responsible for triggering cell-death response in tobacco and barley plants (Maekawa et al. 2011). In maize, identified RGAs are involved in flavone biosynthesis pathway with the trait loci and show resistance against maize disease like corn earworm. The locus of p1 in maize codes for transcriptional activator with three other RGAs accounts for 76% of phenotypic changes for developing resistance against the pathogen (Zhang et al. 2003).

R-genes belonging to NL conserved domains have displayed a significant level of sequence homology compared to other classes of RGAs, and during evolution, these genes indicated the possible gene duplication. Eighty-eight (88) *R* genes, identified

in sugarcane (*Saccharum officinarum*), represent three influential groups: NBS/LRR domain, LR repeats, or S/T KINASE domains. Sequential association of two NBS/LRR RGAs clusters in *Oryza sativa* and *Zea mays* also showed orthologs' polyphyletic origins. This present information suggested that paralogous RGAs in *Saccharum officinarum* have a more considerable degree of divergence than that from an ortholog in a distant species (Rossi et al. 2003). Dicot *R* genes showed shared motifs on peptide sequence comparisons, but monocot showed no evidence for shared motifs in a specific signature.

RGAs mapping revealed linkage to the identified and characterized resistance (*R*) genes which ensured the presence of mixed clusters. This investigation of identified *R*-genes showed nonsystemic mapped locations between cereals like foxtail millet (*Setaria italica*), rice (*Oryza sativa*), and barley species (Leister et al. 1998). The study implies the rearrangement of *R* gene loci, suggesting various NBS and LRR mechanisms for *R* gene evolution compared with other monocot genomes. A string of 6 conserved motifs is shown by comparing 25 genes segments of NL encoding resistant genes from *O. sativa*. The mapping of this gene in *O. sativa* showed linkage to *R* genes. The genes (Xa1, Xa3, and Xa4) showed race-specific resistance against rice blight disease. Barley RGAs also showed linkage with powdery mildew and rust-resistant genes (Leister et al. 1999).

Seah and his colleagues isolated NL gene sequences at the Cre3 locus in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) using specific primer pairs. These sequences showed the resistance against cereal cyst nematode disease of wheat (Seah et al. 1998). It revealed that RGAs of *Hordeum vulgare* and *Triticum aestivum* contain some conserved motifs in identified *R* genes of various crops. At the Cre3 locus, 55–99% protein sequences showed similarity with NBS-LRR conserved domain. Mapping of barley-derived RGAs on chromosome 2H loci (Sharma et al. 2005), 5H (Whitham et al. 1994), and 7H (Liu et al. 2007) was linked to the resistance against CNN and CLA (corn leaf aphids). Sixty cloned fragments were analyzed by Southern blot during amplification of RGAs from Asian gall midge-resistant rice line (Mago et al. 1999) and characterized into 14 categories. Twelve clones were then mapped onto five diverse rice chromosomes with a significant cluster of eight RGAs on chromosome XI. This work indicates that insect and disease resistance genes shared common conserved NBS-motifs.

In the rice genome, scientists selected 68 nonredundant clones of sequences homology to the known *R* gene. They mapped 15 clones on 17 loci on chromosome numbers III, IV, XI, and XII mapped in the rice (*Oryza sativa*). The mapped clones' loci correlate with rice *R* gene against rice blast and blight resistance on chromosomes XI and XII, and some occurred in clusters on chromosome III, which showed correlation with bacterial leaf blight resistance (BLB) (Wang and Xiao 2002). Mohler et al. (2002) also found an association with disease resistance in Triticeae during mapping. In *O. sativa* and *H. vulgare*, powdery mildew resistance genes linked to Pin17 and Mlal were also observed. All RGAs classes were mapped in the genome of *Hordeum vulgare* using PCR-based marker techniques, including RFLP. Moreover, these identified RGAs were near the previously identified disease resistance loci in *H. vulgare* and various cereals. In *Dioscorea alata*, Saranya and his

colleagues identified and characterized the *R* genes against Anthracnose disease by PCR (Saranya et al. 2016).

For testing the association of disease resistance as molecular markers in rice against blast, brown planthopper (BPH), sheath blight (SB), and bacterial blight (BB), the candidate genes are involved in putative defense response experimentation. Scientists derived one hundred eighteen (118) molecular markers from identified resistance gene analogs (RGAs) and putative disease-resistant genes of *Zea mays*, *Hordeum vulgare*, and *Oryza sativ*a (Ramalingam et al. 2003). Upon hybridization, several identified RGAs and disease-resistant genes identified a locus with different copy numbers and mapped mostly on chr11 of *O. sative*. Several known blasts and bacterial leaf blight (BLB)-resistant genes were present. The candidate resistance genes and disease-resistant genes' molecular markers were mainly associated with the pathogen's quantitative trait loci (QTLs).

In *Zea mays*, 11 non-cross hybridizing sequences of resistance gene analogs with NL proteins' identity were identified (Collins et al. 1998). These identified RGAs of *Zea mays* and one RGA of wheat were used to probe for mapping twenty (20) RFLP loci in *Z. mays*. Few of these loci were mapped to fungal and viral resistance genes regions —the identified RGAs co-segregate with maize rust resistance loci, rp1 and rp3. The study results revealed that the RGA probe linked with (rp1) maize rust resistance loci could identify rp1 mutants in *Z. mays*.

The NBS-LRR conserved domain possesses resistance potential against various disease-causing pathogens. By computational biology, 104 NBS-LRR genes were identified and characterized in chickpea (Sharma et al. 2017). Phylogenetic analysis analyzed the deduced RGAs of chickpea and their divergence into TNL and non-TNL types. In silico promoter analysis analyzed four *cis*-regulatory elements, i.e., GCC, DRE, WBOX, and CBF boxes, found in the promoter regions of identified NBS-LRR genes of chickpea. Similarly, Hussain et al. (2020) classified and characterized 3085 *R* genes in *Gossypium arboreum*, 3024 in *Gossypium hirsutum*, and 5355 genes in *Gossypium raimondii* cotton species by computational biology tool. The in silico analysis of promoter elements predicted that the *cis*-regulatory elements were present in different NBS-LRR classes of *R* genes in these cotton species.

RGAs were identified in wheat (*Triticum aestivum*) for developing disease-linked molecular markers (Xie et al. 2008). RGA 200 and RGA390 markers were identified closely associated with Pm31 (powdery mildew *R* gene) and utterly co-segregated with Xpsp3029 (marker allele) linked to Pm31, with a genetic distance of 0.6 cM. RGAs were also used for molecular markers, so, in *Triticum aestivum*, these two identified RGAs (RGA200 and RGA 390) were then incorporated into the previously developed microsatellite map of the Pm31 region. Previous literature identified and functionally validated the importance of conserved *R* genes' domains in plant-pathogen interaction (Grund et al. 2019). WRKY domain, present at the C terminal of NBS from the RPS4 complex, is involved in the detection of AvrRps4 and activation of defense mechanism (Sarris et al. 2015). The WRKY domain is comprised of BLR genes that confer resistance against rust diseases in *Triticum aestivum* (Wang et al. 2020).

Previous research studies support that *R* genes are involved in resistance to significant pathogenic diseases. Lv et al. (2020) identified and characterized *R* genes in *Brassica napus* to improve resistance against Sclerotinia stem rot, clubroot, downy mildew, and Blackleg fungal diseases. Additionally, different identified *R* genes have been used in resistance breeding programs. The eIF (iso) 4E variant transferred into *Brassica rapa* under turnip mosaic virus challenge; as a result, broad-spectrum resistance was observed in transgenic plants (Kim et al. 2014).

The degenerate primers, designed from the conserved P-loop and GLPL regions of NLR, were used to amplify the NBS region from the resistant chili genotype. Naresh et al. (2017) researched to screen the chili genotypes against several viruses from multiple virus-resistant genotypes, i.e., IHR 2451. Moreover, the alignment of deduced protein sequence and phylogenetic analysis grouped conserved domains into TIR and non-TIR clades and confirmed the conservation of kinase-2a, GLPL, P-loop, and RNBS-A motif. Both TIR and non-TIR types are present in dicotyledonous plants. Identified resistant gene analogs (RGAs) showed conserved motif subjected to Blastp, indicating homology with *R* genes such as the Pvr9 gene, which exhibit resistance against potyvirus and RIB-23 gene homology with putative late blight resistance protein (Naresh et al. 2017).

NBS profiling by PCR technique was performed to identify and map RGAs in apple. Degenerate primers were synthesized from the conserved P-loop motif. Identified RGAs were mapped on 10 (out of 17) linkage groups of apple genetic maps residing near the QTL for resistance against mildew and apple scab diseases (Calenge et al. 2005). In another research study, Zhang and colleagues identified and characterized the *R* genes based on the NBS-LRR domain, RPW8 domain in *Dioscorea rotundata*. It is an imperative crop in Africa and is commonly known as yams and sweet potatoes. Different types of pathogens infected the crop during their life span and reduced the quality and quantity of crops (Zhang et al. 2020). The NBS profiling method was also accomplished in potato, tomato, and lettuce to identify RGAs at their transcript level. Therefore, for the generation of the candidate marker genes associated with resistant gene (*R*-gene) in crop plants, the NBS profiling is a significant technique for identifying candidate *R* gene that is transcribed and functional in crop plants (Brugmans et al. 2008).

Resistance gene analogs were identified and characterized by sequence analysis and reannotation analysis. Ren et al. (2020) conducted a research study to characterize the NBS genes under biotic and abiotic challenges in orchardgrass. The sequence analysis showed that 17 NBS genes were expressed under abiotic challenges, whereas 23 NBS genes were differentially expressed under rust challenges. Another fungal disease in common beans (*P. vulgaris*) causes severe losses in the field. The most effective practice for cultivating *P. vulgaris* is to use the resistant cultivars to overcome disease losses. Vaz Bisneta and Gonçalves-Vidigal (2020) identified the 256 nucleotide-binding sites, LRR proteins, and 200 kinases proteins under the fungal disease challenge.

NLR encoding genes are present in angiosperms; however, grasses and monocots lack genes that encode TNL (McDowell and Simon 2006; Guo et al. 2011). The

absence of TNL class from monocots is hypothesized as loss or the failure to amplify these genes in monocot lineage. Moreover, the absence of these genes in monocot might be because of more dependence on CNL proteins than TNL proteins (Kim et al. 2012). The downstream signaling pathways and disease resistance factors differ for CNL and TNL classes (Glazebrook 2001). Mutation in genes encoding for components involved in TNL pathways has caused the genomic shift to genes encoding for CNL in monocots, ultimately resulting in loss of TNL gene functions and conservative selection. It is hypothesized that due to lack of conservative selection, genes encoding for TNL might have never amplified in monocot genomes (Meyers et al. 2002).

Dicots like Arabidopsis possess more TNL as compared to CNL (Yang et al. 2008). Along with TNLs and CNLs, various NBS encoding genes, including N, CN, TN, and NL, are present, which vary in abundance. Many other NBS-LRR like domains, including CNLX, CNXL, CXN, NLX domains in sorghum, CTN and CTNL in apple, and TTNL, TN-TNL, XTNX in Arabidopsis, have been reported (Chelkowski and Koczyk 2003; Cheng et al. 2010; Arya et al. 2014). Many TIR-X RGAs have been reported in various plant genomes, including 67 in cottonwood, 126 in cabbage (*Brassica oleracea* var. capitata), 46 and 92 in Arabidopsis and Medicago, respectively (Yu et al. 2014). Moreover, RLKs members, i.e., 1200 in rice and 600 in Arabidopsis, were also identified (Dardick et al. 2007). RLKs have been reported in wheat (*Triticum aestivum*), cottonwood (*Populus deltoides*), tomato (*Solanum lycopersicum*), and maize (*Zea mays*) as well. In addition to RLKs, RLPs (with TM domain) have been reported in tomato as well as in Arabidopsis.

Crop plants' whole-genome sequencing has aided in the mapping, identification, and characterization of resistance gene analogues. These RGAs having conserved NL domain have been assessed in crop plants, i.e., Arabidopsis, grape, apple, black cottonwood, rice, wheat, Medicago, barley, and sorghum. The number of *R* genes containing conserved domains has been recognized in crop plants against various diseases (Radwan et al. 2008). The NL domain is highly conserved, evolutionarily diverse, and assembled gene families, and it represents the primary class of *R* genes in plants, which contribute to conferring resistance against deadly disease (Porter et al. 2009). Some RGAs are identified as pseudogenes, which have been reported in several plants, including rice (*Oryza sativa*) (Luo et al. 2012), potato (*Solanum tuberosum*) (Lozano et al. 2012), Arabidopsis (Meyers 2003), lotus (*Nelumbo nucifera*) (Li et al. 2010), Medicago (Ameline-Torregrosa et al. 2008), and cottonwood (*Populus deltoides*) (Kohler et al. 2008). Numerous RGAs have been identified in many plant genomes (Table 13.1).

13.7 Conclusion

Phytopathogens are greatly responsible for various plant and crop diseases. The deadly diseases significantly decrease crop productivity, affecting the economies of agricultural countries. Over the decades, phytopathogens have evolved and developed numerous ways of attacking plants and overcoming plant defense mechanisms.

Table 13.1 Resistance gene analogues (*RGAs*) in different plant genomes

Sr No.	RGAs	Plant	Disease	Pathogen	References
1	MB-ClsRCaG1, MB-ClsRCaG2, MB-ClsRCaG3	Mungbean	Cercospora leaf spot	*Cercospora canescens*	Babar et al. (2021)
2	Rev1, Rev7, Rev8, Rev11, Rev12, Rev35, Rev43, Rev45, Rev67, Rev68, Rev84	Chickpea	Fusarium wilt	*Fusarium oxysporum*	Priyanka et al. (2021)
3	RGA-012, RGA-087, RGA-118, RGA-533, RGA-542	Sugarcane	Red rot	*Colletotrichum falcatum*	Parvaiz et al. (2021)
4	PnRGA1, PnRGA3, PnRGA5, PnRGA8, PnRGA11, PnRGA24	Black pepper (Piper nigrum L.)	Phytophthora foot rot	*Phytophthora capsici*	Suraby et al. (2020)
5	OLE 1121/1122 (RGA)	Apple	Powdery mildew	*Podosphaera leucotricha*	Jamalvand et al. (2020)
6	Rdr1 gene	Roses	Black spot	*Diplocarpon rosae*	Menz et al. (2020)
7	RGA003, RGA020, RGA028, RGA035, RGA042, RGA054, RGA055, RGA057, RGA062, RGA068, RGA082, RGA092, RGA100, RGA101a, RGA106, RGA121, RGA140, RGA144, RGA162, RGA199, RGA201, RGA206, RGA207, RGA235,	Peanut	Leaf spot	*Cercospora arachidicola*	Dang et al. (2019)

(continued)

Table 13.1 (continued)

Sr No.	RGAs	Plant	Disease	Pathogen	References
	RGA240, RGA250, RGA260, RGA265, RGA270, RGA286, RGA304, RGA314, RGA315, RGA321a, RGA340, RGA341, RGA348, RGA355, RGA359				
8	RM1, RM6, RM8, RM12 and RM31	Cotton	Cotton leaf curl virus	Whitefly *Bemisia tabaci*	Mushtaq et al. (2018)
9	RGA1-RGA15	Sugarcane	Red rot	*Colletotrichum falcatum*	Hameed et al. (2015)
10	MNBS1-MNBS17	Banana	Fusarium wilt	*Fusarium oxysporum*	Sutanto et al. (2014)
11	RGPM213	Pearl millet	Downy mildew	*Sclerospora graminicola*	Ranjini et al. (2011)
12	Rps1-k-1, Rps1-k-2	Soybean	Root and stem rot	*Phytophthora sojae*	Gao and Bhattacharyya (2008)
13	Ha-NTIR1-Ha-NTIR12, Ha-TIR3, Ha-TIR14, Ha-NTIR15	Sunflower	Downy mildew	*Plasmopara halstedii*	Radwan et al. (2003)
14	GLP1–12, MHD145, MHD98	Grapevine	Powdery mildew	*Uncinula necator*	Donald et al. (2002)
15	RGA1, RGA2	Common bean (*Phaseolus vulgaris*)	Anthracnose	*Colletotrichum lindemuthianum*	López et al. (2003)
16	RGA7	Common bean (*Phaseolus vulgaris*)	Angular leaf spot	*Phaeoisariopsis griseola*	López et al. (2003)
17	Xa21	Rice	Rice bacterial blight	*Xanthomonas oryzae*	Song et al. (1995)

This attribute of pathogens caused devastating effects on crop physiology and production that ultimately resulted in systemic damage. Economic losses and crop damage can be lessened through various crop management and disease control strategies. Agriculturists have employed multiple strategies to overcome the situation. The usage of chemicals poses a negative impact on the environment as well as on all living organisms. To overcome this problem, an eco-friendly, long-term, and sustainable strategy should be designed. In this regard, identifying RGAs and their characterization in different crops against various pathogens has opened new disease management avenues. The knowledge of identified and characterized R genes in plant species provides basic information on genetic and molecular mechanisms involved in the regulation of gene resistance. Resistance gene analogues (RGAs) are candidates for R genes possessing disease resistance potential. Among distinct classes of resistance (R) genes, the most significant class is NBS LRR. NL proteins are involved in conferring defense against fungal, bacterial, and viral infections. Apart from NL, many different R genes have been identified that do not encode NBS LRR, but possess resistance ability against the pathogen, e.g., Xa21 and X26 genes in rice. Recently, numerous resistance genes have been identified in different crops like mungbean (against Cercospora leaf spot), chickpea (against Fusarium wilt), sugarcane (against Red Rot), and black pepper (against Footrot). The identified R genes can be used to design degenerate primers, which can be further used to identify R genes in diverse and distant species. In the future, using RGAs has made it possible to reduce crop productivity losses by developing resistant varieties and controlling diseases in a long-term way.

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Utilization of Biosensors in the Identification of Bacterial Diseases in Maize

14

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Abstract

Nanotechnology is an emerging technological and scientific breakthrough that can transform agricultural sectors by providing novel tools for the molecular

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detection of biotic and abiotic stress, and the rapid detection of phytopathogenic diseases. In plants, it has the potential to enhance their capacity to absorb water and nutrients from the soil. Furthermore, nanobiotechnology improves our understanding of crop biology, yields, and nutritional values. The various applications of nanotechnology in agriculture are (1) energy storage, production and conversion (photovoltaic modules); (2) increased agricultural productivity (nanoporous zeolites for prolonged and efficient release of fertilizers); (3) capsules for the specific release of pesticides; (4) the use of biosensors for monitoring the soil quality and plant vitality; (5) pest and phytopathogen detection biosensors; and (6) pesticide biosensors. Nanosensors and intelligent delivery systems based on nano-products are used in the agricultural sector to combat crop pathogens. This nanotechnology seeks to minimize nutrient losses in fertilization and improve crop productivity by optimizing the use of water and nutrients. Nanotechnology provides a wide range of opportunities to produce agro-products based on nanomaterials such as fertilizers, pesticides, herbicides, and nanosensors. These will make it possible to increase the food yield sustainably, reduce the environmental impact and detect infections in plants. This chapter talks about how nanotechnology can be used in plant pathology and how nanomaterials can be used to make biosensors that can detect the main bacterial diseases in maize.

Keywords

Biosensors · Nanobiotechnology · Nanomaterials · Nanoparticles · Nanosensors

14.1 Introduction

Zea mays is the third most widely cultivated cereals grain in the world, serving as livestock feed, biofuel, human food, and a raw material in the industry. Its commercial impact exceeds US$50 billion. A biosensor is an integrated receptor-transducer device structured by a biological recognition element (cell, tissue, receptor, nucleic acid, enzyme, ribozyme, or antibody, among others), or nanomaterials (nanoparticles and nanocomposites), intelligent materials or biomimetic compounds (aptamers, polymers of intrinsic microporosity, and nucleic acid probes), which is associated with a detection mechanism and interpretation of the variation of optical, physicochemical, and electrical properties, among others, obtained from the interaction between the analyte and the analytical device (Volkov 2000; Turner and Newman 1998). The type of recognition element determines the transducer system, and the physicochemical characteristics of the analyte are determinants for the choice of biological and biometric materials. Biosensors present an analytical approach of greater speed, simplicity, and low economic cost. DNA biosensors based on nucleic acid recognition have applications such as in electrophoresis analysis of amplified DNA. The applications of DNA-based biosensor analysis extend to the field of food control, process control of raw materials, and traceability in industrial processing plants, and in the field of food control, not only for raw materials but also for process

control and traceability in industrial processing plants (Minunni et al. 2005; Mannelli et al. 2003; Bogani et al. 2008). Label-free piezoelectric DNA biosensors present adequate specificity and high sensitivity, allowing rapid and real-time control of DNA hybridisation (Lucarelli et al. 2008; Wu et al. 2007; Sun et al. 2006). Biosensors are designed to detect analytically important molecules such as toxic compounds or pathogens in order to provide reliable, rapid and accurate information about the analyte of interest. Biosensors take part in the important growth of analytical tools useful in the detection of hazardous biological and chemical compounds for health care, food safety and environmental monitoring (Luong et al. 2008; Mascini 2008; Amine et al. 2006). Plant pathogens reduce crop productivity and cause a decrease in food for human and animal consumption. Currently, many methods have been developed to detect crop-dependent phytopathogens of biochemical and molecular types, but they lack speed, reliability, specificity and accuracy, being not suitable for the in situ analysis system. Therefore, there is great interest in developing biosensor systems for early and accurate detection of phytopathogens (bacteria, fungi, and viruses) (Wijesuriya & Rechnitz 1993; Dyussembayev et al. 2021; Ammar 2018).

Climate change and population growth alter agricultural production. Crop engineering is increasingly necessary. Nanoparticle-based biosensors are new tools to advance agricultural practices. As these nanoparticle-based biosensors enter and travel through biofluidic complexes within plants, biomolecules, including proteins, metabolites, lipids and carbohydrates, adsorb onto the surfaces of the nanoparticles, forming a coating known as a "bio-crown". On the other hand, screen-printed carbon electrodes are adapted to different biorecognition elements, including enzymes, antibodies, and aptamers, often with other modifiers, such as mediators and nanoparticles, to produce electrochemical biosensors for a variety of analytes of importance in agri-food safety. Emphasis is placed on biosensor fabrication strategies and device performance characteristics. In addition to biosensors for a range of analytes in different agri-food matrices, there are also those with potential in agri-food safety (Smart et al. 2020; Voke et al. 2021). Of importance is the high specificity and sensitivity to be able to detect physiological and pathogenic molecules, which offers a useful opportunity in the treatment of plant pathogenic disease with early diagnosis. There is also the optical-based biosensor in which a fibre-optic cable is used in the different investigations. Bacteriophages are ubiquitous viruses found wherever bacteria exist. It is estimated that there are more than 1031 bacteriophages on the planet, more than all other organisms on the earth, including bacteria. In recent years, biosensors have been widely recognized as having several potential applications in the food industry (Nasrullah 2021).

Nano-inspired biosensors have acquired a vital role in improving the quality of life through various botanical and environmental applications worldwide. Several nano-inspired biosensors have been reported, ranging from detection of plant infections (fungal, viral, and bacterial), abiotic stress, metabolic content, phytohormones, miRNAs, and genetically modified (GM) plants to transcriptional and genetically encoded biosensors in a very short time. For in vitro and in vivo measurements, with the existing tools and technologies (such as molecularly

imprinted polymers, microfluidics, plasmonic nanosensors, surface-enhanced Raman scattering (SERS), fluorescence, chemiluminescence, quartz crystal microbalance, and advanced electrochemical measurements), together with customizable nanomaterials or nanocomposites, a potential niche has recently been discovered and is being exploited to make nano-inspired plant-based biosensors. Although the research based on plant-based biosensors has gained momentum very recently, few research results are available (Kumar and Arora 2020). There are new emerging biosensor technologies such as isothermal amplification, nanomaterial detection, paper-based techniques, robotics, and lab-on-a-chip analytical devices. However, these constitute a novelty in research and development of approaches for the early diagnosis of pathogens in sustainable agriculture (Ali et al. 2021).

Both bacterial and fungal diseases can be diagnosed with biosensors because of their potential capacity, real-time detection, and advantages, among other analytical techniques. For example, mycotoxins, which are naturally occurring toxic secondary metabolites produced by fungi, can be determined. Biosensors are effective and efficient for the accurate detection of these toxic molecules in food, combining a biochemical recognition element with a physical transducer (Shrivastava and Sharma 2021).

Plant diseases minimize crop productivity. Another very dangerous plant disease is bacterial stalk rot in maize, which disrupts the flow of nutrients from the primary and secondary roots to other parts of the plant, infecting the inner tissue of the stalk until it rots completely. The disease has been reported to attack maize crops in Asia and Europe. Molecular identification results indicated that this disease is caused by the bacterium Dickeya zeae (Patandjengi et al. 2021). The pathogen needs to be identified both in the field and in greenhouse. Current technologies, such as quantitative polymerase chain reaction (Q-PCR), are time-consuming and lack high sensitivity. They require large amounts of target tissue and several assays to accurately identify different plant pathogens. Biosensors are low-cost methods to improve the accuracy and speed of plant-pathogen diagnosis. However, nanotechnology, nanoparticles, and quantum dots (QDs) are essential tools for the rapid detection of a given biomarker with extreme precision. Biosensors, QDs, nanostructured platforms, nanoimaging, and nanopore DNA sequencing tools have the potential to increase the sensitivity, specificity, and speed of pathogen detection, facilitate high-throughput analysis, and be used for high-quality monitoring and crop protection. In addition, nanodiagnostic kits can easily and quickly detect potentially serious and dangerous plant pathogens, allowing experts to assist farmers in the prevention of epidemic diseases (Khiyami et al. 2014; Prasad et al. 2014).

Other biotechnological advances developed are quorum quenching (QQ), which is a technique to control quorum-mediated bacterial pathogens by interfering with population sensing systems, catalysing degradative enzymes, modifying signals, and inhibiting signal synthesis. In many Gram-negative pathogenic bacteria, chemically conserved signalling molecules called *N*-acyl homoserine lactones (AHLs) are studied. AHLs modulate virulence factors in several plant pathogenic bacteria, including Dickeya zeae. Dickeya zeae is a bacterium that causes plant rot in maize, causing economic crop losses. Zhang et al. (2021) isolated an

AHL-degrading bacterial strain W-7 from samples of Pseudomonas nitroreducens. Strain W-7 revealed a superior ability to degrade *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), when it completely degraded 0.2 mmol/L OdDHL in 48 h. By GC-MS, *N*-cyclohexyl-propanamide was identified as the main intermediate metabolite during AHL biodegradation (Zhang et al. 2021).

Food safety and security must be ensured for plant pathogenic microorganisms to become a threat to global food consumption. Also, nanomaterials have chemical and physical properties, which are used for high-throughput, non-invasive detection, and as diagnostic techniques for various plant pathogens. The sensitivity and selectivity are currently improved due to the use of engineered nanomaterials corresponding to molecular and sequencing techniques. This is a biotechnological alternative needed for rapid, in situ diagnostics of diseased plants and long-term monitoring of plant health conditions (Li et al. 2020).

Aflatoxin is a carcinogen secreted by fungi and is found dangerously in some food samples. Many detection methods have been developed to determine traces of aflatoxin. Dyussembayev et al. (2021) developed a specific, cost-effective, and simple colorimetric competitive assay method to detect aflatoxin B1 based on the interaction of gelatin-functionalized gold nanoparticles in a specific enzymatic reaction. The results obtained showed that through this approach aflatoxin could be detected in a linear range of 10–140 pg mL^{-1}, with a detection limit of 4 pg mL^{-1}. The assay on real saffron samples showed a recovery rate of 92.4–95.3%. The analysis should be efficient and highly sensitive in testing to achieve the best detection of pathogens in food as the limit of detection by analyzing the highest amount of volume. Xu et al. (2019) developed a flow-through immunoelectrochemical biosensor to identify two types of bacteria (*E. coli* O157: H7 and *Salmonella*) in food. The electrode was formed with a porous, antibody-coated graphite felt electrode that served as a solid support coated with biorecognition elements for capturing target pathogens as a signal transducer, and large volumes of the aqueous sample can be rapidly exposed to the solid support through gravity flow (Xu et al. 2019). Therefore, this chapter addresses the applications of nanobiotechnology in plant pathology, as well as biosensor platforms based on nanomaterials to detect the main bacterial diseases in maize.

14.2 Biosensors

A biosensor is a device that measures biological or chemical reaction that detects, records, transmits, and provides specific quantitative or semi-quantitative analytical information from its environment using a specific biological recognition element with a physiological change/process in a biological system, providing specific biochemical interactions or reactions, or uses biological materials to monitor the presence of various chemicals in a substance. According to the International Union of Pure and Applied Chemistry (IUPAC) definition, a biosensor is an analytical device used for sensitive and selective biomarkers for the detection of chemical compounds, usually by optical, thermal, or electrical type signals (McNaught and

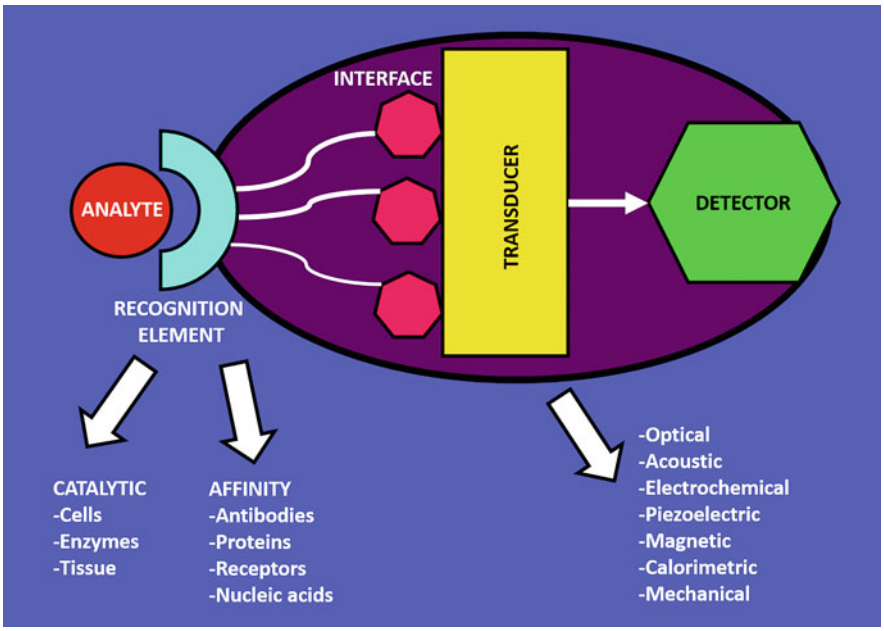

Fig. 14.1 Components of a biosensor

Wilkinson 1997). In most successful biosensors, the principle underlying the determination of a chemical or biological molecule is the specific interaction of that analyte molecule with the biological material present in the biosensor probe device. Figure 14.1 describes the elements of a biosensor.

14.3 Mechanism of Biosensors

Biosensors are devices that combine a bioreceptor, and a suitable transducer, which measures the effect produced by the interaction between the substrate and the bioreceptor and transforms it into an electrical signal. Bioreceptors such as tissues, cells, nucleic acids, artificial binding proteins, monoclonal and polyclonal antibodies, as well as enzymes, among others, bind to a specific compound using higher-order structural elements. Depending on the transduction mechanism, biosensors can be classified as electrochemical, piezoelectric, thermal, optical, etc. The overall reaction/interaction of the bioreceptor and analyte is transduced into a signal that is easily quantifiable by the transducer. The biological recognition element is usually in close contact with a transducer, using an additional element located between the recognition element and the transducer corresponding to an interface composed of hybrid, inorganic or organic materials, with the objective of

improving the functionality of the device, either by providing greater stability or by amplifying the signal (Eggins 2002; Bănică 2012; Thévenot et al. 2001).

14.4 Biosensor Types

14.4.1 Enzymatic Biosensors

The development of new biosensors has been investigated in a variety of biological materials and transduction methods, such as enzymes immobilized as biological material and electrochemical transducers (Volkov et al. 1998; Volkov and Mwesigwa 2001). One of the alternative applications of enzymatic biosensors is to inspect different pollutants present in the environment in an automated, efficient, fast, and economical way. Oxidative enzymes, such as polyphenol oxidases (laccases and tyrosinases) and peroxidases, are interesting, highly functional and versatile enzymes used as analyte recognition elements in biosensors. With these biosensors, contaminants can be detected, as recognition elements mediate the use of oxidative enzymes and detection of contaminants such as toxic compounds and environmental pollutants: pharmaceuticals, heavy metals, phenols, and pesticides (Patel 2002; Rebollar-Pérez et al. 2020).

The generation of electrochemical sensing and biosensors based on the modification of the working electrode is a suitable tool for quality assurance in the food industry (Table 14.1). Petrlova et al. (2007) reported that the process could be used to determine an avidin-modified carbon paste electrode to determine concentrations up to 3 pm in solution and 170 nM in a corn seed extract.

14.4.2 Chemical Biosensors

In 1924, Palmer studied the coherence of contact-free thin filaments induced by electromagnetic waves in the presence of different gases and the correlation between the observed responses and the heat of gas absorption. This was one of the first chemical sensors ever recorded (Datskos et al. 2005). A chemical sensor is defined as a physical transducer (transducer of physical quantities into suitable output signals) and a chemically selective layer so that measurable output signals can be produced in response to a chemical stimulus (Datskos et al. 2005; Liawruangrath et al. 2001). In the design of a chemical sensor, molecule-selective coatings can be used, which means that these coatings can be chemically functionalized with compounds that recognize or interact with other chemical molecules of interest for detection or monitoring, such as sensors used for the detection of polluting particles in the environment or in water, to cite some examples. Another relevant aspect of these sensors are the different transduction modes; basically, these can be thermal, mass, electrochemical, and optical (Fig. 14.2).

