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REVIEW

Glycosyltransferases: key players involved in the modification of plant secondary metabolites

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Abstract Glycosyltransferases are members of the multigene superfamily in plants that can transfer single or multiple activated sugars to a range of plant molecules, resulting in the glycosylation of plant compounds. Although the activities of many glycosyltransferases and their products have been recognized for a long time, only in recent years were some glycosyltransferase genes identified and a few functionally characterized in detail. Glycosylation is thought to be one of the most important modification reactions towards plant secondary metabolites, and plays a key role in maintaining cell homeostasis, thus likely participating in the regulation of plant growth, development and in defense responses to stress environments. With advances in plant genome projects and the development of novel technologies in analyzing gene function, significant progress could be made in gaining new insights into the properties and precise biological roles of plant secondary product glycosyltransferases, and the new knowledge will have extensive application prospects in the catalytic synthesis of glycoconjugates and metabolic engineering of crops. In this review, we summarize the current research, highlighting the possible biological roles, of plant secondary metabolite glycosyltransferases and discuss their potential applications as well as aspects to be further studied in the near future.

Keywords glycosyltransferases, glycosylation, plant secondary metabolite, metabolic engineering

1 Introduction

A huge number of various low-molecular-weight compounds, defined as plant secondary metabolites, naturally occur in plants. Molecular modifications, such as glycosylation, methylation, hydroxylation and acylation, play a very important role in forming the diversity of plant compounds. Glycosylation, in particular, has been observed in a wide range of biological processes of plants. Glycosyltransferases (GTs) are the enzymes responsible for glycosylation of plant compounds. Results from analyzing the entire *Arabidopsis* genomic sequence indicate that more than 100 putative GTs, which might be involved in the modification of plant secondary metabolites, are present in this model plant (Li et al., 2001). Glycosyltransferases can typically transfer single or multiple activated sugars from nucleotide sugar donors to a wide range of small molecular acceptors of plants. Hydroxylated molecules are the most common acceptors, whilst UDP-glucose is the most common donor in the GT catalytic glycosyl-group transferring reaction, thus the name "UGTs" for plant glycosyltransferases, sometimes.

For a long period of time, although glycosyltransferase activities and glycosylated products were known from a variety of plants (Knofel et al., 1984; Hughes and Hughes, 1994; Schneider and Schliemann, 1994), the enzymes and genes involved in the glycosylation failed to be isolated. Their roles in plant growth and development were also uncertain. Until recently, glycosyltransferases were thought to have only limited influence on the basic physiology of plants. Identification of glycosyltransferase genes and their functional characterization has changed that view. In recent years, dozens of glycosyltransferase genes have been identified, and among these, quite a few have been characterized functionally. It is now recognized that the glycosylation of low-molecular-weight compounds of plants, by adding a sugar moiety to the acceptors, usually changes acceptors in terms of their bioactivity, stability, solubility, subcellular localization and binding property to other molecules, and this possibly reduces the toxicity of endogenous and exogenous toxic

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substances (Bowles et al., 2005). Therefore, glycosyltransferases might have an important role in maintaining cell homeostasis and the regulation of plant growth, development and defense response to stress environments (Jones and Vogt 2001; Lim and Bowles, 2004). The study on glycosyltransferases and glycosylation of plant molecules has thus attracted researchers' considerable interest, because understanding the catalytic mechanisms of the enzymes and their physiological roles would be of great significance for *in vitro* designing and synthesizing of valuable glycosides and for *in vivo* metabolic engineering of crops for important agronomic traits (Kristensen et al., 2005; Lim, 2005; Weis et al., 2008).

Herein, we summarize the recent progress in the research of the glycosyltransferase multigene family, involved in the modification of plant secondary metabolites, highlighting the possible physiological roles of glycosylation in plants. In addition, we discuss the potential application of glycosyltransferases in glycoconjugate synthesis and in the metabolic engineering of important crops. Several aspects of glycosyltransferases to be further studied in the near future are also suggested.

