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Recent technologies to improve disease resistance against *Botrytis cinerea*

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Abstract

Gray mold (*Botrytis cinerea*), is a significant postharvest disease of horticultural crops and can cause massive economic losses. It affects various plant organs including fruits, flowers, leaves, storage organs and shoots. Chemical fungicides are used as the first measure by most plant growers. However, due to their impact on the environment, the resistance of the pathogen to various chemicals, and consumer interest in organic consumption, alternative control strategies are increasingly being used. Recent studies are discussed on the improvement of fruit physiological aspects such as plant hormones and their signal transduction pathways playing a significant role in the defense against the necrotrophic fungus. Various phytohormones and plant elicitors can stimulate the defense response and control the outbreak of infection. Thus, these signaling molecules may be responsible for plant resistance and serve as substitutes for fungicides. The use of nanotechnology to enhance plant resistance to pathogens and the omics approach to induce disease resistance are pioneering studies to combat the disease. Crop genomics, metabolomics and transcriptomic studies are making important contributions to the identification of pathogen-plant interactions and the study of control mechanisms. The review suggests that the use of an integrated pest management approach could help overcome the high post-harvest food losses that the world has been facing recently. © 2022 Department of Agricultural Sciences, AIOU

Keywords: Disease resistance, Fungicide, Gray mold, Horticultural crop, Plant hormone

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Introduction

Botrytis cinerea is the anamorph of *Botryotinia fuckeliana* (Heinrich Anton de Bary) and is an airborne pathogen that affects over 200 crop species worldwide (Williamsonet al., 2007). The fungus is a threat to all horticultural crops, causing massive losses in the fields and greenhouse at the pre-and postharvest stages or any developmental stages of horticultural crops. Small fruits such as strawberries are particularly affected by infection with the pathogen (Collado & Viaud, 2016). A genus of fungal species with a wide range of biology, ecology, morphological characteristics, hosts and typically classified as necrotrophic (Hong et al., 2016). *Botrytis cinerea* is the most detrimental to developed or senescent tissues of horticultural crops and usually enters these crops at early stages of crop development and stays dormant for a long term before hastily killing tissues under favorable environment when physiology of the host plant changes resulting in massive losses. Besides *B. cinerea* causes annual economic losses that easily surpass $10 billion, with estimates ranging as high as $100 billion. Currently, the fungus has been ranked as the second most important plant pathogen due to its commercial and scientific significance. Controlling this fungus is difficult due to its wide host range, several attack modalities, and ability to persist in both asexual and sexual stages (Hong et al., 2012). To date, synthetic fungicides have been the primary means of controlling *B. cinerea*. However, due to the emergence of resistant strains and dangers to human health and the environment, standard fungicides are not an appropriate management method. It is required to develop such disease management strategies that are ecofriendly and cost-effective. Various elicitors and their role in defense mechanisms of the host plants have been recognized and important against fight in *Botrytis cinerea* (Abuqamar et al., 2016). Hormones are produced as secondary metabolites by plant pathogenic microorganisms, and a few pathogenic microorganisms may be able to conquer defense responses of the host plant by interfering with hormone signaling of the host using an aggregation of effectors. Plant infections create effectors that directly alter the hormonal level or mechanisms of hormone signaling network, allowing them to control plant resources for their benefit (Ahlem et al., 2012). The nature of these interactions affects the hormone signaling pathways that integrate functions including nutrient acquisition, defense mechanism, ensuring growth, and development, as well as the outcome of host-pathogen interactions. The interactions between plant and microorganism can be altered by directly applying plant hormones or interfering with mechanisms of the hormone signaling network to evaluate responses affecting host production and defense mechanisms (Abuqamar et al., 2016). Therefore, a clear perception of plant elicitor and hormonal responses to *Botrytis cinerea* will facilitate the successful management of grey mold and retains plant productivity as well. This review of recent technologies such as molecular and omic approaches to induce resistance in plants against *Botrytis cinerea*.

