Suppression of UDP-glycosyltransferase-coding Arabidopsis thaliana UGT74E2 Gene Expression Leads to Increased Resistance to Psuedomonas syringae pv. tomato DC3000 Infection

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Plants possess multiple resistance mechanisms that protect themselves against pathogen attack. To identify unknown components of the defense machinery in Arabidopsis, gene-expression changes were monitored in Arabidopsis thaliana under 18 different biotic or abiotic conditions using a DNA microarray representing approximately 25% of all Arabidopsis thaliana genes (www.genevestigator.com). Seventeen genes which are early responsive to salicylic acid (SA) treatment as well as pathogen infection were selected and their T-DNA insertion mutants were obtained from SALK institute. To elucidate the role of each gene in defense response, bacterial pathogen Pseudomonas syringae pv. tomato (Pst) DC3000 was inoculated onto individual T-DNA insertion mutants. Four mutants exhibited decreased resistance and five mutants displayed significantly enhanced resistance against Pst DC3000-infection as measured by change in symptom development as compared to wild-type plants. Among them, member of uridin diphosphate (UDP)-glycosyltransferase (UGT) was of particular interest, since a UGT mutant (At1g05680) showed enhanced resistance to Pst-infection in Arabidopsis. In systemic acquired resistance (SAR) assay, this mutant showed enhanced activation of SAR. Also, the enhanced SAR correlated with increased expression of defense-related gene, AtPR1. These results emphasize that the glycosylation of UGT74E2 is a part of the SA-mediated disease-resistance mechanism.

Keywords: Arabidopsis, Pseudomonas syringae, salicylic acid, systemic acquired resistance, UDP-glycosyltransferase UGT74E2

In nature, plants and pathogens keep up with developing a variety of weapons to attack each other for their successful survival during life time. Plants, as a defender, are under continuous biotic stresses derived from diverse invaders including bacteria, fungi, viruses, and insect herbivores. Plant pathogens can generally be divided into three classes: biotrophs, necrotrophs, and hemibiotrophs according to their lifestyles. Biotrophic pathogens cause minimal damage to the host cell, although disease symptoms usually occur as a result of nutrient exhaustion. Necrotrophs, in contrast, rely upon dead host cell for reproduction and nutrient. However, many pathogens behave as both biotrophs and necrotrophs. Hemibiotrophs are biotrophic in one stage of the infection cycle and necrotrophic in another stage of invasion cycle (Glazebrook, 2005; Hancock and Husiman, 1981; Spoel et al., 2007). In response to those invaders, plants operate complex defense mechanisms at the level of molecular, cellular, and whole-plant (Beckers and Spoel, 2006; Jones and Dangl, 2006). These sophisticated defense responses commonly involve the harmonious transcriptional activation of multiple genes and the phytohormones including salicylic acid, jasmonic acid, and ethylene are involved. The specific disease resistance response often includes the activation of the hypersensitive response (HR) and development of systemic acquired resistance (SAR) (Dangl and Jones, 2001).

Salicylic acid (SA) is a well known crucial component that is associated with plant disease resistance. SA level increases in plant tissue following pathogen infection, and exogenous treatment of SA triggers both induced resistance to broad range of pathogens and the accumulation of pathogenesis-related proteins (Kunkel and Brooks, 2002). Outbreak of attack by certain pathogens generates activation of SA-dependent signaling. Plant resistance to biotrophic pathogens is normally thought to be mediated through SA signaling (Loake and Grant, 2007). In Arabidopsis thaliana, two genes, PAD4 and EDS1, are needed for activating SA accumulation in response to SA-inducing stresses (Falk et al., 1999). Previous reports indicate that the majority of SA is produced by way of SID2 which encodes isochorismate synthase (Widermuth et al., 2001). EDS5 is also essential for production of SA against pathogen attack (Ferrari et al., 2003; Rossi et al., 1998). The experiment of transgenic
NahG, which encodes a bacterial (Pseudomonas putida) salicylate hydroxylase, reveals that SA is required for activation of defense-related genes such as pathogenesis-related gene 1 (PR1) and for induction of SAR (Delaney et al., 1994; Gauffrey et al., 1993). When SA levels are increased, NPR1, which acts downstream from SA, is converted to an active monomer and this signaling regulated the conformation of NPR1 by S-nitrosylation (Mou et al., 2003; Tada et al., 2008). Activated NPR1 then is localized to the nucleus, where it interacts with TGA transcription factor, ultimately leading to the activation of PR1 (Spoel et al., 2003). Even though there are many efforts to dissect SA mechanism in plants, the complete mechanism is not fully defined yet.