2 Multigene families of plant glycosyltransferases

To date, according to substrate recognition, sequence similarity and phylogenetic analysis, GTs that exist in the biosphere can be divided into 91 distinct families, and among these, family 1 has the most number of GTs which have a close relationship with plants. Substrates for GTs of family 1 are low-molecular-weight lipophilic compounds in which single or multiple glycosylations can take place at the -OH, -COOH, $-NH_2$, -SH and C-C groups (Bowles et al., 2005).

In family 1 there are about 50% of members in which each contains a carboxy-terminal consensus sequence called the plant secondary product glycosyltransferase box (PSPG box) (Hughes and Hughes, 1994). This box consists of 44 amino acids close to the *C*-terminal part of the protein, and is believed to be involved in binding of the activated donor sugars (Fig. 1). The amino-terminal regions of GTs are more variable, supporting the hypothesis that the domain is involved in the recognition and binding of diverse acceptors.

Because of the completion of the whole genome sequence of *Arabidopsis thaliana*, multigene families of glycosyltransferases were first comprehensively analyzed in this plant species. In the *Arabidopsis* GT family 1, most of the GTs are UGTs and have the *C*-terminal consensus sequence except for three GTs (Paquette et al., 2003). Through a search of the *Arabidopsisthaliana* genome with the PSPG motif, a very big glycosyltransferase superfamily consisting of 119 putative UGT genes was found. Further phylogenetic analysis of this superfamily showed that all of those UGTs could be divided into 14 distinct groups (Li et al., 2001). When comparing UGT sequences at amino acid level, we found that most UGTs from other plant species share a certain degree of homology with those of *Arabidopsis*. However, with some UGTs from different plant species, even though their activities and substrates are similar, their sequence similarities may be very low. For example, five UGTs responsible for the glycosylation of cytokinins were identified from *Arabidopsis* (Hou et al., 2004). When these sequences were compared to the cytokinin-glycosylating enzymes identified in *Zea* and *Phaseolus*, only low sequence similarity was observed, and the UGTs of *Zea* and *Phaseolus* form a unique branch on the phylogenetic tree containing *Arabidopsis* UGTs (Hou et al., 2004). It is likely that the *Zea* and *Phaseolus* UGTs evolved from a common ancestor distinct from those identified in *Arabidopsis*.

It has been found that the intron distribution in *Arabidopsis* UGT genes is diverse. Although some genes have one or two introns, over half of *Arabidopsis* UGT genes contain no intron. Analysis of the conserved regions and intron positions of the UGT genes of *Arabidopsis* suggests that at least ten independent intron-insertion events and either one or two intron-loss events have occurred during *Arabidopsis* UGT evolution (Ross et al., 2001).

UDP-sugar is the most commonly used donor for family 1 UGTs, but as for the types of monosaccharides, different UGTs use different monosaccharides. UDP-glucose (UDP-Glc) is the most common sugar donor, whilst UDP-rhamnose (UDP-Rha), UDP-galactose (UDP-Gal), UDP-xylose (UDP-Xyl) and UDP-glucuronic acid (UDP-GlcUA) have also been used for some UGTs (Bowles et al., 2005).

The structural information of proteins could be very important for us to understand the evolution and catalytic mechanisms of family 1 GTs. Unfortunately, the protein structural information on family 1 GTs is very limited at the moment. Recently, Shao et al. (2005) and He et al. (2006) presented the crystal structures of a UDP-glucose flavonoid/triterpene glycosyltransferase (UGT) from *Medicago truncatula*. Their results show that plant glycosyltransferase contains two Rossmann folds and the acceptor binds to residues in the *N*-terminal half, whereas activated

N-terminal < WAPQVEVLAHPAVGCFVTHCGWNSTLESISAGVPMVAWPFFADQ > C-terminal

Fig. 1 PSPG-box consensus sequence of plant glycosyltransferases. Highly conserved amino acids are indicated by two asterisks (identity > 80%) or one asterisk (identity > 50%) above the letters of amino acids. PSPG box: plant secondary product glycosyltransferase box.

donor sugars bind to amino acids in the *C*-terminal region. In addition, their findings revealed the key residues involved in the recognition of donor substrates, and mutagenesis confirmed the roles of these key residues in enzyme activity, providing an initial structural basis for understanding the complex substrate specificity and regiospecificity underlying the glycosylation of plant molecules.

