Unraveling Microbial Strategies: Targeting the Plant Ubiquitin - Proteasome Pathway

ABSTRACT

The Ubiquitin-Proteasome System (UPS) stands as a central regulator in the intricate web of plant immune responses, orchestrating the turnover of key immune components. Through the UPS, plants finely tune their defenses, from the initial recognition of pathogens to the activation of defense strategies. Immune receptors, signaling components associated with defense hormones, and transcription factors are among the targets modulated by the UPS, shaping both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). However, pathogens have evolved sophisticated strategies to manipulate the UPS for their benefit. Fungi, bacteria, and viruses deploy effector proteins to exploit the UPS, either by promoting the degradation of host defense elements or by interfering with UPS-mediated processes. This intricate interplay underscores the pivotal role of the UPS in plant-pathogen dynamics, presenting both challenges and opportunities for understanding and manipulating plant immunity. Unraveling the complexities of UPS regulation and pathogen exploitation thereof is crucial for advancing our knowledge of plant-microbe interactions and developing strategies for sustainable disease management in agriculture.

Keywords: Pathogen, Effector, Regulation, Ubiquitination, Immunity

1. INTRODUCTION

In order to combat disease outbreaks triggered due to attacks from plant pathogens, plants have evolved an intricate defense mechanism refined over time through the process of evolution [1]. Fundamentally, the first line of defense hinges on the identification of common microbial components known as pathogen-associated molecular patterns (PAMPs). This recognition occurs through receptors located on the surface of cells recognized as pattern-recognition receptors (PRRs)[2]. Named PAMP-triggered immunity (PTI), this initial defense mechanism can be circumvented by adept plant pathogens. They achieve this by directly injecting effector proteins into host cells, leveraging the host's cellular processes to enhance the pathogen's viability and reproduction [3].

In reaction, plants have evolved an extra level of protection, heightened by the detection of these effectors via internal receptors. This supplementary defense mechanism, known as effector-triggered immunity (ETI), is triggered upon the recognition of substances from microbes by receptors within the immune system [4]. Upon this acknowledgment, downstream defense mechanisms are set in motion through the transmission of hormonal cues and alterations in genetic coding. Consequently, this prompts utilizing substances that fight microbes and/or initiating programmed cell demise to combat the situation intrusion [5, 6]. Termed the zig-zag model, this phenomenon entails complex interplays among diverse cellular pathways and has historically served as the foundation for exploring plant-microbe interactions on a molecular scale [7]. The sophisticated defense mechanisms require a significant level involving the adaptability of proteins, including their creation and breakdown, to regulate processes [8]. As a result, it's not unexpected that pathways responsible for breaking down proteins, like the ubiquitin-proteasome system (UPS), play a crucial role in coordinating plant defense and shaping the outcomes of interactions between plants and microbes [9].

The ubiquitin-proteasome system (UPS), a remarkably preserved pathway, manages the degradation of roughly 80% of proteins within eukaryotic organisms [10]. To enable proteins to undergo recycling via the UPS, they must undergo a process known as poly-ubiquitination. This complex procedure includes a sequence of enzyme-driven stages, which include activators of ubiquitin (E1), carriers of ubiquitin (E2), and attachers of ubiquitin (E3). First, activated ubiquitin connects with an E1 enzyme and is subsequently passed to an E2 enzyme. Following this, the E2 enzyme carries the activated ubiquitin to the E3 enzyme, which aids in linking ubiquitin to a lysine site on the intended protein. Through multiple iterations of through the succession of E1, E2, and E3 enzymes, the target protein acquires one or multiple ubiquitin chains, marking it for identification and subsequent breakdown by the 26S proteasome[11].The 26S proteasome, an immense ATP-dependent protease complex weighing 2.5 megadaltons, comprises the 20S core protease (CP) accompanied by either one or two 19S regulatory components (RPs). Every RP comprises both top and bottom sections. The CP acts as a versatile protease complex with peptidase activity that operates independently of both ATP and ubiquitin. When these subcomplexes unite, they create a capped cylindrical mega structure capable of recognizing, unfolding, and degrading ubiquitinated proteins. The process initiates with the identification of substrates, after that, the removal of ubiquitin and the process of unfolding are

assisted by the upper sections of the RP.After linearization, the protein being targeted is guided by the lower sections of the RP and conveyed to the core particles of the CP. Within the CP, enzymes with active sites similar to those found in trypsin, chymotrypsin, and caspase known as β1, β2, and β5 peptidases exhibit properties that allow them to cleave various polypeptides. The RP sub complex identifies ubiquitinated prepares the CP channel for the entry of intended proteins entry of the unfolded substrate, thus enabling degradation[12].