Chemical sensors have been actively used within the MEMS (microelectromechanical systems) family, especially the simple structures called microcantilevers that have proven to be very useful as transducers of physical,

Table 14.1 Biosensors applied to evaluate food quality

Analyte	Matrix	Recognition enzyme	Transduction system	References
Glucose	Grape juice, wine, juice, honey, milk, and yogurt	Glucose oxidase	Amperometric	Centonze et al. (1997), Ángeles and Cañizares (2004)
Fructose	Juice, honey, milk, gelatin, and artificial edulcorants	Fructose dehydrogenase, D-fructose 5-dehydrogenase	Amperometric	Bassi et al. (1998), Palmisano et al. (2000)
Lactose	Milk	ß-galactosidase	Amperometric	Marconi et al. (1996), Palmisano et al. (2000)
Lactate	Cider and wine	Transaminase and lactate dehydrogenase	Amperometric	Silber et al. (1994), Ramanathan et al. (2001)
Lactulose	Milk	Fructose dehydrogenase and ß-galactosidase	Amperometric	Sekine and Hall (1998)
L-amino acids	Milk and fruit juices	D-amino acid oxidase	Amperometric	Sarkar et al. (1999)
L-glutamate	Soya sauce and condiments	L-glutamate oxidase	Amperometric	Kwong et al. (2000)
L-lysine	Milk, pasta and fermentation samples	Lysine oxidase	Amperometric	Kelly et al. (2000), Olschewski et al. (2000)
L-malate	Wine, cider and juices	Dehydrogenated malate, others	Amperometric	Miertus et al. (1998)
Ethanol	Beer, wine and other alcoholic drinks	Alcohol oxidase, alcohol dehydrogenase, NaDH oxidase	Amperometric	Katrlık et al. (1998)
Glycerol	Wine	Glycerophosphate oxidase and glycerol kinase	Amperometric	Niculescu et al. (2003)
Catechol	Beer	Polyphenol oxidase	Amperometric	Eggins et al. (1997)
Cholesterol	Butter, lard, and egg	Cholesterol oxidase and peroxidase	Amperometric	Akyilmaz and Dinckaya (2000)
Citric acid	Juice and athletic drinks	Citrate lyase	Amperometric	Prodromidis et al. (1997)
Lecithin	Egg yolk, flour, and soya sauce	Phospholipase D and choline oxidase	Electrochemical	Mello and Kubota (2002)

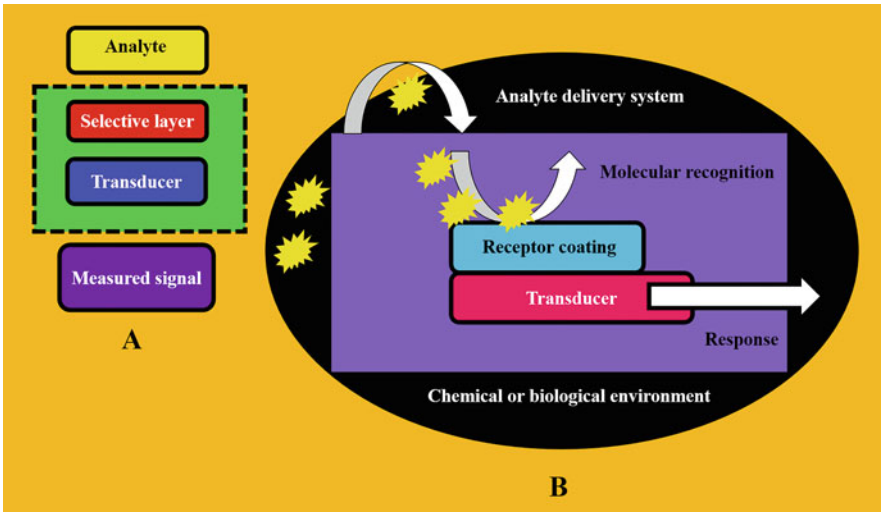

Fig. 14.2 (**a**) Schematic representation of a chemical or biological sensor with an output signal in response to the presence of an analyte source or chemical compound of interest. (**b**) Chemical sensor with a receiver layer that provides a selective response to chemical or biological molecules

biological or chemical stimuli into measurable signals. Sensors based on cantilevers involve measurements of their deflection, resonance frequency, and damping characteristics.

14.4.3 Biological Sensors

A biological sensor has an operating principle similar to that of a chemical sensor, but in this case, specific interactions can occur between biomolecules of the functionalized device, with the biomolecules of interest for detection, such as antibody-antigen, enzyme-substrate (biomolecule) interactions, and DNA strand recognition; even microorganism-culture medium or culture medium interactions can occur to carry out the biodetection of the recognition of the biomolecule of interest (Capobianco et al. 2021). These interactions result in the variation of one or more physico-chemical properties (pH, electron transfer, heat transfer, change of potential, mass variations, and variation of optical properties, among others) that are finally detected by the transducer. This system transforms the response of the recognition element into an electrical signal indicative of the presence of the analyte under study proportional to its concentration in the sample or to the growth of the micro-organism (Velasco-García and Mottram 2003). Biosensors can be classified in four different ways (Gonzalez et al. 2005) according to Table 14.2.

In practice, the choice of biological material depends on the characteristics of the compound to be analyzed, and the choice of the transducer is conditioned by the type of element to be recognized, as this determines what variation in physicochemical properties will occur as a consequence of the interaction (Datskos et al. 2005).

Table 14.2 Biosensors classification

Type of interaction	Characteristic
Between the recognition element and the analyte	Biocatalytic, bioaffinity
Method used to detect such interaction	Direct and indirect
Nature of the recognition element	Enzyme, organelle, tissue or whole cell, biological receptor, antibody, nucleic acids, PNA (peptide nucleic acid), aptamers (single-stranded nucleic acids or chemical antibodies)
Transduction system	Electrochemical, optical, piezoelectric, thermometric, nanomechanical

14.4.4 Mass Biosensors

A mass biosensor is a device capable of detecting the magnitude of mass and transforming this detection into an electrical variable: resistance, capacitance, voltage, current and frequency, among others. Currently, there are systems capable of detecting mass variations in picograms up to sensitivities of 0.18 ag/cm2, in commercial devices, at high frequencies (10–15 MHz) (Qsense 2011). Probably the most widely used biosensor with this function is the quartz microbalance (QCM, Quartz Crystal Microbalance or QMB, Quartz microbalance), which achieves an absolute mass resolution of 0.9 ng^2/cm^2. Quartz balances are used in chemical reaction monitoring, biomedical biosensors, metal deposition monitoring and environmental control. These systems sometimes allow electrochemical measurements in liquid, known as EQCM (Electrochemical Quartz Crystal Microbalance).

The quartz microbalance works by applying an external electrical potential to a quartz disc with two metal electrodes (usually gold), producing an acoustic wave that propagates through the crystal. This wave encounters a minimum impedance when the thickness of the system is a multiple of half the wavelength of the acoustic wave. The quartz crystal disc must be cut with a specific orientation with respect to the crystalline axes. The deposition of thin layers on the crystal surface decreases the frequency proportionally to the mass of the deposited layer. By detecting the variation in frequency, the deposited mass can be determined (O'Sullivan and Guilbautl 1999).

Zhang et al. proposed a system based on a comb microoscillator using parametric resonance amplification with picogram resolution in the air (Zhang and Turner 2005). Ekinci, on the other hand, presents a resonant nano-bridge with magnetic detection that allows absolute mass resolutions in the order of the atogram (Ekinci et al. 2004). This bridge is placed in a perpendicular magnetic field to excite the resonance, and together with the alternating current passing through it, an electromotive force is generated, which is detected through a network analyzer, and the mass changes are known. Devices capable of detecting 7 zeptograms have been designed, taking measurements in ultra-high vacuum and at temperatures below 7 K. Other results from biosensors based on piezoelectric resonant

membranes for biochemical detection indicate that resolutions close to 300 femtograms/Hz can be achieved (Nicu et al. 2005).

14.5 Biosensors to Detect Pathogens

Among the biological sensing components that can be used by optical biosensors are aptamers, which are single strands of DNA or RNA containing include aptamers with a three-dimensional structure, capable of recognizing specific molecules by binding to them (IBIAN 2020; Tombelli et al. 2009). There are several advantages of using aptamers, such as their high affinity and specificity, as they can be synthesized in a customized way (IBIAN 2020), their thermal and chemical stability, their low cost, and, in general, their numerous applications (Song et al. 2012). In plants, optical biosensors have been used for detecting pathogens of agricultural or epidemiological importance, as well as for detecting the presence of substances of interest, including allergens, toxins, and heavy metals (Sadanandom 2010; Michelini et al. 2008). Particularly, aptamer-based biosensors promise to be an ideal technique for the detection of commercially important metabolites, displacing traditional detection methods that can be time-consuming and resource-intensive to perform (Sadanandom 2010; Amini and Saify 2017). An important application of optical biosensors in plant biology is the assessment of the physiological state of a plant according to the content of secondary metabolites present in a given tissue (Coppedè et al. 2017). Secondary metabolites are compounds that play an important role in the interaction of plants with the environment, as their synthesis constitutes a physiological defence response against biotic stress conditions (insect attacks, infections, etc.) or abiotic stress conditions (droughts, extreme temperatures, etc.) (Pagare 2015; Zimdahl 1999; Kumar and Kumar 2018).

14.5.1 Biosensor Applications in *Zea mays*

Goron and Raizada (2016), studied more than 1500 maize seedling leaf extracts, which were treated with different N rates under uptake/assimilation systems. In situ imaging allowed demonstrated in all leaves sampled those multifactorial interactions allow Gln accumulation at the position within each leaf. In situ imaging localized Gln in leaf veins for the first time. These authors reported to GlnLux biosensor, which can measure relative Gln levels inexpensively with tiny amounts of tissue.

Liu et al. (2020) designed an electrochemical DNA biosensor based on nitrogen-dropped graphene nanosheets and gold nanoparticle nanocomposites for event-specific detection of the transgenic maize MIR162. This biosensor exhibits high reproducibility of fabrication, high selectivity, and good stability. The response choice they chose to monitor the target DNA hybridization event was methylene blue differential pulse voltammetry. Under optimal conditions, the peak current increased linearly with the logarithm of the DNA concentration in the range of 1.0×10^{-14} to 1.0×10^{-8} M, and the detection limit was 2.52 × 10–15 M. The

biosensor was effectively applied to detect MIR162 in real samples, demonstrating its potential as an effective and efficient tool for transgenic crop identification analysis.

Zeng et al. (2013) reported a biosensor based on Surface Plasmon resonance (SPR) to detect maize chlorotic mottle virus (MCMV). The effects of coupling reaction time and antibody concentration on detection sensitivity indicated that the developed SPR biosensor showed highly specific recognition for both purified MCMV and crude extracts from real-world samples.

Fumonisins are natural toxins produced by fungi species of the genus *Fusarium*. Fumonisins B1, B2 and B3 (also called FB1, FB2) are found in foods and were discovered in 1988. Fumonisins have health effects on livestock and other animals, contributing to health problems such as cancer or birth defects. The fungi *F. verticillioides*, *F. proliferatum* and *F. fujikuroi* are species that emerge in warm climates and tropical zones, and are the main contaminants of corn. An evanescent wave fiber-optic biosensor, which was competitive for fumonisin B1 and non-competitive for aflatoxin B1 was developed by Maragos and Thompson (1999).

14.5.1.1 Bacterial Detection Biosensors in Maize

Aflatoxin B1 (AFB1) is mycotoxin, carcinogenic, nephrotoxic, and hepatotoxic in humans and animals. Mycotoxins infect maize. Zearalenone is a mycotoxin considered as a xenoestrogen, similar to natural estrogens because it binds to estrogen receptors leading to various reproductive diseases, especially hormone imbalance. ZEN has toxic carcinogenic effects on human health. Valuable electrochemical detection assays based on nanomaterials included several immunodetection studies for the highly sensitive determination of several ZEN families (Sohrabi et al. 2022; Shahi et al. 2021).

Wang et al. (2021) developed an immunochromatographic assay with polystyrene microspheres to detect AFB1 mycotoxin sensitively and quantitatively. The reliability of the microspheres was confirmed with Liquid Chromatography-Tandem Mass Spectrometry.

A wide range of specific biosensors for mycotoxins and bacterial toxins are available for environmental and food control (Guilbault et al. 1993; Carter et al. 1994; Delehanty and Ligler 2002; Palleschi et al. 1997; Tran and Pandey 1992). Boiarski et al. (1996) developed an integrated optical biosensor to analyze aflatoxin B in maize plants to analyze ricin and saxitoxin, based on the impedance of an ultrathin platinum film with an immobilized layer of antibodies against staphylococcal enterotoxin B. On the other hand, Kumar et al. (1994) designed an evanescent wave immunosensors detecting botulinum with ultra-low detection limits while Ogert et al. (1992) obtained a highly specific reaction fiber-optic based biosensor that uses the evanescent wave of a conical optical of a sensitive and rapid immunosensor type to detect *Clostridium botulinum* toxin A by means of a rhodamine label at concentrations of 5 ng/mL.

The technique lateral flow immunoassays are based on gold colloidal nanoparticles for the detection of various plant pathogens, such as potato virus X (Drygin et al. 2012), *Fusarium* species (Xu et al. 2019), and *P. stewartii* subsp. stewartii (Pss) bacteria in maize was also detected (Zhang et al. 2014; Feng et al.

Table 14.3 Biosensors developed for the detection of plant pathogens in *Zea mays*

Biosensor type	Bio-recognition element	Technique	Pathogen	Detection limit	References
Optical	Antibody	Lateral flow immunoassay	*Pantoea stewartia* sbusp. *stewartii*	538 pg/mL	Feng et al. (2015)
Optical	Antibody	Lateral flow immunoassay	*Pantoea stewartia* sbusp. *stewartii*	5.38 pg/mL	Zhang et al. (2014)
Electrochemical	DNA	Quartz crystal microbalance-based detection	Maize chlorotic mottle virus	2.5×10^5 pg/mL	Huang et al. (2014)

2015). The causal agent of late blight in potatoes and tomatoes was detected by a combined lateral flow biosensor (Zhan et al. 2018) and integrated asymmetric PCR, mediated by a universal primer (Table 14.3).

Wen et al. (2015) generated a new low-cost and easy-to-use real-time technology with the objective of detecting biotic stress in the field; this system consisted of a lateral flow detection biosensor integrated into a corn leaf, while microspheres conjugated with analyte-specific and concentration-specific capture antibodies are non-invasively injected. In order to achieve infiltration and immobilization in the corn leaf, the size of the microspheres was optimized. In addition, a fluorescent biomarker, fluorescein, is detected in a living corn plant.

Syringe agroinfiltration is a system for introducing genes into host plants using *Agrobacterium* (Chen et al. 2013). It has been successful in several plant species (Wroblewski et al. 2005) because it uses simple equipment. The method consists of filling a needleless syringe with a solution containing *Agrobacterium* and injecting it manually. The tip is positioned on the dorsal side of an intact leaf. A temporary color change from light green to dark green indicates infiltration of *Agrobacterium* into the leaf (Annamalai et al. 2006). Wen et al. (2015) complemented this biosensor technology using a live corn leaf as a lateral flow "test strip", but injecting and immobilizing antibody-conjugated microspheres in the leaf interstitium (Fig. 14.3).

Detection and identification of plant pathogens are essential to improve crop yields by PCR or ELISA assay, which are time-consuming and destructive to the sample. Raman spectroscopy (RS) is a non-invasive and non-destructive analytical technique to know the chemical structure of the sample. Faber and Kurousky (2018) studied that Raman spectrometer, in combination with chemometric analysis in a stand-alone, portable and sample-independent manner, could distinguish between healthy and diseased maize (*Z. mays*) kernels, as well as in other crops, between different diseases, with 100% accuracy (Faber and Kurousky 2018). Faber and Kurousky (2018) demonstrated that RS can be used to detect and identify plant pathogens in intact maize kernels. These researchers obtained Raman spectra of

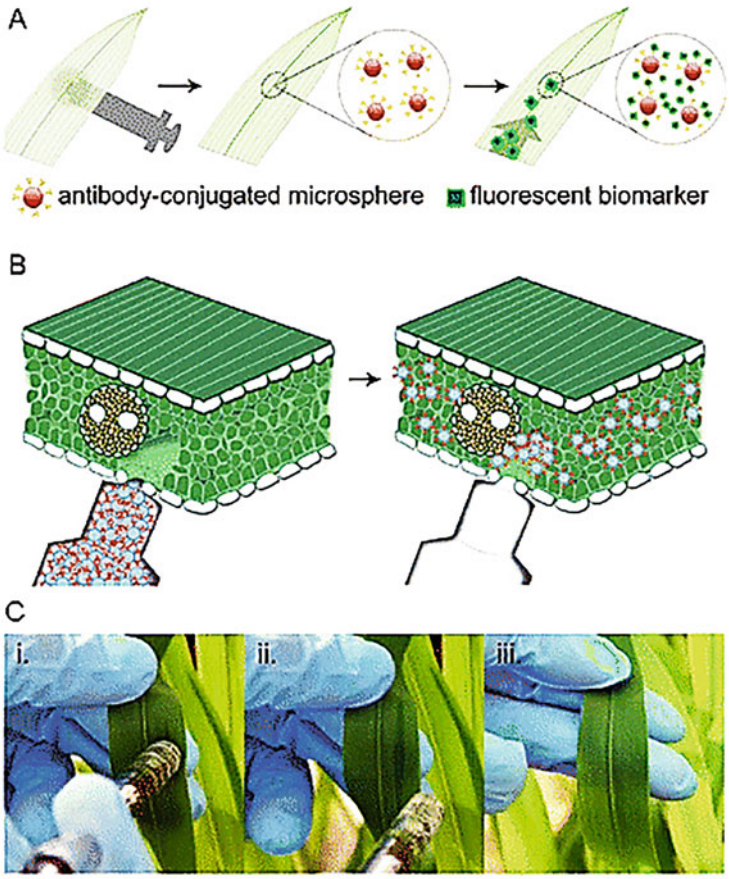

Fig. 14.3 One-step lateral flow detection method of plant-pathogen markers in live maize leaves. Detection of a fluorescent biomarker using antibody-conjugated microspheres (**a**) Detection of non-fluorescent biomarkers by incorporation of stimuli-sensitive colorimetric vesicles, (**b**) Schematic of microsphere infiltration into leaf tissue (left) before infiltration and (right) after infiltration, (**c**) Infiltration into a maize (*Zea mays*) leaf: (*i*) infiltration with a needleless syringe, (*ii*) immediately after infiltration, when the injected buffer solution is visible and (*iii*) 10 min after infiltration, when the injected buffer has evaporated without leaving visible marks. (Source: Wen et al. (2015))

individual maize kernels using a Rigaku Progeny ResQ portable spectrometer equipped with a 1064 nm Nd:YAG laser. These spectra show the average spectra of a healthy corn and the corn infected by the plant pathogenic fungi *Diplodia* spp., *Fusarium* spp., *A. niger*, and *Aspergillus flavus*.

Biosensors are bacterial cells containing a reporter gene (fluorescence marker), such as a green fluorescent protein (GFP) expression cassette (Sorensen et al. 2009). There are a limited number of reporter genes. With this method, using epifluorescent and confocal microscopy, bacterial colonization and activity are detected at the

single-cell level in rhizosphere microsites. Götz et al. (2006) and Germaine et al. (2004) successfully introduced GFP-tagged plasmids to monitor rhizosphere colonization of endophytic bacterial strains as *Pseudomonas putida* PRD16 and *Enterobacter cowanii* strain PRF116. Weyens et al. (2012) investigated the ability and colonization of plant growth promotion by endophytic *P. putida* strain W619 with GFP-tag insertion, without growth promotion. High background fluorescence limits the performance and detection of biosensors as a function of sample preparation and handling.

14.6 Nanosensors

The origin of nanotechnology goes back to research by the American physicist Richard Phillips Feynman, winner of the Nobel Prize in Physics. Important events for the foundation of nanotechnology lie in the 1982 invention of the scanning tunneling microscope by Swiss Gerd Binnig and German Heinrich Ruhrer, which made it possible to observe objects on a nanometer scale. In September 2003, the application of nanotechnology in agriculture and the food industry was discussed for the first time at the United States Department of Agriculture (USDA) (Weiss et al. 2006; Alam et al. 2016; Agrawal and Rathore 2014). Nano-sensors are devices that can treat and detect a fungal or bacterial infection, nutrient deficiency, or any other phytosanitation problem, long before phenotypic symptoms appear in plants (Fraceto et al. 2016; Rai et al. 2012). The application of nanotechnology in agriculture and the food industry receives a lot of attention nowadays. Investments in nanotechnology for food and agriculture are increasing due to its potential benefits, which range from improved quality and safety of agricultural inputs to better processing and higher nutritional value of agricultural inputs (Dasgupta et al. 2015). Agricultural scientists face a wide range of challenges such as stagnant crop yields, climate change, multi-nutrient deficiencies, low macro- and micronutrient use efficiency, reduced availability of arable land, declining soil organic matter, and a shortage of water and labor for the field (Shiva, 2016). Recent research on the use of nanotechnology in plants shows that the incorporation of synthetic nanoparticles can increase photosynthesis and transform leaves into biochemical sensors. The single-walled carbon nanotubes (SWNTs) coated with single-stranded DNA infiltrate the lipid envelope of extracted plant chloroplasts and assemble with photosynthetic proteins. The same occurred when SWNTs were released into the living leaves of *Arabidopsis thaliana* through the stomata. The researchers demonstrated that photosynthetic activity was three times higher in SWNT-containing chloroplasts than in controls due to increased light capture by the photosynthetic molecules. The use of nanotechnology allows the development of potential techniques for disease management in crops. Nanoparticles can be used in the preparation of new formulations such as insecticides, fungicides, insect repellents, and pheromones, which is made possible thanks to the new properties of these materials, such as their reactivity, quantum effects, and electrical conductivity.

14.7 Nanobiosensors

These biosensors have a huge impact on precision agriculture methods. Nanotechnology allows monitoring to be done in real time where biosensors are linked to GPS systems. These biosensors monitor the soil conditions and crop phenological status over large areas of land (Nair et al. 2010). Some commercial biosensors use plant redox enzymes. For example, superoxide dismutase is used to assess antioxidant activity and tyrosinase (monophenol monooxygenase) to monitor phenolic contamination. The enzyme laccase is used to monitor the presence of flavonoids in foods. Some biosensors such as electronic noses are used to analyze volatile organic compounds from diseased and healthy plants in crops such as potatoes and tomatoes.

The work of Pérez and Rubiales (2009) highlighted that nanotechnology is opening new potential applications for agriculture, which are already being explored. These authors also point out the potential of nanotechnology to develop nanodevices and nano-transporters to be used as smart systems to target specific chemical emission sites in plants.

Nanometer gold with sizes from 5 to 25 nm is used to deliver and incorporate DNA into plant cells, while 30 nm iron oxide was used in nano-sensors to detect pesticides at very small concentrations. These functions aid the development of precision agriculture, minimizing contamination and allowing maximizing sustainable agricultural practices (Malsch et al. 2015; Subramanian et al. 2015). N toxicity can be attributed to the following two actions: (1) chemical toxicity based on the release of toxic ions; (2) stress or stimuli caused by surface area, particle size, and/or shape. NPs oxide solubility has been confirmed to significantly affect plant response.

In the studies of Zhang et al. (2014), the phytotoxicity of ZnO NPs on the germination of maize (*Zea mays L.*) and cucumber (*Cucumis sativus L.*) seeds was investigated. Regarding root elongation, all seedlings were affected when exposed to a concentration of 1000 mg L^1. On their side, research by El-Temsah and Joner (2012) determined the phytotoxic potential of iron (Fe) NPs, using three types of particle sizes in the range of 1–20 nm, in the seed germination of barley and flax species.

Researchers at Iowa State University have used 3 nm-sized mesoporous silica (MSN) NPs as carriers and for the delivery of DNA and chemicals inside isolated plant cells. The MSN NPs are chemically coated and serve as gene containers that are then applied to the plants. This coating causes the plant to take up the particles through the cell walls and membranes where they are inserted, activating the biological genes in a precise and controlled manner without causing any toxic side effects afterwards. This technique has been successfully applied to introduce NPs into pumpkins and DNA into tobacco and corn plants (Corredor et al. 2009).

Silver can be integrated into inert materials, such as zeolite, silicate, and clay. Silver zeolite (Ag-zeolite) is produced by replacing Na ions in the zeolite with Ag ions; it is one of the most widely used antimicrobial agents, as it is a broad-spectrum antimicrobial agent that kills bacteria, yeasts, and mycelia, but not the spores of heat-resistant bacteria. Ag-zeolite incorporated into chitosan film shows strong antimicrobial activity against Gram-positive and -negative bacteria. Nanocomposites such

as silver silicate have been produced using a flame spray pyrolysis process and incorporated into polystyrene. This complex showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. A green synthetic approach for the preparation of antimicrobial silver nanoparticles has been suggested, by using carbohydrates from sucrose, or waxy and soluble corn starch (*Zea mays* L.).

Carbohydrates act as reducing agents and as a template for the realization of silver nanoparticles with excellent antibacterial activity.

14.8 Carbon Nanotubes

In the agri-food sector, water intake, crop growth rates, and uptake of essential nutrients are enhanced by the use of multi-walled carbon nanotubes (Scrinis and Lyons 2007). One of the functions of carbon nanotubes is the promotion of plant growth without any inhibitory, toxic or adverse effects on plants (Srilatha 2011). Rameshaiah et al. (2015) have reported that a concentration of 50 μg mL^{-1} with multi-walled carbon nanotubes increased the root and shoot length, and improved the seed germination time and growth of crops such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), groundnut (*Arachis hypogaea* L.), and garlic (*Allium sativum* L.).

14.9 Conclusions

Corn (*Zea mays* L.) is a crop of great importance that is exposed to factors such as the presence of disease-causing phytopathogens, which limit the maximum expression of its productive potential. Nanosensors can prevent the spread of diseases between crops by non-destructively detecting the presence of plant pathogens before symptoms appear.

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Part IV

Nanobiotechnology

Nanomaterials for Integrated Crop Disease Management

15

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Abstract

Because of the rising food demand, climate change, and environmental pollution, the global agricultural system is under increasing stress. In the current era, nanotechnology has demonstrated several applications in a variety of areas, including agriculture, medicine, and drugs. Due to their nano size, the increased surface to volume ratio, and unique morphology, nanoparticles have different characteristics than bulk materials. Nanoparticulated systems are being developed for use as fertilizers, insecticides, herbicides, sensors, and quality enhancers in agriculture. The present chapter discusses the use of nanoparticles (NPs) to improve sustainable agriculture and the environment by managing plant diseases directly as well as indirectly. The use of nanoparticles in plant disease control is a potential method for dealing with global concerns and ensuring sustainable crop production. This chapter will cover the basics of nanoparticles (NPs) and their uses in plant disease control. Plant disease management via the use of non-conventional nano-pesticides and fertilizer can play a pivotal role in mitigating the global food challenges and agricultural pollution concerns.

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Keywords

Nanoparticles · Nano-pesticides · Nano-fertilizers · Nano-fungicides · Bioavailability

15.1 Introduction

Agricultural pests and pathogens are responsible for 20–40% of crop losses each year globally (Worrall et al. 2018; Mesterházy et al. 2020). Despite many advantages, such as high availability, quick action, and effectiveness, pesticides exert negative impacts on non-target species, resulting in insecticide resistance. Furthermore, during or after the application, about 90% of applied pesticides are lost (Ghormade et al. 2011; Willkommen et al. 2021; Spinozzi et al. 2021). So, there is a greater need to produce efficient, high-performance, and low-persisting pesticides that are also environmentally friendly (Hatami et al. 2021). Nanotechnology has helped to make new agricultural ideas and products that have a lot of potential to help solve the problems (Worrall et al. 2018).

Nanoparticles (NPs) possess characteristics that differ from bulk and macroscopic materials, and these differences influence their destiny and impact on the biotic and abiotic components (Klaine et al. 2008; Gonçalves et al. 2021). The nanoparticles (NPs) are nanometer-sized particles that have shown some beneficial properties for sensing and detecting biological activities and structures in living bodies (Singh et al. 2008; Nie et al. 2021). Their size, large surface area, reactivity, absorbance, and aggregation govern their adherence to the soil as well as their subsequent mobility and movement (Borm et al. 2006; Xu et al. 2022). Although the NPs are used as antimicrobial agents against disease-causing bacteria, their overuse is hampering soil biodiversity, which executes important natural functions such as plant development, element cycling, and pollutant breakdown (Molina et al. 2006). As such, nanomaterials (NMs) are an important component of both biotic and abiotic remediation efforts because they interact with soil contaminants, affecting their toxicity, fate, and mobility (Usman et al. 2020). Rapid advances in nanotechnology have prompted concerns about the incidence, distribution, destiny, and mobility of NPs in the environment (Kurwadkar et al. 2015). Nanotechnology can help to ensure food security by improving crop productivity because NPs have the potential to improve plant development and production (Sadak 2019). They act as "magic bullets," holding fertilizers, genes, herbicides, or nano-pesticides, and concentrating their contents on certain cellular organelles in the plant (Siddiqui et al. 2015). NPs may be naturally or synthetically originated (Khan 2020). They can serve as a source of nutrients by ensuring their slow and controlled release, particularly micronutrients, and thus, limiting their access to the surrounding environmental barriers, as plants only require a small amount of these minerals (Tripathi et al. 2015; Dimkpa and Bindraban 2017). The NPs synthesis plays an important role in their properties. That's why several synthesis techniques are being researched to improve their qualities while decreasing the manufacturing costs (Kim et al. 2013; Jamkhande

et al. 2019). Some techniques are modified to improve the mechanical, optical, chemical, and physical characteristics of individual nanoparticles (Cho et al. 2013). A significant advancement in instrumentation has resulted in the enhancement of their characterization as well as their application.

Plants, the most significant part of the terrestrial ecosystem, play an important role in nanoparticle uptake and transport through absorption and bioaccumulation (Monica and Cremonini 2009). The response of plants to nanoparticles is of great interest (Dimkpa et al. 2013; Hernandez-Viezcas et al. 2013), as the use of NPs as nano-pesticides has the ability to revolutionize agriculture (Adisa et al. 2019). Due to their physicochemical properties, NPs have a lot of potential in agriculture. The NPs–plant interactions cause a range of genotoxic, physiological, and morphological changes that must be understood for nanotechnology to be employed effectively in agriculture, especially in integrated disease management (Nair 2016; Elmer et al. 2018). The size of plant tissues and cells is the first requirement for NP penetration. Plants allow NPs with a diameter of 40–50 nm to easily enter and translocate into their bodies (Sabo-Attwood et al. 2011). For penetration, NPs adopt either apoplast or symplast transportation to travel through tissues. Plant cell NPs travel across the extracellular space of the plasma membrane to reach plant cell vessels in apoplast transportation (Sattelmacher 2001). Apoplast transportation enables NPs to travel radially across the plant's vascular system and into the central cylinder of the roots. NPs are transported by cell sieves and plasmodesmata during symplast movements (Roberts and Oparka 2003). This chapter is a brief review of the potential role of nanoparticles in plant disease management.

Owing to the immense potential use of nanotechnology and nanoparticles, their potential in pest and disease management of plants is also being explored, in which metal-based nanoparticles are very important. This chapter is a review of all the potential applications of nanoparticles in plant disease management.

15.2 Nanoparticles: Types, Synthesis, and Classification

The nanoparticles are a diverse class of chemical compounds made in a special way to get particle size on the nm scale. The nanoparticles can be organic (including dendrimers, micelles, liposomes, and ferritin) or inorganic (metal, metal oxide, mixed, metalloid, or beneficial nutrient NPs) in nature. The most widely employed metals for nanoparticle synthesis are aluminum (Al), zinc (Zn), cobalt (Co), silver (Ag), copper (Cu), gold (Au), and iron (Fe). Metal oxide nanoparticles are produced largely for their improved efficiency and reactivity. Magnetite (Fe_3O_4), cerium oxide (CeO_2), iron oxide (Fe_2O_3), zinc oxide (ZnO), silicon dioxide (SiO_2), aluminum oxide (Al_2O_3), and titanium oxide are some of the most frequent metal/metalloid oxide NPs (Tiwari et al. 2008; Salavati-Niasari et al. 2008).

Nanoparticles can be manufactured in several ways, including bottom-up or top-down methods. Bottom-up or constructive material accumulation refers to the accumulation of material from a single atom to clusters, which are subsequently turned into nanoparticles. Sol-gel, biosynthesis, pyrolysis, chemical vapor

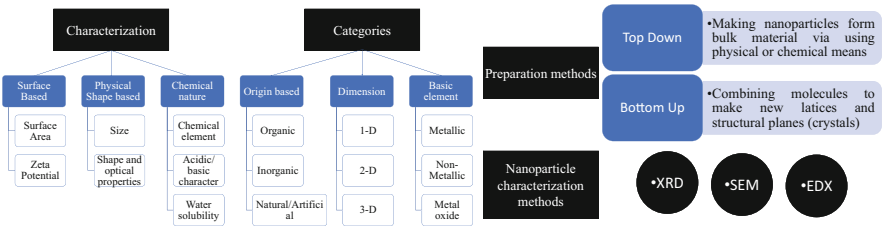

Fig. 15.1 Synthesis, types, and classification of nanoparticles (NPs)

deposition, and spinning are the most frequently utilized bottom-up procedures for nanoparticle production. A top-down or destructive technique is used to reduce a bulk material to nanometric-sized particles. Top-down nanoparticle production methods include mechanical milling, nanolithography, laser ablation, sputtering, and thermal breakdown. In the classification of nanoparticles, shape (2D-3D) and particle size (spherical, rods, crystals, etc.) are important, while the chemical nature of nanoparticles is also used to classify them (organic, inorganic, metal/metalloid/metal oxide-based, etc), as explained in the literature reviews (Sohail et al. 2019, 2021). Figure 15.1 is a pictorial summary of the NPs' preparation methods and classifications.

