

Potential Application of Chitin Signaling in Engineering Broad-Spectrum Disease Resistance to Fungal and Bacterial Pathogens in Plants

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Abstract

Chitin, a polymer of N-acetylglucosamine, is an important structural component in the cell walls of fungal pathogens. Plant chitinases are capable of degrading this component to directly inhibit infection by fungal pathogens. Plants can further detect the chitin fragments released by chitinases to trigger additional defense responses. Recent studies have identified chitin receptors in different plant species and a number of downstream signaling components. The chitin-mediated signal transduction pathway appears to be a unique and promising target for engineering plants for broad-spectrum resistance to various fungal as well as bacterial pathogens.

Fungal pathogens are an important limiting factor to crop production. Growing resistant crops and application of fungicides are currently two major ways to control fungal diseases. However, there are downsides to these practices. For example, resistant crops developed from a breeding program are generally resistant to a narrow spectrum of fungal pathogens or even a particular race or strain, and such resistance can be overcome by fungal pathogens; fungal pathogens can also develop resistance to fungicides over time. Additionally, the application of fungicides also poses certain risks to the environment and humans and livestock. Therefore, alternative ways are needed to combat plant fungal pathogens. Different from other microorganisms, fungi contain chitin in their cell walls. Chitin is a polymer of N-acetylglucosamine, structurally very similar to cellulose in plant cell walls, which is a polymer of glucose. Chitin plays an important role in fungal growth and development, as well as pathogenesis. Therefore, it can be targeted for engineering plants for resistance to fungal pathogens.

Unlike fungi, plants do not make chitin. However, plants contain a large number of chitinases. One major function of these proteins is to degrade chitin present in fungal cell walls to thwart fungal infection, although some of them may also play different roles in plant growth and development [1]. Indeed, various studies demonstrated that plants overexpressing chitinases [2], especially in combination with β -1,3-glucanases are resistant to fungal pathogens [1]. In addition to this direct reaction on the chitin component in fungal cell walls, plants have also evolved a secondary reinforcing defense mechanism: the chitin fragments released by chitinases are further detected and recognized by a Pattern Recognition Receptor (PRR) to trigger additional defense against fungal infection. Recent studies have identified such receptors in different plants. They are all LysM RLKs (Lysin Motif Receptor-Like Kinases) [e.g. 3-6]. And they also appear to interact with other LysM proteins to form a receptor complex for chitin perception. For example, the rice LysM RLK OsCERK1 interacts with the LysM protein CEBiP, OsLYP4, and OsLYP6, which do not have an intracellular kinase domain [5,7,8]; and the Arabidopsis chitin receptor LysM RLK1/CERK1 likely interacts with LysM RLK4 which has a non-functional intracellular kinase domain [9]. As expected, mutations in or silencing of these receptor genes led to enhanced susceptibility to fungal infection, as well as blockage of the induction of defense genes, MAPKs (Mitogen-Activated Protein Kinases), and ROS (Reactive Oxygen Species) by chitin [3,4,9-13]. Interestingly, mutations in or silencing of chitin receptor genes also appeared to enhance susceptibility to bacterial pathogens [6,7,9,14]. Furthermore, these receptors were targeted by the bacterial effector protein AvrPtoB for degradation or inhibition

[6,14]. Therefore, chitin receptors also appear to be involved in defense against bacterial pathogens. This raised an interesting question: Since bacterial pathogens do not contain chitin, what molecular signal is recognized by these LysM RLK receptors to lead to defense against bacterial pathogens? Considering that LysM was originally identified in peptidoglycan binding proteins [15], peptidoglycan is structurally similar to chitin, and bacterial pathogens contain peptidoglycan in their cell walls, therefore, peptidoglycan in bacterial pathogens is a good candidate for these receptors. Indeed, recent studies showed that LysM RLKs function together with other LysM proteins, e.g., LYM1 and LYM3 in Arabidopsis and OsLYP4 and OsLYP6 in rice, to perceive bacterial peptidoglycan to trigger innate immunity [12,13,16]. Interestingly, these receptor complexes appear to play dual roles in the perception of chitin and peptidoglycan to mediate plant defense against both fungal and bacterial pathogens. In addition to these receptors, a number of downstream components have also been identified, for example, heterotrimeric G proteins in Arabidopsis, and OsRacGEF1 and small GTPase OsRac1 in rice [7,8,10,17], and a signal transduction pathway has been established in plants [7,17,18]. This pathway appears to be conserved in different plants and independent of other defense pathways, such as those mediated by SA (salicylic acid), ETH (ethylene), and JA (Jasmonic Acid) [19]. However, resistance mediated by chitin is generally low. The recent identification of the receptors and downstream components opens up great opportunities to manipulate this pathway using transgenic tools to possibly lead to enhanced, broad-spectrum resistance to not only fungal, but also bacterial pathogens in plants.