As more protein structures of plant GTs of family 1 are solved and more biochemical data are available on the activities of plant GTs *in vitro*, hopefully, we could predict the structure of every glycosyltransferase identified in plant and have an in-depth understanding of their evolution and catalytic mechanisms.

3 Approaches to GT identification

Due to the development of new biotechnologies, the studies on plant GTs have advanced much in recent years. Several approaches, including through biochemistry, bioinformatics, molecular biology, and genetics, have been successfully used to clone, identify and analyze genes that encode plant GTs. More often however, identifying GT genes requires an integration of several methods. Therefore, the following approaches are divided basically according to their differences in the first steps of identifying GTs.

3.1 Classical biochemical methods

The isolation or purification of the target proteins directly from plants is usually the first step in these methods when identifying GT genes. However, these methods often encounter difficulties in getting target proteins with high purity because of the complexity of protein components in plants. Once the target proteins are purified, their enzyme activities toward glycosylation of specific substrates can be investigated and the corresponding genes can be cloned by the derived nucleotide sequences from the amino acids. By classical biochemical methods, plant GT gene from *Arabidopsis*, *GT72B1*, has been cloned and the gene product GT72B1 has been confirmed to be capable of glycosylating inorganic pollutant 3,4-dichloroaniline *in vitro* (Loutre et al., 2003). Another plant GT gene encoding limonoid UDP-glucosyltransferase in *Citrus* has also been cloned by this method (Frydman et al., 2004).

3.2 Bioinformatics combined with biochemistry

The development of genomics and bioinformatics greatly facilitates the identification of plant GTs. As mentioned above, through the bioinformatical analysis of many GTs identified from plants, the PSPG conserved box was proposed. This consensus sequence provides a good starting point for searching new glycosyltransferases from a database. After the related expressed sequence tags (ESTs), cDNAs or genes are determined by means of bioinformatics, a series of investigations such as cloning the full length of cDNA, expressing and purifying recombinant proteins *in vitro*, analyzing the substrate-specificity and enzyme activities of recombinant proteins can be sequentially performed. Using these methods, several glycosyltransferases for the plant hormone auxins, cytokinins, brassinosteroids (BR), abscisic acid (ABA) and salicylic acid (SA), have been successfully identified (Jackson et al., 2001; Lim et al., 2001; Hou et al., 2004; Poppenberger et al., 2005).

3.3 Molecular biological methods

Because of the rapid development of molecular biology, many methods could be used in the identification of plant GTs. For example, based on the conserved amino acid sequence of GTs, degenerate primers can be designed and RT-PCR can be carried out using plant RNA material to clone putative GTs. Using molecular biological methods, Moraga et al. (2004) cloned a saffron apocarotenoid crocetin GT, which provides the foundation for producing crocin in heterologous systems.

3.4 Genetic methods

The use of mutants is very important for gene identification and functional analysis. However, the lack of mutants of plant glycosyltransferases makes it difficult to use this method to identify GT genes currently. Quiel and Bender (2003) luckily cloned a GT gene, *UGT74F2*, by screening blue fluorescent mutants of *Arabidopsis*. UGT74F2 could catalyze the glycosylation of anthranilate to form a blue fluorescent anthranilate glucose ester. Different from other methods, the identification of UGT74F2 was merely an accident, because the experiment was supposed to have no relation to GT in the beginning.