Ever since ubiquitin was first identified over five decades ago, the ubiquitin-proteasome system (UPS) has become increasingly recognized as a prominent figure in a vital and indispensable regulator governing numerous cellular processes in eukaryotic organisms [11, 13]. The diversity found in E3 ubiquitin ligases among plants plays a pivotal role in ensuring specificity when targeting substrates, thus granting the UPS significant flexibility to effectively address various changes in the environment. Plant E3 ligases are categorized classified into four primary categories: RING (remarkably intriguing novel gene), complexes containing Cullin-RING ligases (CRLs), HECT (Homologous to the E6-AP carboxyl terminus), and plant proteins with U-box domains (PUB). While RING, HECT, and PUB E3 ligases operate as standalone entities, CRLs form complex assemblies consisting of multiple subunits. Within plant systems, the UPS route, specifically involving E3 ligases, is known to regulate responses to a wide spectrum of internal and external signals[14, 15]. The investigation into the functions of E3 ligases in plant immunity has represented a significant advancement in comprehending defense mechanisms. Nonetheless, recent studies have emphasized the critical importance of additional components of the UPS like the enzyme E1, E2, and the complex comprising the 26S proteasome in coordinating effective plant defense responses. In the context of plant defense mechanisms, pathogens have developed tactics to exploit the UPS for their benefit. This analysis aims to explore the various mechanisms through the manner in which the UPS governs plant defense mechanisms and examine the diverse tactics employed by plant pathogens to disrupt UPS operations, thereby circumventing activating plant protective mechanisms and initiating illness.

2. THE UBIQUITIN–PROTEASOME SYSTEM OVERSEES EVERY PHASESRELATED TO THE IMMUNE SYSTEM OF PLANTS

Within the realm of developmental and stress response pathways, the ubiquitin-proteasome system (UPS) exerts its influence, with one notable area being plant immunity. Its management

influence of plant immunity extends across every phase, starting from identifying pathogens to carrying out defensive measures a wide array of defense mechanisms.

2.1. Key immune components are broken down by the Ubiquitin–Proteasome System

2.1.1. Immune receptors

The initial stage of plant immune reactions involves identifying pathogens, which happens through two mechanisms: the recognition of PAMPs by cell-surface PRRs and the detection of effectors by intracellular receptors. Instances have been documented where both categories of immune receptors serve as subjects for degradation orchestrated by the UPS.

Receptor kinases and receptor-like proteins, which constitute PRRs, are primarily located on the outer membrane of cells. In this location, they detect conserved PAMPs [16]. The regulated breakdown of these proteins is a critical regulatory process, strongly influenced by the UPS, owing to their essential role in initiating plant defense mechanisms. Arabidopsis's sensitivity to flagellin 2 (FLS2) is among the extensively researched PRRs, renowned for its recognition of When detecting flg22, a fragment derived from bacterial flagellin, FLS2 interacts with its co-receptor BAK1 through a series of phosphorylation events enabled by their kinase domains [2].Following the recognition of flg22, FLS2 experiences swift changes degradation, a phenomenon associated with the UPS, as demonstrated by the preservation of FLS2 protein levels when proteasomal degradation is chemically inhibited using MG132 [17]. Additionally, FLS2 is subjected to ubiquitination facilitated by the E3 ligases PUB12 and PUB13. While the precise relationship between FLS2 ubiquitination and proteasomal degradation remains unclear, it is conceivable that it contributes to the turnover of FLS2 through mechanisms that are yet to be fully understood. Among the initial components activated downstream in the signaling pathway initiated by FLS2 is Botrytis-induced kinase 1 (BIK1) engages directly with the FLS2/BAK1 complex, receiving phosphorylation from them, and then relays the signal to different internal cellular elements [18]. Similar to FLS2, BIK1 experiences proteasomal degradation subsequent to ubiquitination, which is mediated by a different duo of PUB proteins, specifically PUB25 and PUB26 [19]. Furthermore, PUB4 has been recognized as another player in the ubiquitin-dependent proteasomal degradation of BIK1. Notably, divergent functions have been observed regarding the phosphorylation of BIK1 [20]. While BIK1 activity is bolstered by phosphorylation from the FLS2–BAK1 complex, its activity is hindered by phosphorylation from Protein kinase 28 activated by calcium ions (CPK28). CPK28, in turn, undergoes

proteasomaldegradation through ubiquitination facilitated by the E3 ligase duo in Arabidopsis consisting of the RING domain proteins ATL6 and 31[21].

The receptor kinase 1 in Arabidopsis responsible for detecting chitin elicitors stands out as another significant PRR. It detects chitin, a common signal molecule found in the cell walls of fungi, initiating subsequent immune signaling by phosphorylating PBL27 [2]. Like FLS2, the persistence of CERK1 protein levels rises in reaction to inhibiting the degradation process carried out by proteasome through chemical means [22]. PUB12 is involved in modulating the association between CERK1 and PBL27, while both PUB12 and 13 engage with CERK1. However, PUB12 exerts a detrimental effect on the stability of CERK1[23]. Interestingly, there have been observations of ectodomain shedding occurring in CERK1[24]. Additionally, PRRs found in monocots, like rice SPL11 cell-death suppressor 2 (OsSDS2), are subject to regulation by the UPS [25]. Taken together, these discoveries highlight the pivotal role of the UPS in overseeing early pathways of immune signaling and underscore the essential contribution of plant E3 ligases in this regulatory mechanism.

