Analyses of the Genes Involved in Disease Development to Construct Disease-resistant Plants by Genetic Engineering

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Abstract:

Many genes responsible for the disease development were identified from transposon-tagging, micro-array, and proteomics analyses. Here, we introduce especially the genes required for the initiation of pathogenic life cycle, the suppression of otherwise induction of resistance responses, massive production of virulence factors in plant pathogenic bacteria and an unique plant gene responsible for the development of canker symptom. From these findings we came to raise some new strategies to construct disease-resistant plants by genetic engineering.

1. Introduction

Plants can be considered to be basically resistant to the attack by any plant pathogens by inducing a series of effective resistance responses. However, the pathogens have been evolved to escape from the induction of this resistance responses exceptionally in their host plant species. This escape can be achieved by suppressing the elicitation of resistance responses. Thus, all of the plants are known to have the gene sets required for such complex interactions between the pathogens and their compatible and incompatible plants. So, it is important to identify such broad-sense pathogenicity genes and the plant genes required for the cross talk among these genes.

2. Results and Discussions

2.1. Genes required for the entrance of the pathogens into pathogenic life cycle

The soft-rotting bacterial group such as *Pectobacterium carotovorum* subsp. carotovorum and *Dickeya dadantii* (syn. *Erwinia carotovora* subsp. carotovora and *Erwinia chrysanthemi*, respectively) commonly possess the genes of two component regulatory systems such as *phoP/phoQ* [1,2,3], and other regulatory genes such as *slyA* [4] and pir [5], [6] for these pathogens to enter into the pathogenicity life cycle. These regulatory genes were known to be important for the pathogens to adjust to the micro-environments typically found in plants (especially in intra-cellular areas of plants) such as low concentration of Magnesium, acidic pH, and low concentration of sugars. The genes and their translational products under the control of these regulators have been identified using microarray and proteomic analyses.

Thus, the new strategy to control the disease incidence by blocking the entry of pathogen into "disease life cycle" may be now possible. The blockage of disease development at this stage may be most effective strategy for the control of plant pathogens. In fact, the mutants deficient in these regulatory genes and/or in their regulons in *Xanthomonas axonopodis* pv. *citri* and *X. oryzae* pv. *oryzae* (pathogen of citrus canker and that of rice bacterial wilt, respectively) were shown to fail in eliciting disease development. Thus, many strategies using genetic engineering to cause the disturbance of these regulatory mechanisms are expected to provide the new strategy of constructing disease-resistant plants.

2.2. Genes involved in suppression of induced resistance in plants

Representative *avr* (avirulence) genes such *avrXa7*, *avrXa10, avrPto, avrB*, and *avrRps4*, which are known to be responsible for elicitation of the cultivar specific resistance response in the host plants containing each of their cognate resistance genes, were surprisingly found to suppress the elicitation of the resistance response in the plants carrying no cognate resistance gene [7], [8] , [9]. In other words, many of *avr* genes were shown to have dual functions (namely elicitor and suppressor of resistance response)(Fig. 1).

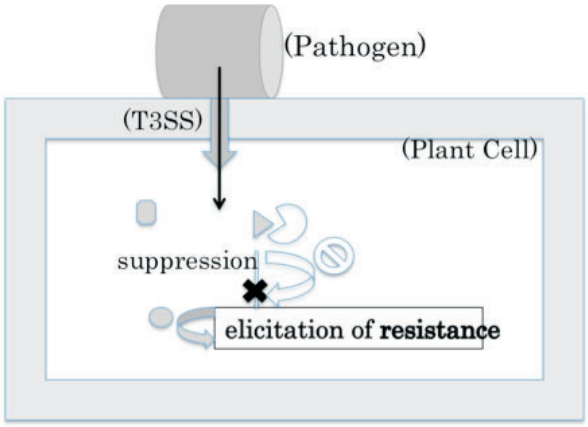

Fig. 1. Strategy to escape from suppression of resistance responses. Pathogen (in this case bacteria) inject (instead of onject) the effectors into plant cells via arrow (ie. Type III Secretion System, T3SS). Some elicit the resistance response and others such as most of avr genes suppress the resistance response. Thus, the block of this suppression should lead to elicitation of the resistance response even in compatible plants.