15.3 Cereal Disease and NPs Interaction

The NPs can be utilized to fight arthropod pests, as well as to develop new insect repellants, insecticides, and insecticide formulas (Barik et al. 2008). The nanoencapsulation technology is used to deliver chemicals such as pesticides to a specific plant as a host, with the goal of controlling insect pests. The nanoencapsulated insecticides benefit plants by absorbing poisons (Scrinis and Lyons 2012). Nanoencapsulation is now seen as the most promising method of shielding host plants against insects and pests. Plants have been observed to absorb a nano-silica-silver silicon composite that helps them cope with stress and sickness (Brecht et al. 2003). Pathogenic bacteria that cause powdery mildew or downy mildew in plants are believed to be effectively suppressed by an aqueous silicate solution. It also increases plant growth and physiological development, as well as stress and disease tolerance (Kanto et al. 2004). Plant nanotechnology also has an important application in gene transfer technology, assisting in the provision of plant protection via chemicals as well as DNA delivery to receptor cells of plants (Wang et al. 2016). In this regard, nanoencapsulation is an important tool for the potentially slow and timely release of encapsulated chemicals for a prolonged time period. This can have a higher efficiency compared to traditional pesticides prone to runoff and leading to the human food chain (Agrawal and Rathore 2014; Khot et al. 2012).

15.3.1 Nano-pesticide

A nano-pesticide is a pesticide formulation or product that contains engineered nanoparticles with biocidal properties as active ingredients (A.I), either as a whole or as part of the designed structure (Kah and Hofmann 2014). In the presence of specific NMs, slow degradation and regulated release of active components may provide long-term pest control (Chhipa 2016). Nano-pesticides are required for the effective and long-term control of a wide range of pests, and they can assist in minimizing the use of synthetic chemicals and the environmental dangers that come with them. Due to their tiny size, nano-pesticides function differently than regular pesticides, and plants may absorb them more quickly (Kah et al. 2019). Kumpiene et al. (2008) suggest that nanoparticles may be transported in two ways: dissolved and colloidal. This explains why they act differently from other forms of solutes.

Rice (*Oryza sativa* L.) is a widespread staple food that is grown on vast swaths of fertile land all over the world (Zhu et al. 2017). Approximately 90% of the world's rice is grown in Asia, while China is one of the world's largest rice producers (Zahra et al. 2018; Li et al. 2015a, b). Plant diseases are the most important biotic restrictions on crop output in agriculture, and they have the potential to cause worldwide food devastation (Khoa et al. 2017). The most frequent bacterial pathogen in rice is Xanthomonas *oryzae pv. oryzae*, which causes bacterial leaf blight (Ryan et al. 2011; Udayashankar et al. 2011). Biogenic silver nanoparticles (AgNPs) have received a great deal of interest due to their exceptional biological, physicochemical, and antibacterial properties in decreasing plant illness (Adil et al. 2015). Wheat, after rice, is regarded as a basic grain due to its great nutritional content and numerous applications (Peng et al. 2011). In spite of other biotic stress-causing agents, various fungi have severely damaged the wheat crop, resulting in a 12.4% yearly yield loss worldwide (Galvano et al. 2001). A nano-pesticide is a pesticide formulation or product that contains engineered nanoparticles with biocidal properties as active ingredients, either as a whole or as part of the designed structure (Kah and Hofmann 2014). In the presence of specific NMs, slow degradation and regulated release of active components may provide long-term insect control (Chhipa 2016). Nano-pesticides are needed for the effective and long-term control of a wide range of pests, and they can assist in reducing the use of synthetic chemicals and the environmental dangers that come with them. Due to their tiny size, nano-pesticides function differently than regular pesticides, and plants may absorb them more quickly (Kah et al. 2019). Because nanoparticles (NPs) may be delivered in two states: dissolved and colloidal, they act differently than conventional solutes (Kumpiene et al. 2008).

Planthoppers are a major threat to world rice production. In China alone, they damage over 20 million hectares of rice-growing land each year (Hu et al. 2019). Engineered nanomaterials (ENM) have the potential to be employed as nano-insecticides in agriculture (Adisa et al. 2019; Sun et al. 2019). The ENMs have also been demonstrated to penetrate rice cells, interact with DNA, and boost relative Os06g32600 expression, resulting in enhanced disease tolerance (Li et al. 2018). Insects have developed resistance to pesticides because of their widespread usage,

raising concerns about the environment (Zhang et al. 2017a, b; Wang et al. 2018). While omethoate, imidacloprid, and acetamiprid have shown to be effective against wheat aphids, their poor persistence makes them unsuitable for use during epidemics. A 40% dilution of Omethoate EC demonstrated that it had no effect on the wheat aphids in a field experiment (Yu et al. 2019). Incorporating nanotechnology into pesticide formulations is a new strategy for prospective organic crop growth that reduces the indiscriminate use of synthetic pesticides, while also offering environmentally friendly applications (Kumar et al. 2019). The United States Food and Drug Administration has given chitin and its derivatives a safe (GRAS) designation as a food additive since they are non-toxic and have been reported to be safe for humans, cattle, and animals. Because of their biocompatibility, biodegradability, and lack of cytotoxicity, nano-chitin components have been widely employed in biomedical manufacturing (Yang et al. 2020). Nano-chitin whiskers are non-toxic at quantities less than 50 g mL^{-1} and exhibit a greater cytocompatibility at 200 g mL^{-1} (Zhao et al. 2019). Chitosan was shown to be the most efficient in pest management, with molecular weights ranging from 2.27105 to 5.97105 g mol^{-1} (Badawy and El-Aswad 2012). As a result, nano-chitin has a demonstrated pro-insecticidal effect on chemical pesticides while causing no harm to non-target populations.

In an investigation by Choudhary et al. (2019), the Zn-encapsulated chitosan nanoparticles were reported to have antifungal activity on maize crops. The potential foliar as well as seed treatment of Zn nanoparticles was also proved to be linked with the control of Curvularia Leaf Spot (CLS) disease in maize. The findings of Wagner et al. (2016) conclude that Zn nanoparticles can act as a non-persistent and economical antimicrobial agent against oomycete *P. tabacina*. Similarly, their toxicity against *Xanthomonas oryzae pv. Oryzae* is also reported by Ogunyemi et al. (2019) in addition to their well-established antifungal properties (Navale et al. 2015; Savi et al. 2015; Wagner et al. 2016). Another important element, silver (Ag) nanoparticles, also has been tested and their antimicrobial activity has been reported as they can interfere with the microbial enzymatic system (Kim et al. 2017).

It is reported that nanoparticles are helpful in controlling pathogens causing diseases like belly rot (*Rhizoctonia solani*), Common Root Rot (*Bipolaris sorokiniana*), rice blast fungus (*Magnaporthe grisea*), grey mould (*Botrytis cinerea*), seedling blight, foot rot, ear blight (*Fusarium culmorum*), cottony soft rot (*Scalrotinia sclerotiorum*), colletotrichum fungal plant pathogens (*Colletotrichum gloeosporioides*), and black-leg of seedlings (*Pythium ultimum*) (Park et al. 2006; Gopal et al. 2011; Rai et al. 2014; Yah and Simate 2015). The Ag nanoparticles have been reported to eliminate the effects of the sun-hemp rosette virus (Jain and Kothari 2014). That is the reason Ag NPs are being used in some commercial fungicides like Kocide® to control *Alternaria solani* (causative agent of early blight disease), as reported by studies (Nejad et al. 2016). The use of Ag NPs against insects is also reported as Ag NPs prepared from green methods exhibited larvicidal and toxicity against the house fly (Abdel-Gawad 2018) and the mosquito (C*ulex pipiens pallens*), respectively (Fouad et al. 2016). The study conducted by Ismail et al. (2016) reported that Se and Cu NPs can be an effective way of controlling the attack of Alternaria solani on tomato plants. The third important

nanoparticle involved in the management of pests in plants is Cu, with its extraordinary antimicrobial properties reported for the control of disease spread by Xanthomonas sp. (Chhipa and Joshi 2016) and are widely being used because of their broad-spectrum antimicrobial properties (Esteban-Tejeda et al. 2009). The Cu nanoparticles have been reported to be effective against diseases like fusarium wilt and early blight, which cause diseases in tomatoes (Saharan et al. 2015). Furthermore, the insecticidal aspects of Cu nanoparticles are also present, as reported by Le Van et al. (2016), with Cu NPs in low concentration increasing the expression of Bt toxin protein, thus improving the pest resistance of transgenic cotton. The fourth important nanoparticle being used as nano-pesticide is silica (Si-NPs) and has been reported by various studies as presented in Table 15.1. The Si-NPs are reported to have lethal properties against *Callosobruchus maculatus* (Rouhani et al. 2012) and are being used in commercial pesticides to control the early blight of tomatoes (Derbalah et al. 2018) and spot diseases in dragon fruit (Tuan et al. 2018; Verma 2018). The role of Si-NPs in the control of various pests is also well reported for the control of lesser grain borer (*R. dominica*), confused flour beetle (*T. confusum*) (Ziaee and Ganji 2016), African cotton leafworm (*Spodoptera littoralis*) larvae (El-Helaly et al. 2016), and cowpea weevil (*Callosobruchus maculatus*) (Rouhani et al. 2012). Moreover, the application of Si-NPs has also been reported to control pests strains and diseases like *P. fluorescens* causing pink eye potato, the bacterial blast caused by *P. syringae* and *P. carotovorum* (Cadena et al. 2018), *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa* (Mohammadi et al. 2016), *Listeria innocua* (Ruiz-Rico et al. 2017), *Escherichia coli* (Mohammadi et al. 2016; Shevchenko et al. 2017), *Staphylococcus aureus, Aspergillus fumigatus* (Song et al. 2018), *B. subtilis, S aureus*, and *P. aeruginosa* (Tahmasbi et al. 2018) is also well known.

15.3.2 Nano-fertilizers

Fortifying wheat with essential micronutrients like zinc and iron is one approach for combating "secret hunger" in a major section of the world's population and is also an integral part of integrated pest management, as a healthy plant can fight diseases very well. The availability of essential nutrients has imparted significant impacts on crop nutrition, health, and output (Chhipa 2016). Nanoparticles improve crop yield and ensure food safety either upon direct application to the soil or as foliar sprays to the plants (Dimkpa and Bindraban 2017). Large amounts of micronutrients used during fertilization can result in nutrient waste and environmental contamination. Therefore, the application of nano-fertilizers to the crops is considered a more efficient method due to the high penetration in the plant. "Nano fertilizers are synthesized or modified forms of conventional fertilizers, which can enhance nutrient use efficiency (NUE) via various mechanisms such as controlled release and target delivery. Moreover, they can release their active ingredients in response to environmental triggers as well as biological demands" (Solanki et al. 2015). The physical and chemical properties of nanoscale materials vary from those of bulk materials (Nel

Table 15.1 Effects of various nanoparticles in plants

Type	Source	Dose	Organism of action	Effect	References
ZnNPs	ZnNPs formed via green synthesis using **Sargassum vulgare**	Variable dose	Aspergillus, Candida *and* Saccharomyces cerevisiae	Potential antifungal activity was observed in the prepared NPs	Karkhane et al. (2020)
ZnNPs	Zn and ZnO	8 and 10 mg L^{-1}	*Peronospora tabacina* (Tabaco infecting pathogen)	Both doses as well as sources were found to be toxic for pathogen germination and growth, suggesting its potential role as nano-pesticide	Wagner et al. (2016)
ZnNPs	ZnO	0–100 mgL^{-1}	*Pathogenic bacteria and fungi*	Strong antimicrobial activity of NPs was observed owing to their capability in ROS production	Navale et al. (2015)
Zn compounds	Zn, ZnO, ZnSO4 and nano ZnO	Various doses	Fusarium head blight on wheat (*Triticum aestivum* L.)	Zn compounds in addition to existing formulations, can help in overcoming the deoxynivalenol formation in wheat plant	Savi et al. (2015)
Chitosan NPs coated with Zn	Zn-chitosan NPs	0.01–0.16%	Maize (*Zea mays*)	Zn-chitosan NPs proved to be helpful in promoting maize growth, disease control and help in nutrient fortification	Choudhary et al. (2019)
Se and Cu NPs	Se and Cu NPs	Foliar application of various doses	Tomato (*Solanum lycopersicum*) under fungal pathogen Alternaria solani attack	The exogenous application of Se and Cu-NPs helped enhance plant growth and control pathogen effect on the plant by improving contents of various inorganic and organic compounds	Ismail et al. (2016)
Carbon	Carbon nanoparticles	Variable doses	*Rice* (*Oryza sativa*)	The carbon nanoparticles helped rice plant in increasing plant growth as well as disease resistance	Li et al. (2018)
Silica and silver	SiO and Ag NPs	1–2.5 g kg^{-1}	Cowpea seed beetle *Callosobruchus maculatus F*	Both NPs have shown a potential effect on larvae mortality, suggesting their pesticide potential	Rouhani et al. (2012)

Silica NPs	Silica gel and silica gel NPs	Variable doses	Moth (*Tuta absoluta*)	Potential toxicity of NPs was observed for tested insects, larvae, and adults	Magda and Hussein (2016)
Silica NPs	Silica	–	Early blight of tomato (Alternaria solani)	The NPs have proven to be better antifungal agents compared to metalaxyl (commercially available fungicide)	Derbalah et al. (2018)
Silica NPs	Silica NPs	1% by wt in PDA media	*Trichoderma harzianum* and *rhizoctonia solani*	Antifungal properties were observed	Verma (2018)
$n\mathrm{SiO_2}$–OC	Oligochitosan (OC) and nanosilica	–	Dragon fruit Brown spot disease caused by *Neoscytalidium dimidiatum* fungus	The NPs treatment enhanced chitinase production and helped in the reduction of disease severity	Tuan et al. (2018)
AgNPs	$\mathrm{AgNO_3}$	500, 1000, 2000 & 4000 mg/L	*Spodoptera litura*	The growth index of lepidopteran species were decreased, damage to the nucleolus by the deposition of AgNPs in midgut cells	Yasur and Rani (2015)
AgNPs	Green synthesized Ag NPs	Variable doses	Cluster bean leaves inoculated with sunhemp rosette virus	The green synthesized ag NPs have shown a successful suppression of viral disease onset showing potential antiviral properties	Jain and Kothari (2014)
AgNPs	$\mathrm{AgNO_3}$	30, 60, 90, 120 & 150 ppm	*Spodoptera litura* & *Helicoverpa armigera*	Damage the epithelial tissues and goblet cells of larval midgut of Spodoptera litua & Helicoverpa armigera	Manimegalai et al. (2020)
Ag and Zn NPs	Ag and Zn NPs prepared	Various doses	House Fly (*Musca domestica*)	The applied doses of NPs have shown positive effects on controlling early staged individuals of houseflies suggesting strong possible use as an alternative pesticide	Abdel-Gawad (2018)

(continued)

Table 15.1 (continued)

Type	Source	Dose	Organism of action	Effect	References
AgNPs	$AgNO_3$	100, 500, 1000 & 1500 mg/L	*Spodoptera litura*	Acute toxic effect on Spodoptera litura larvae, non-significant effect on the activity of detoxification enzymes (glutathione-s-transferase & carboxyl esterases enzymes)	Jafir et al. (2021)
Glass containing Cu NPs	Sepiolite fibres containing monodispersed Cu NPs	Variable doses	*Fungal species*	Ca(2+) lixiviated mediated toxicity to fungal species, suggesting the strong antifungal potential of hybrid nanoparticles	Esteban-Tejeda et al. (2009)
Carbon and Cu NPs	Chitosan, chitosan-saponin and Cu-chitosan nanoparticles	0.001–0.1% doses in invitro study	Phytopathogenic fungi (*Alternaria alternata*, *Macrophomina phaseolina* and *Rhizoctonia solani*)	Model has shown NPs capability in controlling fungal sprawl suggesting its long term and field application as a possible option	Saharan et al. (2013)
Cu-carbon	Cu-chitosan based NPs	Variable doses	*Alternaria solani* and *Fusarium oxysporum* affecting tomato plant	The model demonstrated a potential antifungal effect on both species suggesting the potential applicability of NPs in field conditions	Saharan et al. (2015)
ZnO, Cu, and Cu_2O/Cu	Zn and Cu nanoparticles	Variable doses	*F. oxysporum, F. solani, C. gloeosporioides*	A net inhibition of growth of all fungal species was observed	Pariona et al. (2021)
CuO-NPs	CuO	Variable dose	*Bt Cotton*	The exogenous application of CuO-NPs helped in gene triggering of crops involved in better disease prevention	Le Van et al. (2016)
CuO-NPs	$CuSO_4 \cdot 5H_2O$	100, 150, 200, 250 & 300 ppm	*Triticum aestivum*	Acute toxic effect against *Sitophilus granarius* and *Rhyzopertha dominic*, improves the plant physiology and yields related parameters	Rai et al. (2018)

CuO-NPs	Sigma–Aldrich	100 mg/L	*Galleria mellonella* L	Increases the activity of CAT & GST while decreasing the activity of SOD & AChE in the midgut of *Galleria mellonella* L	Tuncsoy et al. (2019)
CuNPs	NanoSany Corporation (Iran)	50, 100 & 200 ppm	Pepper	Transcriptionally down-regulates the MVK gene involved in the metabolism of terpenoids and upregulated the microR159 in pepper, increased concentration poses the oxidative stress, upregulates the activity of catalase, peroxidase, & polyphenol peroxidase	Tabatabaee et al. (2021)

et al. 2006). Nano-fertilizers penetrate the seeds and increase the nutritional status of seedlings, resulting in healthier and longer shoot and root lengths. Nano-fertilizers are classified as either micronutrient or macronutrient nano-fertilizers, depending on their nutritional status (Chhipa 2016). Plant metabolism is stimulated by nanoscale nutrient forms, which improve development, nutritional quality, and growth (Dimkpa and Bindraban 2017). Nano-fertilizers increase nutrient use efficiency, minimize important nutrient immobilization, and reduce nutrient leaching through agricultural run-off (Liu and Lal 2015). As compared to conventional fertilizers, nano-fertilizers enhance chlorophyll synthesis as well as the rate of photosynthesis, and thereby increase the transfer of the photosynthates to different plant parts and increase crop production (Ali and Al-Juthery 2017; Singh et al. 2017).

In waterlogged conditions, zinc (Zn) is an essential nutrient for rice growth and development (Naik and Das 2007). The foliar application of Zn to plants increases its concentration (Saha et al. 2017). The use of Zn nano-fertilizer benefits rice development by providing nutrients slowly during crucial periods (Yuva Raj and Subramanian 2021). The use of Si and Zn nano-fertilizers boosted the concentrations of essential plant nutrients silicon and zinc in rice plants by around 24% and 21%, respectively (Ghasemi et al. 2014). Nano-silicon fertilizers have high availability because of their small size and strong penetration power, whereas standard silicon fertilizers have low availability. Nano-silicon fertilizers, when compared to traditional Si fertilizers, can minimize silicon (Si) accumulation (Wang et al. 2016). It's a reported fact that micronutrients and beneficial nutrients can be very effective agents for plants to fight against diseases, and exogenous application of these nutrients can help plants in various diverse ways in coping biotic stress (Datnoff et al. 2007; Fones and Preston 2013).

15.4 Bioavailability, Concentration, and Toxicity of the Nanoparticles

Because many NPs contain biotic life, the incorporation of NPs into plant reproductive and eating tissues is of special importance (Rizwan et al. 2016). The absorption and transportation of the NPs depend upon the plant species, cultivars, and developmental stages (Anjum et al. 2015; Shi et al. 2014). Plant tissues' natural micro-meter or nanometer-scale pores allow NPs to attach to and pass through plant surfaces (Schwabe et al. 2015). The uptake of the NP is characterized as an "active-transport mechanism" because it involves a variety of cellular processes such as recycling, signaling, and plasma membrane regulation (Wang et al. 2012). Before adopting the apoplastic way to the epidermis and cortical cells, the NPs adhere to the root surface (Anjum et al. 2015, 2016). When NPs enter plants, they penetrate through the cell membrane and cell wall of root epidermal cells before being guided via the xylem (vascular bundle) by a series of complicated processes before being transported to the stele via the symplast route and are eventually translocated to the leaves. Cell membrane holes tailored to the size of the nanomaterial allow NPs to penetrate through the integral cell membrane (Tripathi et al. 2015). The NPs must be absorbed

by a passive channel via the endodermal apoplast before they can get near to the stele (Judy et al. 2016). Xylem is a plant-based mechanism for allocating and transporting nanoparticles (Aslani et al. 2014).

15.5 Fate and Safety Aspects of Nanoparticles

The fast growth of nanotechnology has prompted concerns about the risks of a wide range of hazardous NPs, and their uncontrolled usage as nano-pesticide and nano-fungicide formulations should be monitored since they can contaminate the soil. In order to establish safe nanomaterial-based technology release mechanisms, the formation of these NPs in soil, and their absorption into the food chain, should be monitored. The NPs have been shown to exert dose-dependent toxicity in agricultural plants in several studies (Li et al. 2015a, b). Pure aluminum NPs, for example, inhibited root development in maize, tomato, cucumber, carrot, cabbage, and soybean plants (Hassan et al. 2013). Plant development is inhibited by alumina (Al_2O_3) NPs, which are contaminants in the environment. Tobacco seedlings demonstrated a continuous and significant reduction in average leaf count, biomass, and root length when exposed to high levels of Al_2O_3-NP (Burklew et al. 2012). Copper NPs were cytotoxic to mung beans, but Ag-NPs were cytotoxic to zucchini and onions. At higher concentrations, multi-walled carbon nanotubes (MWCNTs) have been proven to be cytotoxic in a variety of plants, including Arabidopsis and rice (Nair et al. 2010). These findings emphasize the need to understand the ecosystem's lowest safe NP threshold. Nano-ZnO, for example, is taken from the soil by the roots and accumulated in the edible parts of soybean plants, decreasing food quality. Similarly, nano-CeO_2 lowered soybean plants' capacity to fix nitrogen and hence reduced the yield.

15.6 Conclusion

Nanotechnology is a branch of science that has applications in a wide range of fields. Nanotechnology is undergoing intensive research in an attempt to commercialize it around the world. In agriculture, nanoparticles are used to reduce the use of plant protection chemicals, reduce nutrient losses, and boost yields. Because food demand is increasing every day and staple food crop yields are low, metal nanoparticles must be commercialized for sustainable agriculture. NPs promote plant metabolic activity and act as a plant nutritional fertilizer to boost crop yield. Every day, food demand rises while primary food crop yields diminish. Today, however, increasing the food supply is important in order to feed the world's growing population. Commercialization of metal nanoparticles for sustainable agriculture is consequently required.

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Metallic Nanoparticles and Nano-Based Bioactive Formulations as Nano-Fungicides for Sustainable Disease Management in Cereals

16

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Abstract

The main challenge in disease management is to develop and enhance long-term management strategies that diminish the pathogen's ability to pose a threat in the future. The use of fungicides and the planting of resistant varieties are two of the most common ways to combat blast disease. Natural products, botanical extracts, and nanoparticles have been increasingly used as safer antibacterial treatments against plant infections in recent years. Plant tonics and extracts are environmentally safe goods and there is no risk of resistance to their use, as there is with traditional pesticides. The goal of the present chapter was to focus on the effect of the application of these products on the causal agents of cereal diseases. In vitro and in vivo tests were used to assess the impact of plant products or manufactured nanoparticles on crop disease. Application of plant natural compounds

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suppressed mycelial growth and conidial growing conditions of fungus considerably in vitro, with maximum suppression. Plant tonic application and nanocarbons were likewise the most effective treatments in in vivo settings, resulting in a considerable reduction in the area under the disease progress curve (AUDPC) value when compared to the control. The application of plant tonics and natural products resulted in a higher phenolic compound accumulation and higher activity of peroxidase and polyphenol oxidase enzymes than the control. Plant tonic, natural products, and nano-carbon treated rice plants showed no phytotoxicity when compared to the control. The benefits of plant natural products and nanoparticles in suppressing the rice blast disease were confirmed by the findings presented in this chapter. As a result, their application may aid in the development of appropriate managementmethods and provide the possibility of a cleaner and safer agricultural environment.

Keywords

Antifungal · Characterization · Fungicide · Fusarium · Plant extract · Nanoparticles · Synthesis

16.1 Introduction

Nanotechnology is rising in prominence as a result of its numerous agricultural applications (Chowdappa and Gowda 2013; Ul Haq and Ijaz 2019). Among other disease control measures, nanobiotechnology plays a vital role in early diagnosis, presumed fungicides (nanofungicides), and is effective for fungicide distribution to plants (Mishra and Singh 2015). It is this groundbreaking science that has transformed the green revolution into the green nano-bio revolution. It is centered on two parts: nanomaterial fabrication and implementation (Khan and Rizvi 2014).

Technology enables real-time tracking of agricultural crops for smart farming, resulting in greater production with minimal input (Sharma et al. 2010a, b). Herbicides and fungicides are extensively used, resulting in ecotoxicity and the emergence of novel resistant phytopathogen species (Chen et al. 2015). As a result, there is a pressing need to develop new approaches to managing crop diseases (Vu et al. 2015). The use of environmentally friendly methods that produce less toxic waste is urgently needed on a global scale. Scientists have become more aware of the need to embrace and create "green synthesis" methodologies and techniques as a result of this circumstance. Nanobiotechnology as a green chemistry strategy aims to minimize the manufacturing of hazardous materials using nontoxic and eco-friendly assets. As a result, utilizing biological agents (bio-macromolecules and microorganisms) for nanoparticle production is a unique notion in green chemistry, opening up new paths for studying a wide range of biological species (Chowdappa et al. 2013; Prasad et al. 2018).

Plant extract-based bio-reduction processes for nanomaterial generation involve a variety of biomolecules, including polysaccharides, plant resins, organic

compounds, tannins, pigments, proteins, and enzymes (Nam et al. 2008; Prasad 2014) of green chemistry that connects microbial biotechnology and nanotechnology. Inorganic chemicals are accumulated by microbes either inside or outside the cell, and bio-reduce metals including copper, gold, platinum, silica, and silver to produce nanoparticles (Prasad 2016).

By improving sustainable agriculture, nanoparticles play a critical role in producing better food (Gruère 2012). A wide spectrum of phytopathogens causes damage to crop plants, ornamental plants, and trees, resulting in significant economic damage. Many of them have harmful consequences for human health. In the next half-century, global food demand is predicted to double, posing a significant challenge to food production losses (Tournas 2005).

Because of exhaustion during the application, photodegradation, and off-target deposition, only a trace amount of fungicides and pesticides (0.1%) find the exact site of action; these losses have an impact on the ecosystem and raise production costs. When a fungicide or pesticide is implemented to target pathogens, it may alter their population into new species or strains through genome recombination, resulting in the evolution of new species with resistance to that fungicide or pesticide (Castro et al. 2013; Chowdappa et al. 2013). The best method to deal with this problem right now is to use nanomaterials in illness control, disease monitoring, and precise or controlled dispersion of bioactive agents (Johnston 2010). These nanoparticles are aimed at fixing specific agricultural issues, such as plant protection (disease control) and crop improvement (Ghormade et al. 2011). Nanoparticles' high surface-to-volume proportion makes them more responsive and biochemically active. They attach to pathogen cell walls, causing cell membrane distortion due to high-energy transfer and causing the pathogen to die (Dubchak et al. 2010). These nanoparticles or nanoparticle-based formulations form a robust nanoscale framework that allows agrochemicals to be entrapped and encapsulated for gradual and targeted delivery of their active components while also reducing agrochemical runoff into the environment (Chen and Yada 2011). As a result, this emerging science could play a critical role in global sustainable agriculture. This chapter discusses the importance, production, and properties of nanoparticles (particularly metallic nanoparticles) as well as their use as nanofungicides for long-term disease management in plants (Gruère 2012).

16.2 Cu Nanoparticles (Cu-NPs) Fungicides Against Fusarium

16.2.1 Synthesis and Characterization of Copper Nanoparticles

Copper nanoparticles were created through the use of the cetyltrimethyl ammonium bromide method (Kanhed et al. 2014). The process was optimized for the optimal concentration of copper nitrate and cetyltrimethyl ammonium bromide (CTAB) in terms of nanoparticle stability. The optimization study used 20 mL of copper nitrate at room temperature, with concentration ranges of 0.010–0.100 M for cetyltrimethyl ammonium bromide and 0.0010–0.0100 M for copper nitrate (Bramhanwade et al.

2016). Different concentrations of CTAB solution (0.001–0.01 M) and 20 mL of CTAB solution (0.001–0.01 M) were prepared in isopropyl alcohol. Drop by drop, copper nitrate solution was poured into the CTAB solution while vigorously stirring. Copper nanoparticles have a well-known feature of easily oxidizing in the presence of oxygen. This can be avoided by applying a capping agent to cover the nanoparticles. In this method, the concentrations of copper nitrate and CTAB were adjusted to produce copper nanoparticles. Copper nitrate at 0.003 M was discovered to be the lowest concentration that might support copper nanoparticle production, while CTAB at 0.02 M was determined to be the lowest concentration that might support copper nanoparticle synthesis. Moreover, Kanhed et al. (2014) used a comparable concentration of copper nitrate (0.003 M) but a larger concentration of CTAB to synthesize copper nanoparticles (0.090 M). For the manufacture of copper nanoparticles, different amounts of CTAB were used, including 0.087 M, 0.09 M, and 50% (Zhang and Cui 2009). Upon adding copper nitrate to the CTAB solution with continual swirling and magnetic stirring, the color shift for copper nanoparticles was gloomy violet. Bahadory (2008) attributed the color change to surface plasmonic stimulation in metal nanoparticles. The stability of the CTAB procedure of copper nanoparticle manufacturing was one of its shortcomings (Shah et al. 2014).

To determine the stability of copper nanoparticles, the zeta potential was evaluated. It is based on charge behavior phenomena. Nanoparticles are said to be unstable if their zeta potential value is between −30 and +30. The stability of generated copper nanoparticles was taken into account when measuring the zeta potential of various concentrations of copper nitrate and CTAB.

16.2.2 Antifungal Activity of Cu-NPs Toward Fusarium

The use of nanoparticles in a variety of disciplines has a principal impact on society and the global economy. In a continuous flow mode, copper metals were successfully absorbed from polluted water using an alginate-immobilized water hyacinth, i.e., *Eichhornia crassipes*, which serves as a potential biosorbent in acidic media (Bramhanwade et al. 2016). *Fusarium culmorum*, *Fusarium oxysporum*, and *Fusarium equiseti* belong to the Fusarium species. In barley and wheat, *F. culmorum* causes pre-emergence cotyledon blight, root rot, foot rot, or head blight (Mesterhazy et al. 2005).

Chickpea wilt, Fusarium crown, Fusarium head blight, yellows, black point disease, corm rot, root rot, vascular wilt, or damping-off are all plant diseases caused by *F. oxysporum* in spinach, sugarcane, lettuce, prickly pear, tomato, garden pea, pansy, potato, cultivated zinnia, cowpea, and Assam rattlebox. *F. equiseti* is a soil-dwelling parasite that can infect a range of crop seeds, roots, tubers, and fruits. It causes disease in a broad range of crop plants (Raabe et al. 1981).

Copper nanoparticles were tested in vitro for antifungal activity versus three different crop fungal pathogens: *Fusarium* sp., *Fusarium oxysporum*, or *Fusarium equise*. Copper nanoparticles, interestingly, had a lot of effect against the crop pathogenic fungi that were studied. Amphotericin B was utilized as a conventional

antifungal drug for antifungal action. Copper nanoparticles had the most action versus *F. culmorum*, *F. equiseti or F. oxysporum*. Kanhed et al. (2014) discovered in vitro antifungal activity of chemically generated copper nanoparticles combined with the marketed antifungal drug Bavistin against four different plant pathogenic fungi, including *F. oxysporum*, *C. lunata*, *A. alternata* and *P. destructiva*. Copper nanoparticles have been known to be successful against a wide variety of plant species.

16.3 Iron Nanoparticle Biofabrication and Fungicidal Properties

Iron nanoparticles (FeNPs) are used in magnetic storage devices, ferrofluids, magnetic refrigeration systems (Ahmad et al. 2017), medication administration, hyperthermia, bio-separation, and magnetic resonance imaging (e.g., enzyme-linked immunosorbent assay) (Sophie et al. 2008). FeNPs are used in a variety of applications due to their high magnetism, tiny size, microwave absorption capabilities, and low toxicity (Chang et al. 2011). FeNP has been created using a number of chemical processes. Electrospray synthesis, microemulsions, sonochemical reactions, chemical co-precipitation of iron salts, hydrothermal reactions, sol–gel synthesis, and hydrolysis and thermolysis of precursors are some of the popular methods used for FeNP synthesizing (Albornoz and Jacobo 2006). Numerous scientists have created techniques for green synthesis of Fe_3O_4 nanoparticles in response to the requirement to produce beneficial formulations of bioactive chemicals utilizing nanomaterials that are both environmentally and economically beneficial (Venkateswarlu et al. 2013). Using phytochemicals to generate iron oxide nanoparticles is also a simple, cost-effective, less poisonous, and environmentally friendly method that has previously been used to make other heavy metal nanoparticles. One of the major components in the antibacterial activity mechanism can be active oxygen types created by these metal oxide materials. In this way, nanoparticles of related metal oxides could be good antibacterial agents (Ales et al. 2009).

16.3.1 Plant Extracts Are Used to Produce Iron Oxide Nanoparticles

FeNPs were made utilizing processes that have previously been revealed (Xiulan et al. 2013; Valentin et al. 2014). $6H_2O$ was handled by 10% plant extract in a 1: 2 ratio with around 0.1 M $FeCl_2$. The combination was thoroughly agitated at 100 °C till the greenish hue entirely changed to a deep black solution. The solution was seated for 72 h (on Petri plates) inside a 60 °C oven. Eventually, the blackish dry matter was subjected to several characterization procedures.

16.3.1.1 FeNPs Characterization

Surface plasmon resonance (SPR) uptake transition is the most distinguishing feature of nanoparticles. The yellow-colored reaction mixture turned dark brown

after overnight incubation in the dark. It could be due to the produced nanoparticles' SPR excitation (Gopinatha et al. 2012). The reaction medium of iron chloride and neem extract showed a strong peak at 272 nm, confirming the synthesis of FeNPs. Due to N–H stretching and bending vibration of amine group NH_2 and O–H overlying and stretching mode of soluble neem leaf extract particles, the Fourier transform IR spectroscopy (FTIR) summary of neem extract and FeNPs exhibited continuous spectrum of about 3000 cm^{-1} focused at 3325 cm^{-1}. Methyl C–H stretch is indicated by peaks at 2916 and 2850 cm^{-1}, whereas –CHO group of neem extract is indicated by peaks at 1728 cm^{-1}. The existence of ester linkages is noted by a sharp peak at 1725 cm^{-1}, whereas the peaks at 1522 and 1453 cm^{-1} in neem extracts can be attributed to bending vibrations of aromatic nitro compounds and carbonate ions, respectively. As reported earlier (Gotic et al. 2009), the band around 600 cm^{-1} revealed Fe O extending of FeNPs, indicating the synthesis of nanomaterials. According to the general FTIR image, neem extract has the highest FeNP lowering potential, which has been validated by other investigations.