Physiological aspects of resistance

Identification and modulation of host disease resistance mechanisms is becoming increasingly important to minimize losses as chemical treatment of crops is limited and pathogen resistance to fungicides becomes more common. Pre and postharvest cultural measures can help strengthen natural defenses (Atwell et al., 2015). Other resistance factors include senescence and membrane change, cuticle barrier, cell wall barrier, hormonal role in mitigating *B. cinerea*, limitation of disease within internal tissues of the host, and defense proteins are the major ones. Consequently, resistance to *B. cinerea* infection can be influenced by both genetic resistance and environmental factors that affect the physiological state of host tissues. Any treatments that accelerate the onset of senescence may increase the risk of *B. cinerea* infestation and may also have the opposite effect. Plant hormones and chemicals that alter the oxidative status of tissues can be explained primarily by their effects on senescence and senescence-related processes (Bartlett et al., 2002). To prevent the invasion and spread of diseases, the plant employs several physical and chemical barriers. The balance between host defense and the ability of the pathogen to overcome these barriers is influenced by the environment. However, traits such as senescence rate, the extent of chemical and structural defenses, and cell wall structure are genetically determined (Jiang et al., 2016). Horticulturists, in collaboration with plant breeders, must take steps to limit gray mold losses to crops, each of which tips the balance in favor of host plant resistance. Induced resistance is a very effective and inexpensive plant defense against pathogen attack. Plants activate the intrinsic immune system that occurred as a result of plant-pathogen coevolution to prevent pathogen infection (James et al., 2016). In recent years, many studies have linked plant growth-promoting rhizobacteria (PGPRs) that can improve plant vigor by boosting defenses against a wide pathogenic range (Hong et al., 2012).

Phytohormones and elicitors induce resistance against *B. cinerea*

1. Plant hormones role in the induction of resistance to *B. cinerea*

Plant hormones are substances that occur naturally. Low quantities of plant hormones that control plant growth and development at several growth phases include auxins, gibberellins, abscisic acid, cytokinin, and ethylene. Some hormones that help regulate the plant defense system are salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) that are mainly reported to regulate plant defense against various pathogens and pests. Brassinosteroids (BRs) and abscisic acid (ABA) are the other phytohormones that can enhance plant immunity against fungal attack through transcription factors (TFs) to regulate transcription rate of genetic information flow from DNA mRNA or camalexin biosynthesis (a phytoalexin) and callose deposition (Grant-Downton, 2016).

2. The role of plant hormone signaling pathways in reaction to *B. cinerea*

Resistance to necrotrophs depends largely on the interplay of phytohormones such as JA, SA, ET, BRs and ABA, which can play a fundamental role to control *B. cinerea*. These phytohormones are interwoven in an enormous and multilayered network. Fruit ripening can also influence the disease susceptibility of fruits (Fig. 1). During the ripening process, many changes occur in fruit such as activation of ethylene and synthesis of plant hormones, loosening of cell wall, pH and cuticular changes, and intensification of soluble sugars, deterioration of antifungal complexes that bring gray mold from its dormant state to a necrotrophic pathogenic form. During fruit ripening, significant physiological changes and cell wall models (Wang et al., 2022) occur with decreases in phytoalexin content and biosynthesis of phytoanticipins in the cuticle (Barik et al., 2010), inducible host defense response, pH changes and increase in soluble sugars in response to biotic stress through hormonal changes of ethylene, jasmonic acid, abscisic acid, and salicylic acid (Arya et al., 2020). All phytohormones, ABA and ethylene stimulate the ripening process and can also influence the host defense response with JA and ethylene resistance and induce susceptibility as maturation progresses. Ethylene has a dual opposing role and is considered a key hormone in crop postharvest; it induces fruit susceptibility at climacteric and also takes a part in defense along with jasmonic acid. Therefore, the timing of ethylene release, differentiation, and ethylene levels are expected to be critical for the outcome of susceptibility or resistance (Fig. 2). Signaling molecules may be responsible for plant resistance and act as substitutes for fungicides against fungal attack and postharvest biotic stress, such as jasmonic acid and salicylic acid (SA) (Romanazzi et al., 2016). SA showed a reduction in the incidence of fungal attack and improved the resistance of horticultural fruits. The resistant symptoms showed comparatively smaller lesion diameter, dehydrogenases and different types of proteins (antioxidant proteins and heat shock proteins) were detected (Romanazzi et al., 2016). Tomato fruits treated with exogenous MeJA exhibited higher resistance and antioxidant activity to scavenge excess reactive oxygen species (ROS) (Liu et al., 2021). They also reduced oxidative damage to proteins and stimulated defense responses, etc. Previous studies have shown that plant hormone such as JA (tomato fruits and Chinese bayberries), SA (sweet cherries), Calcium (grapes) can be used as substitutes for fungicides (Romanazzi et al., 2016; López-Galiano et al., 2018; Wu et al., 2020; Chan et al., 2021).