In the defense against adapted pathogens, intracellular immune receptors assume vital functions in plant resistance, with a significant portion part of the family of proteins called nucleotide-binding leucine-rich repeats (NLRs). NLR-mediated immunity frequently results in programmed cell demise, known as hypersensitive response (HR), as a reaction to pathogen intrusion [6]. Hence, it is imperative for plants employ mechanisms to regulate the natural function of these proteins and prevent self-inflicted immune responses. The UPS contributes to this control mechanism, as several investigations have uncovered instances of NLR proteins from diverse plant species, such as Arabidopsis, *Nicotianabenthamiana*, rice, and barley, being marked for degradation via the proteasome [26, 27, 28, 29]. Together, these discoveries highlight the crucial role of the UPS in regulating both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) reactions in a range of plant species confronting different pathogens. Significantly, the UPS serves as both a promoter and inhibitor of immune responses, illustrating the complex and flexible characteristics of this regulatory network.Figure 1 illustrates various types of E3 ubiquitin ligases in the plant system.

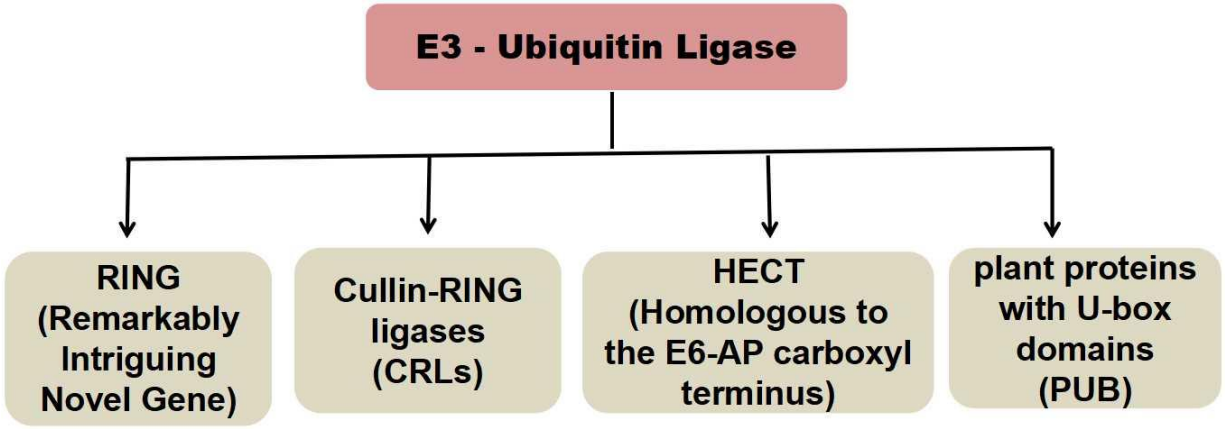

Fig 1 Different types of E3 – Ubiquitin Ligase in Plant System

2.1.2. Components involved in signaling pathways associated with plant defense hormones

When plants perceive pathogens, it initiates a substantial reconfiguration to execute suitable defensive tactics by regulating diverse cellular processes. A vital aspect this reorganization primarily happens through changes in gene expression, involving the signaling of hormonesassumes a central role. Interestingly, hormone-driven alterations in transcription in plants are closely linked to proteasomal degradation. This association is notably apparent concerning two key hormones implicated in immunity: salicylic acid (SA) and jasmonic acid (JA).

Playing a central role in plant defense against biotrophic pathogens, salicylic acid (SA) acts as the primary hormone. The control of SA-triggered defense reactions is managed controlled by the primary transcriptional regulator known as non-expressor of pathogenesis-related genes 1 (NPR1)[5]. The amount of NPR1 protein is carefully regulated through salicylic acid (SA) recognition, which is facilitated by the CRL3NPR3/NRP4 receptor complex[30, 31]. During typical circumstances, NPR1 undergoes degradation orchestrated by the UPS, a process initiated by ubiquitination mediated through the CRL3NPR3 complex.

As salicylic acid (SA) levels rise initially, NPR3's function is inhibited, resulting in the stabilization of NPR1. Conversely, NPR4 acts as a regulatory mechanism, becoming active to induce NPR1 degradation when SA concentrations reach excessive levels [30]. A recent investigation has highlighted the significance of ubiquitination of NPR1 in its role. The study revealed that the initial attachment of ubiquitin molecules to NPR1 by CRL3 enhances its activity, while also triggering further elongation of ubiquitin chains mediated by UBE4, eventually resulting in the breakdown of NPR1 [32]. Additionally, the recent finding that two

HECT E3 ligases, ubiquitin-protein ligase 3/4 (UPL3 and UPL4), play a role in controlling NPR1 introduces further intricacy to the regulation of NPR1 degradation through ubiquitination [33]. In rice, OsNPR1 undergoes degradation via the UPS facilitated by the CRL4 complex, indicating the presence of one of them alternate either the breakdown pathway for NPR1 or differences in evolution among different plant types.