4 Physiological roles of GTs in plants

Up to now, studies on glycosyltransferases and glycosylation towards low-molecular-weight plant compounds have been conducted mainly in *Arabidopsis* and several other plant species. Along with the availability of considerable biochemical data and genomic data on plant GTs, the analysis of the biological roles of GTs in plants has been possible by using the techniques of functional genomics, especially the strategies of gene over-expression and gene knock-out (or knock-down). Recent results obtained with functional characterization of plant GTs indicate that glycosyltransferases might play an important role in plant growth, development and interaction with the environment.

4.1 Taking part in hormone homeostasis

The homeostasis of hormones is crucial to plant growth, development, and adaptation responses to changes in the environment. Many mechanisms have evolved to control precisely the level of different hormones in plant cells and tissues. Glycosylation is thought to be one of these mechanisms, because glycosides of all classical hormones except ethylene have been identified in plant extracts (Creelman and Mullet, 1997; Mok and Mok, 2001; Fujioka and Yokota, 2003; Woodward and Bartel, 2005).

The bioassays of activities of hormone glycosides by exogenous application showed that the activities of hormones could generally be reduced or lost after glycosylation, although the inactivation mechanism is not very clear. It was assumed that glycosylation could alter recognition between acceptors and hormones or change properties of hormones (Kleczkowski and Schell, 1995).

At present, some studies have dealt with endogenous glycosylation of plant hormones. Jackson et al. (2002) cloned *UGT84B1* in *Arabidopsis*, and they revealed that UGT84B1 could endogenously synthesize 1-*O*-indole acetyl glucose ester (IAGlc). Overexpression of that GT in *Arabidopsis* led to a phenotype of aerial parts similar to the auxin-deficient mutant as well as a root system losing geotropism (Jackson et al., 2002; Jackson et al., 2001). In another example, overexpression of UGT73C5 resulted in BR-deficient phenotypes and reduced levels of active BRs in transgenic *Arabidopsis*, suggesting that glucosylation of BRs reduces their bioactivities. On the other hand, silencing UGT73C5 led to a reduction of BR glucosylation, confirming that UGT73C5 is involved in the regulation of the active BR pool in plants (Poppenberger et al., 2005). Recently, Rodo et al. (2008) reported the effects of cytokinin *O*-glucosylation in *maize*. Their results showed that overexpressing *ZOG1* gene (encoding a zeatin *O*-glucosyltransferase from *Phaseolus lunatus* L.) in the roots and leaves of the transgenic *maize* greatly increased the levels of zeatin-*O*-glucoside and produced a similar phenotype to those of cytokinin-deficient plants, leading to growth retardation and tasselseed formation. These results indicate that cytokinin *O*-glucosylation plays an important role in balancing cytokinin levels.

What are the effects of cytokinin *N*-glucosylation? It is believed that *N*-glycosylated cytokinins are irreversibly inactive forms (in contrast to reversibly inactive forms of *O*-glycosylated cytokinins), but the precise *in vivo* function of *N*-glycosylated cytokinins is unknown. Hou et al. (2004) made the first step towards answering this question. They screened the recombinant GTs from *Arabidopsis* and identified two UGTs responsible for the cytokinin *N*-glucosylation for the first time. The identification of cytokinin *N*-glucosylating GTs paved the way for the functional analysis of cytokinin *N*-glucosylation. Further, other GT genes capable of glycosylating ABA (Xu et al., 2002; Lim et al., 2005a) and SA (Taguchi et al., 2001; Lim et al., 2002) have also been identified. It is expected that these genes will further promote the study of the relationship between glycosylation and hormone homeostasis.