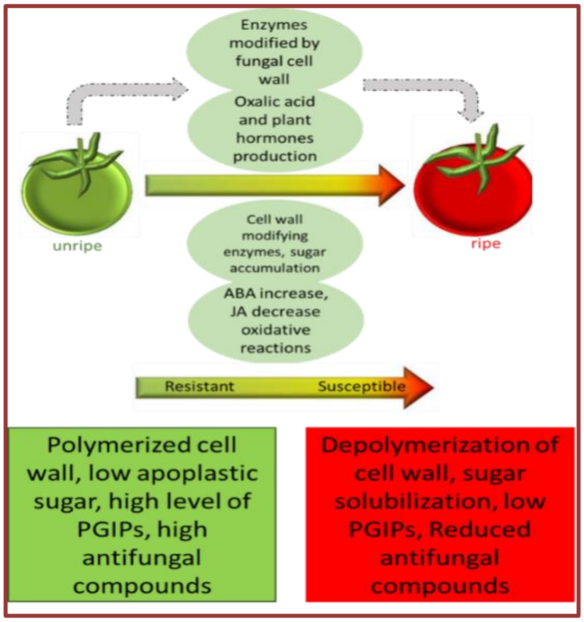

Fig. 1 Fruit changes occur in the ripening and senescence that regulates susceptibility

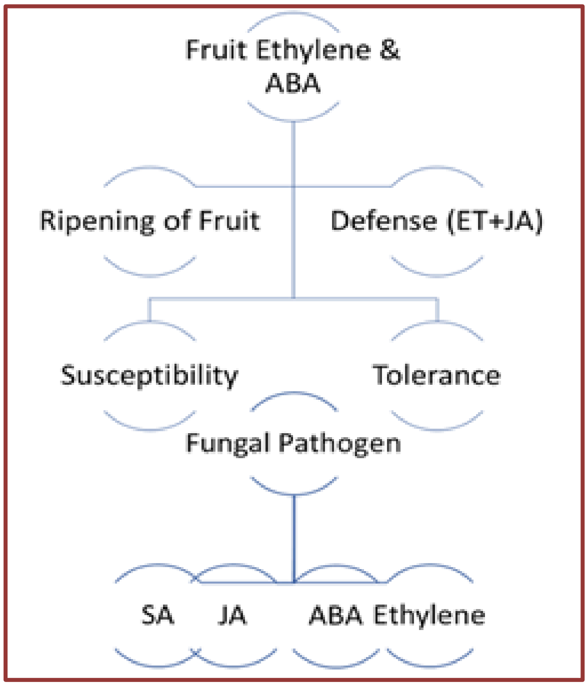

Fig. 2 Role of phytohormones: ET and ABA activate fruit ripening and defense system together with JA. This interaction leads to increased tolerance Fruit defense response to fungal pathogens is denoted by phytohormones such as SA, JA, ABA, and ET. Phtohormone debate may determine fruit tolerance to necrotrophic fungal or biotrophic pathogens. JA and ET are characteristics of tolerance to necrotrophs and SA to biotrophs, respectively

3. The role of different elicitors in the induction of plant resistance to *B. cinerea*

B. cinerea produces a high number of generations and easily develops resistance to different classes of fungicides. Induced resistance consists of the application of treatments that elicit a host defense response and prevent the outbreak of infections (Walters et al., 2015). Several resistance inducers have been used singly or in combination for the management of *B. cinerea* mold (Romanazzi et al., 2016). Resistance to *B. cinerea* can be induced by biological control agents (yeasts, yeast-like fungi, and bacteria), physical agents (UV-C irradiation, heat treatment, and hypobaric treatment), natural and synthetic chemicals (salicylic acid, chitosan, benzo thiadiazole, etc.), or disinfectants (ozone, electrolyzed water, and ethanol) that elicit a range of host responses that activate most of the enzymes involved in host-pathogen interactions. Since *B. cinerea* is capable of infecting a long list of fruits and vegetables (Romanazzi et al., 2016), studies of induced resistance have been conducted on a range of crops and worldwide, providing a list of information and protocols that can be applied in different systems. However, elicitation of resistance also has several positive aspects, as it does not induce resistance to fungicides, covers a broad spectrum, can be long lasting, has no side effects, and can increase the quantity of nutraceutical compounds, which are usually phenolic (Romanazzi et al., 2016). One of the most commonly used elicitors against gray mold on various crops is the biopolymer chitosan, which is derived from crab shells, making it a perfect example of the circular economy. This biopolymer has a triple action: it can trigger host defenses, form a film on the plant surface, and also has antimicrobial activity (Romanazzi et al., 2016). These multiple mechanisms of action against *B. cinerea* were also highlighted by a meta-analysis confirming previous findings (Rajestary et al., 2021).

