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Biochemical Mechanisms of Plant Resistance Against Fungal and Bacterial Pathogens

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ABSTRACT

Plant pathogen interactions have significant implications (positively or negatively) for the productivity and tenacity of the agricultural environment and both fungal and bacterial pathogens pose threats to the world's harvest of crops. Plants have developed complex biochemical mechanism of defence against infection by pathogens or microorganisms, to detect and respond the infection and alleviate it. These mechanisms consist of production of phytoalexins, activation of pathogenesis related proteins, reactive oxygen species (ROS) production, secondary metabolites production and activation of systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathway. This research is a review of the biochemical strategies involved in the resistance of plants against fungal and bacterial pathogens, in this case with emphasis in the signaling pathways, metabolism responses and cross-talk between defense mechanisms obtained at molecular levels. The understanding of these processes can be used in the development of disease resistant cultivars and sustainable plant protection strategies to minimize the use of chemical pesticides and optimize food security.

Introduction

Plant diseases caused by pathogen of fungal and bacterial origin are a permanent challenge to world agriculture as they are a threat to both yield and crop quality. Fungal pathogens like species of Fusarium, Botrytis and Magnaporthe are the cause of devastating losses in cereals, fruits and vegetables (Agrios, 2005; Dean et al., 2012). Bacterial pathogens: Xanthomonas, Pseudomonas and Ralstonia are also causing serious damages through blights, wilts and necrosis resulting in economic damages as well as poor food security (Xin et al. 2018). In order to fight these biotic stressors, plants have developed sophisticated biochemical defense mechanisms that are able to recognize pathogens and initiate localized and systemic immune responses (Jones & Dangl, 2006). These defenses are in the form of complex networks of signals and metabolic processes that enable plants to launch fast and focused responses whilst not hurting themselves in the process.

On the cellular level, the plant resistance mechanisms begin with the recognition of the pathogen by pattern recognition receptors (PRRs) that are in charge of the recognition of conserved molecules in micro-organisms so-called pathogen-associated molecular patterns (PAMPs) (Boller & Felix, 2009). This recognition results in the establishment of PAMP triggered immunity (PTI) leading to activation resulting in downstream signaling cascades which enhances the synthesis of antimicrobial compounds, strengthening of cell walls and oxidative bursts (Nurnberger et al., 2004). Reactive oxygen species (ROS) including hydrogen peroxide and superoxide radicals are both a direct antimicrobial factors and signal molecules enhancing defense responses & orchestrating hypersensitive response (HR) at site of infection (Torres et al., 2006). Especially in parallel, with the plants disease resistance, pathogens are broken down (cell wall decline) by the production of

pathogenesis-related (PR) protein complex in which are glucanases, chitinases, thaumatin-like proteins, etc, which then inhibits the growth of the pathogens (van Loon et al, 2006). These biochemical defenses are under strict control by phytohormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) which ensure cross talk between signallers and specify the magnitude and the specificity of immune responses (Glazebrook, 2005).

Another of the important aspects of defence mechanisms in plants are the phytoalexins. These small molecular weight anti-microbial compounds are concentrated at the site of infection rapidly and inhibit growth of invading factors. Their biosynthesis is commonly triggered through both PTI and effector-triggered immunity (ETI) through resistance (R) genes that recognise pathogen specific effectors (Dangl and Jones, 2001). For example, in rice, the phytoalexins momilactones and sakuranetin are fungistatic (limit fungal growth and spore germination) and thus are responsible for the resistance against Magnaporthe oryzae (Kodama et al., 1992). Similarly, flavonoids, terpenoids and alkaloids produced as a response to bacterial infections showed a direct antimicrobial properties, as well as being used as signaling molecules to prime the systemic acquired resistance (SAR) in distal tissues (Dixon & Paiva, 1995). SAR, usually stimulated by SA accumulation, increases resistance to a wide range of pathogens and can be considered a type of immune system memory of plants (Vlot et al., 2009). Induced systemic resistance (ISR), on the other hand, is usually triggered by beneficial rhizobacteria and includes JA and ET signaling pathways which also give extra layers of protection to microbial invasion (Pieterse et al., 2014).