The FeNPs are grouped and embedded in plant components due to the presence of plant detritus. Nanoparticles were discovered to have an average size of 20–80 nm. SAED (selected area electron diffraction) reveals that FeNPs have a less crystalline structure. Along with FeNPs, bio-coatings were apparent, confirming neem extract's capacity to intervene as a protective coating for FeNPs. In FeNPs, the XRD report reveals a thick downward slope with no sharp peaks. In FeNPs, there were no diffraction pattern peaks associated with the prolonged crystalline form. Rather, a wideband appears, which is characteristic of nebulous and ultra-small crystal structures, with poorly defined diffraction patterns. Previous research on plant extract-based FeNP synthesis has found the same things (Mahnaz et al. 2013; Monalisa and Nayak 2013).

16.4 Green Synthesis of Zinc Oxide Nanoparticles

Research on nanoparticles is being conducted for its potential, especially in biomedical research, converting agriculture and food wastes to fuel and other useful residues via enzyme nano-bioprocessing, and managing phytopathogens in agriculture using various types of nanocides (Joshi et al. 2019; Ahmad et al. 2020; Nandini et al. 2020; Sangeetha et al. 2017), as well as, drug delivery and bioimaging probes, have proved to have a wide variety of functions in molecular diagnostics, detection, and microelectronics (Sangeetha et al. 2017). ZnONPs have a unique feature towards bacterial cellulase, which was discovered by studying the creation of hydrogen peroxide on the exterior of ZnONPs (Sharma et al. 2010a, b). The antibacterial properties of nanomaterials have been shown to be more effective than zinc oxide (Kumar et al. 2014). This is because smaller particles have a higher surface-to-volume ratio, with strong antibacterial properties (Kumar et al. 2014; Sharathchandra et al. 2016). ZnONPs are also excellent photocatalysts, which are used to sanitize wastewater and degrade or decrease herbicides and pesticides. Hydrothermal synthesis (Thilagavathi and Geetha 2014), electrochemical approach, mechano-chemical

method, laser ablation, sono-chemical, and polyol methods are some of the commercial routes for ZnONPs manufacture (Muneer et al. 2015), sol-gel method, precipitation method, microwave technique, and vapor-phase transport method (Wang et al. 2014), and by aerosol process (Ozcelik and Ergun 2014). These approaches can be used to make nanoparticles using either chemical or plant-derived materials. The chemical production of metal nanoparticles necessitates the use of synchronized conditions and specific external catalysts. In the case of plant-derived nanoparticles, plants secrete catalysts in the form of co-enzymes that are non-toxic and environmentally friendly reactants, and the reaction takes place at room temperature.

16.4.1 Biomaterial Preparation

As a bio-reducing agent, *Eucalyptus globulus* leaves have been chosen for preparation. Plant materials have been shade-dried, cleaned in distilled water, sterilized for 30 s with mercuric chloride (0.1%), and washed five times with sterile water, and then shade-dried again. Using a laboratory blender, the leaves were grinded and utilized for additional research. Fifteen gram of leaf powder has been mixed with 200 mL of deionized water in a flask and incubated for 6 h in a shaker at 80 °C and 1500 rpm. The extract was centrifuged at 10,000 rpm for 10 min before being filtered through Whatman No. 1 paper to achieve a completed volume of 100 mL (Ahmad et al. 2020).

16.4.2 Phytosynthesis of Zinc Nanoparticles

In a plant extract solution, 1 mM of zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) was postponed in a 1:2 ratio with continuous stirring and was agitated at 150 °C for 2–3 h after it was completely dissolved, and the supernatant was discarded. The solid was centrifuged two times at 6000 rpm for 10 min each time before being cleaned and dehydrated at 80 °C for 5–6 h. Dry particles have been kept at room temperature till they change color before being used in future studies (Ahmad et al. 2020).

16.4.3 Formation of Zinc Nanoparticles

E. globulus leaf extracts were used to make zinc oxide. The color change from colorless to pale yellow confirmed the production of ZnONPs. Other metals have been reported to have color changes that indicate preliminary confirmation of the creation of nanoparticles (Joshi et al. 2019). The presence of ZnONPs is confirmed by the color change in the reaction mixture caused by surface plasmon resonance (Shekhawat et al. 2014). Without any additives or reactions, plant-derived nanomaterials respond quickly at room temperature (Ahmad et al. 2020). When compared to other techniques like physical, chemical, biological, or hybrid

approaches, which require additional power and may introduce dangerous materials that lose their consistency, this method is simple and best suited for measuring biological activity.

16.4.4 Characterization of ZnNPs

SEM pictures of ZnONPs produced utilizing *E. globulus* extract demonstrate that agglomerations of molecules were more common when this technique of production was used. The presence of biological material in the sample is confirmed by the clustered form of nanoparticles. The shape and size of created ZnONPs were discovered utilizing TEM. The resulting ZnONPs were often circular, with some extended particle sizes ranging from 52 to 70 nm. *E. globulus* extracts have previously been shown to behave as an active template during synthesis, avoiding the agglomeration of nanoparticles generated (Gnanasangeetha and Sarala 2013).

16.4.5 Antifungal Activity of ZnONPs

ZnONPs are inorganic nanoparticles that have multiple functions, including antibacterial capabilities. The rate of antifungal activity of ZnONPs produced with *E. globulus* extract was higher than that of Zn bulk material. The activity of ZnONPs had been dose-dependent; at 25 ppm, there was reasonable to fine suppression, followed by a considerable rise in pathogen inhibition at higher concentrations of 50 and 100 ppm (Sharma et al. 2010a, b). In comparison to synthetic ZnONPs, green ZnONPs demonstrated a significant improvement in biological activity against a variety of diseases. Eman et al. (2013) discovered t hat ZnONPs have antifungal action toward *Microsporum canis*, *Candida albicans*, *Aspergillus fumigatus*, and *Trichophyton mentagrophyte* (Eman et al. 2013). The synergetic effect of ZnONPs and *Eucalyptus globulus* extracts in equal proportion on fungal mycelial growth was assessed. In the instance of *B. dothidea* and *A. mali*, the synergetic activity resulted in a total suppression (100%) of the mycelium at 100 ppm.

16.4.5.1 Fungi Treated with Zinc Nanoparticles Under Microscope

Microscopic examination revealed a rupture at the hyphae tip, which is a site for the generation of new conidia, as well as unconnected conidia in two fungi. The discharge of cellular components could be triggered by damage to the fungal hyphae's surface caused by hyphal contraction. Water-treated hyphae, on the other hand, are unaffected by hyphal injury (Shetty et al. 2019).

16.4.5.2 Effects of ZnONPs on Fungal Mycelia as Examined by SEM

The influence of nanoparticles on developing hyphae was studied under the microscope, and it was discovered that ZnONPs visibly harmed *D. seriata* hyphae, but hyphae handled with water appeared to be unaffected. Under treatment with nanoparticles, distortions and injuries of *D. seriata* hyphal cell wall, degeneration

of sexual organs, and serious damaged hyphal wall layers resulted in severely fractured hyphal wall layers retaining few and shrunken hyphae. Surprisingly, these changes in mycelium structures had no effect on the fungus's life cycle. Villamizar-Gallardo et al. (2016) made a similar observation claiming that produced AgNPs cause significant structural damage to *Aspergillus flavus*, but have no effect on the fungus's life cycle creation.

16.5 Metallic MgO Nanoparticles

As scientific knowledge has advanced, developing unique alternative techniques for managing soilborne fungal infections has become increasingly desirable (Chen et al. 2020). Magnesium oxide nanomaterials (MgONPs) have been acknowledged by the US Food and Drug Administration as safe disinfection agents with no toxic consequences, and they have significant potential in medical therapies and water disinfection (Chalkidou et al. 2011). Furthermore, earlier research has shown that MgONPs can be employed as microbicide in vitro versus gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative (*E. coli*) bacterial and fungal pathogens. MgONPs' antibacterial activity is influenced by their pH, size, concentration, or shape (Parizi et al. 2014).

These toxic effect processes, unlike agro-chemicals, quite probably result from immediate physiochemical deletion upon contact, which prevents the disintegration of vegetative fungal spores by producing malic acid and amino acids. Numerous findings have argued that the production of ROS and their buildup in cells is an actual mechanism of metal nanomaterials' antibacterial pathogen defense; this is especially true since ROS formation directly limits a cell's ability to reproduce (Chen et al. 2014). Disinfection of microorganisms is assumed to be based on direct contact among biological cells and nanomaterials (Zhao et al. 2018a, b). Superoxide ion production on the exterior of MgONPs, for example, disrupts peptide connections in the bacterial cell membrane. *Ralstonia solanacearum*, a medicinal and foodborne pathogen, has shown antibacterial potential, successfully lowering agricultural bacterial and fungal infections (Sierra-Fernandez et al. 2017). Despite this, little study has been done on the impacts of MgONPs on fungal infections or complex antimycotic processes. MgONPs could be antifungal by acting directly on fungal cells. Most importantly, an ideal agricultural microbicide would be free of phytotoxicity, which is critical for environmentally friendly and sustainable agriculture. Foliar spray of MgONPs as nanoscale fertilizers or optical absorption boosters substantially boosted crop growth, which was very exciting (Cai et al. 2018). It also showed that Chen et al. (2020) looked into how MgONPs antifungal mechanisms worked against phytopathogenic fungi. This was in comparison to macroscale MgO (mMgO) antifungal mechanisms.

16.5.1 Synthesis of MgONPs

Under vigorous stirring, 10 mL of *Carica papaya* L. leaf extract was progressively combined with 50 mL of 0.1 M magnesium nitrate solution. As a result, some white precipitates, primarily composed of $Mg(OH)_2$, were identified. To remove any remaining impurities, the material was centrifuged three times with deionized water at 5000 rpm for 10 min. Finally, the precipitate was dehydrated at 100 °C and calcined at 400 °C to yield MgONPs (Oladipo et al. 2017).

16.5.2 Characterization of MgO Nanoparticles

Several methods were used to characterize the MgONPs, including morphological structure and aggregation state analysis. Nanoparticles were irregularly spherical and had a size distribution of 100 nm. Nanoparticles, on the other hand, tended to clump together in stacks, as shown by TEM pictures of the nanoparticle morphology, showing poor dissolvability because of van der Waals (vdW) force (Stabryla et al. 2018). The SAED pattern of MgONPs confirms the material's nanocrystalline structure as well as its ability to be archived into the cubic structure of MgONPs, which is consistent with XRD analysis (Makhluf et al. 2005). In HRTEM image, the interplanar distance between interlayer outskirts is 0.237 nm. The (111), (200), (220), (311), and (222) crystallographic planes of face-centered cubic (FCC)-structured MgO nanoparticles were attributed to only a few strong peaks situated at 36.95, 42.92, 62.30, 74.76, and 78.61, respectively, according to classic XRD spectra (Fig. 16.1a–e).

16.5.3 Fungitoxic Mechanism of MgO Nanomaterials

Among all testing conditions, MgONPs reduced both fungi's mycelial development, exhibiting significant concentration-dependent toxic impacts that were consistent with many other metallic nanomaterials (Sun et al. 2018). On the third day, the mean mycelial size of colonies grown on plates containing 125–500 g/mL nanoparticles were 2.1, 1.32, and 0.63 cm, and on the fifth day, it was 5.84, 3.17, and 0.63 cm for *P. nicotianae*; these were much lower groups (Fig. 16.2a). Untreated samples, on the other hand, obtained values of up to 6.21 and 8.3 cm at similar intervals. *T. basicola* mycelia grew slowly in comparison to controls, and flagellated colony expansion was significantly reduced after 10 and 20 days of incubation, with 1.89 and 3.18 cm after 250 g/mL MgONPs, and 2.09 and 2.96 cm after 500 g/mL MgONPs (Fig. 16.2b). Despite the fact that 125 g/mL MgONPs also had no effect on colony size, closer examination revealed a loosening of the mycelial structure as compared to the control group's thick and dense colony. Both fungi's hyphae developed slowly after 5 and 20 days of incubation. In comparison, we used the same approach to investigate the biocidal activity of mMgO (Makhluf et al. 2005).

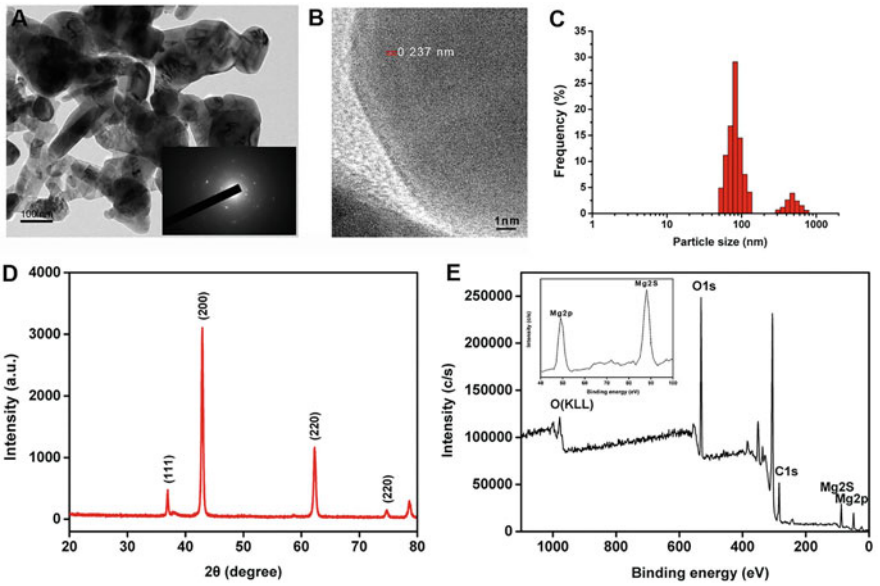

Fig. 16.1 (**a**) Inset of representative transmission electron microscopy (TEM) photos of produced MgO nanomaterials with selected area electron diffraction (SAED) patterns (MgONPs). (**b**) High magnification of MgONPs. (**c**) Size distributions of nanoparticles (D,E) X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) survey spectrum of nMgO. The inset plot indicates the strong XPS scan spectrum of nanoparticles in Mg 2p and Mg 2s spectral areas (Chen et al. 2020)

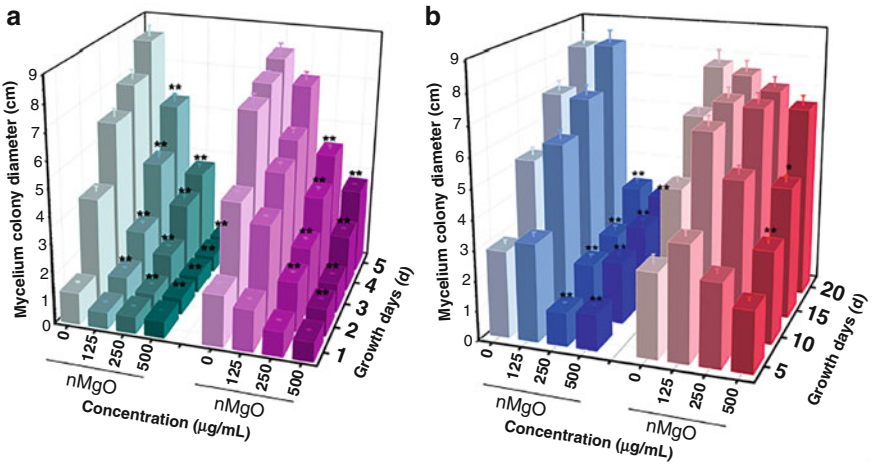

Fig. 16.2 *P. nicotianae* (**a**) *and T. basicola* (**b**) mycelium colony diameter upon 5 and 20 days of application to oatmeal agar (OA) and potato dextrose agar (PDA) media varying concentrations (0, 125, 250, 500 g/mL) of MgO particles or (MgONPs) particles, respectively (Chen et al. 2020)

The results demonstrated that mMgO followed the same concentration-dependent pattern as two types of fungus that were treated with MgONPs. Surprisingly, *P. nicotianae* hyphae growth was substantially hindered, despite *T. basicola* toxicity being quite low. Diameters of two hyphal colonies cultivated for 10 and 20 days in media containing 125 and 250 g/mL mMgO, respectively, compared with those of control were not statistically different, while control exhibited thinner mycelia. Especially for *T. basicola and P. nicotianae* growth inhibition rates were 29.63%, 61.8%, 92.4% and 0%, 60.88%, 63.59%, upon MgONPs treatment for 5 and 20 days, whereas mMgO treatment caused 11.46%, 40.0%, 84.80% and 2.91%, 10.00%, 16.10% inhibition rates. In other words, as the incubation time increased, the growth suppressive impact of nanoparticles became stronger. It's possible that when original normal hyphae came into contact with MgONPs, they were severely injured, and the compromised fungal hyphae kept growing, but at a much slower rate. During the incubation period, it appears that the antifungal activity of nanoparticles diminished progressively. Importantly, the antifungal activity of MgONPs was dose-dependent, similar to other metallic oxide nanoparticles and carbon-based nanomaterials (Chen et al. 2016a). It's worth noting that mMgO fungistatic activity was not as high as that caused by MgONPs. Metal oxide nanoparticles have been shown to be more harmful to bacteria, fungus, and plants than their bulked counterparts (Heinlaan et al. 2002). TiO_2, CuO, and ZnO nanoparticles had also exhibited distinct antifungal action against numerous phytopathogens, including *Gloeophyllum trabeum*, *Lycopersicon esculentum*, *Tinea versicolor*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Pseudoperonospora* (Terzi et al. 2016; Hao et al. 2017). It is the result of increased effective surface area, i.e., compact size that enhances the chances of nanoparticles contacting biological samples, allowing for a broad variety of diverse interactions in nanobiosystems. Further theory holds that when nanoparticles interact with biological cells and membranes, they form a variety of cell-nanoparticle interfaces including protein corona creation, particle encasing, or even intracellular utilization (Nel et al. 2009).

16.5.4 Repression of Conidial Spore Germination and Sporangium Formation

Spores are the smallest propagative components of fungal infections; they significantly contribute to the pathogenic achievement of hosts and have a modest dormant survival potential, such that spore regeneration is required; this is the most important stage in the development of vegetative and reproductive protonema. In the following study, to further test the fungicidal efficiency of nanomaterials, conidial spores of fungal species were assessed for the existence of MgONPs and mMgO (Judelson and Blanco 2005). Microscopy photos of *T. basicola* and *P. nicotianae* spore detentions after incubation with various concentrations demonstrated a significant reduction in spore germination rate, when compared to untreated fungus acting as control samples (approximately complete germination). There was no germination when fungal spores were incubated at their greatest dosage, indicating that they had

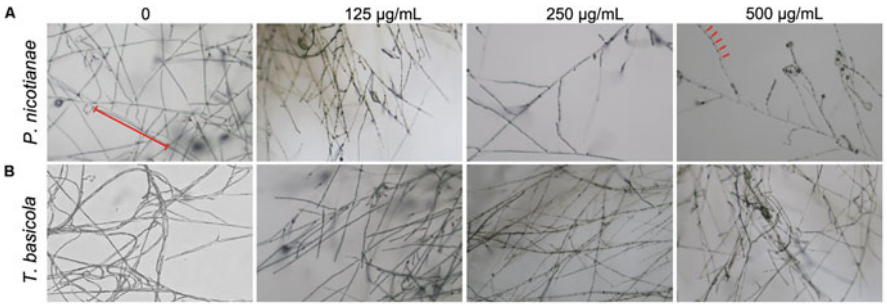

Fig. 16.3 Microscopic photos of *P. nicotianae* (**a**, **b**) and *T. basicola* (**c**, **d**) sporangia after co-culture with tested concentrations of MgONPs and micro-Mgo particles, respectively (Chen et al. 2020)

complete sporicidal effects. The MgONPs have shown a stronger sporicidal effect than MgONPs and that the antagonistic impact on spore development is as strong as the effect on mycelial growth. On the other hand, MgONPs had a significant impact on sporangium production. As shown in Fig. 16.3, sporangia of *T. basicola* and *P. nicotianae* developing in the control group contained a lot of conidia. Nevertheless, the number of sporangia and their morphology pattern were significantly reduced in the MgONPs-exposed group at the concentrations tested, which can be attributed to the hypothesis that profoundly directs nanomaterial-hyphae interaction; this disrupts cellular protein and chemical characteristics that are implicated in sporangium forming (Chen et al. 2016a). The *T. basicola* sporangial wall's outer electron-dense layer had disappeared, and the sporangium's structure was loosening (red arrow). However, after 500 g/mL of mMgO treatment, there was a moderately substantial suppression of sporangia production, indicating that mMgO had a mild fungistatic impact (Fig. 16.3). In vivo and in vitro, metals, metal oxide nanomaterials, single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), and graphene have all been shown in studies to have sporicidal properties against a variety of phytopathogenic fungi (Liu et al. 2017a, b).

Wani and Shah (2012) observed the nanotoxicity of MgONPs on many agricultural pathogenic funguses and considerable suppression of spore development of *Mucor plumbeus*, *Rhizopus stolonifer*, *F. oxysporum*, and *Alternaria alternata*. MgONPs were recently discovered to inhibit sporulation in seven distinct rot-causing fungi (*Aspergillus alternata*, *Aspergillus niger*, *M. plumbeus*, *Trichothecium roseum*, *Penicillium chrysogenum*, *Rhizoctonia solani*, *and Penicillium expansum*) with no reason. Also, a comparative toxicity experiment was performed on metal nanoparticles' antifungal effectiveness toward seven species of major foliar and soilborne plant diseases, including *B. cinerea*, *A. alternata*, *Verticillium dahlia*, *Monilinia fructicola*, and *Fusarium solani*. Copper nanoparticles (CuNPs) have been found to be most impactful on the majority of fungal spores studied, followed by zinc oxide nanoparticles (ZnONPs), which were also more poisonous than advertising fungicide $Cu(OH)_2$ (Malandrakis et al. 2019).

Nevertheless, there was no proof of their antifungal mechanisms. In this respect, we discovered that MgONPs can inhibit sexual reproduction in fungal cells, and future research will look into why MgONPs causes such great sensitivity in fungal cells.

16.5.5 Direct Physical Connection of Nanoparticles with Fungal Cells

Several investigations have shown that manufactured nanomaterials come into direct contact with biological tests such as bacteria, fungi, and cells, as well as exterior adhesion and cell absorption patterns (Rodriguez-Gonzalez et al. 2016). Certain metal oxide nanomaterials bond to the surfaces of harmful bacteria, as predicted (Jiang et al. 2009). The authors used SEM/EDS to examine morphological changes in live cells and visually detect the existence of nanoparticles on hyphae to investigate the impact of MgONPs on fungal hyphae.

In this experiment, two types of vegetative mycelia were generated and cultured for 3 h with varied quantities of nanoparticles before being supported on the grid and observed. *T. basicola* and *P. nicotianae* were treated with MgONPs at 500 g/mL, which resulted in clearly undesirable changes after crumbled morphologies under SEM upon exposure. That preserved a filled, uniform, and well-developed tube-like formation. Sunken and bloated, mycelia developed an aberrant structure. In this experiment, two types of vegetative mycelia were generated and cultured for 3 h with varied quantities of nanoparticles before being supported on the grid and examined. *T. basicola* and *P. nicotianae* were exposed to MgONPs at 500 g/mL, which resulted in a clearly undesired alteration, which preserved a complete, consistent, and well-developed tube-like shape under SEM upon treatment. Mycelia sunk and bloated, and they evolved an abnormal structure. EDS was utilized to evaluate if ether MgONPs was present in or on fungi, or to validate the chemical makeup of associated agglomerates because it could trace the atomic number of every atom in a substance (Rodriguez-Gonzalez et al. 2016).

Furthermore, the presence of MgONPs on the hyphal exterior has been established, resulting in cell membrane local disruption. The presence of MgONPs in the cell membrane was investigated as well. These findings back up the theory that metal-based nanoparticles have particle-specific antifungal mechanisms (Stabryla et al. 2018). Furthermore, TEM photos demonstrated that regulated fungal mycelia had normal dense cytoplasm with regular organelle distribution and typical inner and outer cell wall layers.

In summary, the first steps are thought to be harmful to the exterior cell membrane and downregulation of the cellular membrane; as a result, a sequence of essential reactions occur, including successive nanomaterial uptake and communication to biological components such as lipids, DNA, and protein, leading to apoptosis. Aggregation circumstances, geometry, size, and physical qualities all influence the inactivation effects of nanoparticles (Herd et al. 2013). Numerous studies using nanomaterials which physically coated and permeated bacterial membranes demonstrated that they behaved differently than one's microscale aggregates, such as Al_2O_3 or SiO_2 in comparison to their microscale aggregates (Xue et al. 2014). It

appears that understanding the underlying mechanism requires mechanistic interfacial contact among nanoparticles and biological membranes (Sharma et al. 2015). Cell wall structure and composition of fungus could be to blame for these events. Chitin, 1,3 glucans, and 1,6 glucans, as well as a variety of glycoproteins, make up the hyphal cell wall (Brown et al. 2015). Adhesins, or glycoproteins, play a role in adhesion to inorganic or organic surfaces, as well as host–pathogen interactions. Agglutinin-like sequence (ALS) and glycosylphosphatidylinositol (GPI)-modified cell membrane protein families are two main members (Bamford et al. 2015). Nanoparticles, for instance, can behave like promoters, encouraging direct interaction in the same way as carbon nanotubes (CNTs) drive pathogen agglomeration. Sugar-based ligands have been added to CNTs, which are recognized by receptors on Bacillus spore surfaces (Luo et al. 2009).

16.5.6 Membrane Destabilization in Fungal Cells

In addition, the contribution of glycoproteins to the negative charge of the fungal cell wall cannot be overlooked. Outstanding nanoparticle–cell aggregates that have been found in previous findings of the antibacterial activity of a series of nanoparticles could be mediated by electrostatic contact (Chen et al. 2016a, b). MgONPs and graphene were discovered to be directly bound to phytopathogens, altering cell membrane potential and energy metabolism (Cai et al. 2018). Enhanced adhesion caused by MgONPs adsorption on fungal cells should, in theory, alter membrane potential. Pan et al. (2013) suggest that the Zeta potential of fungal cells has been altered by electrostatic forces among positive-charged MgONPs and fungi, allowing MgONPs to come into close contact with the cell surface and deposit (Pan et al. 2013). Reduced electric repulsive forces resulted in improved antifungal medication adhesion to microorganisms. As a result, as indicated by the SEM and TEM photos discussed above, nanoparticles may be capable of physically harming the cell envelope (Sharma et al. 2015). Leung et al. (2014) discovered that MgONPs effectively conversed with *Escherichia coli*, causing downregulation of membrane proteins like connection porins and ion channel proteins and disruption of proteins associated with membrane lipid metabolism, resulting in cell lysis. Because the internal membrane was directly touched by MgONPs, the nanoparticle–cell interface was extremely diversified. Nanomaterials, afterwards, stimulated vibrant physiochemical conversations that were motivated by adhesion forces that could emerge from specific or non-specific conversations like electrostatic, hydrophobic forces, twisting, vdW, and deforming membranes and rising cytoplasmic membrane permeability to nanomaterials (Wu et al. 2015).

16.6 Fungal Cells' Oxidative Stress Responding

More research is needed to investigate if nanoparticles generate subcellular or cell membrane oxidative stressors, which has been previously assumed to be the most conceivable method for nanomaterials in living organisms, given the significant

activity of MgONPs versus fungal cells in response to direct interaction. After treatment with modest concentrations of MgONPs, bacterial *Ralstonia solanacearum* cells accumulated ROS (Cai et al. 2018). This is due to the fact that metal nanoparticles' free radicals can damage lipids in bacterial cell membranes (Lopes et al. 2016). However, when fungal pathogens react with nanoparticles, oxidative stress has not been examined. Various species are reported to be the most prominent indicators of oxidative stress erupting in cellular components, including $O_2^{\bullet -}$, H_2O_2, and ROS (Rispail et al. 2014). While two kinds of fungal hyphae have been subjected to a variety of MgONPs levels, H_2DCFH-DA fluorescence was generated inordinately compared to the control. Once the concentration of MgONPs has been improved, the creation of fluorescence improved, demonstrating that MgONPs do indeed induce the generation of ROS.

16.7 Bimetallic Nanoparticles: Flow Synthesis and Fungicidal Activity

AgNPs (Długosz et al. 2021) are the most widely characterized nanomaterials. They are particularly active against bacteria and can be applied to a wide range of various products (Peszke et al. 2017). Despite nanosilver's numerous advantages, like small doses adequate to restrict bacteria growth, a vast variety of options, and simplified techniques for generating steady suspensions, substances which would operate well for biocide while restricting nanosilver's negative effects are also being sought (Ahmed et al. 2016). CuNPs which have strong antibacterial and antifungal properties are instances of particles with similar properties to AgNPs (Chatterjee et al. 2014). In addition, CuNPs are less costly and easier to find than AgNPs (Asghar et al. 2018). Basic disadvantage of utilizing CuNPs is the challenge of establishing good suspension with sufficient nanoparticle concentration to guarantee adequate bactericidal activity. The technique of generating CuNPs would be time-consuming, and nanomaterials themselves are often bigger than AgNPs, which could reduce CuNPs biocidal potential (Tan and Cheong 2013).

The combination of AgNPs antibacterial capabilities with CuNPs antifungal qualities allows for the creation of material with a broad spectrum of antimicrobial activity (Kalinska et al. 2019). It is feasible to lower quantities of individual metals while keeping similar antibacterial action by synthesizing a product that contains both components. Single-stage or multi-stage techniques can be used to create bimetal molecules or multi-stage core-shell molecules (Liu et al. 2017a, b). The biological activity of the final substance is affected by the ion reducing sequence. Furthermore, the biocidal characteristics of nanoparticles are dependent on the donation of particular metals to item and molecule form. Hikmah et al. (2016) investigated the microstructure or morphology of silver-copper core-shell nanoparticles as a function of Ag to Cu molar proportions. Depending on the metal concentration, nanoparticles varying in size between 25 and 50 nm were created. The magnitude of CuNPs grew significantly as the proportion of copper in

the material increased. This could be due to CuNPs reduced stability, whereas AgNPs remained unaffected by process conditions, and their size stayed unaltered.

Utilization of copper, silver, and bimetallic nanomaterials results in slow mobile ions into a scheme that is important in antibacterial action. Metal ions release ROS that, among other things, impair the action of cell respiratory enzymes. The presence of thiol groups –SH makes it easier for silver ions to interact with each other, enhancing ROS production. On the one hand, AgNPs interact with the bacterial membrane of cells, injuring it, and on the other side, it helps silver ions enter the cell, deactivating it (Sreeju et al. 2016).

Metal nanoparticle suspensions were created in the microwave reactor's flow system. The experimental system's schematic diagram was previously published (Banach and Długosz 2019). In a continuous microwave flow reactor, metal and bimetallic nanoparticles were synthesized (CMFR). Solutions have been hyped through microwave (Samsung, 100–800 W, 2.45 GHz frequency) using an HPLH dosing micropump (model pulse free). The length and diameter of the glass pipe were 550 mm and 70 mm, respectively. To generate nanomaterials, a flow of metal saline solution has been coupled with a flow of tannic acidic media, followed by the flow of hydroxide solution. The overall current of mixture ranged from 171.5 to 343.0 lm^3/s depending on residence time. Metal ion solution, tannic acid solution or alkaline solution had reagent volume ratios of 5:2:3. The final volume of nanomaterials has been 500 mg/dm^3.

16.8 Pectinase-Responsive Mesoporous Silica Nanoparticle Carriers (MSNPs)

To protect crops from pests, a great array of chemical pesticides is used in agriculture around the world. However, upwards of 90% of pesticides are misplaced throughout application due to deterioration parameters such as hydrolysis, light, microbes, temperature, and others. Furthermore, non-systemic pesticides are quickly washed into groundwater by rain and are influenced by immediate exposure to external elements like temperature or ultraviolet rays (Zhu et al. 2018). All of these difficulties pose serious risks to the environment and non-target creatures and the increasing expense of agricultural applications. As a result, it's critical to get insecticides to the right target location in plants without decreasing its potency (Kumar et al. 2014). Nanotechnology can currently improve pesticide transfer and distribution in plants, resulting in increased use efficiency (Kumar et al. 2014). Mesoporous silica nanoparticles (MSNPs) offer a lot of potential as nanocarriers for delivering chemicals to plant cells because of their simplicity of fabrication and surface alteration, high surface area, maximum load performance, bioactivity, and general stability (Sun et al. 2014). As a result, MSNPs have been used in a variety of applications, including the packing of molecules like nucleic acids (Kamegawa et al. 2018), proteins, drugs, or pesticides (Shao et al. 2018). On the other hand, MSNPs still need to be improved in terms of control safety and effectiveness. Initial pesticide discharge from MSNPs, for instance, and lower service performance could both lead

to low control systems (Manzano and Vallet-Regí 2020). As a result, developing MSNPs predicated on an encapsulation strategy could indeed help to avoid the untimely secretion of packed cargo in MSNPs.

Smart stimuli-reacting materials are stimulated by light, redox potential (Tryfon et al. 2019; Liang et al. 2020), pH (Xiang et al. 2018), temperature (Gao et al. 2020), or enzymes (Kaziem et al. 2018). They are good for putting pesticides inside nanoparticles so that they stay stable and can be released for a long time. Because of their biodegradability, eco-friendliness, and ease of availability, natural polymers such as chitosan, cellulose, alginate, pectin, and hyaluronan have been widely used in a variety of sectors (Xu et al. 2018; Pang et al. 2019). Because of its abundance of functional groups that could be altered to convey unique physicochemical characteristics, pectin, or polysaccharide, was used as an intermediary for content delivery methods. Moreover, coating a vehicle with pectin, which could be broken down by plant pathogen-secreted enzymes like pectinase, enables pesticide release over a lengthy period of time. Pathogens which induce apoptosis, besides damaging plant cell walls, frequently use the pectin secretion mechanism (Fan et al. 2017).