4.2 Taking part in defense response

There are several examples that implicate the role of glycosylation of secondary metabolites in plant defense response to biotic stress. For instance, TOGTs are *tobacco* glycosyltransferases with the highest *in vitro* enzyme activity towards hydroxycoumarin, scopoletin, and hydroxycinnamic acids. It was found that reducing the expression of TOGTs in transgenic *tobacco* substantially decreased the levels of scopoletin glucoside and simultaneously impaired the resistance to *Tobacco Mosaic Virus* (TMV) (Chong et al., 2002). However, overexpression of TOGT1 in transgenic *tobacco* caused enhanced resistance to *Potato Virus Y* (Matros and Mock, 2004). Both of these studies suggested that glycosyltransferases might play an important role in glycosylating scopoletin and enhancing plant resistance to pathogens by some unknown mechanisms.

Another example is the C-3 oligosaccharide chain of saponins. It is believed that the oligosaccharide chain consisting of Glc, Gal, arabinopyranose (Ara), GlcUA, Xyl or Rha attached to the C-3 of saponins may be crucial for resisting fungal pathogens. Removing these sugar residues often leads to loss of bioactivity. Interestingly, fungal pathogens could produce hydrolases to attack the C-3 oligosaccharide chain of saponins in order to detoxify saponins after their invasion. For example, the oat root-infecting pathogen *Gaeumannomyces graminis* produces avenacinase, a kind of β-glucosidase, to detoxify the triterpenoid avenicin saponins by removing the Glc from the C-3 oligosaccharide chain. *Tomato* pathogens could also detoxify steroidal glycoalkaloids in the same way (Sandrock and VanEtten, 1998).

4.3 Taking part in detoxification

As described above, fungal pathogens could detoxify plant glycoside molecules by hydrolyzing the glycosidic bonds. However, in the battle between plants and pathogens, plants seem to take an opposite strategy, namely forming glycosidic bonds to detoxify the toxicity of pathogens. The evidence comes from the detoxification of trichothecene deoxynivalenol (DON) which is produced by the fungous *Fusarium,* one of the most familiar fungi of cereal species such as *wheat*, *barley* and *maize*. DON is not only harmful to plant growth but also to human health, and it is considered as a virulence factor in fungal pathogenesis. *In vitro* experiments indicate that the recombinant protein UGT73C5, a putative glycosyltransferase of *Arabidopsis*, could catalyze DON to form DON-3-*O*-glucoside. Glycosylated DON could lose toxicity, and overexpressing UGT73C5 in transgenic *Arabidopsis* could enhance the

resistance of transgenics to DON (Poppenberger et al., 2003), suggesting a role of glycosylation in the detoxification of plants.

Further, GTs can also detoxify exogenous chemical compounds such as herbicides, insecticides, pollutants, as well as xenobiotics (Lim et al., 2002; Loutre et al., 2003). For example, overexpression of either GT BX8 or GT BX9 in *Arabidopsis* reduced the toxic effects of 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) applied on transgenic plants (von Rad et al., 2001). Several UGTs of *Arabidopsis* have also been confirmed to be involved in the detoxification of the xenobiotic 2,4,5-trichlorophenol (TCP) (Messner et al., 2003) or the pollutant 3,4-dichloroaniline (DCA) (Loutre et al., 2003).

4.4 Taking part in biosynthesis and storage of secondary metabolites

In the long-term process of evolution, plants have developed many metabolic pathways to synthesize a vast multitude of secondary metabolites for environmental adaptation. Many studies have shown that glycosyltransferases and glycosylation reactions could be involved in the biosynthesis, modification, transportation and storage of secondary metabolites.

In the lignin biosynthesis pathway, lignin monomers (coumaryl, coniferyl and sinapyl alcohols) need to be translocated from the cytosol to the cell wall, where they are polymerized into lignin. The glucosides of lignin monomers have been considered as the transport forms. The recombinant glycosyltransferases, UGT72E2 and UGT72E3, of *Arabidopsis* display strong activities of 4-*O*-glucosyltransferase to phenylpropanoids, in particular, the coniferyl and sinapyl alcohols (Lim et al., 2005b), suggesting their involvement in the biosynthesis of lignin. Down-regulating the expression of these glucosyltransferases in transgenic *Arabidopsis* plants could severely reduce the glucoside levels of lignin monomers (Lanot et al., 2006), but the relationship of monolignol glycosylation and lignin synthesis has not been clarified yet.

