Glycosylation of plant secondary metabolites: the regulation from chaos to harmony

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Abstract

The Bible of life is homeostasis. Plants respond to stresses by forming a coordinated defence process from a seemingly chaotic response. Plant molecular glycosylation is the most common and most extensive type of modification reaction in plants. Glycosylation is also an indispensable step for secondary metabolites to produce stable, soluble, storage, detoxification or inactive forms through conjugation with sugars. The present review adds to the description of glycosylation and corresponding glycosyltransferases (GTs) in plants during defence versus growth, which, in comparison to other plant defence regulations, are relatively uncharted. Growth inhibition under stress conditions is related to the redirection of plant resources (such as energy and metabolism) from primary metabolism and growth to activate defence mechanisms in resource-limited environments. A variety of small molecule compounds colored by GTs play an important role in buffering the effects of biotic and abiotic stresses on plants. Autotoxicity defense compounds are stored in the form of inactive glycosides in the defense reaction to release toxic aglycones and to produce cascade effects. All told, glycosylation of secondary metabolites is of first importance to balance growth and defence in an efficient and energy-saving way, but still far to go.

1 INTRODUCTION

Nature today bestows rewards upon plants who are comfortable with change, and it may punish those who are not. In the long evolutionary history of plants, surrounding environmental factors have provided an impetus for the adaptive growth of plants under natural selection, forming a two-way influence between plants and the environment. Plants will change their growth and development (by changing plant height, leaf curling, increasing branching, etc.) to adapt to constantly changing light signals, temperature signals, and osmotic pressures and contents of metal ions in the soil, as well as other external environmental queue. Moreover, these environmental signals will also stimulate the synthesis and shunting of secondary metabolites in plants. Secondary metabolism is the result of plants interacting with biotic and abiotic factors during long-term evolution processes.

Although secondary metabolites in plant immunity have long been considered one-dimensional chemical warfare agents, emerging evidence has revealed that secondary metabolites may play a novel role in plant defence responses. For example, indole glucosinolates (IGs) and benzoxazinone glucosides (BXs) regulate the deposition of callose and the closure of stomata in the defence response (Bednarek, 2012). In *Capsella rubella* , a cruciferous plant that does not produce IGs, there is no defect in the deposition of callose triggered by flg22 (Bednarek et al., 2011), indicating that changes in metabolites that regulate defense responses may be relatively rapid. However, why and how plants use specific compounds to control conservative defence responses remain to be determined. Another important research area is to determine the possible alternative functions of specific metabolic pathways in plant immunity. In plants where IG biosynthesis or metabolism is affected after pathogens attack, it is observed that the accumulation of callose is not affected, indicating that IG metabolites may have an additional role in plant immunity, although the regulation of corpus callosum

deposition was observed after microbial-associated molecular pattern (MAMP) recognition. Research on IGs and camalexin further shows that different subclasses of phytochemicals, even if they come from the same precursor, can mediate specific functions at different stages of infection. Our understanding of the spatial organization of metabolic pathways and the transport of phytochemicals in plant cells is still very limited. However, for specific loss-of-function mutations, genes involved in the synthesis of secondary metabolites have been able to be associated with the presence of these compounds and resistance to specific pathogens. This method has been successfully used to prove the defence function of several secondary metabolites in the plant body. Such as benzoxazinones (Frey et al., 2009), which are mainly related to the defence effects of gramineous and dicotyledonous plants, glucosinolates in cruciferous plants (β-glucosinolate-N-hydroxysulfate) (Fan et al., 2011), and camalexin et al., 2010) produced by *Arabidopsis thaliana* and other related species.

The biosynthesis, metabolism and secretion of secondary metabolites triggered by pathogenic bacteria are some of the oldest responses of plants to pathogenic microorganisms (Bednarek et al., 2009; Dixon, 2001). Interestingly, highly diverse pathogen-induced metabolic pathways can be activated in different branches of the plant phylogeny, leading to the accumulation of thousands of different metabolites. Therefore, in plant immunity, it is still a major challenge to determine the biosynthetic pathways of secondary metabolites and the functions of their corresponding products. In the past few decades, great progress has been made in understanding the innate immune system of plants (Dodds & Rathjen, 2010; Panstruga et al., 2009). Research progress on plant immunosensor-mediated non-self-perception mechanisms includes the recognition of MAMPs through pattern recognition receptors and the detection of pathogen effectors through resistance proteins (Jones & Dangl, 2006; Reimer-Michalski & Conrath, 2016; Yu et al., 2017). In addition, significant progress has been made in elucidating the signal cascade leading to the transcriptional activation of defence-related genes(Kourelis & Van, 2018). However, the mechanism for directly preventing the development of pathogens is still unclear. It is speculated that the production of ROS, the strengthening of the cell wall (including the deposition of β-1,3-glucan polymer callose), the production of antimicrobial peptides, and the production of low molecular weight secondary metabolites are all related to this phenomenon. These processes contribute to plant immunity and participate in the prevention of pathogen growth.