Rice (*Oryza sativa* L.) is the significant yield that feeds over half of the world's inhabitance. Rice blast disease, induced by *Magnaporthe oryzae*, is among the most damaging diseases to rice, resulting in 80–100% production losses in epidemic areas (Hendy et al. 2019). Rice blast can affect different sections of the rice plant, including leaf collars, pedicels, panicles, seeds, leaves, or necks, causing symptoms and lesions. Rice blast is combated with a variety of pesticides, including nonsystemic insecticides. Efficiency of insecticides against rice blast could be increased by using transport features of nanomaterials in plants. Among other cell wall components, *M. oryzae* produces enzymes that break down cellulose, hemicellulose, cutin, and pectin (Quoc and Bao Chau 2017). Scientists have utilized these enzymes as stimuli throughout investigations on the sustained releasing of pesticides on regular basis (Liang et al. 2020).

Prochloraz (*N*-propyl-*N*-(2-(2,4,6-trichlorophenoxy)ethyl)-imidazole-1-carboxamide) (Pro) is an imidazole fungicide that is proudly utilized to protect plants from a wide range of fungi, including *M. oryzae* (Quoc and Bao Chau 2017). This substance is a 14-demethylation inhibitor that inhibits the CYP51 enzyme encrypted by the CYP51 gene. Pro is a nonsystemic fungicide of low plant uptake, due to ineffective use of field capacity (Zhao et al. 2018a, b). The purpose of this study was to look into the migration and allocation of Pro-loaded MSNPs that had been cross-linked by pectin (Pro@MSN-Pec) throughout rice plants. MSNPs have been produced or fluorescein isothiocyanate (FITC) labeled tracking the carriers' migration through rice plants utilizing optical microscopy. Pro@MSN-Pec and/or commercial formulation antifungal and hybridization characteristics were examined further. Upon revealing rice leaves to Pro@MSN-Pec and commercial formulations, the pro-content in rice plant organs has been determined using high-performance liquid chromatography (HPLC). Utilizing ultrahigh-performance liquid chromatography/mass spectrometry (UPLC/MS), the ultimate residue quantities of Pro in the field have been evaluated.

16.8.1 Pro@MSN-Pec Synthesis and Characterization

MSN nanoparticles were produced by condensing silica prelude TEOS in the existence of CTAB, resulting in a configuration that served as a pattern for nanomaterial formation. Because of the reactivity of silica, the exterior of nanomaterials is protected by a large number of available –OH groups, providing a platform for transplanting multipurpose polymers onto the exterior and inner streams of nanomaterials. In this study, APTES was used to alter the surface of MSN to amino ($–NH_2$) clusters via organic silane clusters (Hussain et al. 2013). In addition, Pro in hexane has been packed into MSNPs. Morphology of MSNPs and Pro@SN-Pec was classified using SEM or TEM. SEM and TEM were used to classify the morphology of MSNPs and Pro@SN-Pec. MSNPs have been found to have stable appearance or spherical shape, with noticeable mesoporous configuration. MSNPs ranged in size from 20 to 50 nm. TEM and SEM assessments revealed changes in particle morphology and size between MSNPs and Pro@MSN-Pec after Pro-loaded MSNPs were transplanted with pectin. Shell structure of Pro@MSN-Pec differed from those of MSNPs, implying that pectin overlay was powerfully encased onto the exterior of MSN to great miscibility and unified form. Particles' sizes ranged from 19 to 110 nm, with an estimate of 70.89 nm. FTIR spectroscopy has been used to explore structural maledictions that occurred following the initial transplantation of particles to different functional clusters. FTIR spectrum of MSNPs revealed intense peak at 1087 cm^{-1} (asymmetric Si–O–Si stretching), 975 cm^{-1} (Si–O stretching), 833 cm^{-1} (symmetric Si–O–Si stretching), or 462 cm^{-1} (bending vibrations) (Hussain et al. 2013). Absorption band at 1643 cm^{-1} confirmed that $Si(OH)_4$ remained the dominant Si species in MSNPs. In the spectrum of MSN-NH_2, a novel absorption peak of the amino ($–NH_2$) cluster has been noted at 1535 cm^{-1}, along with the absorption band of the methylene ($–CH_2$) group at 2980 cm^{-1}, demonstrating that $–NH_2$ cluster was effectively connected on MSN exterior. In MSN-NH-pectin spectrum, sharp peaks for the amide (–CONH–) bond, including at 1458 cm^{-1} (C–N), emerged, confirming the creation of conjugate from the reaction between amino clusters of MSN-NH_2 and carboxyl clusters of pectin (Liang et al. 2018). Internal plant pathogen stimuli, like pectinase, might dissolve the pectin protective layer around MSNPs, triggers the production of Pro from Pro@MSN-Pec at a specified location and also triggers the delivery mechanism via pectin-cross-linked MSNPs. Pectinase is relatively stable at room temperature and under neutral conditions. Addition of pectinase resulted in significant accumulated discharge of Pro.

16.8.2 Pro@MSN-Pec Fungicidal Activity

After 7 days, fungicidal activity results revealed that Pro@MSN-Pec has been more effective than Pro EC and technical Pro (Table 16.1). After 14 days, Pro@MSN-Pec had greater fungicidal activity than Pro EC or technical Pro at the same concentrations (Abdelrahman et al. 2021), which was most likely due to

Table 16.1 Fungicidal activity of Pro@MSN-Pec, Prochloraz EC, or Prochloraz technical material toward *Magnaporthe oryzae* (Abdelrahman et al. 2021)

Pesticides	Days following treatment	EC50 ± SE (mg/L)	95% Confidence limits (mg/L)	EC90 ± SE (mg/L)	95% Confidence limits (mg/L)
Pro@MSN-Pec	7	0.113 ± 0.004	0.092 ± 0.134	0.657 ± 0.050	0.401 ± 0.906
Prochloraz EC		0.151 ± 0.013	0.085 ± 0.218	1.197 ± 0.113	0.636 ± 1.759
Prochloraz technical		0.196 ± 0.017	0.112 ± 0.280	1.911 ± 0.164	1.096 ± 2.725
Pro@MSN-Pec	14	0.248 ± 0.012	0.191 ± 0.306	2.301 ± 0.049	2.060 ± 2.542
Prochloraz EC		0.453 ± 0.007	0.419 ± 0.486	3.352 ± 0.014	3.283 ± 3.421
Prochloraz technical 7		0.725 ± 0.024	0.606 ± 0.844	6.339 ± 0.393	4.385 ± 8.294

Notes: EC50 inhibitory level which inhibits 50% of exposure fungus, EC90 inhibitory level which inhibits 90% of exposure fungus, or SE is standard error (all values are mean of triplicates)

Pro@MSN-Pec's ability to decrease active substance deterioration and thus prolong its efficient period. Furthermore, as a result of response to intensifying stimuli, active ingredient's release may become higher and faster over time, resulting in an increase in Pro's fungicidal activity (Liang et al. 2018). In comparison to Pro EC and technical Pro, stimuli-responsive Pro loaded into pectin covered MSNPs had superior and longer-lasting fungicidal efficacy against *M. oryzae*. The percentage of recovery was calculated using the quantity of Pro injected into blank samples. In blank samples spiked with Pro at three fortified concentrations of 1, 10, and 100 mg/kg, correctness of the analytical technique was explored. Pro recoveries in leaves ranged from 70.1% to 86.5%, in stems from 88.7% to 98.8%, or in roots from 91.6% to 97.9%. Relative standard deviation (RSD %, $n = 3$) was utilized to convey the reproducibility of the current process, with an RSD of 8% in all instances. Agilent software determined detection limit (LOD) as observed signal-to-noise ratio (S/N of 3).

16.8.3 MSNPs Translocation in Rice Plants

FITC has been transplanted on the exterior of MSNPs and samples have been investigated under fluorescence microscopy to clearly show MSN diffusion in rice plant organs. MSN-FITC has been used to treat rice plant seedlings in hydroponic systems. To cure rice plants, two application methods were used: the first was to cure leaves to guarantee diffusion of MSNPs via leaves to other sections of the rice plant, and the other would be to cure roots to guarantee the transition of MSNPs by roots to various parts of the rice plant. These findings suggest that MSNPs can be utilized as pesticide commercial vehicles for plants, which is consistent with reports that MSN-FITC can act quickly via plant parts (Zhu et al. 2018). Furthermore, previous research has demonstrated that MSNPs can transport particles inside plants (Sun et al. 2014).

16.8.4 Pro Distribution in Rice Plants

Pro@MSN-Pec allocation conduct showed that Pro might be transmitted via rice plant organs like stems, roots, or leaves. The content of Pro throughout leaves handled by Pro@MSN-Pec has been greater than in leaves handled with advertising Pro over a duration of 4 h to 14 days (Abdelrahman et al. 2021). Furthermore, the concentration of Pro in handled leaves peaked on the first day of diagnosis and afterwards gradually declined from 1 to 14 days. From 4 h to 14 days, fungicide was detected through stems or roots. Such findings suggest that Pro might be transmitted through various regions of rice organs. In terms of uptake and accumulation in rice leaves, stems, or roots, Pro@ MSN-Pec outperformed traditional Pro EC. Furthermore, Pro quantities in stems or roots peaked on the third day of diagnosis and subsequently declined for 3–14 days. Several studies found that pectin encasing all over Pro-loaded MSNPs might preserve or extend the active ingredient's

Table 16.2 Last residue amounts of prochloraz in rice plant stems, roots, seeds, leaves, or soil (Abdelrahman et al. 2021)

Compound	Residues (mg/kg)				
	Stems	Roots	Rice seeds	Leaves	Soil
Pro@MSN-Pec (1 g/L)	0.004	0.015	0.004	0.004	0.004
Pro@MSN-Pec (2 g/L)	0.004	0.017	0.004	0.020	0.004
Pro@MSN-Pec (4 g/L)	0.004	0.026	0.011	0.027	0.004
Prochloraz EC (2 mL/L)	0.009	0.015	0.004	0.006	0.004

efficient period, particularly after the third day to 14 days, when contrasted to Pro EC treatment. Particles smaller than 100 nm may also be easily transported into plant tissues (Zhao et al. 2018a, b; Avellan et al. 2019). These particles may contain compounds that plants are unable to absorb, such as pesticides, particularly nonsystemic insecticides, which may enhance their game and extend the active ingredient's lifetime in field treatments against target pests (Zhu et al. 2018).

16.8.5 Pro Residues in Various Sections of Rice or Soil Below Field Conditions

Prior to harvest, pro content was evaluated across several areas of the rice plant, including stems, leaves, roots, seeds and soil (Table 16.2). Residue quantities in rice stems, leaves, or roots have been marginally greater than in rice leaves, stems, or roots handled to advertise Pro EC 44%, but residue amounts in seeds or soil are the same. There was a slight difference in the Pro@MSN-Pec levels in rice plants when different Pro@MSN-Pec levels were compared. The highest residue levels found in the 2RD treatment residue limit (MRL) for Pro through rice calculated by the European Union, Japan, China, and Hong Kong were 0.5, 1, and 0.5 mg/kg, respectively. When residue values were contrasted to MRLs, it was found that final Pro concentrations in rice were below maximum allowable concentrations, implying that Pro@MSN-Pec treatment on rice organs presented a minimal risk.

16.9 Conclusion

Plant breeding and IPM are currently insufficient agricultural approaches, and innovative alternative solutions that can fulfill our present and future food demands are needed. Investing in cutting-edge agronanotechnology research that is just a couple of decades old is worthwhile. We could save money on plant protection chemicals, reduce yield losses, and increase agricultural productivity by using NPs. The method is sufficient for dealing with issues such as rising chemical input costs,

ineffectual pesticide use, and pesticide contamination of land and groundwater. Because zero-valent iron nanoparticles have a strong attraction to organic molecules or heavy metals, they could be used to remediate pesticide-infested soil. Additionally, FeNPs, like $CaCo_3$, have excellent soil-binding properties. Furthermore, in order to reduce the environmental impact of NM manufacturing, greater emphasis needs to be placed on using agricultural residues as raw resources. Advances in nanobiotechnology, such as the use of green chemistry to synthesize nanoparticles from living tissues and plant extracts, provide guarantees of environmental protection. The diverse potential of nanoparticles includes one's use as vehicles for active targeting of antimicrobial substances and, moreover, one's inherent antimicrobial impacts and properties, both of which prove their own utility when used as nanopesticides or nanofungicides toward plant pathogens.

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Applications of Nano-Biotechnological Approaches in Diagnosis and Protection of Wheat Diseases

17

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Abstract

Wheat (*Triticum aestivum*) is a major staple food crop, plays a crucial role in food security, and is grown on an area of 221.6 million hectares (Mha) in multi-environments throughout the globe. Annual wheat production was recorded at 778.6 million metric tons in the years 2020–2021. Regardless of the abundant growth of wheat, people are facing food crises in some parts of the world because of the unavailability of food grains. The ever-growing population of the world is creating a new challenge for farmers and researchers. By the year 2050, the global need for agricultural products will have risen by 50%. To make it more challenging, biotic and abiotic factors become constant reasons for wheat yield losses. Continuously, the wheat crop suffers from a plethora of diseases (pests, insects, fungi, and bacteria). To deal with the challenges given above and meet future food needs, there is a strong need for new and cutting-edge technologies that can keep wheat farming sustainable and boost wheat production from current cropping systems and changing climates.

Nano-biotechnology is an emerging and rapidly evolving field of science that possesses immense potential to revolutionize the sector of wheat crop production.

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The huge applications of nanoparticles in agriculture and other related sectors can counteract the future challenge of food security. Integration of nano-biotechnology into wheat farming envisages modernizing the present scenario of natural resources-based cropping to precision agriculture of advanced systems, with increased material use efficiency and targeted applications. Real-time surveillance, monitoring, and management of diseases that cause significant yield loss can be achieved by using nanoparticles inserted inside the wheat plant cells. Nevertheless, several plant-related, environmental, and health hazards are also associated with the application of NPs in wheat cropping. Researchers reported wider applications of nano-biotechnology in wheat farming, and it is being used for developing a number of precise tool sets (nano-sensors, nano-pesticides, nano-fertilizers, nano-herbicides, and smart delivery systems for controlled release of agrochemicals and other NPs). Although the research on nanotechnology in wheat disease detection and protection indicated that intervention is still in its early stages, it has a bright future in the coming era. In the present chapter, we focused on the versatile roles of nano-biotechnological approaches in the surveillance, detection, monitoring, and protection of wheat diseases.

Keywords

Wheat · Nano-biotechnology · Nanoparticles · Food security · Disease diagnosis · Protection

17.1 Introduction

Wheat (*Triticum aestivum*) is the second most-produced food crop after rice, and it plays an important role in food security due to its 20% contribution to total energy and protein in the human diet (Lata et al. 2021). Wheat and wheat-based products could account for 20% of global protein and calorie consumption per capita. Moreover, wheat is considered the main food source in many countries, and the global population is estimated to increase from 7.3 billion to 9.7 billion by the year 2050. The global need for agricultural products will have risen by 50% by 2050, as expected by the UN-FAO. So, the production of wheat has to be doubled to meet the anticipated requirement (UN 2015). To meet this demand there is a strong need for the improvement of traditional cultivars along with contemporary best management practices and innovative technologies that will revolutionize the production of wheat (Beres et al. 2020). Approximately 80% of poor people in rural areas work in agriculture as their primary occupation, and they play an important role in improving the country's economy by providing food, improved livelihoods, and income to the rest of the population. The world faces the challenge of meeting the heavy dietary needs of an ever-increasing population. To achieve this challengeable goal, a significant increase in rates of genetic gain in grain yield will be obligatory for crops such as wheat (*Triticum aestivum* L.). According to records of FAO (2017), the current rate of gain (ca. 1% p.a.) should be increased by a rate of 30–40% to meet this

demand (Cassman and Grassini 2020). Nevertheless, biotic and abiotic stresses due to climate change will be an additional challenge to hamper productivity (Scott and Ron 2020). Biotic stresses, including plant pathogens, pests, weeds, and insects, cause significant reductions in crop production worldwide, with an estimated global loss of 20–40% annually. Among the biotic stresses, rust diseases are the major threat to wheat production (Lata et al. 2021). Stem rust, along with stripe rust, can cause a 100% loss, while leaf rust results in a 50% loss of wheat yield under favourable conditions (Bhardwaj et al. 2019). Other significant diseases such as powdery mildew, spot blotch, Karnal bunt, and wheat blast also impede wheat production to some extent (Kashyap et al. 2020). In addition, today's climate change results in major and regular pests of wheat. Several insects, such as pests, aphids, borers, termites, and insects like grasshoppers, also caused significant losses of wheat, pre or post-harvest. Existing pest management strategies are based mainly on the application of pesticides, such as fungicides, insecticides, and herbicides. These practices have some advantages like quick action, reliability, and easy availability, but have some detrimental negative impacts and negative concerns due to health hazards, the resurgence of the pest population, the problem of groundwater contamination, food safety, herbicide-resistant weeds, and protection of endangered species (Yadav et al. 2021). Moreover, it is a rough estimate that during or after application, 90% of applied pesticides are lost. So, there is a huge demand to develop attractive technologies in terms of cost-effectiveness, less harmful to the environment, and high and quick-performing pesticides.

Wheat production and productivity are vulnerable to biotic stresses by several means. Wheat production is expected to fall by 6% for every degree Celsius increase in temperature and will become even more difficult as space and time pass (Asseng et al. 2015; Templ and Calanca 2020). Furthermore, the prospective weather forecast predicts an increase in the number of hot days as well as an increase in moderate temperatures and their effects on global wheat production. One of the major wheat-producing regions in India and the world is the Indo-Gangetic Plain (IGP). With the change in climate, yields of wheat will be affected by changes in temperature and rainfall in this region, as access to irrigation water has also declined. These effects raise serious concerns about national and international food security (Daloz et al. 2021). Another important cropping system is rice-wheat cropping, a predominant pattern in South Asia, which is under heat stress and poor soil health. This problem is predominant due to climate change, over-exploitation of natural resources, high cropping intensity, and puddling for rice production (Joshi et al. 2007; Jasrotia et al. 2018). Another possible obstacle to wheat production could be deficiencies in macro-nutrients (iron, zinc, sulfur, manganese, and boron). This is increasing day by day because of over-mining of essential plant nutrients, imbalanced fertilization, burning of crop residues, and similar cropping patterns in various parts of northern India, Nepal, Pakistan, and Bangladesh (Chatrath 2004). The water crisis has become alarming for global wheat production due to the depletion of water sources and the water table going down. Less recharge from monsoon rains makes it a more serious issue (FAO 2017). Salinity stress is another prevalent abiotic stress for wheat production globally. Around 20% of cultivated land is salt-affected, and it will reach

50% by 2050, expectedly. If we look at the Indian scenario, about 6.73 Mha are occupied by salt-affected soils. Salinity stress is an ever-increasing problem for agriculture throughout the globe that is affecting the most productive crop areas (Mann et al. 2021). Nevertheless, proper drainage system development and effective soil reclamation technologies play a crucial role, but the salt stress is a difficult task to combat. Consequently, to address the aforementioned hurdles in wheat productivity, there is a great motivation to develop quick-performing and cost-efficient strategies, which show fewer detrimental impacts on the environment. There is a need for some advanced technology for quick diagnosis and management of wheat diseases to achieve the goals of regional and global food security.

One of the emerging technologies, nanotechnology or nano-biotechnology, has led to the progression of new concepts and is visualized as a swiftly developing area that has great potential to transfigure wheat production and counteract the food security challenge of the present day, and in the near future (Kashyap et al. 2015). Nano-biotechnology has significantly advanced in pharmaceutical, medical, and medicinal sciences but has received relatively less attention for agricultural applications. There are several fields where nano-biotechnology is devised and being explored, such as seed germination, transfer of target genes, plant hormone delivery, nanosensors, water management, nano-barcoding, and controlled release of pesticides/agrochemicals (Mathivanan 2021). Productivity or the yield of wheat can be enhanced in two ways. Firstly, by reducing the yield loss caused by several factors such as biotic and abiotic stress (adverse environmental factors) and secondly, by developing proper disease management strategies. To improve the production and productivity of wheat, the applications of nano-biotechnology can be deployed in both the strategies. Among the different nano-biotechnological approaches, the nano-biosensors possess enormous potential for the detection of wheat diseases quickly in the early stages of the wheat crop. Nano-biosensors may help the wheat crop detect and fight against different pests and pathogens. Further, under disease management strategies, nano-structured catalysts increase the efficiency and potential of commercially available insecticides and pesticides, along with a reduction in the level of doses required for crop plants. Frequently recurring diseases of wheat, such as, rust diseases, bacterial spot diseases, Karnal bunt, wheat grains infected by Fusarium, and spot blotch disease, are considered the most important factors that limit crop productivity. It is only possible to eradicate the root cause of the aforementioned diseases if they can be detected at an early stage of development and can be diagnosed with plant diseases before the effects of pathogens are truly visible to them. Various nanoparticles have shown bactericidal, pesticidal, insecticidal, and herbicidal activities, which can be deployed in disease management strategies for wheat crops. Hence, nano-biotechnology has immense potential in wheat production and protection (Fig. 17.1). Although the practical application of nano-biotechnology in wheat disease diagnosis and management practices is negligible at the moment, it has great potential in the near future to improve agricultural practices above conventional farming at various stages. In the present chapter, we will highlight the role of nano-biotechnology in the detection and management practices for wheat diseases and various applications and their possible potential in wheat crop improvement.

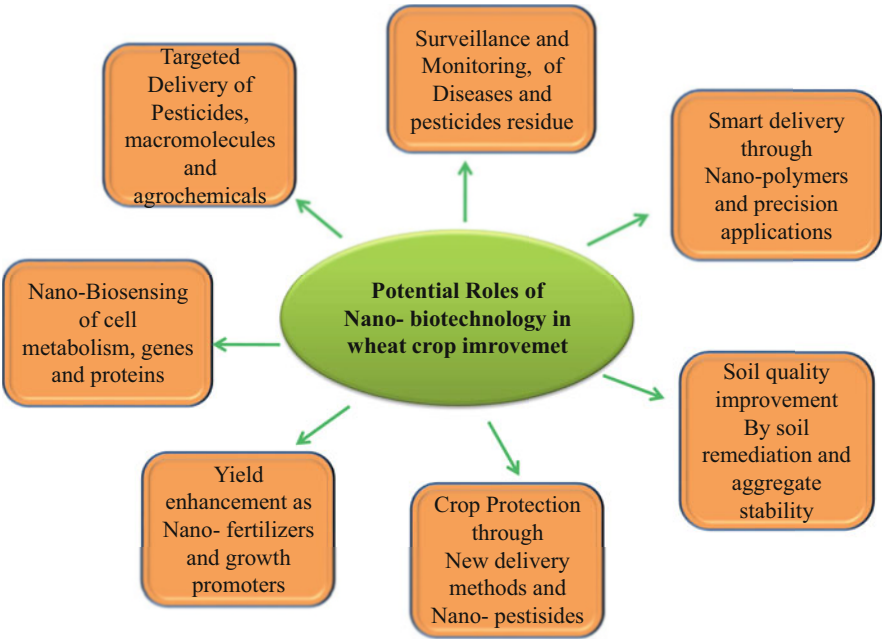

Fig. 17.1 Roles of Nano-biotechnology in wheat production and protection

17.2 Nano-Biotechnology Concept and Advancement

Integration of nanotechnology with biology became nano biotechnology. Nano-biotechnology is an interdisciplinary field of research that involves the submission of new and emergent approaches. Nano-biotechnology refers to the use of nanotechnology to modify living organisms and enable the amalgamation of biological and non-biological materials. Lynn W. Jelinski, a biophysicist at Cornell University in the United States, coined the term nano-biotechnology, and the term "nano" refers to a scale of 1–100 nm (Fig. 17.2). The prefix "nano" is taken from a Greek word which means "dwarf". The branch of science in which we deal with the study of characters of smaller structures than 100 nm (nanometers) is known as "nano-science." Furthermore, the development and conniving of particles in this nano-size range and their combination with living cells or products of cells, along with their applications in a specific area of science are called "Nano-biotechnology". According to the definition of the Royal Society, nanotechnology is the "design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nano-meter scale."

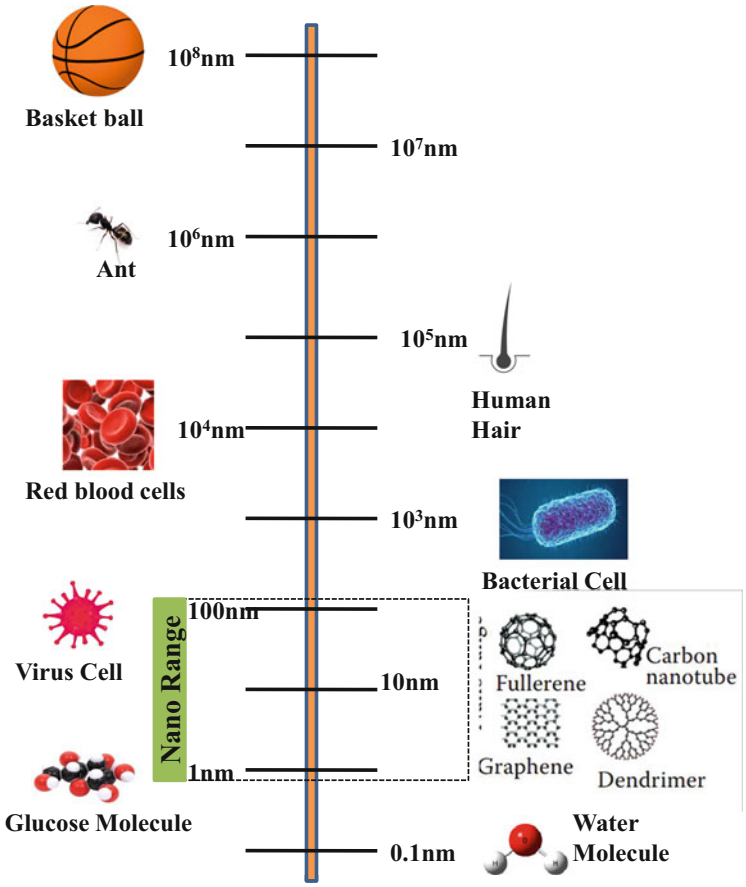

Fig. 17.2 Illustration of nano-size ranges relative to generally known materials

17.2.1 Types of Nanoparticles

They hold a unique place in nanoscience and nanotechnology, not only because of their unique features resulting from their small size but also because they are prospective building blocks for more sophisticated nanostructures. The particle materials ranged between 10 and 100 nm and were designed with exclusive properties (physical, chemical, and biological) that showed differences from their molecular and bulk counterparts and thus are categorized as nanoparticles (Yang et al. 2008). Photosynthesis, seedling vigour, root and shoot growth, etc., are all influenced by the administration of nanoparticles. Plants have to deal with different disease-causing pathogens in real time and exhibit various stress reactions including changes in molecular processes and stress-responsive gene expression, to combat the stress posed by these pathogens (Rejeb et al. 2014). Through various mechanisms, plants attempt to maintain a balance between their response to stress and the

determinantal effect on their viability (Scott and Ron 2020). The relevance of nanoparticles stems from the fact that they provide an effective technique for relaxing this defence mechanism, or in other words, they help plants. In plants, for disease diagnosis and protection, nanoparticles alone have the potential to combat several pathogens. Moreover, nanoparticles can be applied directly to foliage, seeds, and roots of plants for the protection and management of pests and pathogens (such as fungi, insects, bacteria, and viruses). Several metal nanoparticles (copper, silver, titanium dioxide, and zinc oxide) are known for their antibacterial and antifungal properties (Yadav et al. 2015) and have been intensively studied for their antiviral properties (Worrall et al. 2018). Major nano-materials used in agriculture and associated sectors include metal dioxides, carbon nano-tubes, quantum dots, zero-valent metals, dendrimers, and nano-polymers having different types of properties such as nano-sheets, nano-fibers, nano-wires, nano-emulsions etc. (Punia et al. 2021). We focus on some nanoparticles in this section that have known anti-pathogenic properties. Recently, quantum dots, nanoparticles made of indium, cadmium, and silicon with semiconductor properties, were found to aid in the identification of Phytoplasma aurantifolia. Other nanoparticles, such as zinc oxide, titanium oxide, and silver nanoparticles, are commonly utilized in plant tissue culture to limit microbial activity (Singh et al. 2021). Among these, the silver nanoparticles proved to be more focused due to their exceptional physical, chemical, and biological properties associated with huge applications. Moreover, its "green synthesis" made it possible to avoid hazardous by-products (Rafique et al. 2017; Ibrahim et al. 2020). For the treatment of fungal and bacterial pathogens, silver nanoparticles have enormous potential for plant disease management. However, some associated hurdles are also reported with them, such as toxicity, production, and soil interaction.

Another popular nanoparticle is chitosan, which has shown some constructive biological properties like biocompatibility, biodegradability, antimicrobial activity, non-allergenicity, and less toxic effects on animals. Chitosan nanoparticles exhibit viral resistance, antimicrobial, and antifungal properties in plant tissues by protecting them against several viral infections. The application of 1000 and 5000 ppm concentrations of chitosan nanoparticles showed maximum inhibition of radial mycelial growth of Fusarium head blight disease of wheat (Kheiri et al. 2016). In another study, it was found that chitosan nanoparticles helped to mitigate possible oxidative stresses in durum wheat (Picchi et al. 2021). Several metal nanoparticles (gold, copper, titanium dioxide, etc.) have gained popularity at present due to their potential and effective role in diagnosis and protective function against plant diseases (Omar et al. 2021; Satti et al. 2021). Gold nanoparticles have various applications and are used in a number of biosensors, PCR-variants, barcoding and genomic technologies. Copper and titanium dioxide nanoparticles are more frequently utilized as fertilizers despite being less explored as disease management tools. Moreover, aluminium nanoparticles showed insecticidal properties, while titanium dioxide nanoparticles used as nano-fertilizers provide additional protection from viruses and bacterium. Under biotic stress, the effects of biosynthesized titanium oxide were assessed in wheat plants for morphological traits (plant weight,

fresh as well as dry weight, the surface area of leaf, root, and grain yield), physiological traits (membrane stability index, RWC, and concentration of chlorophyll), and for some non-enzymatic metabolites (soluble sugar, protein, soluble phenol, and flavonoid content). It was determined that an effective concentration of titanium oxide can lead to mechanisms for reducing biotic stresses and hence demonstrate the significance of biosynthesized titanium oxide nanoparticles in combating wheat fungal diseases, with the broad aim of yield improvement (Satti et al. 2021).

Another important class of nanoparticles includes silica nanoparticles, which act as a novel silica source that can be used to improve plant resistance to various plant pathogens. It is already established that silica is important for plant nutrition since a lack of it makes plants weaker and more vulnerable to biotic and abiotic stresses (Rafi et al. 1997), and it is also the most widely distributed material on our planet, after oxygen. Silicon is considered to be between essential and non-essential elements because it is not only required for plant survival but is also required for plant benefit (Luyckx et al. 2017). It is relatively easy with a controlled shape, size, and structure, which makes it incredibly suitable as delivery agents or carriers. Several researchers reported that Si aids in the activation of the host defence system and has been found to be beneficial in the control of a variety of plant diseases. Si will improve plant resistance to fungus, bacteria, viruses and nematodes (Khan and Siddiqui 2020). Si is found to modulate the signalling systems of plants associated with defence-related genes, genes related to antimicrobial compound synthesis, genes responsible for structural modification of cell walls, hormones, and genes related to hypersensitivity responses (Rajput et al. 2021). The significance of selenium nanoparticles in ameliorating abiotic stresses has already been established by Soleymanzadeh et al. (2020). They demonstrated that elevated salt tolerance was afforded by accumulating proline, preserving ionic equilibrium, improving the antioxidant system, and increasing levels of different phyto-propanoids, resulting in osmotic adaptations. As selenium particles are showing a significant impact on abiotic stress management, we may expect similar results for biotic stress protection too.

One important type of nanoparticle is cerium nanoparticles, which are also known as nano-ceria. Depending on the exposure concentration, coating, surface charge, plant species, and growing circumstances, nano-ceria has a wide range of effects on plant health, both positive and negative (Milenković et al. 2019). In the biomedical industry, cerium oxide nanoparticles are widely applied as antioxidants, which belong to the nano-ceria family of nanoparticles. Another potential class of nanoparticles that can be indirectly used for biotic stress amelioration in plants is zinc oxide-based nanoparticles. Their significant role has already been proven for abiotic stress tolerance, as evidenced by Adrees et al. (2020). Zinc nanoparticle foliar exposure increased leaf chlorophyll content, decreased oxidative stress, and increased leaf superoxide dismutase and peroxidase activities in wheat. These zinc nanoparticles can improve the overall health of plants under stress as many pathways of plant biotic and abiotic stress defence mechanisms are interrelated with each other. These nanoparticles have enormous potential and could be used directly or as common carriers for plant disease management tools for diagnosis and protection.

The nanoparticles are reported to play a role in wheat disease protection in several ways and could be utilized as nano-biosensors, carriers for RNAi, and gene targeting and delivery systems for insecticides, fungicides, and herbicides.

17.2.2 Nano-Biotechnology: Potential Roles in Wheat Diseases Management

The most important and major issue is the detection of disease at the right stage of plant growth for efficient disease management. Generally, it is observed that plant diseases are actually visible at later stages of infection when it becomes very difficult to control. Farmers and researchers applied conventional pesticides and fungicides only after the appearance of symptoms. At this stage, there was a noteworthy loss of crops. Consequently, to reduce the significant crop losses, it is essential to dissect the plant disease at an early stage of infection. Farmers, on the other hand, can use nano-biotechnological tools to quickly diagnose the location of viral, parasitic, and bacterial sicknesses at early stages for proper disease management and yield loss. An insightful synchronization between nano-biotechnology and plant pathology could provide a promising solution to a tough task. The Nano-biotechnology toolkit helps in the diagnosis and management of wheat diseases in several ways, for example, the Nano/bio barcode assay, Quantum Dot, Nano-pore sequencing tools, Bio-nano materials, Nanoparticles, Nano-diagnostic kit, Nano-biosensors.