Flavonols, monoterpenoids and hydroxybenzoic acids usually accumulate as both aglycones and glycosides. In the case of flavonols, Jones et al. (2003) identified two GTs (UGT73C6 and UGT78D1) and Tohge et al. (2005) identified three GTs (UGT79B1, UGT75C1 and UGT78D2) involved in the biosynthesis of flavonol glycosides in *Arabidopsis*. Glycosylation is usually the last step of flavonol biosynthesis metabolism, probably indicating a requirement of stabilization, reactivity or translocation. Multiple additions of sugar moieties to a given compound, in parallel or in chains, give rise to a broad spectrum of secondary metabolites, thus contributing to their unique properties. For example, one single flavonol, quercetin, has 300 different glycosides naturally occurring in plants. The biosynthesis of some flavonol or anthocyanin glycosides plays an important role in the occurrence of fruit and flower color, fruit flavor and the protection of plants from damage due to ultraviolet radiation (Bowles et al., 2005). Glycosylation also strongly affects the bioavailability of these dietary compounds, some of which display antioxidant or anticancer activity.

The biosynthesis of steviol glycosides is another example. *Stevia rebaudiana* accumulates a mixture of at least eight different steviol glycosides, kinds of compounds that are unique in the plant world because of their intense sweetness (300 times sweeter than sugar) and high concentration in leaf tissue. The majority of the glycosides are formed by four glucosylation reactions that start with steviol, producing the mixture of mono-, di-, tri- and tetra-glycosides. The tri-glycoside stevioside and the tetra-glycoside rebaudioside A represent the majority of the steviol glycosides present in *S. rebaudiana* leaves. The first step of stevioside biosynthesis takes place in plastids. After glucosylation of the C-4 carboxyl position of steviolbioside, the glycosides are transported into the vacuoles of leaf cells. The glycosylation of the C-4 carboxyl position appears to be critical for glycoside transport into the vacuole, because glycoside accumulation only occurs following that step. Using functional genomics strategy, Richman et al. (2005) found three glucosyltransferases involved in the synthesis of the major sweet glucosides of *Stevia rebaudiana*, and their *in vitro* regioselective glucosylating activities towards steviol were confirmed by the recombinant enzymes.

5 Potential applications of plant GTs

As more and more plant glycosyltransferases are identified and their biological roles are revealed, increasing attention is paid to glycosyltransferases due to their potentials in practical applications. At the first stage, it appears that the use of plant glycosyltransferases is mainly in the *in vitro* biochemical synthesis of some valuable glycoconjugates. For example, Karim and Hashinaga (2002) cloned a GT from *pummelo* and by using enzyme immobilization, they have successfully converted the limonoids, the bitter components of lemon juice, into tasteless glycosides. ABA can exist as two enantiomers, i.e. (±)-ABA. Both enantiomers occur in chemical preparations. It was found that UGT71B6 of *Arabidopsis* enantioselectively glucosylated only (+)-ABA, thereby it could be used to separate (+)-ABA from (–)-ABA, offering an alternative to chemical synthesis for the production of pure (+)-ABA (Lim et al., 2005a). Weis et al. (2008) exchanged the *N*-terminal domain and the *C*-terminal domain of two *Arabidopsis* GTs, 71C1 and 71C3, to construct chimeric mutants. This engineering of proteins provides a basis for creating novel GTs and for improving the substrate affinity of the enzymes. Enzymatic synthesis of glycosides has many advantages over conventional chemical methods, for

example, synthesizing stereospecific glycosides without the use of hazardous chemicals as blocking and deblocking reagents, large scale production in fermentation by using the microbial whole-cell systems, with fewer synthetic steps and lower cost. Therefore, the potential application of glycosyltransferases as biocatalysts in *in vitro* glycoconjugate synthesis has attracted researchers' considerable interest in recent years. On this topic, Lim (2005) has given a detailed discussion in his review.