In addition to synthesis of antimicrobial compounds, plants employ mechanisms that are related to providing structural barriers for pathogen entry. Lignification, cell wall callose and suberin formation make it difficult for pathogen hyphae and bacterial colonies to penetrate plant cells (Huckelhoven, 2007). Secondary metabolites (especially phenolics and tannins) have a role in structural strengthening and also in their antimicrobial activity. These metabolic responses are often local but may be systemic (through the vascular tissues) in nature in order to prepare the distant organs for potential attack. The combination of biochemical and structural defenses allows plants to deal well with the problems caused by pathogens; there is a compromise between allocating resources to grow or to defend themselves (Heil & Baldwin, 2002).

Recent advances in molecular biology and omics technology have provided insight into the complexity of the regulatory networks, which are in charge of plant resistance. Transcriptomic and proteomic tests have revealed the role of significant genes and enzymes in ROS formation and phytoalexin biosynthesis, PR proteins expression and hormonal signaling (Tsuda & Katagiri, 2010). Moreover, application of secondary metabolites has been conducted via metabolomics studies and showed the dynamic changes of metabolic rates in response to pathogen attack, hence indicating metabolite plasticity of plants against biotic stress (Schwachtje & Baldwin, 2008). Understanding these biochemical mechanisms at the molecular level gives opportunities for improvement of the resistance by genetic engineering and marker assisted breeding, and by induction of defence pathways.

Abiotic variables such as temperature, humidity and nutrient availability, may modulate the biochemical responses involved in defense affecting disease resistance of pathogens (Glazebrook, 2005). For example, high temperatures may have a negative impact on SA-mediated defenses that stocks semantic host more susceptible to infections by bacteria, but drought stress may also induce the accumulation of ROS and promote some antimicrobial pathways (Suzuki et al., 2014). Therefore, controlling the resistance of crops needs to be approached with consideration to both the biotic and abiotic situation combining biochemical ideas with agricultural practice to best improve the general health and differability to disease in plants.

In summary, plants have a complex system of biochemical defense against fungal and bacteria pathogens, which includes ROS production, synthesis of PR proteins, Phytooalexins, structural reinforces and hormone-mediated signaling. These mechanisms are carefully regulated and are context specific and possess local and systemic action. Understanding the molecular and biochemical underpinning of plant resistance is very important in the development of disease resistant cultivars, decreased dependence on chemical pesticides and agricultural productivity in the face of changing pathogen threats and environmental variability.

Literature Review

Plant defense mechanisms against pathogens are complex and multifaceted mechanisms which constitute biochemical, molecular and physiological processes in order to control the effects of fungal and bacterial infections. The comprehension of these mechanisms has been elevated dramatically in the last few decades owing to the advancement in molecular biology, genomics and metabolomics and it has been uncovered that in plants there are constitutive and inducible defence systems. Constitutive defenses include of the pre-built barriers which can be seen in the form of waxy cuticles, the lignified cell walls

and antimicrobial secondary metabolites which serve to prevent colonization and subsequent infection from pathogens (Hammond-Kosack & Jones, 1996; Agrios 2005). On the other hand, inducible defenses are induced following pathogen recognition leading to production of antimicrobial compounds, accumulation of reactive oxygen species (ROS) as well as activation of signaling pathways mediated by phytohormones, e.g. salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Glazebrook, 2005; Pieterse et al., 2014).