Use of nanotechnology to enhance resistance against *B. cinerea*

Nanotechnology is among the recently developed disciplines that has various potential applications. It is supposed to offer propitious solutions to assist farmers in treating diseases and controlling pests. Nanotechnology has been used to develop fertilizers, herbicides, and fungicides that have no significant impact on the environment. Moreover, nanotechnology has been used to detect plant diseases and pests in the fields via nano kits, nano-sensors and nano-capsules and these diseases can be controlled in time. Some of the nanomaterials have been used to increase yields by enhancing the plants capability to absorb minerals and nutrients (Rai & Ingle, 2012). Nanotechnology is used to produce nano-particles and use them in combination with fungicides or alone to protect plants from diseases. In addition, encapsulation of fungicides with nanoparticles improves the ability of plants to properly absorb the fungicides. Metals including gold (Au), silver (Ag), titanium (Ti), zinc (Zn) and copper (Cu) have been noted for their various antimicrobial activities against fungi as nano fungicides. The broad surface area of these nano fungicides allows excellent connection of microorganisms with their surface, which is why there has been a recent increase in interest in the production and research of nano fungicides. Most studies have focused on the inhibition of fungal growth, but their impact on plants and plant pathogens has not been adequately explored. Recently, silver nano fungicides such as AgNPs are widely used against fungal and bacterial plant diseases. These nano fungicides are used because they exhibit high reactivity by affecting nutrient transport systems and ion efflux because of the silver (Ag^+) ions production (Malarkodi et al., 2014; Balachandar et al., 2022) which penetrate vigorously into pathogen cells even at little quantities (Kim et al., 2009; Balachandar et al., 2022). Moreover, these nano fungicides can destroy nucleic acids, lipids, and surface proteins of pathogenic cells by generating Ag ions that consequently form the injurious reactive oxygen species (ROS) (Storz & Imlay, 1999; Hwang et al., 2008). Silver nano fungicides have been used against *Botrytis cinerea*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Pythium ultimum*, and *Magnapor the grisea* and were found to be effective fungicides (Park et al., 2006). Though, little studies have been conducted on the antifungal activities of nano fungicides against *Botrytis* spp. to date. Besides silver nano fungicides, polymer-based copper nano fungicides have also been used against *B. cinerea* (Costa et al., 2022).

Other studies showed that microbial cells of *B. cinerea* were damaged by Ag-SiO_2 nanoparticles. In another study, silver NP, plant extracts, and microbial culture filtrates were observed to be efficient against grey mold disease alone or in combination with the fungicide (Draz et al., 2022). Ag-chitosan nano fungicides were found to be efficient in protecting fruits from *B. cinerea*. The antifungal silver titanate nanotubes (AgTNTs) were also found to be effective in inactivating *Botrytis cinerea* within 20 minutes. A component of AgTNTs such as $H_2Ti_3O_7$ nanotubes functionalized with silver nanoparticles (AgNPs) inactivates *B. cinerea* by invagination of the plasma lemma because of oxidative stress through reactive oxygen. It also increases cytotoxicity and damages conidial morphology leading to the death of *B. cinerea* (Rodríguez-Mansilla et al., 2016). In some studies, zinc-oxide nano fungicides were used, which reduced conidial germination and also disrupted cell functions and deformed the morphology of mycelial partners of *B. cinerea* (Musarrat et al., 2010). Other nano fungicides such as nano-CHT composite caused destruction and lysis of fungal mycelia (Moussa et al., 2013). In another study, the essential oil of *Zataria multiflora* was encapsulated in chitosan nanoparticles and reduced disease severity and *B. cinerea* incidence when applied (Mohammedi et al., 2015).