Phytoalexin is a low-molecular-weight compound with antipathogenic activity that is produced and accumulates on the infected part of the plant and its surroundings when plants are infected by pathogenic microorganisms. Numerous studies have shown that after a plant has undergone pathogenic infection, there is an obvious increase in the release of phytoalexin, suggesting that this release represents a plant defence reaction to previous pathogens, which plays an important role in plant resistance to diseases (Dixon, 2001). There are many chemical species of phytoalexins, including phenols, terpenes, furan acetylenes, steroidal glycosides, alkaloids, sulfur-containing compounds and indoles. Studies and reviews on the regulation of biosynthesis, biological activity, structure-activity relationship, and metabolism of phytoalexins by microorganisms have been published (Harborne, 1999; Jeandet et al., 2013). A large number of phytochemicals have been isolated and identified from various plants, such as camalexin in cruciferous plants, capsidiol and scopoletin in Solanaceae, pisatin in legumes (Ahuja et al., 2012), aucuparin in apple subfamily plants (Jia et al., 2018), and N-acyltryptamine phytoalexin in rice (Peng et al., 2004). Some of the isolated and identified phytoalexins are also the main active ingredients in medicinal plants. The antibacterial properties of many pathogenic plant metabolites identified in vitro make these compounds candidates for plant antibiotics. However, in vitro detection of antibacterial activity may be misleading, and certain phytochemicals may have other functions in plant immunity. As a kind of "chemical defence" substance, phytoalexin needs to reach a certain concentration in the plant body to exert a bacteriostatic effect.

An important strategy to maintain the concentration of active metabolites is the chemical modification, which can change the bioavailability and activity of the compound. The glycosylation of plant small molecule compounds is a common physiological phenomenon, and the glycosylation of small molecule lipophilic receptors has been proven to be a key regulatory mechanism of plant cell metabolic homeostasis. Plant glycosyltransferases (GTs) are enzymes that are specifically responsible for catalysing this glycosylation reaction. GTs convert the active sugar group from nucleotide sugars, usually from uridine diphosphate-glucose (UDPG), that are transferred to a series of plant small molecule compound receptors such as hormones, secondary

metabolites, pathogen infestation, and plant internal and external toxic substances. GTs contain a common sequence, which is believed to be involved in the binding of nucleotide sugars and act as a donor in the transfer reaction; the acceptor contains a variety of molecular structures and biological functions; but the enzymes that recognize these compounds can also recognize structurally related receptors produced in other organisms or the environment. The role of GTs and the evolution of the multigene GT family have enhanced the plasticity of plant development and metabolism, which is a result of the sedentary lifestyle of plants. Although the content of these glycosides is high under stress conditions, the biological significance of the formation of these compounds is still elusive. Glycosylation is an important hormone metabolism modification that can affect hormone activity, regulate hormone dynamic balance, and control plant growth and development. Moreover, glycosylation plays an important role in cell "housekeeping" and buffers the effects of biotic and abiotic stresses on plants, and the response of plants to adverse stresses involves a large number of glycosylation modifications. What's more, glycosylation will change the biological activity, water solubility, stability, intracellular and overall plant transport characteristics, subcellular localization, and mutual recognition of small molecule compounds, as well as their binding characteristics with receptors, and this process can also reduce/remove the toxicity of endogenous and exogenous substances (xenobiotics) (Eng-Kiat et al., 2004). GTs perform a variety of functions and are involved in many biological activities. They work with a wide range of substrates. Meanwhile, glycosylation can also produce cascade effects. Therefore, the effect of glycosylation on small molecule compounds affects many aspects of plant growth and development.