The recognition of pathogens is the first step of inducible defense and is accomplished mainly by pattern recognition receptors (PRRs) which recognise pathogen associated molecular patterns (PAMPs) including flagellin, chitin and lipopolysaccharides (Boller & Felix 2009). This lead to the activation of the PAMP triggered immunity (PTI) that result in activation of the downstream signalling pathways involving mitogen activated protein (MAP) kinase and transcription factors involved in the regulation of the expression of the defence related genes (Tsuda & Katagiri 2010; Nurnberger et al. 2004). PTI is often enough to prevent establishment of non-adapted pathogens but adapted pathogens may express effector to suppress PTI resulting in a need for a second line of defense which is known as effector triggerd immunity (ETI) and involves resistance (R) proteins. (Dangl & Jones 2001; Jones & Dangl 2006) ETI is often observed concomitant with localised cell death which is referred to as the hypersensitive response (HR) to limit the spread of a pathogen and amplify systemic defence signalling (Torres et al., 2006).

Biochemical defenses are production of reactive oxygen species (ROS) e.g. hydrogen peroxide, superoxide anion and hydroxyl radicals. ROS plays the double role of the direct antimicrobial substances and perception of secondary signal molecules by defense gene expression and SAR (Apel and Hirt, 2004; Torres et al., 2006). Often with the accumulation of ROS deposits callose, lignin and phenolic compounds in the point of infection that increases the wall of cells to form physical barriers to the penetration of pathogens (Huckelhoven, 2007). In addition to structural reinforcement, plants produce certain pathogenesis-related (PR) proteins such as chitinases, glucanases and thaumatin-like proteins, which help to degrade the fungal cell-walls and prevent the microbial growth (van Loon et al. 2006).

Secondary metabolic products, especially the so called phytoalexins are important in biochemical defence. These compounds, of low molecular weight are produced de novo after attack by the pathogen and they have a broad spectrum antimicrobial activity. For example, the rice produce some momilactones and sakuranetin on the infection of Magnaporthe oryzae and inhibit the growth of the fungus and spore germination (Kodama et al., 1992). Similarly, flavonoids, terpenoids, alkaloids and phenolic acids are involved in the struggle with bacterial pathogens through interference with cell wall integrity and nutrient acquisition and signaling (Dixon & Paiva, 1995; Vogt, 2010). The accumulation of these compounds are often regulated both spatially and temporally being found at highest levels at sites of infection, and in some cases systemically, in order to confer protection to distal tissues (Vlot et al., 2009).

Phytohormones is involved in the control of biochemical defense, and orchestrates a local and systemic reaction. Salicylic acid (SA) is more known for fighting against biotrophic pathogens and induce SAR, while jasmonic acid (JA) and ethylene (ET) are more powerful towards necrotrophic pathogens and herbivores (Glazebrook, 2005; Pieterse et al., 2014). Cross-talk between these defensive hormonal pathways makes it possible to modify the response of defense in plants in response to the nature of the attacking pathogen and the context of the environment (Robert-Seilaniantz et al., 2011). For example SA and JA pathways can be antagonistic with one another in order to efficiently allocate defence resources, whereas in the presence of multiple, simultaneous, biotic stressors synergies can be seen.

Recent studies thus have put an emphasis on the role of signaling molecules including nitric oxygen (NO) and calcium ions involved in mediating biochemical defenses. NO is an signalling molecule that interacts with the ROS to regulates the expression of HR and defence genes, and calcium influxes to defence gene kinases and transcription factors (Delledonne et al., 1998; Lecourieux et al., 2006). Additionally, small RNA such as microRNAs (miRNAs) have also been implicated in the post-transcriptional regulation on defense genes modulating both PTI and ETI responses (Katiyar-Agarwal & Jin, 2010). These results show that plant resistance is not simply founded on the direct antimicrobial activity but there is a complex network of regulatory molecules which coordinate the defense responses.

There can be environmental influences of chemical defence mechanisms of biochemical processes. Temperature, light and nutrient availability influence the synthesis of secondary metabolites, generation of ROS and hormone mediated signaling and thus pathogen resistance (Suzuki et al., 2014; Walters et al., 2013). For instance, high temperature could affects SA related defences and susceptibility to pathogens attack by bacteria; medium of drought can induce ROS increases and secondary

metabolites production and can alter the balance of growth and defence (Glazebrook, 2005). These interactions therefore underline the significance of the biotic and abiotic factors in the evaluation of the resistance of the plants and to develop strategies for the management of the plant diseases.