Firstly, the activity of chitinases can be increased by overexpressing these genes derived from different organisms [2]. Many studies have demonstrated that overexpression of chitinase genes in plants can

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indeed lead to enhanced resistance to fungal pathogens. Since different chitinases may have different enzymatic properties toward chitin degradation, two or more different types of chitinases can be expressed together to increase chitin degradation efficiency. Additionally, a β -1,3-glucanase genes can be co-expressed to further enhance resistance to fungal pathogens, since the combination of a chitinase and a β -1,3-glucanase was shown to confer a stronger resistance to fungal pathogens than expressed alone [1]. Similar to chitinases, β -1,3-glucanases are also involved in degrading β -1,3/1,6-glucans in fungal cell walls to directly inhibit fungal infection, and the released glucan fragments can serve as an elicitor to elicit further defense responses [2]. Therefore, the co-expression of a chitinase and a β -1,3-glucanase may produce a synergistic effect on defense against fungal pathogens.

Increased expression of chitinases will not only increase the direct destruction of chitin in fungal cell walls to cause direct inhibition, but also increase the production of chitin signal to be detected by chitin receptors to activate further defense. To accommodate such an increased signal input, the level of the chitin receptor can be increased by overexpression, since PRRs are generally expressed at a low level. As mentioned above, chitin receptors have been identified in different plants, and they are all LysM RLKs and appear to function together with other LysM proteins in a receptor complex. Therefore, it may be necessary to co-express these genes in the same plant to fully accommodate the increased chitin signal input. Additionally, chitin receptors can be fused to other defense proteins to form a chimeric receptor to increase defense and/or spectrum. For example, the rice chitin receptor component CEBiP was fused with the intracellular region of XA21 [21], which mediates strong resistance to the rice bacterial leaf blight disease. Enhanced resistance to the fungal pathogen *Magnaporthe oryzae* that causes the serious rice blast disease was achieved by combining high affinity for chitin from CEBiP and the HR- (hypersensitive reaction)-like strong resistance from XA21 [21]. Similar chimeric receptors can be explored using LysM RLKs with other appropriate defense proteins to enhance resistance to fungal and/or bacterial pathogens.

Although chitin and bacterial derived elicitors flg22 (derived from flagellin) and elf18 (derived from bacterial elongation factor Tu, EF-Tu) are perceived by different PRRs [22], there are a large number of genes commonly regulated by these different elicitors, suggesting that the pathways elicited by these signals may converge downstream of their initial perception by individual receptors [4,17]. It will certainly be nice to regulate some components shared by different defense pathways to engineer plants for resistance to both fungal and bacterial pathogens. A recent study has succeeded in revealing one of these components, showing that heterotrimeric G proteins likely serve as a converging point in defense signaling activated by chitin and bacterial elicitors in Arabidopsis [8]. It can be foreseen that more such components will be found and manipulation of these components will likely affect plant defense against both fungal and bacterial pathogens.

Other down-stream signaling components, such as MAPKs and transcription factors (TFs), especially the latter due to their important roles in regulating gene expression, can be further regulated too to enhance resistance to fungal and/or bacterial pathogens. In Arabidopsis, more than 100 TF genes were regulated by chitin [23]. Many of these TF genes, e.g., WRKY22, WRKY33 and WRKY53, were also regulated by flg22 and other defense signals [4]. Therefore, manipulation of these TFs will likely affect defense against different types of pathogens. Depending on their expression or induction patterns during defense or plant-pathogen interactions, TF genes can be overexpressed or silenced to achieve their defense function. Additionally, transcriptional repressor

domains, such as the EAR (ERF-associated amphiphilic repression) domain [24], can be used to regulate downstream gene expression of a particular TF to enhance defense responses.

With current available gene manipulation and plant transformation techniques, it's possible to manipulate multiple genes to enhance resistance in strength and in spectrum. Ideally, overexpression of these genes should be under the control of inducible and/or tissue-specific promoters of appropriate strength to make these proteins available in a spatially and temporally controlled manner to reduce possible fitness penalty potentially caused by constitutive expression of these proteins. Therefore, it certainly is beneficial to identify or engineer a number of promoters strictly regulated by chitin or fungal pathogens and employ them in such genetic engineering work. Gene expression data [e.g., 4,11] can be utilized to screen for such promoters.

Concluding Remarks

With the advances in plant molecular biology and biotechnology, we can foresee that manipulation of multiple components involving in the chitin-mediated defense pathway (chitin degradation and signal generation, signal perception and amplification, and downstream signaling components, especially the components shared by different defense pathways) will be a promising way to engineer plants for enhanced resistance to diverse fungal and bacterial pathogens. Since multiple components with different roles in the pathway are manipulated, the resultant resistance is likely not easy to be overcome by pathogens. The resultant resistance is also likely to be strong, but supposedly not to be as strong as that mediated by typical resistance genes; therefore, it is not likely to force pathogens to evolve fast. Fitness penalty should be minimal if inducible and/or tissue-specific promoters are used. Additionally, due to the presence of chitin in insects and nematodes (in egg shells), such engineered resistance might have a serendipitous inhibitory effect on these pathogens too.

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