2 GLYCOSYLATION OF SECONDARY METABOLITES AND PLANT METABOLISM HOMEOSTASIS

2.1 Coordinating defence versus growth

To cope with constant biotic and abiotic stresses, plants have a series of defence response mechanisms that result in the accumulation of thousands of different metabolites. However, in autoimmune mutants such as snc2-1D, npr1-1 and s3hs5h, there is a trade-off between defence and growth. These mutants accumulate high levels of defence hormones and exhibit a severe dwarf phenotype (Zhang et al., 2017). However, long-term and structural defence responses, such as those activated during systemic acquired resistance (SAR), cannot be sustained because they require resources and energy for growth and reproduction (Karasov et al., 2017). Other mechanisms have gradually been revealed to play a role in coordinating defence versus growth (DvG). To balance growth and defence responses, plants constantly monitor and adjust the homeostasis of these compounds. Dynamic changes in the level of immune signalling molecules enable plants to respond quickly and appropriately to danger (Hartmann & Zeier, 2019; Huang et al., 2019). Therefore, the biosynthesis, transportation and homeostasis of signalling molecules are strictly regulated, and most of the signalling molecules involved in SAR induction can be found in the phloem after infection (Fu & Dong, 2013).

2.1.1 Regulating the dynamic balance of metabolites to maintain plant growth

The dynamic balance of hormone contents in plants plays a vital role in the growth, development, and environmental response of plants. Plant hormones are continuously synthesized, metabolized and transported, regulating hormone levels in different organs at various stages and maintaining normal growth and development. In addition to ethylene, glycosides of other classic hormones have been found in plants, so glycosylation is considered to be one of the mechanisms that precisely regulates the contents of different hormones in different tissues and cells in plants Bonnie, 2005). It is generally believed that glycosylation can reduce or even eliminate the biological activity of phytohormones. The reason for the inactivation of hormones by glycosylation is not clear. It may be that glycosylation affects the receptor's recognition of hormones, or glycosylation may change other aspects of hormones. A growing number of GTs have been shown to participate in the regulation of plant life through the glycosylation of hormones. IAA is an important plant hormone. The ability to synthesize glucose conjugates of IAA is considered to be a common feature of all vascular plants (Sztein et al., 1995). This synthetic pathway was revealed in maize; during the maturation period of maize, IAA and UDPG synthesize the glucose ester of IAA (IAGlc). When screening the members of glycosyltransferase family 1 for IAA substrate activity, it was found that UGT84B1 could

transfer glycosyl groups from UDPG to IAA. Overexpression of UGT resulted in a phenotype of reduced IAA levels in transgenic *Arabidopsis thaliana* , weakened gravitational growth of roots, and loss of apical dominance. Moreover, the glycosylation products of IAA in plant extracts have also been shown to be significantly increased in transgenic compared to wild-type plants, indicating that glycosylation would affect the steady state of IAA (Jackson et al., 2002). Brassinosteroids (BRs), as signalling molecules, play a vital role in the regulation of plant growth and development. BRs play a local role near their synthesis site, so the homeostasis mechanism must work at the cellular level to balance the concentration of BRs. UGT73C5 and UGT73C6 in *Arabidopsis thaliana* have been shown to catalyse the glycosylation of brassinolide (BL) (Poppenberger et al., 2005). Transgenic plants overexpressing UGT73C5 and UG773C6 showed BR deficiency, and the content of BRs was reduced. Overexpression of the glycosyltransferase ZOG1 in transgenic tobacco can convert zeatin (ZT) in tobacco to high levels of zeatin-O-glycoside. In tobacco transgenic leaf disc culture, the inducible promoter is used to promote the expression of ZOG1. The transgenic culture needs 10 times more ZT than the nontransgenic culture to form buds from the callus, indicating that ZT is inactivated due to glycosylation in the transgenic culture (Martin et al., 2001). In addition, Hou et al. (2004) found through in vitro biochemical analysis that *Arabidopsis thaliana* cytokinin (CK) glycosyltransferases are a multigene family with at least five members. Among them, UGT76C1 and UGT76C2 can catalyse the N position of CK and produce N-glycoside; the UGT76C2-overexpressing body is less sensitive to CK in terms of main root elongation, lateral root formation, and chlorophyll and anthocyanin accumulation. UGT85A1, UGT73C5, and UGT73C1 catalyse glycosylation at the -OH position of CK and produce O-glycosides. It is generally believed that the N-glycoside of CK is the permanently inactivated form of the hormone, and the activity of the hormone cannot be reversed, while the O-glycoside is the storage form of the hormone, which can restore CK activity through deglycosylation under certain conditions. Xu et al. (2002) cloned an ABA glycosyltransferase from adzuki bean, and in vitro recombinant protein experiments showed that the gene product can specifically glycosylate trans-abscisic acid. Lim et al. (2005) also identified the ABA glycosyltransferase UGT71B6 in *Arabidopsis thaliana* , and this gene product can recognize naturally occurring cis-abscisic acid. The glycosylation of SA has been studied thoroughly in tobacco, and some SA-induced glycosyltransferase genes have been found, such as Is5a and Is10a (Horvath et al., 1998) and TOGT1, TOGT2, SAGT, NtGT1a and NtGT1b. UGT74F1 and UGT74F2 in *Arabidopsis thaliana* are active against SA. The glycosylation of lignin monomers has also been shown to contribute to the growth and development of plants. The lignin of higher plants provides mechanical support for plant stems and realizes the conduction of water from roots to leaves, which is very important for plant growth and development. Glycosyltransferase UGT72B1 has been shown to catalyse the glycosylation of lignin monomers in plants. Mutant plants shows lignin accumulation, secondary cell wall thickening, dwarfing, severe anthocyanin accumulation in the stem tip, and growth inhibition. Glycosylation of lignin monomers is very important for maintaining normal cell wall development and lignin synthesis.