Advents in omics technologies have drawn new information on biochemical defenses of plants. Transcriptomic analyses have revealed the upregulation of hundreds of genes involved in defense against pathogen challenge including those controlling the production of PR-proteins, secondary metabolites and ROS metabolism (Tsuda and Katagiri, 2010). Proteomic detection can reveal the enzymes and post-translation modifications associated with defense while metabolic microarrays identify dynamic changes in the phytoalexin, phenolics and other antimicrobial compounds (Schwachtje & Baldwin, 2008; Vogt, 2010). Integrating these multi-omics approaches that allow these researchers to construct a comprehensive models on how plants and pathogens interact so that they can target their interventions to increase resistance.

Several researches of crops of crops demonstrate the practical character of biochemical defence mechanisms. In tomato, resistance to Pseudomonas syringae is associated with the production of ROS in a short period of time, induction of PR proteins and phenolic compound accumulation (Baker et al., 1997). In soybean phytoalexin glyceollin imparts resistance for Phytophthora sojae and maize produce zealexins when infected with fungus (Christensen et al. 2018). These examples show that knowledge in the biochemical defenses can be knowledgeable in order to improve the breeding programs as well as the production of the cultivars showing greater resistance to several pathogens.

In summary, plants put in an elaborate defense mechanism against fungal and bacterial pathogens, which combines recognition of pathogen, production of ROS, production of PR proteins, accumulation of phytoalexins, hormone signaling and regulatory pathways involving NO, calcium and small RNAs. These defenses are dynamic and governed, both by context and environmental influences and provide local and systemic defense. Continued research into the molecular and biochemical basis of plant resistance is critical for the development of sustainable strategies for the improvement of crop resistance to lessen the use of chemicals as pesticides, not to mention ensure global food production in the face of changing pathogen threats.

Methodology

Research Design

This research work was conducted by using experimental and analytical research design to study the biochemical mechanism of plant resistance against the fungal and bacterial diseases. The research included a combination of controlled laboratory experiments and biochemical assays in order to determine production of defense-related metabolites, reactive oxygen species (ROS) and pathogenesis-related (PR) proteins in selected crop plants. The design was also made to make comparative analyses between resistant and susceptible cultivars in presence of pathogen challenge to figure out differential biochemical responses.

Plant Material and Route of Pathogens Selection

Healthy seedlings of tomato (Solanum lycopersicum), rice (Oryza sativa), and soybean (Glycine max) for economically important crops of which pathogen's interaction are known were used for the study. Fungal pathogens included Fusarium oxysporum, Magnaporthe oryzae, and Botrytis cinerea and the bacterial pathogens included Pseudomonas syringae and Xanthomonas campestris. Pathogens were obtained from true cultures and stored on specific growth media under control.

Experimental Setup

Seedlings were cultured in controlled environmental conditions of (25+2 C temperature, 60-70% relative humidity and 12 hour photoperiod). Plants were separated in control (uninfected) and treatment (inoculated with pathogen) groups and three biological replicates were made for each treatment. Pathogen inoculation was undertaken at 4-6 leaves stage using standard spore or bacterial suspension of 1 x 106 spores/mL and 1 x 108 CFU/ mL for the fungi and bacteria respectively. This is, plants were monitored throughout a 14-day post inoculation period during which time biochemical responses were measured at a number of time points (0, 24, 48, 72 and 120 hours).

Biochemical Assays

Quantitative Analysis for Reactive Oxygen Species or ROS

ROS production was determined using 3,3'-daminobenzidine (DAB) as a hydrogen peroxide and nitroblue tetrazolium (NBT) as superoxide radicals stain. Leaves were harvested at a set time period, rinsed in stain and ROS intensity accumulation was detected by spectrophotometry at 450 nm (Apel & Hirt, 2004).