Jasmonic acid (JA) orchestrates defensive responses against pathogens that thrive on dead tissue. The signaling pathway of JA operates through primarily restraining transcription, mainly under the control of the JAZ family of proteins, which act as suppressors of transcription factors (TFs) MYC2, MYC3, and MYC4 [34]. After detecting pathogens, jasmonic acid (JA) levels increase and are detected inside the nucleus by the SCFCOI1 assembly. This initiates the ubiquitination and subsequent breakdown of the JAZ repressors, resulting in the release of the MYC transcription factors mentioned earlier [35, 36]. As a result, COI1, a constituent of the SCFCOI1 complex, is also directed for degradation via the proteasome, highlighting the UPS's multifaceted involvement in JA signaling [37].

2.1.3. Components situated further along in the immune signaling pathway

Plant immunity operates on an intricate molecular structure that harmonizes external triggers like pathogen identification and intrinsic signals like hormones to coordinate appropriate defense reactions. At the core of this coordination lies the control of protein levels and functionality. This control primarily occurs through post-translational alterations (PTMs) like Phosphorylation, acetylation, SUMOylation, and ubiquitination are all methods used by cells to modify proteins. Ubiquitination, in particular, serves as a mechanism for the UPS to control the stability of its targets.

2.1.3.1. E3 ubiquitin ligases

The significance of E3 ligases in governing plant defense mechanisms is apparent from their role earlier discussions. Intriguingly, certain E3 ligases associated with immunity experience degradation through the proteasome themselves, notably members of the PUB family. For example, in standard conditions, PUB22 undergoes self-interaction and self-ubiquitination, resulting in its breakdown. However, during infection, modification through the action of mitogen-activated protein kinase 3 (MAPK3) impedes this degradation process, thereby preserving PUB22 [38]. This preservation empowers PUB22 to engage in ubiquitination of its target, the exocyst subunit EXO70B2, a pivotal step in suppressing PAMP-triggered signaling

[39].PUB17 holds significant importance in governing the plant cell demise and aids in plant defense against the oomycete *Phytophthorainfestans*, as well as resistance conferred by RPM1 and RPS4 against *Pseudomonas syringae*[40, 41]. Its control mechanism entails ubiquitination facilitated by the POZ–BTB protein 1 (POB1) element of the CRL3 E3 assembly, marking PUB17 for breakdown through the ubiquitin-proteasome system (UPS) [42]. The participation of PUB17 counterparts in immune responses has been recorded in multiple varieties of plants like tobacco, potato and cotton, emphasizing the crucial role of PUB17 as a conserved regulator of immunity across various plant families facing diverse types of pathogens [43, 44].

2.1.3.2. Proteins involved in signaling pathways and other aspects of immune response

Phosphorylation serves as a crucial mechanism in regulating signaling pathways, especially those implicated in defense reactions. An example of this is the pivotal role of MAPK cascades within the communication routes involved in the immune response triggered by pathogen-associated molecular patterns (PAMPs)[2]. A recent investigation revealed that the E3 ligase known as KEEP ON GOING (KEG) stimulates the process of ubiquitination and subsequent degradation of MAPK kinase 4 and 5 (MKK4 and 5)[45]. Curiously, it appears that KEG may experience degradation subsequent to a fungal invasion, demonstrating its function as a suppressor of immune responses [46].

Another vital category of signaling proteins comprises GTPases and their affiliated counterparts. For example, in rice, the protein SPL11-interacting protein 6, which interacts with the Rho GTPase-activating protein (OsSPIN6), assumes a crucial function in overseeing hypersensitive response (HR) against fungal and bacterial pathogens. The degradation of OsSPIN6 is controlled by ubiquitination followed by proteasomal breakdown, a mechanism facilitated by the rice PUB13 ortholog OsSPL11 [47]. In rice, several proteins are essential for the plant's defense against fungal pathogens. For instance, the circadian clock modulator OsELF3–2 undergoes degradation via the ubiquitin-proteasome system (UPS) following ubiquitination catalyzed by OsAPIP6[48]. Another instance involves the regulator of cell demise in rice, associated with the BCL2-related protein anthogen 4 (OsBAG4), which imparts wide-ranging resistance and experiences breakdown via the proteasome system through ubiquitination catalyzed through increased resistance to blight and blast (OsEBR1) under typical circumstances to avert autoimmunity [49]. Furthermore, organelle signaling plays a role in immunity, demonstrated by the control maintaining the equilibrium of chloroplastic TRX-like 1 (TRXL1) via both the UPS

and degradation processes within the chloroplast, enhancing defense against *Pseudomonas syringae*[50].