2.1.2 Participating in the plant defence response

2.1.2.1 Responding to biotic stress

The glycosylation of small molecules catalyzed by GTs plays an important role in the response of plants to biotic stress. Usually, when plants defend against pathogens, they trigger SA and JA-mediated signaling pathways. They usually act in an antagonistic way through mutual inhibition. Glycosylation of small plant molecules by UDP glycosyltransferases (UGTs) plays an important role in regulating the activity of signalling molecules, such as IAA (Jin et al., 2013), ABA (Chen et al., 2020), CK (Hou et al., 2004), SA(Dean & Delaney, 2010) and JA (Song, 2005). Blocking SA glycosylation has been shown to enhance disease resistance (Noutoshi et al., 2012). It has been reported that UGT76B1 uses the intermediate metabolite of isoleucine (2-hydroxy-3-methylvaleric acid) as a glycosylation substrate. It is an intermediate link between the SA and JA pathways, and it also proves that the connection between amino acid-related molecules and plant defence is mediated by small molecule glycosylation. In the absence of pathogenic bacterial infection, UGT76B1 overexpression impedes the SA-dependent plant defence pathway, promotes the JA response, and delays senescence. These mutants lead to enhanced resistance to*Pseudomonas syringae* and accelerated senescence but increased sensitivity to necrotizing *Streptococcus cuprous* (von et al., 2011). UGT73B3 and UGT73B5

are inducible genes of SA, and their mutants show ROS accumulation, which causes a hypersensitive reaction (HR) response after inoculation with pathogenic bacteria, during which cell death increases (Simon et al., 2014). UGT74F1 and UGT74F2 are SA glucosyltransferases; UGT74Fl only forms SA sugar (SAG), while UGT74F2 forms both SAG and SA glucose ester (SGE) (Dean & Delaney, 2010). Compared with wild-type plants, plants with UGT74F1 and UGT74F2 mutations displayed altered levels of SAG and SGE, to a certain extent, but the effects in the face of pathogen infection were different. Correspondingly, the mutants showed enhanced resistance to pathogens (Boachon et al., 2014). The main catabolic forms of SA, 2-3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA), are bound to glucose and xylose through UGT76D1. After infection with DC3000, the levels of DHBA glycosides and SA in UGT76D1 mutants decreased, and the immune response was delayed. Overexpression of UGT76D1 leads to a large accumulation of SA and a lesion phenotype similar to HR (Huang et al., 2018). MeSA, as a mobile signal for plant systems to acquire resistance, also undergoes glycosylation modification in plants. UGT71C3 specifically catalyses MeSA glycosylation but has no activity on SA. In UGT71C3 mutants, after local infection with pathogenic bacteria, the glucose ester of MeSA decreased compared with the wild type, and the free SA level in the distal leaves increased, thereby increasing SAR (Chen et al., 2019). According to reports, several UGTs are closely related to plant disease resistance. For example, in *Arabidopsis thaliana* , UGT73B3 and UGT73B5 are necessary for resistance to *P. syringae* pv tomato (Pst) DC3000 (Langlois-Meurinne et al., 2005). UGT84A2/BRIGHT TRICHOMES 1 (BRT1) is required for the nonhost resistance of *Arabidopsis thaliana* to*Phakopsora pachyrhizi* (Langenbach et al., 2013). In addition, UGTs can recognize some defence-related metabolites as substrates and modify them into inactive forms. UGT74F1 is a negative regulator that converts SA to SA β-glucoside, the inactive form of SA (Boachon et al., 2014). In tobacco, the glucosidic form is transported from the cytoplasm to the vacuole, indicating that SA β-glucoside is a storage form of SA.