17.3 Nano-Biotechnological Approaches for Diagnosis of Wheat Diseases

Successful disease management requires an accurate diagnosis of plant disease and plant pathogen detection. In the last few decades, the demand for highly sensitive, rapid, and high throughput assays for plant pathogen detection has increased. Integrated molecular diagnostics with nanotechnology are now being used for the identification of plant pathogens (Fig. 17.3). A number of nano-devices and nano-sensors are used to investigate the DNA sequences to diagnose diseases. Also, nanotechnology is serving the development of chip-based systems for pathogen detection. A summary of major nano-biotechnological based approaches and nano-materials used for disease diagnosis is presented in Table 17.1.

17.3.1 Quantum Dot Nanoparticles-Based Approach

Quantum dots (QDs) are a class of nano-crystals containing luminescent semiconductors that get excited and radiate light at particular wavelengths (Edmundson et al. 2014). Khiyami et al. (2014) define QDs as basically inorganic fluorophores in nature that are used as probes or markers for nucleic acid or protein detection. Many properties of QDs, such as narrow emission peak, longer

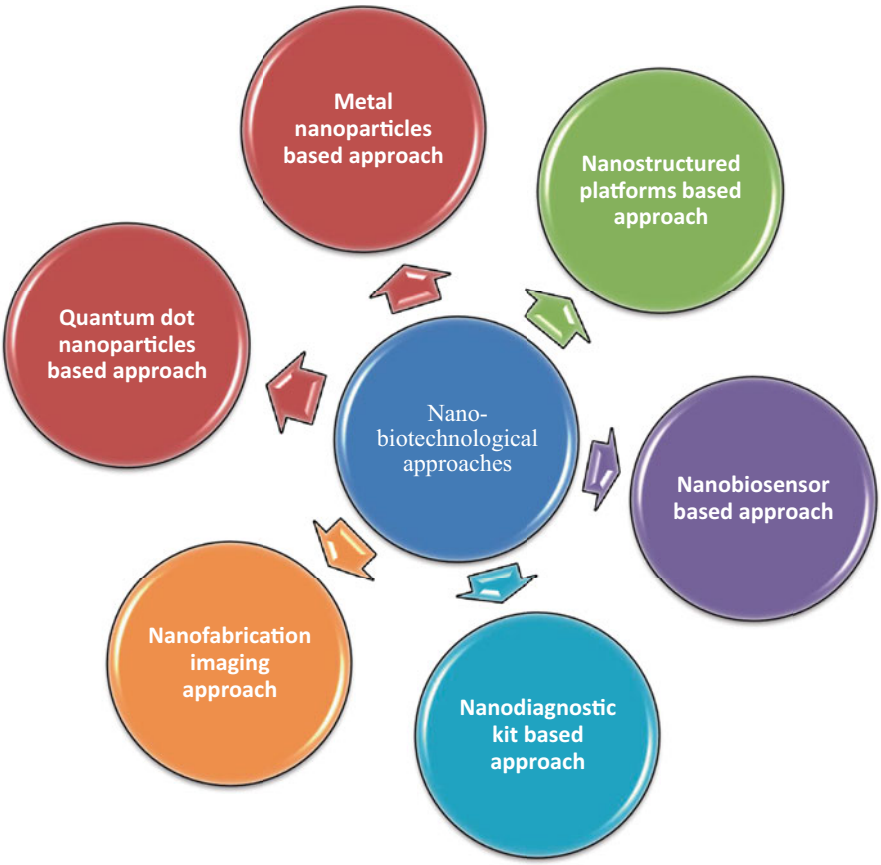

Fig. 17.3 Overview of major nano-biotechnological based approaches for diseases diagnosis of wheat

fluorescence lifetime, 10–100 times higher extinction coefficient, and photo-bleaching resistance, allow them to be multi-coloured quantum dots (Zhao and Zeng 2015).QD-based nano-sensors are being used in agriculture and allied sectors due to their advantages in detecting nucleic acid and enzyme activities. The first report of myco-synthesized semiconductor nano-materials was introduced by Dameron et al. (1989), where crystallites of cadmium sulphide were produced by yeast in response to heavy metal cadmium stress. For the biosynthesis of cadmium sulphide, a number of microbes have been used, but only limited studies have reported the fluorescent properties (Kashyap et al. 2016). When incubated with the $CdCl_2$ and $SeCl_4$ mixtures, *Fusarium oxysporum* (a wheat pathogen) produced highly fluorescent myco-mediated Cadmium sulphide QDs (Syed and Ahmad 2013; Kumar et al. 2007). For possible agricultural applications, carbon quantum dots feature simple synthesis, high stability, high water solubility, high biocompatibility, strong photoluminescence, adjustable surface functions, and low toxicity. Su

Table 17.1 An overview of approaches and nano-materials used for plant pathogens detection

Approaches	Nano-materials used	Plant pathogen detected	Reference
Quantum dot nanoparticles based	Crystallites of Cadmium sulphide	*Fusarium oxysporum*	Kashyap et al. (2016)
	Crystallites of Cadmium sulphide	*Tristeza virus*	Safarnejad et al. (2017)
Metal nanoparticles based	Gold nanoparticles	*Fusarium oxysporum*, *Dematophora necatrix*, *Alternaria aternata*, *Sclerotium rolfsii*, and *Colletotrichum capsici*	Thakur and Prasad (2021)
	Silver nanoparticles	*Fusarium graminearum*, *F. avenaceum*, *F. poae*, and *F. sporotrichioides*	Yugay et al. (2021)
Nanostructured platforms based	Chitosan nanoparticles	*Fusarium graminearum*	Kheiri et al. (2016)
	Colloidal gold nanoparticles	*Pseudomonas syringae*	Lau et al. (2017)
Nanofabrication imaging	Silver nanoparticles	*Fusarium graminearum*	Jian et al. (2021)
Nano-diagnostic kit based	Tetraplex antibody	*Fusarium* species	Lattanzio et al. (2012)

et al. (2018) found carbon nano-dots to be significant in improving the tolerance of peanuts against drought stress (Su et al. 2018). In the future, these quantum dots will have significant potential as nano-tools for improving crops under biotic stresses too.

17.3.2 Metal Nanoparticles-Based Approach

Metal nanoparticles are being used in biosensors, which allow the use of several novel signal detections to identify pathogens. For a specific recognition between a target pathogen and nanomaterials, various strategies such as adhesion-receptor, antigen-antibody, recognition of complementary DNA sequences, and so on have been used (Fan et al. 2003). AuNPs offer distinctive physical and chemical properties i.e. high surface-to-volume ratios, lower detection limits, higher sensitivity etc. which makes them an excellent component for a wide range of bio-sensing techniques. Because of its unique qualities, including form, size, and flexibility, silver has been regarded as the most promising NP in terms of electrical and antibacterial activities. These Ag nanoparticles have also been shown to prevent fungal growth. The silver nanoparticles are effective against a broad range of fungal pathogens such as *A. brasiliensis*, *C. glabratus*, *P. oxalicum*, etc. These nanoparticles exhibit various mechanisms against pathogenic infection, such as

altered membrane structure, cellular content leakage, dysfunction of mitochondria, disruption of protein structure, and oxidation of lipids inside the pathogen cell.

AuNP consists of two components coated with thio-oligonucleotides. These two components bind with target DNA and aggregate, leading to the change in colour from red to blue, which is a well-known property of AuNPs (Kashyap et al. 2016). Thakur and Prasad (2021) synthesized gold nanoparticles through bacterium *Bacillus sonorensis* and explored their toxicity potential for plant pathogens such as *Dematophora necatrix*, *Fusarium oxysporum*, *Alternaria aternata*, *Alternaria mali*, *Sclerotium rolfsii*, *and Colletotrichum capsici*. It was witnessed that after 1 week of incubation, synthesized AuNPs caused the 70% inhibition in growth of *Fusarium oxysporum*.

17.3.3 Nano-Structured Platforms-Based Approach

The employment of nanostructure as a detecting material is a result of the development of nanotechnology and biotechnology. The main advantages of nano-structures are their high surface-to-volume ratio, the possibility of device miniaturization, and size-dependent electrical properties (Sertova 2015). Also, the main aim of such nano-structured platforms is the detection of pathogens in less time. Nanomaterials such as carbon nano-tubes, grapheme, nano-wires and nano-structured metal oxide play an important role in mycotoxin and pathogen detection. Some of the nano-structured platforms are based on microscopic fluidics systems that detect pathogens in real time with high sensitivity. Some platforms use various nanoparticles, which can be visualized in different colours when they get infected with pathogens (Bhattacharya et al. 2007). For the first time, Stadler et al. (2010) investigated the insecticidal activity of nano-structured alumina against two insect pests, namely, *Sitophilus oryzae* and *Rhyzopertha dominica*. These two insects are serious pests in stored food supplies all over the world. Exposure of these pests to alumina-treated wheat leads to substantial mortality. So, it can be concluded that compared to commercially available pesticides, inorganic nano-structured alumina may provide a cost-effective and dependable option for insect pest control, and such research may open new doors for nano-particle based pest management solutions. Kheiri et al. (2016) reported that the application of chitosan and chitosan nanoparticles showed significant inhibition of mycelial growth and the number of colonies formed against *Fusarium graminearum*. Likewise, Lau et al. (2017) developed a nano-structured electrochemical biosensor to rapidly detect plant pathogen (i.e. *Pseudomonas syringae*) DNA with high sensitivity. The assay is based on the rapid electrochemical assessment of amplified target DNA through gold nanoparticles.

17.3.4 Nanofabrication Imaging Approach

Nanofabrication imaging approach involves the technologies used to diagnose the different plant pathogens by looking inside or outside the plant tissues. This

approach allows us to accurately adjust the physical and chemical properties of target materials to prevent toxicity problems. Also, how the pathogens make a way to the plant tissue and colonize the tissue can be visualized using nano-imaging technologies with an electron beam and photolithography techniques. González-Melendi et al. (2008) stated the visualization of carbon-coated magnetic nanoparticle transport and deposition inside the plant host using imaging methods. Similarly, Rispail et al. (2014) studied the communication between the quantum dot and super-paramagnetic nanoparticle with pathogenic fungi *F. oxysporum*. They visualized the fungal hyphae incubated with SiO_2-Magnetic NPs through transmission electron micrographs. Recently, Jian et al. (2021) reported the antifungal activity and mechanisms of silver NPs against *Fusarium graminearum* strains to conclude the effects on mycotoxin deoxynivalenol production using the nanofabrication imaging approach. They examined the morphological changes of fungus caused by AgNPs using SEM, TEM, and fluorescence microscopy and evaluated the potential of silver NPs for fusarium head blight disease management in the field.

17.3.5 Nano-Biosensor Based Approach

Nano-biosensors are the result of a collective approach of nanotechnology and biology. It is an altered form of biosensor that may be explained as a tiny systematic tool that integrates biological elements with physio-chemical transducers (Turner 2000). Nano-biosensor-based approaches have increased sensitivity, which leads to a reduced response time to sense diseases in crops. Currently, nano-biosensor-based approaches are employed to identify the minute quantities of contaminants i.e. bacteria, viruses, fungi, and their toxins (Kashyap et al. 2016). Additionally, the on-site detection of pathogens by using these approaches can help in designing strategies to control the disease spread. They have been significantly used in the diagnosis of soil diseases caused by various bacteria, fungi, and viruses. It is based on the differential consumption of oxygen by good and bad bacteria. The quantitative analysis of this oxygen consumption reveals the types of microbes causing various diseases in the soil. Apart from that, it can also be anticipated whether or not a soil disease will emerge in the examined soil ahead of time. As a result, it is important to note that the biosensor provides a unique method for diagnosing soil conditions using a semi-quantitative approach. These nano-biosensors are GPS-based for real-time monitoring of diseases.

17.3.6 Nano-Diagnostic Kit-Based Approach

Nano-diagnostic kits are state-of-the-art tools in nano-biotechnology. These are the lab-in-a-briefcase devices, which include reagents, power supplies, and other utilities such as microarrays, and provide portable, rapid, and highly accurate diagnostic tools, allowing for early disease detection and epidemic control. It involves placing of sensitive measuring devices, required reagents, power supply,

and other features that now take up laboratory space into a parcel no larger or heavier than a briefcase (Goluch et al. 2006). A briefcase-sized kit is transported to a crop-growing field to look for diseases that might infect the crop and limit its productivity. This is a simple and accurate process. Experts can assist farmers in preventing disease epidemics by using nano-diagnostic kit technology to detect possible hazardous plant diseases swiftly and easily (Pimentel 2009).

The application of nano-diagnostic assay for detection of various pathogens is now a common analytical practice. Fungal plant pathogens such as *Fusarium* species can easily be detected using nano-diagnostic assay (Khiyami et al. 2014; Lattanzio et al. 2012) developed the immunoassay for detection of mycotoxins (T-2/HT-2, ZEA, DON, FB1/FB2) in wheat, corn, oat, and barley. This nano-diagnostic assay kit is a 4mycosensor which is fast, low-priced, easily accessible, and appropriate for the detection of pathogens in cereals.

17.4 Protection/Management of Wheat Diseases Through Nano-Biotechnological Approaches

A number of organic and inorganic salts have been utilized since the beginning of agriculture to protect and save the crops from pests, insects, bacterial, and fungal diseases (Abdollahdokht et al. 2022). Due to their high surface-to-volume ratio, nanoparticles exhibit unique chemical, physical, and optical properties. Nano-biotechnology is emerging as a new field in the sector of agriculture with a bunch of applications. However, it has been a growing science for the past couple of years, but the focus and continuous research in the exploration of NPs in the management of plant infection will be increased with the passage of time. Plant disease management mainly involves any of these ways: (1) Nanomaterials are used in bio-sensing devices to make nano-biosensors for the detection of plant diseases at early stages; (2) Nanoparticles are used directly as pesticides and are applied to plants for disease control; (3) NPs are used as carriers for other pesticides and molecules, such as miRNA, for targeted delivery. The effective way for the application of nanomaterials is the priming of seeds or foliage to control pathogens at the site of entry.

17.4.1 Nanoparticles: Relocation in Wheat Plants

Direct application of NPs and coupling with other formulations with conventional pesticides results in wheat plants either through roots or foliar parts. After the migration of nanoparticles in the plant cells, these particles move through the xylem and phloem and reach different parts of the plant at the site of action. The entry of nanoparticles into plant cells has been studied by several researchers. As per their findings, the uptake mechanism of nanoparticles can be predicted (Yang et al. 2008). Nevertheless, the exact mechanisms of entry, absorption, and relocation of these nanoparticles in the plants of wheat have not been studied so far, but a general

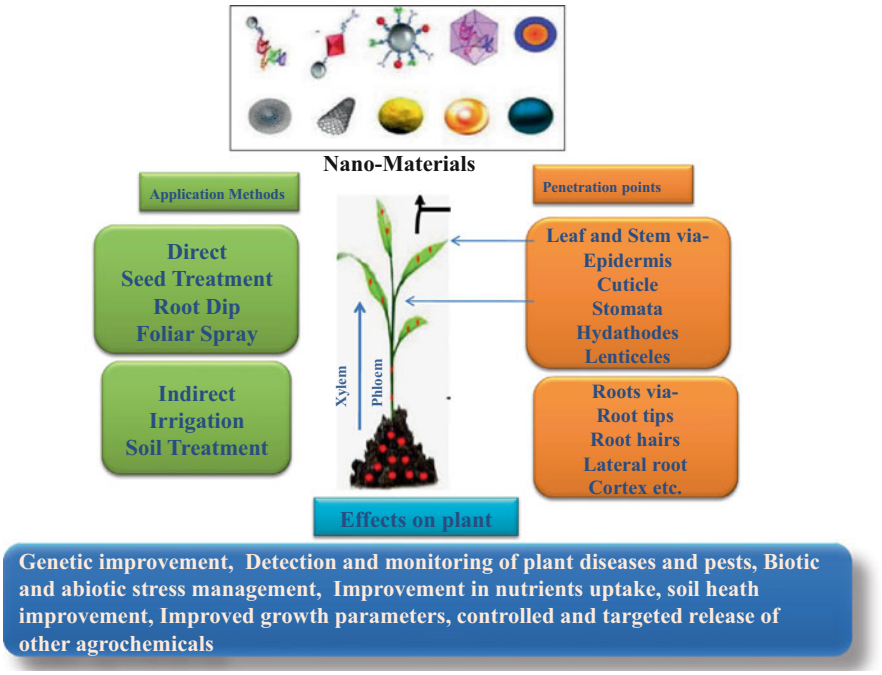

Fig. 17.4 Predicted route of nanoparticles penetration, uptake, and relocation in the wheat plants

mechanism could be predicted based on literature findings (Fig. 17.4) (Kashyap et al. 2020).

17.4.2 Nanoparticles Towards Protection of Wheat Diseases

Researchers have studied the effects of NPs on various stages of wheat plants and have concluded the fate of NPs. Germination, emergence, seedling, leaf emergence, tilering, differentiation, flowering, grain filling, and maturity are the various growth stages of the wheat plant, varying their behaviour towards nanoparticles. Wheat disease control with nanoparticles—Conventional wheat disease control methods—are based on chemical fungicides, pesticides, and insecticides. However, as nanomaterial science progresses, NPs create a gap in wheat disease control approaches. Several studies have reported various successful examples of nano-formulations based on wheat management strategies. In Table 17.2, a list of several nanoparticles and their modes of action in controlling the different wheat diseases are given. In the present segment, we briefly discuss the functions and roles of nanoparticles in the protection of wheat diseases.

It has been clearly demonstrated that among the metallic nanoparticles, silver nanoparticles are the most effective in activating the antioxidant-based defence

Table 17.2 Example of several nanoparticles and their mode of action in controlling the wheat diseases

Type of nanoparticle	Disease	Action	Reference
Silver nanoparticles	Yellow rust	Biological control	Sabir et al. (2022)
Silver nanoparticles (AgNPs)	Bacterial and fungal disease of wheat	Suppress growth and germination of pathogen	Mansoor et al. (2021)
Copper nanoparticles (CuNPs)	Several fungal and bacterial pathogens of wheat	Antimicrobial activity	Banik and Pérez-de-Luque (2017)
Copper nanoparticles (CuNPs)	Bacterial and fungal pathogen of wheat	Antimicrobial activity and growth-promoting behavior	Yasmeen et al. (2017)
Chitosan-nanoparticles (CSNPs)	Several wheat pathogens	Induces plant defence responses at the infection sites	Saharan et al. (2015), Abd-Elsalam et al. (2017)
Copper nanoparticles (CuNPs)	*Fusarium* sp.	Fungal growth inhibition	Viet et al. (2016)
Nano-structured liquid crystalline particles (NLCP)	*Raphanus raphanistrum* L.	Negative impact on pathogen growth	Nadiminti et al. (2016)
Chitosan-nanoparticles (CSNPs)	Fusarium head blight (*Fusarium graminearum*)	Agglutination at penetration sites	Kheiri et al. (2016)
Fe_3O_4 to ZnO/AgBr	Lentil-vascular wilt and head blight diseases of wheat	Antifungal activities	Hoseinzadeh et al. (2016)
Copper nanoparticles (CuNPs)	Fungal pathogen of wheat	Antimicrobial activity and growth-promoting behaviour	Hafeez et al. (2015)
AgNPs	Spot blotch infection in wheat	Showed strong antifungal activity at germination stage	Mishra et al. (2014)
Gold nanoparticles (AuNPs)	Stem rust (*Puccinia graminis*)	Active antifungal behavior	Jayaseelan et al. (2013)
AgNPs	Fungal diseases of what	Act as pre-planting fungicide	Karimi et al. (2012)
AgNPs	Karnal bunt (*Tilletia indica*)	Active antifungal behaviour	Singh et al. (2010)
Nano-silica	Insects' pest of wheat	Absorbed into the cuticular lipids and killed insect pest	Barik et al. (2008)
Silicon	Larvae of Hessian fly (*Phytophaga destructor Say*)	Less infestation	Miller et al. (1960)

mechanisms in plants. Furthermore, because of its green synthesis, it has been an extensively researched nanoparticle in wheat disease management by researchers. Green synthesized nanoparticles have a lot of antibacterial power and might be used instead of toxic fungicides. There are several molecular mechanisms reported for AgNPs for their antimicrobial activity. These nanoparticles have a high affinity for membrane-bound targets. Moreover, with surface binding, these NPs also penetrate inside the bacterial cell. Recently, a study conducted by Sabir et al. (2022) has highlighted the role of silver nanoparticles in the biological control of yellow rust disease in wheat. They used *Moringa oleifera* leaf extract as a reducing and stabilizing agent for green synthesis of silver nanoparticles. Different concentrations of silver nanoparticles were applied by foliar spray on wheat plants that were already inoculated with *Puccinia striiformis*, which causes stripe rust disease in wheat. They demonstrated that the foliar application of silver nanoparticles improved morphological and physiological characters in wheat as well as reduced the non-enzymatic compounds. Hence, the silver nanoparticles may be used as a potential source for biological control of yellow rust. Mishra et al. (2014) found the powerful antifungal behaviour of AgNPs against wheat pathogen *Bipolaris sorokiniana* (causative agent of spot blotch infection in wheat). Silver nanoparticles biosynthesized by *Serratia* spp. in different nano-size ranges exhibited strong antifungal activity towards *Bipolaris sorokiniana* at the germination stage. Greenhouse studies of wheat plants showed that conidial germination of *B. sorokiniana* was totally inhibited by the application of particle and spherical shaped AgNPs (10–20 nm sizes ranged) at concentrations of 2, 4 and 10 μg/mL, while under control conditions, the germination was 100%. Silver nanoparticles did not affect the seed germinability when coated on wheat seeds and provide similar protection like a conventional pre-planting fungicide under testing (Carboxitiram). Moreover, no harmful effect was found on soil conditions after the application of AgNPs coating. So, Karimi et al. (2012) suggested that nano-coating of AgNPs may be considered a potential pre-planting fungicide due to its comparable effects with conventional pre-planting fungicide.

Gold nanoparticles (AuNPs) synthesized through green synthesis act as an active antifungal agent towards a number of fungal pathogens. AuNPs size ranges between 45 and 75 nm, tested towards *Puccinia graminis tritici*, fungal agent of economic important disease of wheat (stem rust) in a concentration (Jayaseelan et al. 2013). These NPs showed active antifungal behaviour in controlling the stem rust disease of wheat along with several fungal spp. (*Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans*). Singh et al. (2010) also documented the effective role of silver nanoparticles in the detection and management of *Tilletia indica*, the causal agent of Karnal bunt disease in wheat plants.

Copper nanoparticles exhibit antifungal activities in many diseases towards several wheat pathogens. Fungal growth of *Fusarium* sp. was inhibited up to 94% by the use of copper nanoparticles in a concentration of 450 ppm under 9 days of treatment (Viet et al. 2016). In an additional study on wheat published by Banik and Pérez-de-Luque (2017), the authors revealed the significant antimicrobial activity of CuNPs towards a number of fungi and bacteria. Hafeez et al. (2015) published the

effect of CuNPs in different concentrations (10–50 ppm and 30 ppm) on the growth and yield parameters of wheat. Incubation with 30 ppm copper nanoparticles showed improvement in yield significantly in terms of leaf area, number of grains/spikes, Chl content, number of spikes/pot, 1000-grain weight, and final grain yield as compared with the untreated plants. Yasmeen et al. (2017) also reported similar findings that CuNPs exposure increases the quantity of grains per spike as well as 1000 grain weights. In addition to these findings, the experimental varieties showed additional resistance toward diseases.

In a greenhouse experiment, it was found that most resistant varieties of wheat had dark shapes of silicon depositions in their leaf sheaths and contained a comparatively intense and grainy covering of silicon. Miller et al. (1960) reported another interesting factor: wheat varieties containing high amounts of silicon present in stems showed less infestation by Phytophaga destructor Say larvae (Hessian fly). Barik et al. (2008) documented the unique properties of nano-silica and found antifungal activity. Plant-silica has been deployed to develop several nano-pesticides for plant disease management, including wheat. The mechanism of pest death purely relies on the fact that insect pests use a number of cuticular lipids, which are taken from plant cells and prevent death from desiccation by protecting their water barrier. However, when nano-silica is applied through a foliar application on wheat leaves and stems, it gets absorbed into the cuticular lipids and kills insect pests.

Nanostructured liquid crystalline particles (NLCP) also showed a negative impact on *Raphanus raphanistrum* L. (weed wild radish) under wheat field trials. NLCP mixed with phytantriol (18% w/w) in a size range of ~250 nm with 0.22 polydispersity index having a zeta potential of −15 mV applied as a nano-formulation. This nano-formulation minimizes the effect on epicuticular waxes when used as a carrier to deliver 2,4-D to weeds. Nadiminti et al. (2016) found that there is no significant change in yields of wheat crops when these nano-formulations are used in low concentrations of 0.03% and 0.06%. Moreover, the comparison with commercially available herbicide formulations like Estercide 800 has a similar impact on weeds.

Chitosan-nanoparticles are another effective nanoparticle, and these NPs are effective against plant pathogen spectra, while being non-toxic to humans and animals. In a greenhouse study, it was found that wheat plants sprayed with CS and CSNPs at the stage of anthesis protected the plants against *Fusarium graminearum*, causing Fusarium head blight disease in wheat. The poly-cationic properties of CS and CSNPs disorganized hyphae formation, which directly caused inhibition of fungal growth as a result of causing membrane permeability and leakage of cellular contents. CS applied to plant tissues creates its agglutination at penetration sites; it forms the physical barrier which inhibits the pathogen's spread in healthy tissues and prevents the pathogen/disease from spreading in wheat crops (Kheiri et al. 2016). Chitosan acts as a powerful inducer of signalling cascades towards the fungal disease of wheat. It induces plant defence responses at the infection sites and alleviates systemic alert for healthy tissues of plants. These responses include early signalling events and the synthesis of defence-related metabolites and proteins such as PR-proteins and phytoalexins to cope with pathogens (Saharan et al. 2015; Abd-Elsalam et al. 2017). Wheat seed priming

with chitosan and its nano-formulations showed an increase in lignin synthesis and accumulation in plants, which directly provides disease resistance for fungal pathogens.

Nano-formulations of Fe_3O_4/ZnO/AgBr was prepared with diverse weight ratios of Fe_3O_4 to ZnO/AgBr by using facile microwaved-assisted technique and investigated for their antifungal activities towards *Fusarium oxysporum* and *Fusarium graminearum* causative agent lentil-vascular wilt and head blight diseases of wheat (Hoseinzadeh et al. 2016). The nano formulations deactivate both fungi in a short duration of time, about 60–90 min. Moreover, nano-biotechnology produces bionic plants by setting and inserting nanoparticles inside the plant cells and organelles. These plants are more responsive towards sensing or imaging objects and infrared devices and have great potentials in precision farming. NPs integrating plants show self-power and act as light sources to other communicating devices.

Spherical-shaped silicon dioxide nanoparticles in size ranging between 9.92 and 19.8 nm biosynthesized using saffron extract by Abdelrhim et al. (2021). Authors introduced these nanoparticles against *R. solani* to protect wheat seedlings and used it as a potential alternative therapeutic solution. SiO_2 nanoparticles exhibited a strong antifungal activity against *R. solani* and reduced mycelial radial growth up to 100%. A clear reduction was observed in pre-, post-emergence damping-off, fresh and dry weight of mycelium, and severities of root rot. Along with this, SiO_2 NPs showed a positive impact on the growth of wheat seedlings and correlated with disease suppression. These nanoparticles increased the amount of chlorophylls and carotenoids (photosynthetic pigments) and salicylic acid, and other defence-related compounds. SiO_2 NPs enhanced the content of enzymatic (POD, SOD, APX, CAT, and PPO) and non-enzymatic (phenolics and flavonoids) compounds and alleviated the oxidative stress by activating the antioxidant defence machinery. Moreover, the application of SiO_2 NPs enhanced the germination, vigour indexes, and vegetative growth of wheat seedlings infected with *R. solani*. Nevertheless, these nanoparticles have no phytotoxic effect on wheat seedlings (Fig. 17.5).

17.5 Adverse Effects of Nanomaterials

The field of nano-biotechnology has various applications in the detection, diagnosis, and protection of wheat diseases. Several research teams are collaborating to determine the role of nano-biotechnology in agriculture and related industries. Nevertheless, with the positive effects of NPs, several reports have been found on the negative behaviour of these NPs on plant growth, animals, humans, soil, water and the environment. This aspect of nanomaterials is collectively known as nano-toxicology. It is a sub-discipline of toxicology that attempts to deduce the interaction mechanisms of a nanostructured material with a living organism, including plants and animals (Hobson 2016; Paramo et al. 2020). Because of the growing demand for nanoparticle-based goods in manufacturing, waste management, and water treatment facilities, these compounds are quite easy to release into the environment. There are some studies showing the adverse behaviour of nanoparticles on wheat crops.

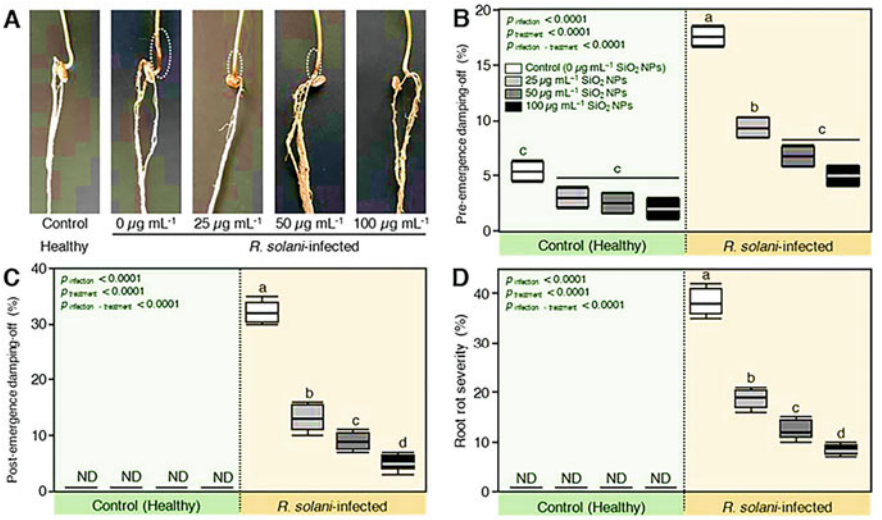

Fig. 17.5 The influence of silicon dioxide nanoparticles (SiO_2 NPs) on the severity of damping-off in wheat seedlings infected with *R. solani*. (**a**) Healthy (control), *R. solani*-infected (positive control), and SiO_2-treated wheat seedlings; (**b**) pre-emergence damping-off; (**c**) post-emergence damping-off; and (**d**) root rot severity. Whiskers represent the minimum and highest values, thick, horizontal lines represent the medians, and boxes represent the interquartile ranges (25th to 75th percentile of the data). According to Tukey's honestly significant difference test ($p < 0.05$; $n = 5$), different letters indicate statistically significant differences between treatments, whereas the same letters imply no significant changes between them (Abdelrhim et al. 2021)

Karunakaran et al. (2013) found the harmful impacts of copper nanoparticles on soil nutrients and other soil parameters (soil pH and electrical conductivity). Authors also reported a minor variation in the amount of macronutrients in NPK and the quantity of organic matter after application of CuNPs but in a considerable way. Phytotoxicity and genotoxicity of silver NPs were studied in wheat by Vannini et al. (2014) and found the alternation in cell proteins and metabolites. CuNPs showed the toxic effects on wheat and mung beans documented by Lee et al. (2008). In another study, Dimkpa et al. (2013) found the phototoxic attitude of CuONPs in wheat, resulting in a reduction in root length. Banik and Pérez-de-Luque (2017) reported the negative impacts of CuNPs on the germination index, shoot dry weight, root length, and seed metabolic performance in wheat compared to control. Application of TiO_2 and ZnONPs showed the inhibition of soil enzyme activity (catalase, protease, and peroxidase) in a wheat experiment (Du et al. 2011). These nano-formulations negatively affect the soil quality and health, along with having a negative impact on the wheat biomass. Therefore, carrying out extensive and focused research in this direction is strongly required.

17.6 Conclusion and Future Perspectives

Wheat diseases could have immense potential in the field of detection, diagnosis, and protection through nano-biotechnology. The advancement of nanotechnology combined with biotechnological approaches could transform wheat production to the next level. This improvement in the agricultural sector is directly associated with the living standards of the developing world in terms of feeding a rapidly growing global population. As a result, nano-biotechnology has been significantly less explored in the area of wheat farming as compared to other areas of agriculture such as nano-fertilizers and pharmaceutical and medicinal sciences. There are several fields where nano-biotechnology needs more attention and is being explored, for example, field-level studies in wheat. Nevertheless, wheat farming integrated with nano-biotechnology has enormous potential to combat the challenges of climate change associated with food production and sustainability for the world. Moreover, despite the revolutionizing image of wheat production integrated with the immense potential of nano-biotechnology, there are several risks associated with this area that should also be studied. Subsequently, there is a strong need to build focussed and extensive research efforts in some areas of nano-biotechnology.

- A major challenge in front of researchers coupled with nano-biotechnology is the firm understanding of the fate and environmental impacts of nanomaterials on non-targeted crops and animals. Therefore, it is essential to carefully monitor the impacts of NPs on non-targeted plants and animals. The environmental persistence of NPs should be monitored properly with soil and water studies.
- Wheat plant interactions with nanoparticles vary with several conditions; type of NPs, stage of development and genotype, time of treatment, and so forth. Hence permissible limits of nanoparticles' dosage and safety limits should be defined with experimental validations. That's why these facts should be scrutinized at the time of doing experimentation with NPs while setting tolerable limits and recommendations.
- The study of various non-targeted molecules that are present inside plant cells, un-related pathways, protein function and gene expression is also required to find out the interaction of NPs. Furthermore, the study of the correlation between wheat rhizosphere and experimental NPs should be observed critically to define the possible positive and negative effects on the wheat-agro-ecosystem. The associated microbiome has an immense positive correlation with plant growth and sustainability.
- Cost-effectiveness is another major factor of technology to be popular among the common man. Because wheat is the principal food crop throughout the globe, wheat farming integrated with nano-biotechnology should be cost-effective.