Recognition of pathogen often causes a localized resistance reaction, including HR, a programmed cell death (PCD), at site of infection. Previous report showed that this local resistance spreads to the non-infected site (Ross, 1961). SAR requires transmittance of a signal from infected tissue to the systemic leaves. At first, SA was thought to be a SAR-inducing signal because application of exogenous SA triggers defense response including expression of pathogenesis-related (PR) genes, HR, and restriction of pathogen movement (Dempsey et al., 1999). However, the grafting experiment in tobacco showed that transgenic root stock, although unable to accumulate SA, was completely capable of delivering a signal that rendered non-transgenic scions resistant to secondary pathogen infection (Vernooij et al., 1994). Moreover, previous studies suggested that signaling might occur through the conversion of SA to the volatile compound methyl salicylate in tobacco (Park et al., 2007; Shulaev et al., 1997). These results indicate that SA itself is not a mobile signal for SAR.

In plant biological processes, modification of phytohormones, including salicylic acid, jasmonic acid, and ethylene, by glycosylation, methylation, and amino acid conjugation, is regarded as an integral control process.

It was known that most pathogen-induced SA is glycosylated by uridine diphosphate (UDP)-glycosyltransferase (UGT) to form non-toxic SA 2-O-β-D-glucoside (SAG). Recent studies suggest that methylation and amino acid conjugation as well as glycosylation of SA play a specific role in plant defense (Dean et al., 2005; Loake and Grant, 2007). UGTs convey the transfer of glycosyl residues from activated nucleotide sugars to a wide range of acceptor molecules such as secondary metabolites including SA and phytoalexins. Secondary metabolites accomplish multiple functions in plants, including lignifications, UV protection, herbivore protection, and disease resistance. It is supposed that UGTs are involved in bioactivity, solubility and transport of such secondary metabolites within the cell and throughout the plant. This protein constitutes a large gene family in higher plants. It was reported that there are over 120 and 165 UGT genes in Arabidopsis thaliana and Medicago truncatula, respectively. The UGTs are classified by the presence of a carboxy-terminal 42 amino acid consensus sequence that is thought to be involved in binding of the protein to the UDP part of the sugar nucleotide. Recently, the PSPG-box, that is a signature sequence of glycosyltransferase, was found in Dorotheanthus bellidiformis. This consensus sequence also can be identified in ORFs from animal, plant, yeast, and bacterial genomes. A phylogenetic analysis reveals the presence of 14 distinct groups (A to N) of UGTs in Arabidopsis. Among these family members, at first only group D was reported to be associated with disease resistance against some pathogens in Arabidopsis. A UGT group D encompasses 13 members in Arabidopsis. And it was reported that expression of UGT73B3 and UGT73B5 genes is necessary for resistance to Pseudomonas syringae pv. tomato in Arabidopsis. In recent years, a number of UGTs were characterized in Arabidopsis including UGT73C5, UGT73C6, UGT73B2, and so forth. And also, down-regulation of tobacco glycosyltransferase gene (TOGT1) showed decreased resistance to Tobacco mosaic virus (TMV). (Chong et al., 2002; Gachon et al., 2005; Langlois-Meurinne et al., 2005; Ma et al., 2007; Ross et al., 2001). Despite of these efforts for connecting UGTs in plant defense mechanism, the exact role of UGTs remains unclear.

In this study, we performed a reverse genetic screening for finding SA- and Pst- inducible genes, and functional analysis of one of Arabidopsis UGT gene family members, UGT74E2. UGT74E2 was obtained throughout T-DNA insertion mutant screening of bacterial growth assay by using Arabidopsis-Pseudomonas interaction as a model system during the process of local- and systemic-resistance. Our finding suggested that UGT74E2 plays an important role in local and systemic resistance in Arabidopsis.

Materials and Methods

Plant materials and treatments. Arabidopsis thaliana ecotype Columbia-0, 21 different kinds of T-DNA insertion mutants, nap1-mutants, and NahG transgenic plants were grown in pot containing soil. The plants were grown in growth room with 16 h light and 8 h dark (long-day condition) at 23-25°C for 4-5 weeks before carrying out experiments. For sterile culture, seeds were surface-sterilized in 70% ethanol for 3 min, treated with 25% commercial clorox containing 0.05% Tween 20 (ICI Americas Inc., USA) for 5 min, and rinsed five times with sterile water. Then, the seeds were planted onto MS medium and grown in growth chamber for 3-4 weeks before bacterial inoculation. The pots and the plates were stored at 4°C for 72 h to ensure uniform germination.