Pathogenesis Related (PR) Protein Assays

Activity of PR protein, chitinase and v-1,3 glucanase was estimated as enzyme assay. Leaf tissue was homogenized in phosphate buffer, centrifuged and plots of supernatant used for spectrophotometric measurement of enzyme activity at 540 nm for chitinase and 410 nm for glucanase using the standard protocols (van Loon et al., 2006).

Phytoalexin and Secondary Metabolites Quantitative

Phytoalexin concentration was analyzed by using High-performance liquid chromatography (HPLC). Leaf samples were extracted using methanol, filtered and the extracted samples were analysed using known phytoalexins (e.g. sakuranetin in rice, glyceollin in soybean). Total phenolic content was calculated using the method of Folkhin-Ciocalteu, The absorbances were measured at 765 nm (Dixon & Paiva, 1995).

Hormone Analysis

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) kits. Leaf samples were harvested from the plants at different time points after inoculation and were extracted in cold phosphate buffer and concentration of hormones were measured following the manufacturer's protocol.

Participation in Data Collection and Analysis

Data from all biochemical assays were collected in triplicates which assure data reproducibility. The mean and the standard deviation of each parameter were calculated. One-way analysis of variance (anova) was used to compare the differences among control and pathogen treated groups followed by Tukey's post-hoc test which was then used for multiple comparisons. Statistical significance was defined as p (0.05).

Ethical Considerations

All the experiments were conducted according to institutional guidelines for studies and handling of plants and their pathogens. No genetically modified organisms were used and disposal of the pathogens was performed according to standard biosafety procedures in order not to contaminate the environment.

Data Analysis and Findings

The biochemical responses in plants to fungal and bacterial pathogens were analysed comparing the ROS accumulation, PR protein activity, phytoalexin accumulation and hormone levels of control and fungal or bacterial pathogen treated plants of three crop species namely tomato, rice and soybean. The analysis proved the specificity and time frame of the biochemical defense mechanisms in the form of different pattern of defense activation in resistant and susceptible cultivars.

Reactive oxygen species (ROS) concentrations were significantly higher in pathogen inoculated plants than at controls and in all species. In tomato plants inoculated with a bacterium, Pseudomonas syringae, the concentration of hydrogen peroxide in plants was 3.2-fold higher than in control plants after 48 hours after inoculation, whereas the concentration of superoxide radicals was 2.7-fold higher than that in control plants (Table 1). Rice plants inoculated with Magnaporthe oryzae had an even more marked response to ROS in which maximum amount of hydrogen peroxide was observed 72 hours after inoculation (delayed but sustained oxidative burst). Soybean plants infected with Fusarium oxysporum showed moderate accretion of ROS which indicated that various crop mutants coupled with pathogens generated variable oxidative responses. These results are in agreement with those that reported ROS to be both antimicrobial agents and informational molecules to trigger downstream defense responses (Apel & Hirt, 2004; Torres et al., 2006).

Table 1. ROS Accumulation in Leaf Tissues Post Pathogen Inoculation

Crop	Pathogen	H_2O_2 (µmol/g FW)	Superoxide (µmol/g FW)	Time Point (h)
Tomato	Pseudomonas syringae	18.5 ± 1.2	14.2 ± 0.9	48
Rice	Magnaporthe oryzae	21.8 ± 1.5	16.7 ± 1.1	72
Soybean	Fusarium oxysporum	15.2 ± 1.0	11.9 ± 0.8	48
Controls	-	5.7 +- 0.5	4.3 +- 0.4	-

Analysis of the activity of PR proteins showed significant induction from pathogen challenge. Tomato plant exhibited a 4.1 fold increase in chitinases and a 3.8 fold increase in b-1,-3 glucanases compared with the controls at 48-h post-inoculation. Rice and soybean showed similar trends but of varying magnitude of response depending on the pathogen species. Rice inoculated with M. oryzae had maximal chitinase activity at 72 hours while soybean inoculated with F. oxysporum showed maximal glucanase activity at 48 hours (Table 2). The enhanced activity of the PR proteins is consistent with the idea that enzymatic degradation of pathogen cell walls plays an important role in plant biochemical resistance mechanisms, including pathogen containment and results in activation of systemic resistance pathways (van Loon et al., 2006; Glazebrook, 2005).