2.1.3.3. Transcription factors

An essential aspect of the protective mechanisms in plants operates at the transcriptional level, where various transcription factors (TFs) are involved. Consequently, TFs become targets of the activity of the ubiquitin-proteasome system (UPS) throughout the process regulation of immune responses. One noteworthy group of TFs in this context is the unique WRKY group found in plants. Multiple members of the WRKY family have been identified as subjects for proteasomal breakdown acrossin various plants. For example, rice contains WRKY45, which aids in protection against *Magnaporthegrisea*, experiences proteasomal degradation under standard conditions [51, 52]. Furthermore, in pepper plants, the WRKY40 protein, analogous to Arabidopsis WRKY40, operates as a suppressor of PAMP-triggered immunity (PTI), and it undergoes breakdown by the proteasome. This degradation mechanism functions to control stomatal immunity when encountering the bacterium *Xanthomonaseuvesicatoria*[53]. Withinwild grape, the E3 ligase named VpEIRP1, induced by *Erysiphenecator*, enhances immunity against bacterial and fungal invaders by promoting proteasome-mediated breakdown of VpWRKY11 [54].

Another notable category of one of the transcription factor groups unique to plants is the NAC family. For example, tomato's NAC1 functions to enhance protection against Pseudomonas syringae and undergoes breakdown through the ubiquitin-proteasome system (UPS)[55]. In a recent finding, it was revealed that the rice vascular plant factors termed VOZ1 and VOZ2 undergo degradation via ubiquitination mediated by the E3 ligase OsAPIP10. This process plays a crucial role in modulating Piz-t-mediated immunity. Notably, OsAPIP10 is also implicated in the ubiquitination and subsequent breakdown of Piz-t itself, thus acting as a pivotal regulator in rice's responses to both PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI)[27, 56].In essence, the significance of proteasome-mediated breakdown in regulating proteins associated with immunity highlights its fundamental contribution to plant immunity. This regulatory process spans from the earliest detection of pathogens to the activation of defense strategies, emphasizing its comprehensive role throughout the immune cascade. Through orchestrating meticulous and nuanced modifications, proteasomal degradation empowers plants to orchestrate targeted defense reactions that address distinct challenges effectively.

2.2. Active Participation of the Ubiquitin-Proteasome Pathway in Plant Immune Responses

2.2.1. Functions of the Complex Containing the 26S Proteasome in Immune Defense

At the core of the UPS lies the 26S proteasome complex. Initially regarded as genes responsible for routine cellular maintenance, contemporary studies indicate a dynamic control over proteasome subunits across various fronts, encompassing transcriptional, translational, and post-translational regulatory mechanisms [12]. Despite this comprehension, the exact mechanisms dictating the assembly of proteasome and the regulation of their activity, as well as any prospective roles of individual subunits beyond processes reliant on proteasome, remain inadequately elucidated.

2.2.1.1. Contribution of Proteasome Subunits to Plant Immune Responses

Upon detecting pathogens, plants often display a tendency to boost the production and presence of proteasomal components. For instance, when exposed to fungal signals, Arabidopsis plants ramp up the production of PBB1, whereas viral invasion triggers an uptick in pepper RPN7 transcription. Likewise, when infected by *Pseudomonas syringae*, plants experience an increase in the levels of PBA1 and RPT2, and exposure to salicylic acid prompts the activation of the tobacco RPT6 gene [57, 58, 59, 60]. Plants with a deficiency in RPT2a, a key element of the proteasome assembly, demonstrate alterations in the durability of NLR proteins, reduced effectiveness in systemic acquired resistance (SAR), and heightened vulnerability to *Pseudomonas syringae* infection[61, 62]. Similarly, different genetic variations resulting in deficiencies in proteasome components lead toheightened vulnerability to infection by cauliflower mosaic virus (CaMV)[63].

2.2.1.2. Complete enzyme complexes and their control mechanisms

The synchronized formation of diverse proteasome subunits and structures requires the collaborative effort of multiple elements, some of which directly impact the immune response. Take OsUMP1, for instance, a controller of 26S proteasome construction in rice, which enhances universal resistance to *Magnaportheoryzae*, regardless of race, by regulating H_2O_2 concentrations [64]. Moreover, the involvement of the influence of proteasome regulator 1 (PTRE1) also encompasses the degradation of the NLR protein suppressor of npr1–1 constitutive 1 (SNC1), thereby shaping the immune reactions [65]. Furthermore, the proteasome sub-complexes exhibit the potential to influence immunity even prior to their formation. Notably, sunflower 20S complexes have been observed to harbor endonuclease capabilities, enabling them

to break down RNA from lettuce mosaic virus and tobacco mosaic virus [66].Figure 2 illustrates the Ubiquitin–Proteasome System (UPS) and its function in plant-pathogen interactions.