The study of enzymatic activities and biological roles of plant GTs will also provide the basis for applications in crop improvement in the near future. Several GT-encoding genes, such as those involved in hormone homeostasis, defense response and detoxification, may be suitable candidates for insertion into a variety of plants with the aim of improving crop plants. For example, the recent identification of UGTs acting on ABA, auxins, cytokinins and BRs opens new perspectives on how to manipulate the levels of active hormones in plants and on how to control growth and development of plants. Other applications may involve the metabolic engineering of GTs to detoxify pesticide residues and pollutants so as to increase food security, enhance plant resistance to biotic and abiotic stress and increase the content in food of glycosides with antioxidant or anticancer activities. There is also the possibility that overexpressing specific GTs in plants could change flower colors or fruit flavors.

6 Conclusions

Generally speaking, the present study on plant GTs is still in its initial stages. Although the enzymatic activities and substrate specificities of many GTs of plants are known through *in vitro* analysis, their biological functions *in vivo* need to be confirmed. Encouragingly, the biochemical characteristics of most plant GTs identified so far are consistent with their physiological ones in plants. Therefore, *in vitro* identification of enzymatic activities of plant GTs provides a good starting point for the *in vivo* functional analyses. As a widespread modification of metabolites, glycosylation and its responsible glycosyltransferase multigene families might take part in numerous processes of plant growth, development and response to the environment. However, knowledge of their physiological roles is very limited at present.

Here, we propose that the following aspects deserve to be further studied: (1) GTs that are involved in hormone homeostasis. Given that glycosylation is regarded as one of the hormone homeostatic mechanisms, it is meaningful to further identify the GTs involved in the balancing of all of naturally occurring hormones in plants and further clarify their molecular mechanisms regulating hormone homeostasis. (2) GTs that are involved in plant resistance to abiotic stress. Several studies have confirmed that GTs participate in plant defense reaction to biotic stress, but we do not know whether GTs take part in the plant response to abiotic stress such as salinity, drought, high temperature and low temperature. In the authors' laboratory, we have found that the expression of two GTs of *Arabidopsis* can be strongly induced by salt (unpublished data), and a further study on transgenic plants is in progress. (3) GTs that are involved in plant signal transduction. Whether or not the glycosylation of small molecular compounds has a role in plant signal transduction has not yet been clarified to date. Considering that glycosyltransferases have a vast multitude of substrates and that glycosylation usually alters the small molecular compounds in their bioactivity, stability, solubility, subcellular localization and binding properties, glycosyltransferases may play a role in plant signal transduction, which remains to be determined. (4) GTs that exist in important crops or trees. So far, studies on glycosyltransferases and glycosylation towards plant compounds are carried out mainly in the model plant *Arabidopsis* and several other plant species. Little is known about the GTs of important crops such as *rice*, *wheat*,*tomato* or trees such as *poplar*. Comprehensive studies of GTs in these economically important crops or trees will identify promising genes for their improvement by genetic engineering. Recently, Cao et al. (2008) constructed a *rice* glycosyltransferase phylogenomic database and identified *rice*-diverged glycosyltransferases. These results will greatly facilitate the study of GTs in *rice* and other crop plants.

Glycosyltransferases constitute a large class of enzymes that have numerous members and directly participate in the diverse pathways of plant secondary metabolism, and thus potentially play critical roles in plant growth and development. Without a doubt, making thorough and extensive studies on plant secondary metabolite glycosyltransferases would be of great significance from both basic and practical points of view. More and more biological functions of plant GTs are being characterized, which will provide new insights into the molecular mechanisms of cell homeostasis and integral regulations of plant growth and development. Furthermore, plant GTs will open up a vast range of prospects not only in the enzymatic synthesis of valuable glycoconjugates but also in terms of crop improvements.

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