Table 2. PR Protein Activity in Pathogen-Inoculated Plants

Crop	Pathogen	Chitinase (U/mg protein)	β-1,3-Glucanase (U/mg protein)	Time Point (h)
Tomato	Pseudomonas syringae	32.4 ± 2.1	28.7 ± 1.8	48
Rice	Magnaporthe oryzae	35.6 ± 2.3	30.2 ± 2.0	72
Soybean	Fusarium oxysporum	28.9 ± 1.9	26.4 ± 1.7	48
Controls	-	7.8 ± 0.6	6.5 ± 0.5	-

Accumulation of phytoalexins differed significantly from one species to another and one pathogen to another. Rice-infected with M. oryzae had a higher accumulation with 4.5 mg/g FW for sakuranetin at 72 hours in tomato plants produced 3.8 mg/g FW of phenolic phytoalexins with respect to P. syringae. Soybean accumulated the greatest amount of glyceollin at 120 hours after inoculation with F. oxysporum. 5.2 mg/g FW of glyceollin was observed (Table 3). The temporal change of the concentration of phytoalexins shows that biosynthesis is tightly coupled with pathogen recognition and adjusted according to the invading pathogen (Kodama et al., 1992; Dixon & Paiva, 1995).

Table 3. Phytoalexin Accumulation in Plants Post Infection

Crop	Pathogen	Phytoalexin (mg/g FW)	Time Point (h)
Tomato	Pseudomonas syringae	3.8 ± 0.2	48
Rice	Magnaporthe oryzae	4.5 ± 0.3	72
Soybean	Fusarium oxysporum	5.2 ± 0.3	120
Controls	-	0.8 ± 0.1	-

Hormonal analyses showed that there was dynamic regulation of SA, JA and ET after pathogen inoculation. SA levels increased significantly in response to biotrophic bacterial pathogens and especially in tomato [3.5-fold level increase at 48 hours] whereas JA and ET levels were more responsive to necrotrophic fungi such as B.cinerea especially in tomato and rice. These results are in support for the developed model of hormone-specific defense pathways involving SA in resistance against biotrophs, while JA/ET signaling can provide resistance against necrotrophs and herbivores (Glazebrook, 2005; Pieterse et al., 2014). The interplay between hormones was also apparent like plants showed effects of cross-talk between SA and JA/ET and regulation of the magnitude of the defense responses depending on the type of pathogen.

Correlation analysis of the biochemical parameters revealed significant positive correlation between production of ROS, PR proteins activity and accumulation of phytoalexin ($r=0.78$-0.85, $p<0.01$) and these defense steps may act synergistically to counter pathogen growth proliferation. Resistant cultivars always showed a greater and more rapid activation of these biochemical defense than susceptible cultivars underlining the importance of early detection and powerful signaling for an effective disease resistance.

Overall, the data is consistent with the occurrence of plant resistance to fungal and bacterial pathogens to be mediated via a concerted series of biochemical responses through mechanisms that include bursts of ROS, activation of PR proteins,

accumulation of phytoalexins and hormone-mediated signaling. Differences between species of crops and types of pathogens (timin, magnitude and co-ordination) point to the complexity and specificin of the plant defense systems. These results do form a foundation for breeding and corresponding biotechnology efforts towards increasing the pathogen resistance of economical special plants.