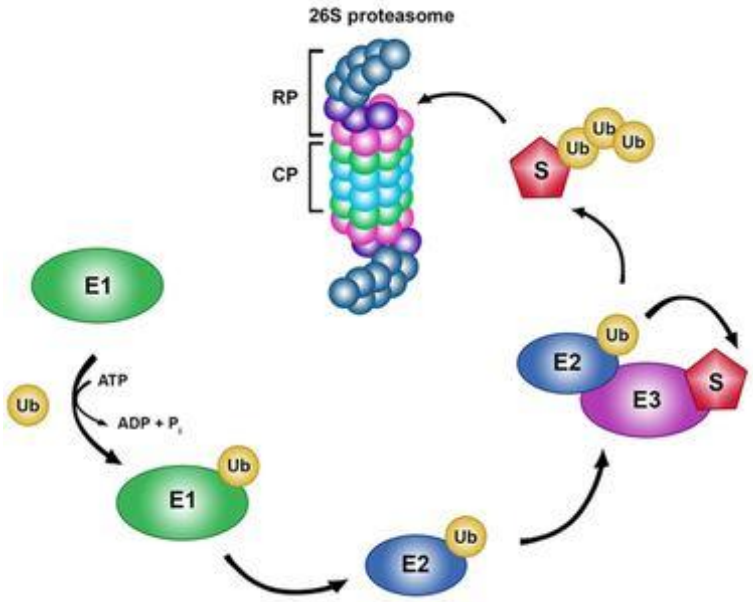

Fig 2 The Ubiquitin–Proteasome System (UPS) and its role during plant-pathogen interactions. In this cascade, ubiquitin molecules are activated and bind to E1 before being transferred to E2. The E2 enzyme then carries the activated ubiquitin to E3, which aids in attaching ubiquitin to a lysine residue on the target protein (S). This polyubiquitination marks the target protein for degradation by the 26S proteasome. This proteasome consists of a 19S regulatory particle (RP) and a 20S core subunit (CP), which together degrade the polyubiquitinated proteins.

2.2.2.Functions of additional elements and routes within the protein degradation mechanism involving ubiquitin relation to the immune response

A mutation in a gene that impacts one of the two ubiquitin-activating enzymes (UBA1) in Arabidopsis leads to heightened susceptibility to *Pseudomonas syringae* invasion[67]. On the contrary, in tomato plants, it's only when UBA2 is silenced, not UBA1, that susceptibility to the same pathogen, *Pseudomonas syringae*, rises, underscoring species-specific differences. Moreover, in both *Nicotianabenthamiana* and rice, ubiquitin-like peptide 5 (UBL5) aids in bolstering the defense against viral pathogens, acting as a positive regulator in these contexts[68]. Furthermore, UPL E3 ligases are newly recognized regulators situated before the

proteasome in the regulatory hierarchy, forming associations with it to amplify its functionality and bolster plant immune defenses [69].

An independent facet of the ubiquitin-proteasome system functions within the endoplasmic reticulum (ER) is a crucial site within cells where essential functions such as protein production and organization take place. Known as ER quality control (ERQC), this process depends on specific pairs of E2 and E3 enzymes associated with the ER. These enzymes facilitate the breakdown of ER-resident proteins via a phenomenon referred to as ER-associated degradation (ERAD) within the proteasome [70]. Some theories propose that ER-associated degradation (ERAD) could serve a dual purpose, not just eliminating misfolded proteins within the endoplasmic reticulum (ER), but also participating in the breakdown of receptors involved in immunity, like the Arabidopsis EF-Tu receptor (EFR) or barley Mildew Locus O (MLO)[71, 72]. Another facet of the ubiquitin-proteasome system (UPS) associated with immunity is the N-degron pathway. This pathway functions by enzymatically modifying the N-terminal ends of target proteins, making them vulnerable to recognition targeted by particular E3 ligases to be broken down later. Additionally, genetic alterations affecting particular elements within the N-degron pathway, including E3 proteolysis 1 and 6 (PRT1 and 6) or arginine transferases 1 and 2 (ATE1 and 2), exhibit varying impacts on the plant's ability to resist or succumb to different pathogens[73, 74, 75, 76].

3. MANIPULATION OF THE UBIQUITIN-PROTEASOME SYSTEM BY PLANT PATHOGENS

Microbes often target the ubiquitin-proteasome system (UPS) for manipulation, recognizing its pivotal function in orchestrating plant defense mechanisms. By tampering with this system, pathogens disrupt cellular functions, inducing disorder within the host environment and fostering their own propagation. Viruses, bacteria, fungi, oomycetes and nematodes utilize diverse tactics to exploit the UPS, dampening plant immune defenses and fostering their own persistence and multiplication.