Integration of nano-biotechnological approaches with wheat research would be incomplete without these factors being studied. Therefore, the aforementioned points should be kept in mind to intend experimentation in this field. Hence, collaborative and multidisciplinary research would be crucial in devising efficient,

multifunctional, cost-effective, environment-friendly, easily applicable, and quick-performing nanomaterials. This would help to depict the clear role of NPs in terms of function, behavior, fate, agro-toxicity, and impacts on non-targeted species.

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Nanomaterials for the Reduction of Mycotoxins in Cereals

18

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Abstract

Mycotoxins are secondary metabolites secreted by certain genera of molds. However, their synthesis is controlled by several biotic and abiotic factors. The presence of mycotoxins in unprocessed or still processed foodstuffs poses major economic and health problems. The daily ingestion of food contaminated by doses higher than the doses recommended by specialized services leads to the development of several mycotoxicoses, some of which are very serious. Most of these mycotoxicoses arise from the high oxidative power of mycotoxins in poisoned living cells. Although recent studies indicate that no food is immune to these toxins, cereals remain the most contaminated food category due to their composition rich in complex sugars and nitrogen compounds. This composition makes cereals a favorable environment for the synthesis of mycotoxins. This alarming situation forces global organizations and control services to adopt several strategies to minimize the economic and health damage caused by mycotoxins and/or their sources of origin. Although conventional methods for the removal of mycotoxins and toxigenic molds continue to advance, current research trends aim to create nanoscale structures capable of offering more promising, cost-effective, and less expensive solutions. Nanostructures based on carbon, zinc, copper, silver, gold, and iron are the most promising nanomaterials. Polymeric nanoparticles doped or substituted with substances or chemical groups are also recommended. Inhibition of mold growth, adsorption of mycotoxins, and reduction of the toxic effect of mycotoxins in poisoned cells are

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the three main strategies by which nanostructures reduce mycotoxins. The current chapter deals generically with the main classical techniques and nanomaterials in the elimination of mycotoxins in cereals.

Keywords

Aflatoxins · Nanoparticles · Antifungal activity · Wheat · Maize

18.1 Introduction

Nowadays, cereals and cereal by-products are the most consumed food sources by humans in several countries, and in particular in developing countries. It brings several nutrients to the diet. They are composed of trace elements such as iron, copper, manganese, phosphorus, zinc, sodium, potassium, and calcium. Cereals are also an important source of carbohydrates, dietary fiber, polyunsaturated fatty acids, and protein. Cereals also contain vitamin B6, thiamin, riboflavin, niacin, and folic acid (Laskowski et al. 2019). Given the rising standard of living and ever-increasing demographic exposure, however, it will be very difficult to balance future world population and food demands in the face of dwindling natural resources and unmet food security, and an increased need for yield and minimum loss of grain crops is required (Mannaa and Kim 2017). According to approximations, a population estimated at more than nine billion by 2050 must be fed, while guaranteeing the health of humans and the planet (Cole et al. 2018). To meet future global food demands for cereals, agronomic research and food processing unit operations play a key role in the success of established strategies. The new strategies granted will ensure an increase in production, stabilization of yields, and guaranteed crop protection.

Mycotoxinogenic molds and/or their mycotoxins are the main contaminants of cereals and their derivatives (Wan et al. 2020). These secondary metabolites are produced by different genera of phytopathogenic filamentous fungi (Palumbo et al. 2020), some of which are abundantly toxic and cause serious illness in humans and animals (Bennett and Klich 2003). Among the mycotoxins detected on cereals, aflatoxins, ochratoxins, fumonisins, deoxynivalenol, and zearalenone are the most abundant. According to Lee and Ryu, the incidences and maximum levels in raw cereal grains were 55% and 1642 μg/kg for aflatoxins, 29% and 1164 μg/kg for ochratoxin A, 61% and 71,121 μg/kg for fumonisins, 58% and 41,157 μg/kg, for deoxynivalenol, and 46% and 3049 μg/kg for zearalenone (Lee and Ryu 2017).

In the field and during the different stages of growth, the contamination of cereals by mycotoxins can only occur after an alteration by a fungal flora of the field. In post-harvest, contamination occurs during transport and long storage periods (García-Díaz et al. 2020). The storage period remains the determining stage of the quality of cereals, and the degradation is strongly due to the drop in quality following the infestation of the fungal flora of storage (Mannaa and Kim 2017). Mycotoxin biosynthesis is influenced by environmental factors such as climate, pest infestations

(Omotayo et al. 2019), water activity (Aw), temperature, and light and eco-physiological requirements (Mannaa and Kim 2017; Priesterjahn et al. 2020). Mycotoxin biosynthesis is also influenced by an optimal pH and carbon to nitrogen (C:N) ratio (Brzonkalik et al. 2012). Moreover, the proper functioning of enzymatic pathways and the safety of biosynthetic gene clusters play a crucial role in the biosynthesis of mycotoxins. It has been reported that the establishment of a good interconnection between environmental signals and regulations is a determining point in mycotoxin biosynthesis (Caceres et al. 2020). In addition, the interaction between fungal species of contaminating mycoflora plays a key role in fungal incidence and the production of mycotoxins. The production of aflatoxin B_1 by *Aspergillus flavus* is stimulated by *Fusarium graminearum*; however, the co-presence of *Aspergillus flavus* considerably reduces the biosynthesis of fumonisin and deoxynivalenol (Giorni et al. 2019). Species interaction can also lead to overexpression of mycotoxins, such as overexpression of mycotoxins following the interaction of *Tribolium castaneum* and *Aspergillus flavus* in maize flour (Duarte et al. 2021).

Among the various mycotoxins known to date, aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, and patulin are classified as serious contaminants from an agro-economic and sanitary point of view. Exposure to these mycotoxins leads to mycotoxicoses, some of which are serious and irreversible and can lead to death (Omotayo et al. 2019). Prolonged exposure to mycotoxins, especially aflatoxins (AFs) and ochratoxin A (OTA), is an important way to increase the incidence of hepatocellular carcinoma (HCC) (Felizardo and Câmara 2013). In addition, ochratoxin A (OTA) is considered a powerful mycotoxin implicated in the development of other types of cancers (Sorrenti et al. 2013). Fumonisins (FBs) induce liver and kidney tumors, esophageal cancers, and neural tube defects (Wild and Gong 2010). In animals, zearalenone (ZEA) induces reproductive disorders, as it is also capable of causing hyperestrogenic syndrome (Rai et al. 2020). Deoxynivalenol (DON) induces disruption of cell function by preventing protein synthesis, DON also affects immune function and growth (Pestka and Smolinski 2005).

Reducing the risk of consumer exposure to food contaminated with mycotoxins relies on two basic strategies. The first strategy aims to prevent pre-harvest contamination, while the second aims to prevent post-harvest mycotoxin production (Guo et al. 2021a). In pre-harvest, the application of good agricultural practices, the management of plant diseases (Karlovsky et al. 2016), and the use of chemical agents such as fungicides, herbicides, and insecticides contribute to the reduction of toxigenic molds and, therefore, a reduction of mycotoxins biosynthesis (Edwards and Godley 2010; Lehoczki-Krsjak et al. 2010). Despite the expenses incurred to achieve the success of prevention strategies, these policies considered are not always effective in preventing the biosynthesis of mycotoxins. The failures recorded in pre-harvest prevention involve the intervention of one or more post-harvest treatments. The appropriate practices and the respect for storage conditions can reduce and guarantee the health quality of the stored product (García-Díaz et al. 2020). In addition, the storage of cereal raw materials may be preceded by treatments

to eliminate mycotoxins and/or their sources of origin. These treatments are classified into several categories in which the physical elimination methods are the most effective, citing as an example, automatic sorting. Food processing unit operations such as grinding seeds and applying high temperatures also reduce mycotoxin content. In contrast, processes in food processing units significantly reduce mycotoxin concentrations but do not eliminate them completely (Milani and Maleki 2014). It is also possible to get rid of mycotoxins with chemical or enzymatic treatment (Karlovsky et al. 2016).

Despite the application of several protocols and techniques aimed at preventing, decontaminating, and detoxifying mycotoxins, these toxins are constantly detected on cereals and their derivatives, which points to the failure of the methods applied, and the persistence of health and economic damage. At present, no method is strongly recommended to completely prevent the contamination of cereals and their derivatives by mycotoxins (Wan et al. 2020), leaving a big void, which offers a new platform for nanotechnology research focused on reducing mycotoxins in cereals. The first part of the current chapter deals generically with the main classical techniques for the elimination of mycotoxins in cereals. In the second part, the chapter deals in depth with the detoxification mechanism exerted by the nanomaterials (NMs) applied during the treatment of mycotoxins, or toxigenic molds in cereals. Optimization of factors involved in detoxification is also discussed.

18.2 Occurrence of Mycotoxins in Cereals

Despite compliance with agricultural practices and the use of fungicides in fields, no grain product is immune to contamination by mycotoxinogenic molds and their mycotoxins. Practices applied in the field and over long storage periods aim to prevent severe fungal damage and minimize contamination. Contamination can occur in the field, during harvest due to damaged grain, during maritime transport due to high humidity, and during long periods of storage. The evolution of contaminating mycoflora can be exponential following non-compliance with storage conditions. Spoilage of grains can affect grain derivatives and by-products due to the stability of mycotoxins and the inability of different processing and detoxification methods to remove them.

According to published studies species belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, and *Claviceps* are the main contaminants of cereals and their by-products (Zhang et al. 2020). Similarly, aflatoxins, fumonisins, ochratoxins, T-2 toxin, deoxynivalenol, and zearalenone are the most abundant mycotoxins in this food category. Table 18.1 gives a general overview of the contamination of cereals or their by-products in different regions of the world. The detection methods used to reveal the mycotoxins, the percentage incidence, and the range of contamination are also reported in Table 18.1.

Table 18.1 Worldwide occurrence of mycotoxins in cereals and cereal-based foods

Country	Detection method	Cereal type	Mycotoxin type	Incidence rate (%)	Max/Range (μg/kg)	Reference
Albania	LC-MS/MS	Wheat	DON	23	1916	Topi et al. (2020)
		Maize	FB1, FB2	76	16.97	
			DON	24	799	
			T-2	2.2	106	
			ZEA	4.4	263	
China	UPLC−MS/MS	Wheat kernel	AOH	31.40	21.10–102.38	Jiang et al. (2021)
			AME	4.90	7.20–40.90	
			TeA	62.70	13.20–3634.80	
		Flour	AOH	10.00	16.19–33.86	
			AME	10.00	1.60–2.35	
			TeA	90.00	10.00–172.00	
Spain	UPLC−MS/MS	Oat kernel	ZEA	66	28.1–153 ng/g	Tarazona et al. (2021)
			HT-2 toxin	47	4.98–439 ng/g	
			DON	34	19.1–736 ng/g	
			FB_1	29	63.2–217.4 ng/g	
			T-2 toxin	24	12.3–321 ng/g	
Argentina	HPLC	Barley	AOH	64	712 μg/kg	Castañares et al. (2020)
			TeA	37	1522 μg/kg	
Vietnam	LC-MS/MS	Paddy and white rice	Afs, FBs, ENN-B, ROQC, STERIG, AME, NIV, OTA, DAS	45	2.3–547 μg/kg	Phan et al. (2021)
Nigeria	ELISA	Maize	AFs	100	3.5–173.3	Ekpakpale et al. (2021)
		Rice		75	1.75–22.8	
Zimbabwe	Neogen AccuScan Lateral Flow Device HPLC	Maize	AFs	51.2	ND to 1369	Akello et al. (2021)
			FBs	88.9	ND to 40,000	
		Sorghum	AFs	25.0	ND to 4.3	

(continued)

Table 18.1 (continued)

Country	Detection method	Cereal type	Mycotoxin type	Incidence rate (%)	Max/Range (µg/kg)	Reference
			FBs	31.7	ND to 2800	
Poland	HPLC with fluorescence detection (HPLC-FLD)	Cereal products from organic farms	DON	26.3	199.60 ± 149.82	Mruczyk et al. (2021)
Brazil	HPLC-MS	Breakfast cereals	FB_1	26.7	105	Mallmann et al. (2020)
			ZEA	14.8	17	
			DON	10	44	
		Infant cereals	FB_1	27.8	55	
			ZEA	6.9	3	
			DON	10.3	36	
Poland	LC-MS/MS	Rye	DON	90	354.1	Kosicki et al. (2020)
			T-2 toxin	63	6.63	
			HT-2 toxin	57	29.8	
			ZEA	45	10.2	
Egypt	HPLC	Wheat	AFB_1	33.33	24.11–62.17	Hathout et al. (2020)
			OTA	/	<LOD	
Lebanon	HPLC	Wheat	AFB_1	65.7	0.04–6.21	Daou et al. (2021)
			OTA	100	0.02–63.3	

LC-MS/MS liquid chromatography coupled with tandem mass spectrometry, *FBs* fumonisins, *FB_1* Fumonisins B_1, *FB_2* Fumonisins B_2, *AOH* alternariol, *AME* alternariol monomethyl ether, *TeA* tenuazonic acid, *UPLC–MS/MS* ultra performance liquid chromatography–tandem mass spectrometer, *TEN* tentoxin, *Afs* aflatoxins, *ENN-B* enniatin B, *ROQC* roquefortine C, *STERIG* sterigmatocystine, *AME* alternariol methylether, *NIV* nivalenol, *OTA* ochratoxin A, *DAS* diacetoxyscirpenol, *ELISA* enzyme-linked immunosorbent assay, *DON* deoxynivalénol, *ZEA* zearalenone, *DAS* diacetoxyscirpeno, *AFB1* aflatoxin B1

18.3 Toxicities of Mycotoxins in Human Organism

Ingestion of mycotoxins is the most common mode of exposure. However, exposure by inhalation of mycotoxins in the air or by a dermal route is no longer negligible. Long-term exposure at high doses causes nephrotoxicity, hepatotoxicity, neurotoxicity, gastrointestinal toxicity, immunotoxicity, genotoxicity, and teratogenicity. AFB1 is currently considered genotoxic and mutagenic for living cells; the toxin induces aberrations in DNA (Bárta et al. 1984; Madrigal-Santillán et al. 2010) and leads to decreased cell viability by an increase in fragmented DNA levels (Golli-Bennour et al. 2010). Human hepatocellular carcinoma (HCC) is linked to a distinct mutation of TP53 by transversions of G to T in the second guanine of codon 249 (Besaratinia et al. 2009). AFB1 negatively affects the embryonic development of skeletal muscles (Oznurlu et al. 2012; Gündüz and Oznurlu 2014). In chicks, AFB1 induces decreased weight, disturbs the biochemical characteristics, and increases liver weight with perilobular inflammation and vacuolar hepatocyte degeneration (Denli et al. 2009). In THP-1 and RAW 264 cell lines, AFB1 affects macrophage functions, it induces ROS-mediated autophagy (An et al. 2017). The kidney is an action site for AFB1, in addition, the toxins decreased the rate of glomerular filtration and tubular reabsorption of glucose (Akao et al. 1971). Another study in rats demonstrated that repeated administration of AFB1 leads to degeneration of the central and peripheral nervous systems (Ikegwuonu 1983). The majority of these diseases result from alteration of repair systems and activations of molecules responsible for inflammation and apoptosis induced by ROS formed after the intoxication of cells. Table 18.2 and Fig. 18.1 present some toxic effects of aflatoxins, fumonisins, ochratoxins, deoxynivalenol, and zearalenone.

18.4 Conventional Methods of Managing Mycotoxins in Cereals

In order to reduce the contamination of cereals, strict procedures are applied in the field just before cultivation, and other procedures continue throughout the stages which follow the cultivation of cereals (Fig. 18.2); these strategies help to minimize the appearance of mycotoxins on the harvested products. Good practices include the right choice of seeds, especially seeds resistant to fungal infections, the choice of fertilizers and irrigation waters, the selection of fungicides, the prevention of damage during harvest, the appropriate drying, and good storage practices. Once the contamination of cereals begins to appear, it will become inevitable because mycotoxins are very stable compounds. Several physical, chemical, and biological techniques are applied to minimize contamination.

Table 18.2 Some toxic effects of aflatoxins, fumonisins, ochratoxins, deoxynivalenol, and zearalenone

Mycotoxins	Selected organism	Sex	Age	Weight	Dose/ administration route	Exposure time	Biological sample	Damage	References
AFB_1	Mice	Male	4 weeks	–	0.75 mg/kg b.w	15 days	Liver	AFB_1 induced oxidative stress and liver injury	Rajput et al. (2021)
AFB_1	Broiler	Male	5 weeks	397.35 ± 6.32 g	100 µg/kg	4 weeks	Bursa of Fabricius	AFB_1 decreased the relative weight of bursa of Fabricius and antioxidant enzymes activities	Guo et al. (2021b)
AFB_1	Mice	Male	6 weeks	35.5 ± 1.53 g	450 µg/kg b.w	28 days	Kidney	AFB1 induced oxidative stress, an increase in apoptotic cells, and liver injury	Zhao et al. (2021)
AFB_1	Mice	–	5–6 weeks	–	450 µg/kg b.w	28 days	Liver	AFB_1 induced oxidative damage and apoptosis in the livers	Li et al. (2021a)
AFB_1	Mice	Male	6 weeks	23–28 g	0.75 mg/kg	30 days	Spleen	AFB1 induced oxidative stress and splenic apoptosis	Xu et al. (2019a)
AFB_1	Mice	Male	6 weeks	23–28 g	Lycopene (5 mg/ kg b.w) + AFB1 (0.75 mg/kg b.w)	30 days	Kidney	AFB_1 exposure increased the serum concentrations of blood urea nitrogen and serum creatinine and caused damage to the renal structure	Yu et al. (2018)
AFB_1 AFM_1	Mice	Male	–	18–22 g	AFB1 (0.5 mg/ kg) + AFM1 (3.5 mg/kg)	28 days	Kidney	Aflatoxins activated oxidative stress and caused renal damage	Li et al. (2018)

AFB_1	Mice	Male	6–8 weeks	18–20 g	10, 20, and 40 μg/kg b.w. AFB1 i.p. daily + H1N1 virus	15 days	Lung and spleen	AFB_1 exposure aggravates Swine influenza virus replication, inflammation and lung damage by activating TLR4-NFκB signaling	Sun et al. (2018)
OTA	Mice	–	6 weeks	20 ± 2 g	Orally/5 mg/kg b.w	27 days	Kidney	OTA increased levels of serum uric acid and blood urea nitrogen OTA induced degeneration of tubular epithelial cells OTA decrease the levels of antioxidant enzymes	Li et al. (2020a)
OTA	Mice	Male	9 weeks	16–18 g	5 mg/kg b.w	27 days	Heart	OTA decreased both body weight and heart weight OTA induced a decrease in heart rate OTA decreased tissue concentrations of antioxidant enzymes	Cui et al. (2020)
OTA	Rats	Male	10 weeks	230–270 g	Orally/125 and 0.250 mg/kg b.w	3 weeks	Liver and kidney	OTA induced oxidative stress	Rašić et al. (2019)
OTA	Chicken	Male	240 days	–	1.0 or 2.0 mg/kg	42 days	Bursa of Fabricius	Immunotoxicity	Bhatti et al. (2018)

(continued)

Table 18.2 (continued)

Mycotoxins	Selected organism	Sex	Age	Weight	Dose/ administration route	Exposure time	Biological sample	Damage	References
OTA	Rats	Male	10 weeks	230–270 g	Orally/0.125 and 0.250 mg/kg b.w	21 days	Kidneys and liver	OTA induced oxidative stress and reduced kidneys glutathione, and increased kidneys and liver malondialdehyde	Rašić et al. (2018)
OTA	Mice	Male	–	21 ± 4 g	Intraperitoneal injections/3.5 mg/kg b.w	–	Brain	OTA caused a significant alteration in the proliferation process, a decrease in glial cells and a significant decrease in the number of neuroblasts	Paradells et al. (2015)
FBs	Mice	Female	8 weeks		10 mg/kg b.w	28 days	Liver	Hepatotoxicity	Régnier et al. (2019)
FBs	Pig	Male	28 days	–	Orally/10 mg/kg	4 weeks	Liver and the jejunum	FBs induced an increase in weight and displayed a higher sphinganine/ sphingosine ratio	Régnier et al. (2017)
FBs	Turkeys	Female	10 weeks old	–	15 mg/kg FB1 + FB2	14 days	Serum	Disturbance of sphingolipid metabolism	Masching et al. (2016)

ZEA	Mice	Male	6 weeks	25–30 g	Orally/40 mg/kg b.w	4 weeks	Serum	ZEA induced an increase in pro-inflammatory factors, including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α	AbuZahra et al. (2021)
ZEA	Gilts		42 days	12.84 ± 0.26 kg	Orally/1.04 mg/kg	35 days	Small intestines	ZEA induced villus injuries of the duodenum, jejunum and ileum	Zhang et al. (2021a)
ZEA	Mice		6–8 weeks		4.5 mg/kg b.w	1 week	Colon	ZEA induced intestinal inflammation	Fan et al. (2018)
DON	Pigs	Male and female	–	6.87 ± 0.41 kg	Orally/1.3 and 2.2 mg/kg	60 days	Cerebral cortex, cerebellum, and hippocampus	DON induced destruction of hippocampal cell ultrastructure DON caused oxidative damage in the cerebral cortex, cerebellum, and hippocampus	Wang et al. (2020)
DON	Chickens		1 day		0, 2.5, 5, and 10 mg DON per kg die	5 weeks	Cecum	DON induced an alteration in cecal bacterial diversity and composition	Lucke et al. (2018)
DON	Chicken	Male	1 day		0.27, 1.68 and 12.21 mg/kg	36 days	Brain	DON caused lipid peroxidation, neurotransmitters secretion and affect the balance of calcium homeostasis	Wang et al. (2018)

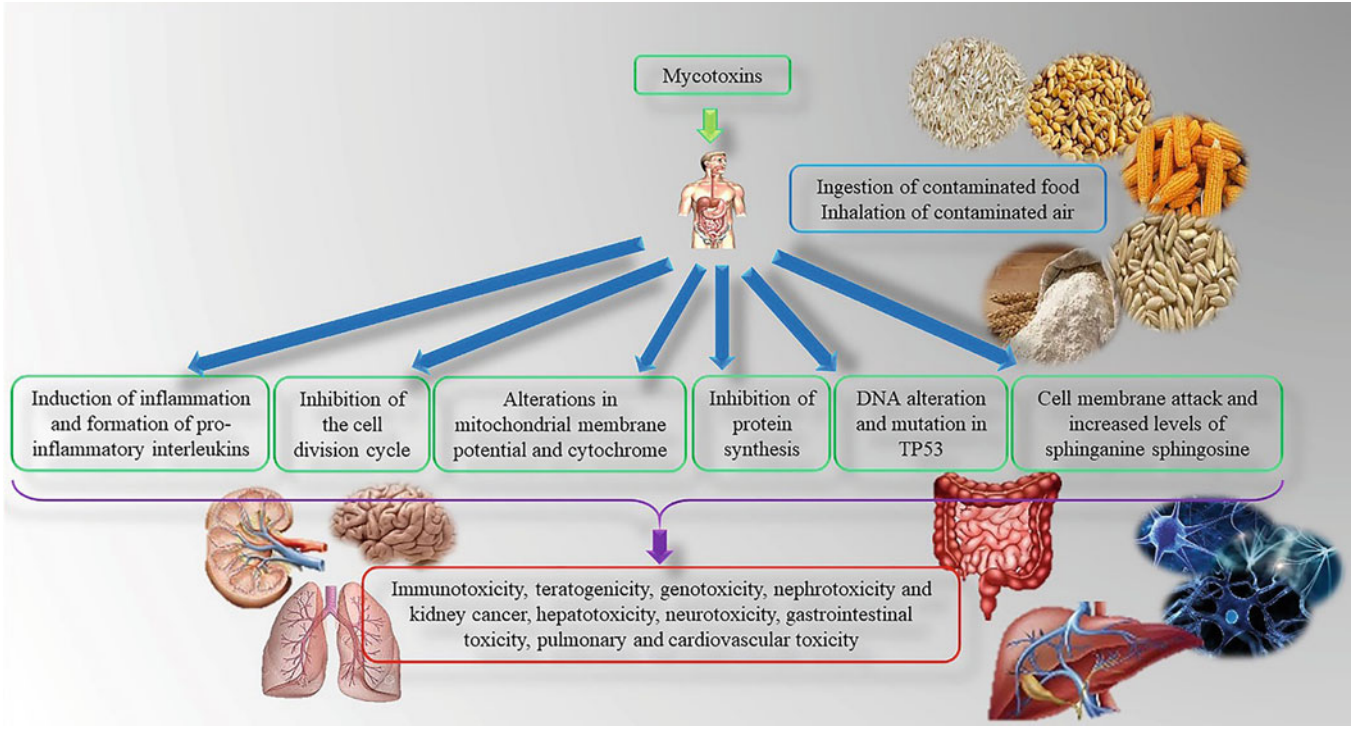

Fig. 18.1 Some toxic effects of mycotoxins in human organism

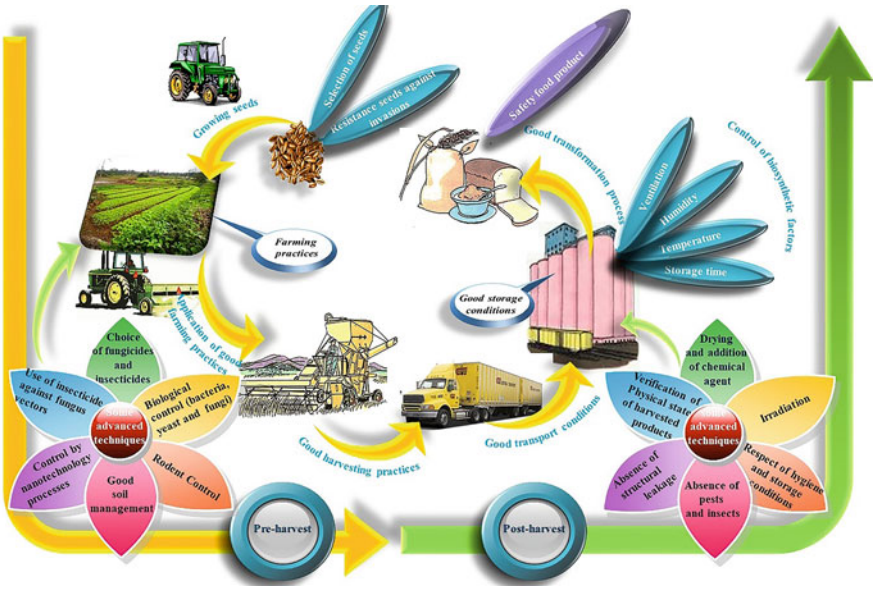

Fig. 18.2 Some necessary preventative processes before and after harvest and during the storage of agricultural products. (Reprinted from Gacem MA, Gacem H, Telli A, Ould El Hadj Khelil A (2020) Chapter 8 - Mycotoxins: decontamination and nanocontrol methods. In: Rai M, Abd-Elsalam KA (Eds) Nanomycotoxicology, Treating Mycotoxins in the Nano Way, 1st edition. Academic Press, pp 230–257)

18.4.1 Biological Methods

The detoxification of mycotoxins by biological processes has been the subject of several studies. So far, these methods are still considered promising approaches due to the absence of harmful effects. Plants and microorganisms, including bacteria, yeasts and molds, and their bioactive substances, have an excellent ability to inhibit the growth of toxigenic molds and reduce the synthesis of mycotoxins. In the case of microorganisms, some species of the genus *Bacillus* have the ability to transform DON into a less toxic compound called de-epoxy DON (DOM-1) (Li et al. 2011). In another study, *Bacillus subtilis* designated *B. subtilis* SG6 isolated from wheat kernels and plant anthers recorded a significant antifungal effect on the mycelial growth of *F. graminearum*, and this mold is one of the agents responsible for yield and quality losses in wheat and barley. *B. subtilis* SG6 is able to reduce sporulation and DON production by *F. graminearum* (Zhao et al. 2014). In Argentina, in vivo tests of two species: *B. subtilis* RC 218 and *Brevibacillus* sp. RC 263 demonstrated a significant reduction in the growth of *F. graminearum*. In comparison with the control plots, whose contamination reaches 1372 µg/kg of DON, the reduction in the growth rate of *F. graminearum* in the assays is accompanied by an absence of accumulation of DON in the ears of wheat (Palazzini et al. 2016). On peanut kernels, volatile organic compounds of *Streptomyces yanglinensis* are able to inhibit mycelial

growth and expression of aflatoxin biosynthesis by *Aspergillus flavus* and *Aspergillus parasiticus* (Lyu et al. 2020); the same result was observed in the volatile compound of *Streptomyces philanthi* RL-1-178 used as a fumigant to protect soybean seeds against *A. parasiticus* and *A. flavus* (Boukaew and Prasertsan 2020). In *A. flavus*, volatile compounds of *Alcaligenes faecalis* N1–4 isolated from tea rhizosphere soil inhibit the expression of 12 important genes in the aflatoxin biosynthesis pathway; additionally, complete inhibition of aflatoxin contamination is recorded for stored peanuts, corn, rice and soybeans (Gong et al. 2019). Besides *Bacillus* and *Streptomyces*, *Shewanella algae* (strain YM8) is highly effective against aflatoxin biosynthesis in maize samples stored at different water activity levels. *S. algae* also has significant antifungal potential against *A. parasiticus*, *A. niger*, *A. alternate*, *Botrytis cinerea*, *F. graminearum*, and *F. oxysporum* (Gong et al. 2015). Meanwhile, the phenolic compounds of *Spirulina algae* (strain LEB-18) reduced mycotoxin biosynthesis; an average reduction of 68% has been recorded for the trichothecene, deoxynivalenol and nivalenol produced by *F. graminearum* (Pagnussatt et al. 2014). These results suggest that detoxification by microorganisms is an effective approach to eliminate the negative effects of mycotoxin (Li et al. 2011). The secondary metabolites of plants have a very active fungicidal power against toxigenic molds. The essential oil of *Ocimum sanctum* L. has fungicidal and antimycotoxinogenic activity on the growth and production of zearalenone (ZEA) of *F. graminearum* (Kalagatur et al. 2015). Alternatively, ergosterol has an inhibitory effect on ZEA and DON produced by *F. graminearum* and *F. culmorum* in maize seed (Perczak et al. 2019). Nowadays, molecular techniques and genetic engineering also play a key role in the fight against toxigenic molds and their mycotoxins. Transgenic wheat expressing a barley UDP-glucosyltransferase (HvUGT13248) exhibited significantly higher resistance to disease and transformed DON to DON-3-*O*-glucoside (D3G) (Li et al. 2015), transgenic wheat expressing UDP-glucosyltransferase also converts nivalenol into the non-toxic nivalenol-3-*O*-β-D-glucoside (Li et al. 2017).

18.4.2 Chemical Methods

Several chemical methods are recommended for the same purpose, eliminate and/or prevent the appearance of mycotoxins in cereals. These techniques and practices are strictly applied to prevent their failure, increase production costs, reduce nutritional value, and prevent the generation of more toxic residues or by-products (Peraica et al. 2002). Ozone (O_3) is a powerful antifungal; it is able to destroy the fungal cell. Cell destruction is accomplished by two main pathways, oxidation of amino acids and breakdown of cell wall fatty acids. O_3 fumigation is also able to reduce spore germination and mycotoxin biosynthesis. This reduction is linked to the modification of mycotoxins structures by reaction of the functional groups with ozone (Afsah-Hejri et al. 2020). In wheat, the application of O_3 reduced DON by up to 29% (Piemontese et al. 2018). Ammonia is also a process used in the detoxification of mycotoxins, and ammonia is capable of completely decomposing OTA, however,

this process causes remarkable changes in the quality of the treated materials, in particular, color changes and a decrease in nutritional value (Omotayo et al. 2019). Chemical agents such as acids (sulfuric acid, hydrochloric acid, phosphoric acid, and acetic acid), salts (sodium chloride and sodium sulfate), and alkaline compounds (sodium bicarbonate) have an excellent aflatoxin and ochratoxin reduction potential (Jalili et al. 2010). Lactic acid is very effective in the degradation of AFB1. Even if the degradation is not complete, a decomposition of 85% is obtained at 80 °C during a treatment of 120 min. The decomposition reaction generates two by-products that are characterized by a very reduced cytotoxicity on HeLa cells line (Aiko et al. 2016).

18.4.3 Physical Methods

Among the most applied physical methods, the sorting of cereal grains is a very effective process in the detoxification of mycotoxins; in maize grains, this process can eliminate <6% of aflatoxin B1 and <5% of fumonisin B1 (Matumba et al. 2015). The reduction is strongly due to the elimination of inferior raw material. Mechanical dehulling methods of maize have the capacity to reduce more than 50% of FBs (Fandohan et al. 2006). In addition to these techniques, grain washing is a process capable of removing water-soluble mycotoxins (Wan et al. 2020). Irradiation techniques also have their place in grain detoxification. Electron Beam Irradiation (EBI) is a non-thermal method of cereal decontamination. It has less harmful effects on the environment and the nutritional value of detoxified food materials (Mousavi Khaneghah et al. 2020), in contrast, EBI reduces the germination rate of treated barley grains (Kottapalli et al. 2006). Gamma-rays also have a strong ability to destroy ochratoxin A, aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2 (Di Stefano et al. 2014).

18.5 Nanomaterials as Mycotoxin Detoxification Tools in Cereals

Nowadays, the elimination and detoxification of mycotoxins have become a challenge for the food industry. Indeed, a large number of control and prevention strategies are applied. Despite the success recorded for conventional methods of detoxification in its early stages, the limitations noted and the requirements demanded proved the failure of these methods. These methods suffer from disadvantages, such as the generation of toxic residues for humans and the environment. Moreover the biological methods require selection and a long period of growth without neglecting the high cost during their applications. The inability of conventional methods to remove mycotoxins has prompted research to innovate more potent techniques capable of destroying toxigenic fungal cells and/or blocking their mycotoxin biosynthetic pathways. In recent years, several studies have reported the advantage of NMs in the detoxification of mycotoxins; several types of NMs

have been the subject of a fungicide capable of inhibiting toxigenic molds and/or their toxin. In this part of the chapter, several types of nanomaterials are reported.