In another study, 3 general carbon nanomaterials viz, fullerene, reduced graphene oxide, and multi-walled carbon nanotubes, and 3 marketable metal oxide NP viz, iron oxide (Fe_2O_3)-NP, titanium oxides (TiO_2)-NP, and copper oxide

(CuO)-NP were individually applied @ 50 and 200 mg/L into water-agar filled petri plates. The 6 nano fungicides suppressed the mycelium of grey mold, but various concentrations showed varying inhabitation percentage. In conclusion, nano fungicides inhibit *B. Cinerea* disease and can be effectively applied as antifungal agents during plant growth and development of different flowers including roses. Recently, the antifungal activity of ZnO nano fungicides against *Aspergillus niger* and *B. cinerea* was studied. These nano fungicides altered the reproductive structures (conidiophores) and hyphae of both fungi. In conclusion, the nano fungicides proved their efficacy against *B. cinerea*, especially silver and copper (Mohammedi et al., 2015). Moreover, unlike commercial fungicides, the nano fungicides proved to be aggressive antifungal agents against *B. cinerea*, even at low doses. Moreover, they do not have any significant impact on soil and the environment like commercial fungicides. There is a need to conduct further studies to understand the toxicity mechanisms of nano fungicides against *B. cinerea*. Such studies would be extremely useful for developing new strategies to control *B. cinerea*. In addition, the effect of nano fungicides on fruit quality should also be examined (Mohammedi et al., 2015).

Omics approaches to induce resistance to *B. cinerea*

Botrytis cinerea is a necrotrophic fungus that destroys the cells of its host plants before colonizing the dead tissue. For many years, high doses of fungicides have been used to treat infections caused by this disease throughout the crop cycle in a season (De Miccolis Angelini et al., 2014). Recent research methods have made it possible to investigate the processes underlying host mechanism to infection by whitefly and regulate infection development events and pathogen detection using omic approaches (Shiratake & Suzuki, 2016), accomplishing it promising to integrate this information into a framework based on systems biology (Fig. 3). The techniques of HT-NGS extending from RNAseq to whole-genome sequencing are rapid, complex, and reliable methods for identifying the genome of *B. cinerea* in symptomatic or non-symptomatic host plants and for studying defense system related with fungal pathogen (Smith et al., 2014). In addition, the techniques of HT-NGS show potential for the molecular study of interaction between Plant and *B. cinerea*. From the plant of *B. Cinerea*, the genomic availability of the fungus, its host range, diversity, and evolution were known. The availability of omic data will help us better understand the infection tactics of *B. cinerea* and predict plant responses to gray mold disease in the future (Jiang et al., 2016).

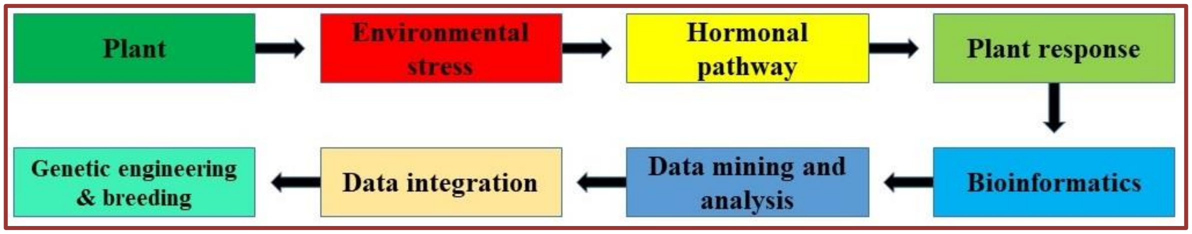

Fig. 3 Resistance/tolerance of plants to biotic and abiotic stressors is improved by omics methods

Patho-genomics

Pathogens employ a variety of effector proteins and toxins that aid in the alteration and repression of host plant defense mechanisms during infection (Fillinger & Elad, 2016). The oomycete pathogen *Phytophthora infestans* has roughly 500 effectors, whereas *Pseudomonas syringae* bacterium has a basic core of tem to forty effectors. The matching of resistance proteins with effector proteins has been aided by yeast-two-hybrid (Y2H) systems. Succeeding the identification and characterization of Pto in tomato. The bacterial effectors were recognized i.e., AvrPto and AvrPtoB as its interacting partners using a Y2H system. Using a Y2H approach, the bacterial effectors AvrPto and AvrPtoB were identified as Pto's interaction partners after it was discovered in tomato (Kim et al., 2009). Besides the Y2H system's initial success, future research attempting to couple R proteins and effectors were unsuccessful. In retrospect, this happened as direct R proteins-effectors interaction seems to be the exception with accessory proteins often serving as a bridge between the two. Recent -omics research backs up the assumption that direct interaction between effectors and R proteins is the exception rather than the rule (Li et al., 2013).