3.1. Fungi

Mounting evidence underscores the capacity of eukaryotic invaders to disrupt the normal function of the plant's protein degradation system. Notably, specific effectors synthesized by these pathogens directly assail host proteins, prompting their breakdown by the proteasome and consequently bolstering virulence. An exemplar of such an effector is HaRxL44, originating

from the parasitic oomycete *Hyaloperonosporaarabidopsidis*, which binds to and destabilizes the plant protein MED19A, depending on the activity of the proteasome [77]. Through its interaction with MED19A, *H. arabidopsidis* manipulates the mediator complex, a crucial player in transcriptional regulation. This manipulation results in a shift in the balance between the signaling pathways involving jasmonic acid (JA) and salicylic acid (SA), favoring JA and consequently heightening the plant's susceptibility for biotrophic pathogens. Likewise, the fungal protein AvrPiz-t, generated by *Magnaportheoryzae*, undergoes ubiquitination by rice E3 ligases OsAPIP6 and OsAPIP10, marking it for degradation by the rice proteasome [27].Interestingly, by targeting these E3 ligases, AvrPiz-t essentially initiates their own degradation via the proteasome, employing a self-destructive strategy. This maneuver undermines the basal defense mechanisms of the rice plant. Another fungal effector, OSP24, derived from *Fusariumgraminearum*, instigates the breakdown of the wheat protein TaSNRK1a through proteasomal activity. It accomplishes this by surpassing the host kinase stabilizer TaFROG in a competitive manner [78].In addition to manipulating specific elements within the UPS, eukaryotic pathogens possess the ability to directly disrupt the normal functioning of this system. They achieve this by targeting regulatory mechanisms involved in proteasomal assembly or function. Moreover, pathogenic molecules can interfere with the functional capability of the proteasome enzymes itself. For example, the potent fungal toxin higginsianin B, derived from *Colletotrichumhigginsianum*, effectively blocks the chymotrypsin- and caspase-like functions of the proteasome. This hindrance results in the preservation of JAZ proteins, disrupting JA signaling and ultimately benefiting the pathogen [79]. Csn5, a subunit of the COP9 signalosome, controls pathogenicity in *Magnaportheoryzae* via autophagy. A Study found that Csn5 deficiency reduced pathogenicity and increased autophagy due to MoToroverubiquitination and degradation. MoCsn5 was identified as an interactor of MoAtg6, promoting K48-linked ubiquitination of MoAtg6, which reduced its protein levels and inhibited autophagy. These disruptions in ubiquitination and autophagy led to various defects in growth, development, stress resistance, and pathogenicity [80].A study identified MoCand2 as an inhibitor of ubiquitination in *Magnaportheoryzae*, affecting autophagy and pathogenicity. Deletion of MoCand2 increased ubiquitination, while its overexpression reduced ubiquitinated proteins. MoCand2, a subunit of Cullin-RING ligases (CRLs), blocks CRL assembly and regulates autophagy by influencing MoTor degradation and MoAtg6 ubiquitination. This imbalance led to defects in growth,

conidiation, stress resistance, and pathogenicity. The high conservation of Cand2s in other fungi highlights the broader relevance of this regulatory mechanism [81].*Pucciniastriiformis* f. sp. *tritici* (Pst) uses the effector protein PstGSRE4 to manipulate wheat host processes. PstGSRE4 interacts with TaGAPDH2, involved in ROS signaling, to regulate ROS levels. Silencing TaGAPDH2 increased ROS and reduced Pst infection, while overexpression decreased ROS and enhanced Pst infection, indicating TaGAPDH2 as a negative regulator of plant defense. PstGSRE4 stabilizes TaGAPDH2 by preventing its degradation via the 26S proteasome. These findings suggest that Pst exploits TaGAPDH2 to suppress wheat immunity and facilitate infection [82].

3.2. Bacteria

In order to bypass plant defense mechanisms, bacteria that cause plant diseases have developed a highly conserved mechanism known as the type III secretion system (T3SS). This system allows them toinjecting type III effectors directly into the cellular environment of the host. These effectors are then directed towards different parts of the cell and serve essential functions in promoting disease progression by suppressing various aspects of the plant's immune responses [3]. While initial studies primarily emphasized their capacity to inhibit PTI and ETI reactions, recent findings suggest that T3Es also aim at essential mechanisms like the UPS to interfere with cellular processes within the host organism [9, 83]. AvrPtoB, a key effector protein originating from *Pseudomonas syringae*, possesses E3 ligase capabilities once inside plant cells. This enzymatic function is crucial for marking and breaking down targets within the host via the action of the 26S proteasome, enabling AvrPtoB to manipulate the UPS and enhance its virulence during bacterial invasion. AvrPtoB interacts with multiple E2 ligases within the host, facilitating the breaking down several PRRs such as FLS2, and CERK1 as well as other proteins associated with PRRs like BIK1 and BAK1[22, 84, 85].XopK, a type III effector (T3E) derived from *Xanthomonasoryzae*, has a distinctive function of focusing on a pattern recognition receptor (PRR) by functioning as an E3 ligase. Through its interaction with rice OsSERK2, XopK attaches ubiquitin molecules, marking it marked for breakdown by the 26S proteasome [86]. Through the removal of OsSERK2, XopK efficiently quells numerous PRR signaling pathways, providing a calculated strategy to bolster virulence.