18.5.1 Detoxification by Targeting Mycotoxinogenic Molds or Adsorption of Mycotoxins

The treatment of corn with zinc oxide nanoparticles (ZnO-NPs) under the experimental conditions, described by Hernández-Meléndez and his team in 2018, demonstrated that at 100 μg/g of ZnO-NPs, significant inhibition of the growth of *A. flavus* and mycotoxin synthesis are recorded. The untreated grains presented a fungal invasion of 67%; on the other hand, the treated grains presented a moderate fungal invasion (30%). Aflatoxin production in control and treated grains was 45 ng/g and 14 ng/g, respectively (Hernández-Meléndez et al. 2018). In a more recent study, the antiaflatoxinogenic efficacy of ZnO-NPs was 93.80% in stored maize grains, and this result is recorded in the presence of 1.13 μg/kg of AFB_1 (Yousif et al. 2019). Similar results were observed with 100 μg/mL of ZnO-NPs,. More than threefold reduction (12 ng/L of AFB_1) is observed on treated samples compared to controls (46 ng/L) (Nabawy et al. 2014). It should be noted that the reducing agents used in the synthesis of ZnO-NPS play a very important role in the efficiency of NPs. The ZnNPs derived from the reducing agent NaOH exert a large antifungal potential and high efficiency in DNA disintegration of maize fungal pathogens (Kalia et al. 2021). In a liquid culture medium, ZnO-NPs prepared from *Syzygium aromaticum* demonstrated their efficacy in the regression of DON and ZEA of *F. graminearum* (Lakshmeesha et al. 2019). Anti-aflatoxigenic activity is highly dependent on reactive oxygen species (ROS) generation, Zn^{2+} release, hyphal damage, lipid peroxidation, and antioxidant response (Zhang et al. 2021b).

Silver nanoparticles (Ag-NPs) are also effective in inhibiting AFB_1 synthesis; Ag-NPs can gain access inside the fungal cell and alter genes responsible for mycotoxin biosynthesis. Deabes and his team confirmed this principle that Real Time-polymerase chain reaction (qRT-PCR) proved that Ag-NPs alter *O*-methyltransferase gene (*omt-A*) in the gene cluster responsible for the biosynthesis of AFB_1 (Deabes et al. 2018). Damage to genes responsible for mycotoxin biosynthetic pathways leads to downregulation or complete blockage of mycotoxin synthesis.

Copper trace elements are essential in cereal crops. Cu deficiency may be the cause of higher *Fusarium* incidence in wheat. The treatment of wheat kernel with powdered CuO-NPs prepared as a superabsorbent polymer demonstrated that at a dose of 200 g/ha Cu, the NPs are able to imbibe water and slowly release nutrients. This result suggests that the Cu uptake capacity of the plants has improved. CuO-NPs improve the fat, crude fiber and cellulose content of wheat grain. Note that an absence of DON was recorded on the samples treated with CuO-NPs (Kolackova et al. 2021). In another study, the ion-exchanged zeolites with Li^+ and Cu^{2+} demonstrated an excellent antifungal effect against *A. flavus* and inhibition capacity of AFB_1 (Savi et al. 2017). However, the use of CuO-NPs can be toxic to

agricultural crops; Rajput et al. demonstrated that the application of CuO-NPs cause toxicity in barley (*Hordeum sativum* distichum). Harmfulness is characterized by the formation of electron-dense materials in the intercellular space of cells and a reduction in root length (Rajput et al. 2018).

Some polymers are also considered as fungicides, chitosan is among the most studied polymers, and this compound has very good antifungal and antimycotoxinogenic activity. When applied alone, chitosan nanoparticles with an average size of 3.00 ± 0.70 nm are able to inhibit AFB_1. Chitosan is able to adsorb AFB_1 by interacting positive charges of the amino group with the negative charges of the oxygen atoms of the aflatoxins (Cortés-Higareda et al. 2019). Chitosan also has the ability to incorporate metallic NPs and other fungicides, and the resulting NMs have an excellent antifungal and antimycotoxigenic potential. Copper-chitosan nanocomposite-based chitosan hydrogels (Cu-Chit/NCs hydrogel) prepared using a metal vapor synthesis exhibits an excellent antifungal activity against *A. flavus* associated with peanut meal and cotton seeds. The activity depends on the fungal strain and the concentration of NPs (Abd-Elsalam et al. 2020). Application of these NPs at different concentrations in maize grains (under laboratory conditions and incubated at 28 °C for 28 days) inhibited *F. graminearum* growth and DON and ZEA synthesis. The encapsulation of the essential oil in NPs gives it an excellent stabilization by increasing the lifetime antifungal activity of CMEO by a gradual release of antifungal constituents of Ce-CMEO-NPs (Kalagatur et al. 2018). In another study, Chitosan nano-biopolymer-entrapped Coriandrum sativum essential oil (Ce-CSEO-NPs) with a size ranging between 57 and 80 nm exerts good antifungal activity against several stored rice contamination molds; complete inhibition is recorded against *A. flavus*, *A. niger*, *A. fumigatus*, *A. sydowii*, *A. repens*, *A. versicolor*, *A. luchuensis*, *Alternaria alternata*, *Penicillium italicum*, *P. chrysogenum*, *P. spinulosum*, *Cladosporium herbarum*, *F. poae*, and *F. oxysporum* Chitosan nanoemulsion showing insignificant inhibition of AFB_1 secretion (13.06%) (Das et al. 2019). Other essential oil encapsulates in Ce-NPs have also proven to be effective in inhibiting mycotoxin synthesis. Fumigation of two samples of maize (150 g) with 1.0 and 2.0 µL/mL of *Origanum majorana* essential oil encapsulated into chitosan nanoemulsion (Ce-OmEO-NPs) and inoculated with 10^6 spores/mL of *A. flavus* demonstrated relevant results. A total absence of AFB_1 is recorded in the two maize samples after a storage period of 6 months, however, the controls recorded contamination of approximately 26.17 and 25.37 µg/kg (Chaudhari et al. 2020). Propolis, known for its medicinal properties, can also be incorporated into chitosan. The application of coatings based on chitosan and propolis on figs under semi-commercial conditions have shown encouraging results. After 12–15 days of storage of figs infected with spores of *A. flavus* and treated with NPs, a decrease in aflatoxin synthesis of <20 ppb is obtained compared to the control which recorded a level of 250 ppb. The sensory quality was acceptable, however, the antioxidant activity increased (Aparicio-García et al. 2021).

Carbon-based nanostructures have impressive advantages in the fight against mycotoxins. Graphene oxide GeO is among one of these promising materials, and it has excellent adsorption property. In vitro, tests have demonstrated that GeO

(10 μg/mL) is capable of adsorbing AFB_1, ZEA, and DON. The maximal removal efficiency was attained at 65% for 25 ng/g DON and 90% for 6 and 0.5 ng/g of ZEA and AFB_1, respectively. The adsorption capacity of GeO was 1.69 mg/g, 0.53 mg/g, 0.045 mg/g for DON, ZEA, and AFB1, respectively (Horky et al. 2020). Reduced graphene oxide-gold nanoparticle (rGO-AuNP) also exhibits good capacity and selectivity in the adsorption of AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2 from wheat and maize samples. The recovery of mycotoxins is related to the concentration of the nanocomposite, and it increased from 48.5% to 106.6% when the quantity of rGO-AuNPs increased from 5 to 15 mg. However, a decrease in recoveries is recorded above 15 mg of rGO-AuNPs (Guo et al. 2017).

The application of magnetic nanomaterials in the removal of mycotoxins in food matrices could be attractive. These types of NMs have good adsorption and separation ability due to magnetic susceptibility (Targuma et al. 2021), and are used in the detection of mycotoxins in food samples, suggesting their ability in the removal of mycotoxins. Iron oxide carbon nanocomposites prepared from bagasse with a size range of 60–300 nm have an excellent capacity in adsorbing AFB_1, of 200 ppm, and the equilibrium time was 115 min and 150 min at pH 3 and pH 7, respectively. According to the researchers, the prepared adsorbent can be used as an alternative to activated carbon to detoxify poultry feed (Muhammad and Khan 2018). Magnetic carbon nanocomposites prepared from maize wastes have good capacity in the removal of AFB_1. Nearly 90% removal of AFB_1 was achieved, the equilibrium time depending on pH is 96 min and 180 min at pH 7 and pH 3, respectively (Zahoor and Khan 2016). In another study performed on oil system with an initial concentration (0.2 μg/mL) of AFB1, magnetic mesoporous silica prepared from rice husk is able to adsorb 94.59% of mycotoxin (Li et al. 2020b). Magnetic nano-zeolite (MZNC) can adsorb mycotoxins better than the natural zeolite. 50 mg of the nanocomposite removed >99% of Afs, 50% of OTA, 22% of ZEA, and 1.8% of the DON from the contaminated sample (Karami-Osboo et al. 2020).

18.5.2 Detoxification of Mycotoxins by Photocatalysis

The photocatalytic nature of NMs enabled these materials to be involved in the degradation of mycotoxins. The process of mycotoxin degradation by photocatalytic reactions is very encouraging, this process is characterized by its low cost and respect for the environment. The process of photocatalysis involves a chemical reaction after absorption of photons, and the reaction is based on the process of generating pairs of electrons ($\boldsymbol{e}^-$) and holes ($\boldsymbol{h}^+$) in the NM (photocatalyst) exposed to light. The electrons and the holes formed lead to reduction and oxidation reactions of the molecules adsorbed on the surface of the NMs. Along with this process, it is observed that during photocatalytic degradation, other reactive oxygen species (ROS) can also form, it is superoxide ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) (Murugesan et al. 2021). Formation of ROS species is shown in Fig. 18.3.

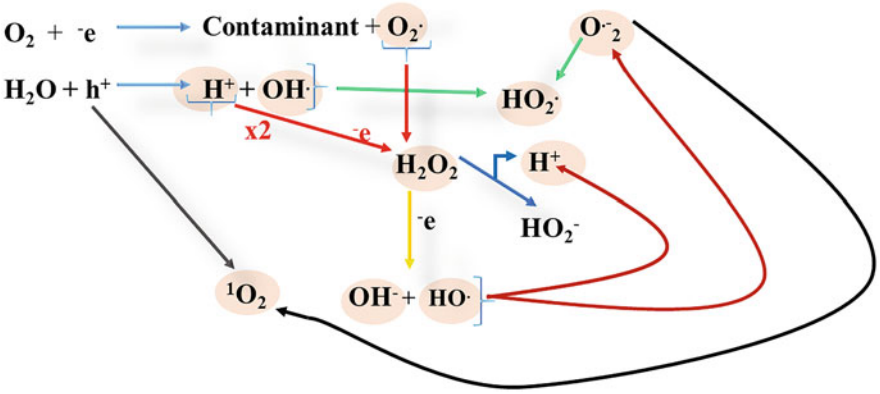

Fig. 18.3 Formation of ROS Species

He developed a very effective nanomaterial, it is composed of titanium dioxide (TiO_2) doped with cerium (Ce) (Ce-TiO_2). Under ultraviolet irradiation (λ = 254 nm), Ce-TiO_2 nanomaterials improve the photocatalytic activity for DON in aqueous solution ($\lambda = 254$ nm). Different levels of Ce doping on pure TiO_2 demonstrated different Ce-TiO_2 photocatalytic degradation effects. The degradation rate can reach 96% using 0.5Ce-TiO_2 after 240 min. The exploration of the main ROS active in the process of DON degradation indicates that the hole plays a crucial role in the photocatalytic reaction than $OH^{\bullet}$. Moreover, the small CeO_2 particles produced on the TiO_2 particles caused by Ce doping play a co-catalytic effect on DON degradation following the generation $O_2^{\bullet-}$. The two possible degradation intermediate products are $C_5H_8O_3$ and $C_{17}H_{18}O_6$ (He et al. 2021). A Schematic illustration of DON degradation under ultraviolet irradiation is shown in Fig. 18.4a. The degradation of DON in an aqueous solution by the photocatalytic activities of the dendritic-like α-Fe_2O_3 under visible light irradiation ($\lambda > 420$ nm) demonstrated that dendritic-like α-Fe_2O_3 could adsorb more DON than that in commercial α-Fe_2O_3. The degradation rate is estimated at 90.3% in 2 h at an initial concentration of 4.0 μg/mL of DON. During the photoreaction over α-Fe_2O_3, the morphology of the dendritic-like α-Fe_2O_3 absorbs more sunlight and provides more electrons and the holes. These lead to the formation of active radicals such as $O_2^{\bullet-}$and $OH^{\bullet}$, which could react with the active site of DON and form two intermediate products (Wang et al. 2019). In another study, three degradation products were identified, namely $C_{15}H_{20}O_8$, $C_{15}H_{20}O_7$, and $C_{15}H_{20}O_5$, under simulated sunlight using UCNP@TiO_2 (6 mg/mL). $OH^{\bullet}$, h^+ and $O_2^{\bullet-}$ are the main ROS produced in the photocatalytic reaction. The application of UCNP@TiO_2 in the reduction of DON in wheat demonstrated a decrease in the detoxification compared to that of standard DON. It may be due to the combination of mycotoxin with starch, protein, and other macromolecules of wheat. On the other hand, the light cannot be evenly diffused on the contaminated wheat, which also affects the degradation efficiency (Wu et al. 2020b). The principle and process of photocatalysis reaction between UCNP@TiO_2

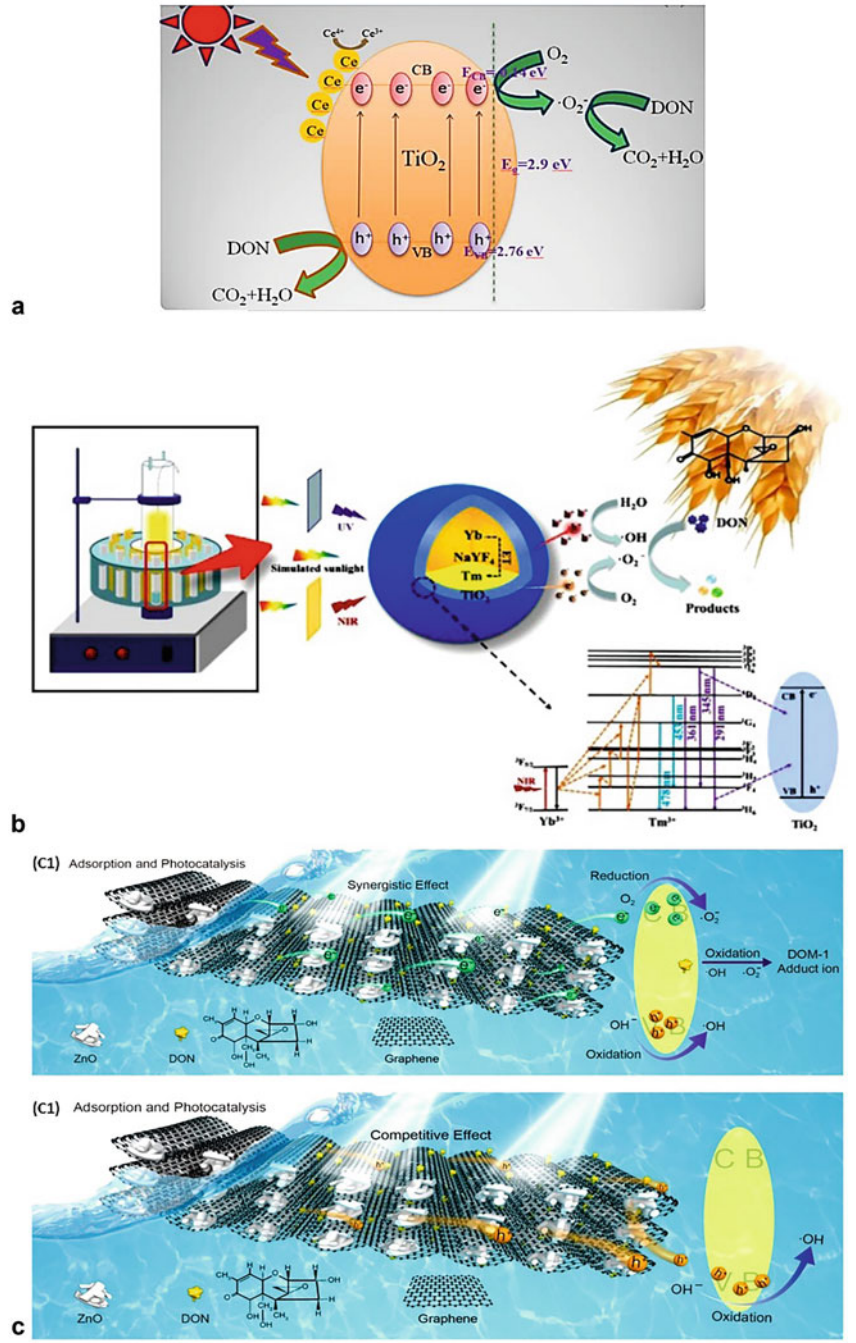

Fig. 18.4 (**a**) Schematic illustration for the charge separation and transfer of Ce-TiO_2 in the process of DON degradation under UV light irradiation (Reprinted from He P, Zhao Z, Tan Y, E H, Zuo M, Wang J, Yang J, Cui S, Yang X (2021) Photocatalytic degradation of deoxynivalenol using cerium doped titanium dioxide under ultraviolet light irradiation. Toxins 13: 481. Open

and DON are described in Fig. 18.4b. Additionally, the photocatalytic degradation of DON using graphene/ZnO hybrids in aqueous suspension is described in Fig. 18.4c.

A recent study investigated the photocatalytic activity of ZnO-NPs in the degradation of AFB_1 in an aqueous solution under UV light. Complete removal of AFB_1 (10 μg/L) by 0.10 mg/mL of NPs has been recorded after 60 min under UV irradiation. Application of NP in soymilk (5 mg ZnO-NPs to 50 mL soymilk with 10 μg/L of AFB_1) completely removed AFB_1 (91.53%) after 60 min under UV irradiation with no significant effect on its overall acceptability, which suggests its application in liquid foodstuffs (Raesi et al. 2022). Similarly, the study of the effect of ZnO-NPs on fumonisin accumulation by *F. proliferatum* both in vitro and in situ demonstrated a significative result. With ZnO-NPs concentrations of 0.8 and 8 g/L at 25 °C, 21 days, and under darkness or photoperiod incubation, a high reduction (84–98%) occurred after 14 days under photoperiodic incubation. Under the in-situ assay, the evaluation of the effect of ZnO-NPs on FB_1, FB_2, and FB_3 rates on irradiated maize grains (adjusted to 0.995, 0.98, and 0.97 aW) in darkness at 25 °C during 21 days demonstrated a reduction of FBs rates. At 0.8–2 g/kg and 0.98–0.995 aW, ZnO-NPs reduce total FBs accumulation by 71–99%, suggesting that ZnO-NPs could be applied in maize grains to control phytopathogenic and toxigenic fungi such as *F. proliferatum* and to reduce fumonisins accumulation (Pena et al. 2022). The photocatalytic graphitic carbon nitride ($g\text{-}C_3N_4$) could induce photocatalytic effect on ZEA UV lamp ($\lambda = 254$ nm). Under experimental conditions, g-C3N4 degrades at a rate of 50% of ZEA in real powder samples (Li et al. 2021b). Patulin degradation in an aqueous solution is also possible by nitrogen-doped chitosan-TiO_2 nanocomposite under UV; 500 μg/kg was completely degraded within 35 min. This improved degradation compared to TiO_2 nanoparticles and chitosan-TiO_2 nanocomposite is linked to the reduction of the average particle size of TiO_2 nanoparticles due to: (1) the introduction of nitrogen and chitosan, the structure obtained facilitates the movement of ^{-}e from the structure to the surface, thereby reducing the probability of recombination with holes; (2) the introduction of nitrogen and chitosan increase the surface of the nanocomposite which is beneficial to the adsorption of toxin; (3) the introduction of nitrogen and chitosan improved the photoresponse ability of TiO_2 nanoparticles and enhanced its photocatalytic activity (Huang and Peng 2021). Activated carbon-supported TiO_2 catalyst (AC/TiO_2) has an excellent efficiency for degradation of AFB_1 under UV–Vis light in comparison

Fig. 18.4 (continued) Access journal). (**b**) Principle and process of photocatalytic degradation of DON using $NaYF_4$:Yb, Tm@TiO_2 nanoparticles (Reprinted from Wu S, Wang F, Li Q, Wang J, Zhou Y, Duan N, Niazi S, Wang Z (2020) Photocatalysis and degradation products identification of deoxynivalenol in wheat using upconversion nanoparticles@TiO2 composite. Food Chem 323: 126823). (**c**) Schematic drawing illustrating synthetic route and the mechanism of charge separation and adsorption-photocatalytic process over graphene/ZnO hybrid photocatalysts under UV light irradiation (Reprinted from Bai X, Sun C, Liu D, Luo X, Li D, Wang J, Wang N, Chang X, Zong R, Zhu Y (2017) Photocatalytic degradation of deoxynivalenol using graphene/ZnO hybrids in aqueous suspension. Appl Catal B 204: 11–20)

with TiO_2. $OH^{\bullet}$ and h^+ play an important role in the degradation of AFB1 (Sun et al. 2019). Magnetic graphene oxide/TiO_2 nanocomposite (MGeO/TiO_2) is able to reduce AFB_1 in corn oil, and the quality of the nanocomposite-treated oil was acceptable after 180 days of storage (Sun et al. 2021). Table 18.3 demonstrates the efficiency of nanomaterials in the elimination of mycoroxins in food.

18.6 Factors Affecting Mycotoxin Detoxification by NMs

18.6.1 Effect of Temperature

Temperature plays an essential role in the adsorption and photocatalytic reactions of mycotoxin detoxification. In most of the cases studied, the rate of elimination of mycotoxins increases with the rise in temperature, suggesting that this factor contributes to the photocatalytic degradation of mycotoxins by enhancing the adsorption and activity of free radicals by increasing their interactions with mycotoxins (Huang and Peng 2021), AFB_1 removal by magnetic graphene increased from 81.60% to 95.64% with an increase in temperature from 25 to 60 °C (Ji and Xie 2020). However, in the case of magnetic mesoporous silica, a higher temperature can have a significant effect on the specific surface area and adsorption of AFB_1. A temperature greater than or equal to 100 °C affects the formation of micelles and increases the degree of aggregation of silicates, consequently a decrease in the specific surface and the opening rate of magnetic mesoporous silica (Li et al. 2020b).

18.6.2 Effect of the Nature of the NMs and Their Quantity

The efficiency of mycotoxin elimination by NMs strongly depends on the type of NM and its nature. This difference in elimination resides in the difference between the binding patterns established between the NM and the mycotoxin. In addition, in Raesi's study, ZnO-NPs demonstrated better AFB_1 removal compared to other metallic NPs (Fe_2O_3, MnO_2 and CuO). High generation of free radicals due to the higher band gap energy of ZnO-NPs is the reason why these NPs have better photocatalytic activity. However, the low adsorption of mycotoxins could be highly reversible and, therefore, a reduction in elimination (Raesi et al. 2022). It is also reported that ZnO contains a higher photocatalytic activity than TiO_2 due to its high capacity for generating electrons and holes compared to TiO_2 (Štrbac et al. 2018). For a better detoxification of mycotoxins, the quantity of NM must be adjusted in an optimal way; to not waste the NMs and to establish a maximum adsorption, as demonstrated in the Karami-Osboo study, the percentage of mycotoxins' recovery is no longer perceptible when the quantity of magnetic zeolite nanocomposite exceeds 50 mg (100 and 150 mg) (Karami-Osboo et al. 2020), similar results are observed in Raesi's study. An increase in the concentration of ZnO-NPs beyond 10 mg/mL does not lead to a significant change in the degradation of AFB_1 (Raesi et al. 2022). During the photocatalysis of mycotoxins, the increase in the quantity of the

Table 18.3 The effectiveness of some nanomaterials in the elimination of mycotoxins in food

Mycotoxin	Nanomaterial	Synthesis method	Percentage of elimination	Removal time	Sample matrix	Detoxification mechanism	References
DON	Ce-TiO_2	Sol-gel method.	96	4 h	Aqueous solution	Ultraviolet light irradiation	He et al. (2021)
AFB_1	Magnetic graphene oxide/TiO_2 (MGO/TiO_2) nanocomposite	Hydrothermal synthesis	96.4	120 min	Corn oil	Ultraviolet light irradiation	Sun et al. (2021)
Patulin (PAT)	Aerogel doped by sulfur-functionalized graphene oxide	/	89	9 h	Apple juice	Adsorption	Liu et al. (2021)
PAT	Triethylene tetramine-modified water-insoluble corn flour caged in magnetic chitosan resin (TETA-WICF/MCR)	/	92.86	/	Apple juice	Adsorption	Guo et al. (2020)
Afs OTA ZEA DON	Magnetic zeolite nanocomposite (MZNC)	/	99 50 22 1.8	/	Barley flour	Adsorption	Karami-Osboo et al. (2020)
AFB_1	Magnetic mesoporous silica (MMS)	Heat treatment	94.59	2 h	Oil system	Adsorption	Li et al. (2020b)
DON	Upconversion nanoparticles@TiO_2	/	74.15% o	30 min	Aqueous solution	NIR light	Zhou et al. (2020)
Ustiloxin A	Wormlike graphitic carbon nitride (g-C_3N_4)	Pyrolysis method	86.1	80 min	Aqueous suspension	Visible light irradiation	Wu et al. (2020a)
AFB_1	CdS/WO_3	Hydrothermal method	/	100 min	Aqueous solution	Visible light irradiation	Mao et al. (2019)
AFB_1	AC/TiO_2	Hydrothermal method	90	30 min	Aqueous solution	Mercury lamp	Sun et al. (2019)

(continued)

Table 18.3 (continued)

Mycotoxin	Nanomaterial	Synthesis method	Percentage of elimination	Removal time	Sample matrix	Detoxification mechanism	References
DON	Dendritic-like α-Fe_2O_3	Hydrothermal method	90.3	2 h	Aqueous solution	Visible light irradiation	Wang et al. (2019)
PAT	Magnetic multi-walled carbon nanotube (MWCNT)	/	88.2	60 min	Aqueous solution	Adsorption	Zhang et al. (2019)
AFB_1 AFB_1	TiO_2 immobilized on a glass support	/	≥99.4 ≥99.2	4 min	Peanut oil	UV and visible irradiation	Magzoub et al. (2019)
PAT	UiO-66(NH_2)@Au-Cys	Heat treatment	87	70 min	Apple juice		Liu et al. (2019)
DON	Upconversion nanoparticles@TiO_2	High-temperature thermal decomposition method	72.8	90 min	Wheat	Xe lamp (200–2500 nm)	Wu et al. (2019)
AFB_1	ZnO-NP	/	93.80 96.42		Stored maize Sea maize grain		Yousif et al. (2019)
AFB_1	Photocatalytic reactor consisting of a glass tube coated with TiO	/	60.41	/	Peanut oil	UV light	Xu et al. (2019b)
AFB_1	Nanosized g-C_3N_4 sheets	Heat treatment	~70.2% t	~20 min	Aqueous suspension	Visible light irradiation	Mao et al. (2018a)
AFB_1	Flower-shaped zinc oxide (ZnO) nanostructures			1 month	Maize		

		Simple aqueous precipitation strategy at room temperature					Yousif et al. (2019)
AFB_1	WO_3/RGO/g-C_3N_4	Hydrothermal method and photocatalytic reduction	92.4	120 min	Aqueous solution	Visible light irradiation	Mao et al. (2018b)
DON	Graphene/ZnO hybrid	Hydrothermal method	99	30 min	Aqueous suspension	Irradiation of UV light	Bai et al. (2017)
DON ZEA HT-2 T-2 FB1 and FB2	Magnetic graphene oxide nanocomposites	/	69.57 67.28 57.40 37.17	5.2 h	Palm kernel cake	Adsorption	Pirouz et al. (2017)
AFB_1	Sc-doped $SrTi_{0.7}Fe_{0.3}O_3$	Complex precursor route	88.2	2 h	Water	Visible light	Jamil et al. (2017)
PAT	Magnetic Fe_3O_4@CTS nanoparticles were coated with inactivated *C. utilis* CICC1769 cells	/	91.5	15 h	Orange juice	Absorption	Ge et al. (2017)
PAT	Chitosan-coated Fe_3O_4 particles	/	99.95	60 min	Fruit juice	Absorption	Luo et al. (2017)

photocatalyst (NMs) leads to a continuous elimination of patulin, however, an excessive concentration of NM results in agglomeration and a dispersion or decrease of incident light and, therefore a reduction in the rate of mycotoxin elimination (Huang and Peng 2021). In addition, the accumulation of metal oxide NPs can give rise to particles of microscopic size and cause a decrease in elimination (Raesi et al. 2022).

18.6.3 Effect of UV Irradiation

UV light plays a very important role in the detoxification of mycotoxins as this light participates in the formation of ROS, such as hydroxyl ($OH^{\bullet}$) and superoxide ($O_2^{\bullet -}$) radicals, and therefore, mycotoxin degradation occurs through reactions with ROS (Raesi et al. 2022). Moreover, during photocatalysis, an increase in the power of UV light increases the degradation of mycotoxins. This phenomenon is attributed to the nature of nanocomposites which require more energy in order to increase the generation of photogenic electrons and holes (Huang and Peng 2021).

18.6.4 Effect of Initial Mycotoxin Concentration

Knowledge of the concentration of mycotoxins in the samples to be detoxified is one of the key success factors for detoxification. As demonstrated in Raesi's study, detoxification efficiency decreases with increasing AFB_1 concentration. 100% and 87.72% of rate elimination are recorded at initial concentrations of 10 and 30 μg/L of AFB_1, respectively (Raesi et al. 2022). The correlation between mycotoxin concentration and photocatalytic performance of NMs is related to the adsorption of mycotoxins on the surface, i.e., the decrease in detoxification may be due to a confined vacant amount of superficial active sites for adsorption and/or reaction with toxins. Moreover, increasing the concentration of mycotoxins can lead to the formation of organic intermediates, as these compounds can defy the adsorption of active sites (Jamil et al. 2017). During the elimination of AFB_1 by magnetic graphene and magnetic graphene oxide, an increase in the concentration of the mycotoxin in the reaction medium leads to the saturation of surfaces of the adsorbent by the occupation of the available active sites, and this leads to a decrease in the elimination efficiency of AFB_1 (Ji and Xie 2020).

18.6.5 Effect of pH

The pH of the reaction medium affects several parameters, such as the nature of the mycotoxins, the localized charges on the surface of the NMs, and the aggregation of the NMs. Upon detoxification of AFB_1 by Sc-doped $SrTi_{0.7}Fe_{0.3}O_3$ in the visible light, the continuous increase in pH also increases the rate of toxin reduction; during this reaction, the maximum reduction is obtained at pH 9. Beyond this pH, a

progressive decrease in detoxification is noticed. The reduction in detoxification rate is explained by the impact of pH on (1) the charge of NM surfaces, as negative charges on the surface of NMs cause electrostatic repulsion and decrease adsorption, (2) the rate of adsorption of mycotoxins on the NMs, (3) the generation of ROS. It should also be noted that the weak detoxification in the acid medium is due to the strong adsorption of mycotoxins on the NMs. The accumulation of mycotoxins on the NMs prevents the photoexcitation of the particles by decreasing the access of visible light to reach the surface of the catalyst (Jamil et al. 2017). In the study by Li et al., magnetic mesoporous silicas are significantly influenced by changes in pH. The latter affects the polymerization of the silicate, which has an impact on the structure of the NMs channel. At pH 11, the unit adsorption capacity and adsorption rate of AFB_1 were maximal (Li et al. 2020b).

18.6.6 Effect of Reaction Time

The effects of the reaction time during the preparation of the NMs considerably influence the structure of the NMs as well as their ability to adsorb mycotoxins. The preparation of the magnetic zeolite nanocomposite demonstrated that a preparation time of 24 h was the optimal duration for maximum adoption of AFB_1. The increase in time increases the specific surface, the unit adsorption capacity, and the adsorption rate of AFB1. In addition, the increase in duration improves the stability of NMs and leads to an increase in adsorption stability (Li et al. 2020a, b). Additionally, adjusting time during detoxification reactions enhances mycotoxin removal. As demonstrated by Ji and Xie when removing AFB_1 in oil, the increase in the contact time allows the superficial sites of the NMs (Magnetic graphene) to be occupied by the toxins and thereafter, the adsorption becomes progressively slower until saturation (Ji and Xie 2020).

18.7 Conclusion

The contamination of cereals by mycotoxins has become a worldwide concern, and the deterioration has caused inestimable economic losses without neglecting the toxicity caused to human and animal health. The alteration is not limited only to the raw materials but spread through food processing chains. Food scarcity is one of the major challenges the world is expected to face in the near future due to climate change, environmental pollution, and population explosion. Faced with these problems, the main objective of food processing industries is the development of better food processing, preservation, and storage techniques in order to provide safe and high-quality food. These challenges have driven researchers and research to develop effective, healthy, and less expensive technologies capable of destroying these contaminants and their origins. Despite the high costs incurred and the numerous research and scientific publications affirming the success of conventional approaches, these techniques suffer from several drawbacks, in particular, the

generation of toxic residues in foodstuffs, the reduction in the nutritional value of foodstuff, the impossibility of reducing mycotoxins completely or to acceptable levels, and being disrespectful to the environment. Other classic techniques are encouraging, but they only apply to model systems and depend on consumer confidence and opinion.

This marked failure of conventional techniques has pushed researchers towards new, very modern strategies. The new strategies innovated by science are based on the different structured NMs. Nanoparticles could constitute a new antifungal ingredient likely to be added in the agri-food sector for the control of toxigenic fungi and their main associated mycotoxins. Therefore, nanobiotechnology is an opportunity capable of overcoming the problem and helping to provide high quality, stable, nutritious, and safe food products. In addition to removing mycotoxins, nanotechnology can ensure a sustainable future by providing crops with better growth, nano-fertilizers and nano-pesticides, and improved crop yield.

Currently, the main concerns of food and nutrition scientists and regulatory and control agencies are the advantages and disadvantages of NMs for the consumer and his health because there is little information on the absorption, distribution, metabolism, and excretion after oral administration of NMs. It is also essential to test the toxicity of NMs on human health and the risks related to their exposure, and to test the toxicity of NMs on the environment. This requires more effort and investment to achieve the commercialization of cereals treated with nanomaterials.

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