Genomics

Both traditional Sanger dideoxy nucleotide sequencing and pyrosequencing are suitable for de novo sequencing and confirmatory sequencing, and DNA sequencing methods are still affordable (Pareek et al., 2011). Ion Torrent Personal Genome Machine (PGM), Pacific Biosciences (PacBio), and Illumina or Solexa, have transformed genetic and genomic study with their next-generation sequencing (NGS) technology (Heather and Chain, 2016). *B. cinerea* has emerged as a model to understand the intricacy of necrotrophy and pathogenicity in diverse hosts. *B. cinerea* strains might be able to withstand a range of environmental conditions that limit or promote infection to the host (Ahlem et al., 2012). A gapless genome sequence of *B. cinerea* strain B05.10 was recently generated through a combination of Illumina and PacBio sequencing techniques (van Kan et al., 2017). The diversity and range of secondary metabolism gene clusters (SM) of two tested pathogenic species genomes differ significantly due to their different environmental conditions, as do the sequence and structure of the mating locus (MAT), the sexual reproductive regulation, and the material of transportable elements between the two species. Nevertheless, the genomes of the two species are very similar in sequence and gene structure, suggesting that *B. cinerea* and *S. sclerotiorum* lack distinguishing features that can be interpreted as unequivocal indication of their destructive nature and multigenic pathogenesis.

The environmental and genetic basis of *B. cinerea*-host plant specificity can be determined by comparative genome sequence analyses of *B. cinerea* strains (Atwell et al., 2015). The genomes of thirteen various isolates of *B. cinerea* were sequenced to assess their genetic diversity, demonstrating the wide range of the host species and its promising adaptability to new plant hosts. Arabidopsis is a flowering plant of great importance in genetics and molecular biology. (The Arabidopsis Information Resource) TAIR1 is a public database containing the entire Arabidopsis genome and genomic maps, as well as physical and genetic markers, gene expression and structure, genetic material, DNA, and seed stocks. The Arabidopsis genome is very flexible and permits adaptation to a broad environmental range. This has been confirmed by the 1001 Genomes Project, which examined the whole-genome sequence diversity of one hundred Arabidopsis ecotypes from various zones (Gloss et al., 2022). The availability of genome sequences of *B. cinerea* and its host plants has facilitated the discovery of contender genes for virulence of *B. cinerea* and potential target genes for host plant resistance. Genome sequencing enables breeders in deciphering the "blueprint" of a plant so that a resistant hybrid can be developed. Through the analysis of whole-genome gene expression the detection of crucial factors in the pathogenicity of *B. cinerea* and pathogen-derived effectors, molecular events related to infection mechanism in host, and mechanisms of disease resistance in hosts and the fungal genomes and its host have been sequenced.