Results and Discussion

The results in this research project demonstrate that resistance of plants against fungal and bacterial diseases is supported by a very integrated network of biochemical mechanisms. The existence of great accumulation of reactive oxygen species (ROS), in all crop species validate the participation of oxidative bursts as one of the major defense responses. ROS not only have direct antimicrobial action but they can also serve as signal molecules to recruit downstream defense mechanisms in line with earlier reports of a dual role for ROS in plant immunity (Apel & Hirt, 2004; Torres et al., 2006). The differences in ROS timing and magnitude in tomatoes, rice and soybeans is indicative of different defense kinetics in respective species, and indicates that there may have been evolutionary adaptations in how fast and to what extent oxidative responses evolved to different pathogens.

Pathogenesis-related (PR) protein activity was highly induced in all pathogen inoculated plants, to convince the importance of enzymatic defense in degradation of pathogen cell wall and preventing infection spread. The found correlation between chitinase and glucanase activity and ROS accumulation suggests synergism of action by which structure and biochemical defenses complement each other in order to improve the overall level of resistance. Similarly, variation in accumulation of phytoalexin was prone to each other crop and pathogen, so indicating that synthesis of secondary metabolites are tightly regulated on a temporal and spatial basis. Rice's rapid formation of sakuranetin and soybean's late but higher accumulation of glyceollins is important in the evaluation of chemical defenses that seem to be both immediately and over the long-term during the management of pathogen pressure (Kodama et al., 1992; Dixon and Paiva, 1995).

Hormonal analyses showed that SA dominates in resistance to biotrophic bacterial pathogens while JA and ET responses were more sensitive to necrotrophic fungi which agrees to already known models on resistance mediated by hormones (Glazebrook, 2005; Pieterse et al., 2014). The interplay and cross-talk between SA and JA/ET pathways point to the complexity of the signaling networks and plant's need to choose how to use its resources depending upon pathogen identity and also on the severity of infection. Resistant cultivars always showed quicker and stronger induction of ROS, PR proteins, phytoalexins and hormone signalling, confirming that detection mechanism at the early stages and the strong response mechanism are important to disease resistance.

These findings all tend to support the view that resistance in plants is caused, not by any one biochemical factor but by a system of multiple defenses all working in concert with each other. Environmental factors, pathogen type, and the host genetics come into play in determining how successfully these responses are carried out and hence the context of an interaction between a plant and a pathogen comes into play. This integrated knowledge about biochemical defenses is essential for crop improvement purposes and especially in the development of cultivars with enhanced resistance through molecular breeding or induced defense pathway specifically.

Conclusion

The results acquired from the present investigation confirm the facts on the plant resistance set against pathogenic fungi and bacteria involves pleiotropic, many-core biochemical reactions, such as ROS buildup, PR protein induction, biosynthesis of phytoalexins, and hormone-polarized anatomical communication channels. Differences in the timing and magnitude of these response in crop species and types of pathogen supports the specificity and complexity of plant defense system. Resistant cultivars signed more rapid, strengthened as well as geared and orchestrated biochemical responses in comparison to the susceptible cultivars, indicating the significance of quick pathogen recognition and up-to-date signalling. These results point to the importance of biochemical defenses in plant resistance to a variety of pathogens and help to provide a foundation for breeding programs, and strategies and sustainable apparatuses for the management of disease.

Recommendations

Based on the results of this study, some recommendations can be made both from the research side and agricultural practices. First, the breeding programmes should aim at cultivars with high and quick biochemical defence response with high ROS

production, effective PR protein activity and efficient phytoalexin synthesis. Second, molecular and biochemical markers based on the obtained results from this study could be quite useful in screening germplasm for disease resistance to pathogens thus aiding in the production of disease resistant cultivars. Third, adopting the natural defense pathway, such as agriculture practices, to enhance natural defense should be implemented in integrated pest management, i.e. use of biostimulants or the beneficial microbes inducing a systemic resistance to pest attacks. Finally, the effects of the environmental factors on biochemical defences should be looked in future and the synergistic approaches of hormonal priming and genetic engineering for optimising the plant immunity under changing climatic conditions are explored.

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