While mimicking E3 ligase activity proves effective degradation of host proteins by a T3E is one method of eliminating immune-related elements, it's not the only approach. Some bacteria that

cause plant diseases have developed F-box effector proteins, which function as connectors in the Skp1-cullin 1-F-box (SCF) E3 ligase complex. For instance, the group of T3E proteins known as the RipG family, present in *Ralstoniasolanacearum*, interacts with the SCF complex in host and is thought to act as E3 ligases. Similarly, VirF originating from *Agrobacterium tumefaciens* and, in recent discoveries, XopI originating from *Xanthomonasoryzae*have been found to function within the complex of SCF. They facilitate breaking down the rice thioredoxin OsTrxh2 and transcription factor VIRE2-interacting protein 1 (VIP1), respectively, to evade plant defense mechanisms[87, 88].*Pseudomonas syringae*pv. *actinidiae* (Psa) causes kiwifruit bacterial canker, a severe threat to kiwifruit production. A study identified the U-box type E3 ubiquitin ligase PUB23 as a negative regulator of immune responses to Psa in kiwifruit. PUB23 interacts with the trihelix transcription factor GT1, suppressing its expression. Silencing PUB23 increased immune responses, including up-regulation of defense genes PR1 and RIN4, and higher accumulation of hydrogen peroxide and superoxide anion, suggesting PUB23 inhibits PTI in kiwifruit. These findings demonstrate PUB23's negative regulatory role in kiwifruit immunity against Psa [89].

3.3. Virus

Emerging research highlights the substantial contribution of the plant UPS in facilitating connections between viruses and plants[90]. Whilst the UPS can hinder viral pathogenesis, viruses have evolved mechanisms to exploit it. Despite their small genomes with limited protein-coding capacity, viruses can impact a multitude of targets, providing a highly adaptable response to plant defense mechanisms. They employ diverse tactics to influence the UPS system in the host, which involves directly affecting the proteasome, modulation of UPS component expression and activity, or disruption of host protein degradation. For instance, the viral effector HcPro, renowned for its gene silencing suppression, is present in papaya ring spot virus. It engages directly with the enzymatic components of the 20S proteasome, hindering its endonuclease function and overall proteasome activity[66, 91]. As a result of this inhibition, viral accumulation is enhanced, indicating a mechanism that favors viral replication. Similarly, in the case of potato virus X, HcPro interacts with components of the 20S proteasome, although the precise details of this engagement remain unclear, requires further clarification[92].

Although no viral proteins that degrade host proteins directly have been discovered, certain viral proteins exhibit this capability to prompt the expression of native E3 genes. For example, the C4 protein from beet severe curly top virus triggers the expression of related to KPC1 (RKP) in

Arabidopsis. Subsequently, RKP functions on the kinase inhibitor protein (KIP)-related protein 2 in the cell cycle, fostering an environment favorable for viral replication [93]. Viruses have the capability to directly interfereby focusing on SCF complexes; it enhances E3 ligase activity within the host serve as central hubs for viral protein interaction. Multiple viral proteins interact with utilize these complexes to facilitate viral reproduction through diverse mechanisms. As an example, C2 proteins found in geminiviruses interfere with ubiquitination by suppressing CSN function, consequently Inhibiting JA signaling by interacting with SCF complexes[94-97].A study examined interactions among wheat, barley yellow dwarf virus (BYDV), and its aphid vector, discovering that the BYDV movement protein (MP) aids viral infection by promoting the 26S proteasome-mediated degradation of wheat catalases (CATs). Overexpression of BYDV MP in wheat increased ROS accumulation and viral infection, reduced wingless aphid proliferation, and increased winged aphid numbers. Silencing CAT genes had similar effects, while overexpressing TaCAT1 showed opposite results and improved grain size and weight. BYDV MP's interaction with PSMD2 facilitates CAT degradation in a ubiquitination-independent manner, highlighting the virus's manipulation of host ROS production for infection and transmission [98].

3.4. Protist

Clubroot, caused by the soil-borne pathogen *Plasmodiophorabrassicae*, severely affects Brassica crops globally. The infection mechanisms of this biotrophic pathogen are not well understood. PbE3-2, a RING-type E3 ubiquitin ligase in *P. brassicae*, shows E3 ligase activity and has a functional signal peptide. Overexpressing PbE3-2 in Arabidopsis increases susceptibility to *P. brassicae* by reducing chitin-triggered ROS burst and salicylic acid signaling. PbE3-2 ubiquitinates the host protease RD21A, compromising immunity. RD21A-deficient plants exhibit similar susceptibility. Other *P. brassicae* RING-type E3 ligases also target RD21A, highlighting a virulence strategy where these ligases degrade host proteases to weaken immunity [99].

4. CONCLUSION

New research emphasizes the essential role of the UPS in the defense mechanisms of plants, serving as a key regulatory mechanism for the turnover of immune components. Pathogens have developed sophisticated tactics to manipulate the UPS, influencing the plant's defense responses. Effectors can either stimulate or hinder the breakdown of proteins involved in immune

responses, providing valuable insights into the participation of the UPS in plant-related processes-pathogen dynamics. This interaction is complex; the UPS plays dual roles in both bolstering plant defense and promoting pathogen proliferation. Unraveling the intricate regulation of the proteasome and its associated components is crucial for a deeper understanding of microbial-UPS interplay.

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