Metabolomics

Metabolomics can reveal the phenotypic consequences of stressors on plants by estimating the number of metabolites genomic downstream, proteomic and transcriptomic changes, allowing dynamic assessment of phenotypic responses to environmental stimuli (Hong et al., 2016). Mass spectrometry (MS) or Nuclear magnetic resonance (NMR) techniques including liquid chromatography-MS (LC-MS) and gas chromatography-MS (GC-MS) are commonly used for metabolome profiling (Gathungu et al., 2014). Carbohydrate and metabolite microarrays can be used to detect pathogens, enzyme activity, carbohydrate-binding screening, and protein/antibody (Yadav et al., 2015). If plants are exposed to unfavorable environmental conditions, metabolomic techniques can screen a wider range of minute compounds (Zhao et al., 2016). The major metabolites playing fundamental roles in plant responses to abiotic stresses include amino acids, sugars, and intermediates of Krebs cycle. The failure of photosynthesis and osmotic re-adaptation is considered to be the reasons of their changes (Arbona et al., 2013). Secondary metabolites that respond to specific stresses include pathogens, ROS scavengers, coenzymes, regulatory compounds, and antioxidants. Secondary metabolites produced by various abiotic stressors can help bridge the gap between biotic and abiotic stress responses and provide for biotic cross-protection. According to Moura et al. (2010), flavonols have been accumulated in Arabidopsis plants that were treated with UV-B and the flagellin effector flg22. They were more resistant to *B. cinerea*, suggesting that the induced flavonols may protect plants from biotic stressors. *B. cinerea* produces nonspecific phytotoxins, such as secondary metabolites, which serve as lethal weapons against plant cells, similar to another necrotrophic fungus. *B. cinerea* has about 40 gene clusters responsible for the production of terpenes, alkaloids, non-ribosomal peptides, and polyketides that means it could produce a wide variety of metabolites (Collado & Viaud, 2016). The use of 1H NMR to assess global metabolites shows that healthy and botrytized grape berries have significant metabolic differences (Hong et al., 2012). *Botrytized* berries absorbed large amounts of glutamate, alanine, proline, and arginine, while sucrose-producing glycerol, phenylpropanoids, succinate, flavonoids, and gluconic acid, were significantly destroyed compared to healthy berries. Camañes et al. (2015) associated *B. cinerea* infection with considerable changes in major tomato main and secondary metabolism in tomato, suggesting extensive metabolic reprogramming. In Arabidopsis, grape, and tomato, *B. cinerea* infection leads to metabolic changes in both the host and the pathogen.

Transcriptomics

To obtain information on stress inflections of gene expression in plants, relative studies of gene expression aid to obtain regulatory data acquired by transcriptomic techniques. High-throughput transcriptomic methods based on hybridization (microarray technology) and sequencing (RNAseq) can be used to study transcriptomes in both model and non-model species (Warren et al., 2007). Early cell responses to one or more stressors can often be detected using transcriptome methods. Plant responses to the pathogen result in transcriptional reprogramming, implying that DEGs comprise approximately 12% of the Arabidopsis genome, with 1498 (7%) and 1138 (5%) genes activated and repressed by *B. cinerea*, respectively (Sham et al., 2015). To produce tagged mutants for reverse genetics, genetic mutagenesis and transcriptomic have been recently introduced (Fig. 4). Transcriptomics can be used to find potential candidate genes for plant defense.

On the other hand, mutant lines having deleted and overexpressed features enable us to determine their function in the defensive response. Botrytis-induced kinase 1 (BIK1), expansin-like A2 (EXLA2), pentatrico peptide repeat protein for germination on NaCl (PGN), and responsive to dehydration 20 (RD20), were found to be *B. cinerea*-sensitive genes, while mutants of these genes showed differential susceptibility to necrotrophic infection (Sham et al., 2015). Using RNAseq, the Mediator 18 (MED18) gene was found to regulate immunological and hormonal responses in plants. Such *B. cinerea*-responsive genes play a role in both disease and abiotic stress responses in the (Lai et al., 2014). These results suggest that the plant response to *B. cinerea* and abiotic stress are linked, with multiple hormone signaling pathways affecting photosynthetic performance. These results show stress in plants, revealing multiple signaling hormone pathways that affect photosynthesis (Abuqamar et al., 2016). Biomarkers for and nonpathogenic stresses are identified by integrating genomes, transcriptomic and proteomics. This is done by merging at least two separate omic datasets (transcriptome and proteome) into a reference dataset with the same functional perspective (Haider and Pal, 2013). This method can lead to the creation of an efficient model of biological systems or pathways that would include proteins and transcripts.