Furthermore, we found that some of these *Avr* proteins can bind to a specific protein of specific plant. For example, *Apl1* (the translational product of *apl1*, a member of the *avrBs3* gene family of *X. axonopodis* pv. *citri* (a causative agent of citrus canker), was shown to bind

specifically to pectin methyl esterase (PME) of Citrus but not of tomato and tobacco [10]. Binding of *Apl1* to PME was shown to alter the localizations of both *Apl1* and PME (analyzed the localization using their fusions with fluorescent protein(s) using confocal laser microscope) and to be involved in alteration of the activities of both proteins.

Thus, the blockage of the suppressor function of *Avr* protein may be released by binding to its these binding proteins so that the resistance response should remain effective to escape from the disease development (Fig. 1).

2.3. Bacterial genes involved in massive production of virulent factors

In soft rot causing bacteria, the major virulent factor was shown to be pectate lyase (PL). In fact, the purified PL was shown to macerate the plant tissues, the major symptom of soft rot disease, and the treatment of plant blocks with PL inhibitors resulted in failure for pathogen to show soft-rot symptom. We found that multiple regulatory mechanisms are involved in the complex regulation of PL: (1) "Product Induction Mechanism"[11], in which induction can be initiated by the metabolic product of the substrate; (2) "Self Catabolite Repression", in which the break down products of the substrate act as the signal for the repression at high concentration [12], (3) "Hyper-induction by plant components", in which plant components (turned out to be sugars) at low concentration in addition to the inducer of product induction mechanism are responsible hyper-induction of PL [13]. Beside these regulatory mechanisms, the cell density dependent induction by homo-serine lactone, the post-transcriptional regulation by determining the stability of mRNA and others have also been found. Thus, the failure of these regulations especially of hyper-induction of PL by genetic modification of plants, by application of chemicals and by cultivation and postharvest devices may also be promising ways of controlling the soft rot diseases.

2.4. An unique plant genes involved in hyperplasia symptom development

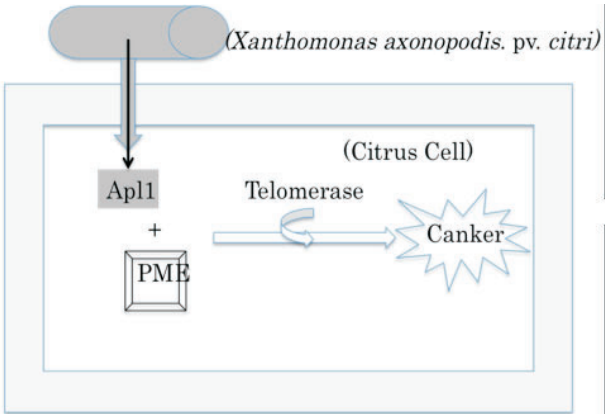

Fig. 2. Apl1 is trafficked from X.a. pv. citri via T3SS into a Citrus cell and binds to PME (pectin methyl esterase). This binding leads to elicit canker symptom with the help of telomerase.

As telomerase, which is the enzyme responsible to stop the reaction of shortening the end part of chromosome (i.e. telomere) upon each cell division, has been thought to be involved in cancer development in animals. In the case of citrus canker, we found that telomerase is also involved in the development of canker symptom where abnormal cell divisions and cell elongation are seen as in the canker cells in animals [14], [15]. Namely, when the expression of *TART* (gene for telomerase) was suppressed by introducing the RNA interference (RNAi) constructs in *Citrus*, the development of canker symptom was severely affected. Thus, this enzyme was thought to be good candidate for the control of Citrus canker disease, though we have to keep in mind that this enzyme is known to be involved in normal physiology of plants too. Thus, the special caution not to disturb these normal functions.

3. Conclusion

From the facts that complex cross talks between plants and pathogens are required for the establishment of pathogenicity, many targets for the control of plant diseases were considered. Here, we showed the findings the genes involved for the pathogens enter into disease cycle and the regulatory genes involved in complex regulation of virulent factors were the good candidates to device new control measures of plant bacterial diseases. Also, we showed the involvement of telomerase of plants are involved in hyperplasia diseases such as a canker symptom. Thus, the control of telomerase may be effective way for the control such diseases.

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