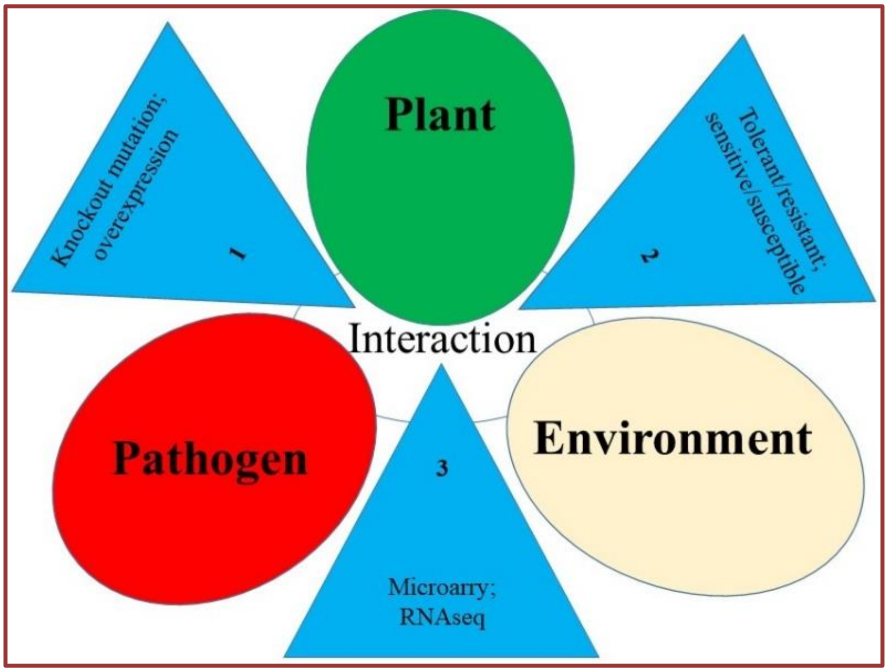

Fig. 4 The interactions between plant-pathogen-environment interactions form a disease triangle. The sequence of a gene is known through reverse genetics, but the function is unknown. Reverse genetic analysis involves three steps: (1) identification and functional characterization of candidate genes (e.g., overexpression, knockout), (2) identification of phenotypic effects of potential genes (resistance/tolerance or susceptibility/sensitivity) and (3) Identification of differentially expressed genes through transcriptome studies during plant-pathogen interactions

Proteomics

Several protein identifications can be performed to determine the availability, unavailability, or total amount of a protein. Proteomic methods help to detect alterations in protein levels under unfavorable environmental conditions. For example, calmodulin and calmodulin-like protein interactions have been detected using protein microarrays (Popescu et al., 2007). Proteins are made by post-translational amendments and folding to preserve their functions on-chip, which is a challenge when creating protein microarrays (Popescu et al., 2007). Proteins are detached by chromatography (MALDI-TOF and MALDI FTICR imaging MS with high mass resolution) or electrophoresis on one-dimensional (size-only) or two-dimensional (charge and size) protein gels (Spraggins et al., 2016). The housekeeping enzymes including glyceraldehyde dehydrogenases and malate were found to have a key role in the differential virulence of two fungal strains (Fernández-Acero et al., 2007). Shotgun proteomics method was used for finding 126 proteins that had changed in the *B. cinerea* proteome, including 13 pectinases that contribute to cell wall degradation (Shah et al., 2009). The secretomes of *B. cinerea* strain B05.10 showed significant differences between pH 4-6 (Lai et al., 2014). Proteolysis-related proteins increased at pH 4, while enzymes responsible for cell wall degradation were aggregated at pH 6. Proteomics showed variation in 186 proteins in mature green fruit of wild tomato infected with *B. cinerea*, but not in red ripe (RR) wild tomato or ripening inhibited (RIN) mutant fruit. Defence-related proteins were less altered in mature green wild tomato fruit compared with RR and (RIN) fruit (Shah et al., 2012).

Conclusions and future perspective

B. cinerea is a polyphagous fungus attacking a broad range of crops in a wide range of climates. The fungus is also capable of developing in response to storage temperatures and is the most prevalent postharvest pathogen of many fruits and vegetables. Nanotechnology and omic technologies help to induce resistance in hosts. Therefore, an integrated approach to the management of its infections is imperative as it is essential to decrease infection inoculum in the field with a range of agronomic, physical, biological, and chemical treatments that promote the hurdle concept, each treatment may also not be fully effective but will help reduce incidence. Disease management must take into account new research findings; as old fungicides have already been or are at risk of being withdrawn from the market. New fungicides are highly specific and the pathogen develops resistance in a relatively short time. Accurate management can take into account the use of predictive models based on recording latent infections that can predict the extent of disease that may develop over time in the field and during postharvest storage. In addition, an integrated approach is required by retailers who specify high-quality fruit with no or very low fungicide residues, and growers and plant doctors face the challenge of finding alternatives to synthetic fungicides that are effective and increase sustainability of production.

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