N-Hydroxypiperidine acid (NHP) is a key signalling factor for the construction of SAR, and the ratio of its glycosylated forms (NHP/NHPG) is essential for coordinating the DvG response of *Arabidopsis thaliana* . One study found that UGT76B1 catalyses the transfer of a hexose to the NHP backbone. UGT76B1 has an inhibitory role in anti-disease regulation by altering the homeostasis of NHP and NHPG. In addition, UGT76B1 knockout plants exhibited dwarfing and early senescence phenotypes, while UGT76B1-overexpressing plants showed larger rosettes and delayed leaf senescence, indicating that the ratio of NHP to NHPG plays a vital role in plant growth. The loss of function of the NHP biosynthesis gene FMO1 in UGT76B1 knockout plants supports this finding (Mohnike et al., 2020). Glycosylation of NHP to NHPG through UGT76B1 reduces the free NHP pool, thereby inhibiting the expression levels of ALD1, SARD4 and FMO1. This forms a "hand brake" for the accumulation of NHP during pathogen attack, and works together with other mechanisms that affect NHP biosynthesis, transcription regulation, and subcellular transport. Based on this phenomenon, some scholars have proposed a working model that includes NHP glycosylation and SAR responses during plant growth: under normal circumstances, UGT76B1 can produce a small amount of NHP and quickly convert it into NHPG, which is the main component of UGT. When pathogens attack, the NHP pathway is activated, and NHP is rapidly induced in local tissues. As a mobile signal, NHP is transported to tissues throughout the body and triggers an SAR response. High levels of NHP lead to an enhanced SAR response, premature ageing and inhibition of plant growth. When the NHP level reaches a certain threshold, UGT76B1 will also be upregulated by NHP, which will accelerate the glycosylation of NHPG, resulting in a decrease in NHP delivery to system tissues. In whole body tissues, UGT76B1 further glycosylates NHP to NHPG, which leads to a further decrease in NHP levels, thereby weakening the SAR response. High concentrations of NHPG can also promote plant growth, delay senescence, and inhibit cell death. The homeostasis of NHP and NHPG regulated by UGT76B1 is essential to coordinate plant defence and growth.

In vitro biochemical experiments showed that TOGT, a glycosyltransferase from tobacco, can glycosylate hydroxycinnamic acid, hydroxycoumarin and hydroxymethoxycoumarin, and the expression of this enzyme can upregulate SA during allergic reactions (Fraissinet-Tachet et al., 1998). Chong et al. (Chong et al., 2002) used an antisense strategy to suppress the expression of this gene in tobacco. The results of the study showed that with the decrease in the content of hydroxymethoxycoumarin glycosides, transgenic tobacco

showed more sensitivity to TMV and virus infection. The necrotic plaques of the tissues increased, and the resistance decreased. Matros and Mock (Matros & Mock, 2004) used the same gene to overexpress TOGT in tobacco and found that the overexpression of TOGT could also increase tobacco resistance to potato virus Y. The C-3 position of saponins usually has an oligosaccharide chain composed of glucose, galactose, arginine, glucuronic acid, xylose or rhamnose. Moreover, some saponins are connected to a glucose residue at C-26 or C-28. It is generally believed that the oligosaccharide chain at the C-3 position of saponins plays a role in transmembrane transport and antifungal activity, and the removal of the oligosaccharide chain will result in the loss of biological activity of such compounds. Interestingly, fungal pathogens synthesize hydrolase to hydrolyse the oligosaccharide chain at the C-3 position of saponin to protect themselves after infecting plants. For example, after infecting oat roots, the parasitic bacterium *Gaeumannomyces graminis* will produce a β-glucosidase, which can hydrolyse the terminal glucose on the triterpene saponin C-3 oligosaccharide chain and eliminate its biological activity. Similarly, in solanaceous plants such as tomatoes and potatoes, pathogens use the same strategy to inactivate glycosylated steroid alkaloids. For example, many tomato pathogens produce a hydrolase that acts on the C-3 oligosaccharide chain of α-lycopene to inactivate it after the pathogen enters the tomato plant (Sandrock & VanEtten, 1998). The natural resistance phenotype of white spruce to spruce aphids was found in a tree population in eastern Canada. Metabolite analysis showed that there are two phenolic compounds in the leaves of white spruce, 3,4-dihydroxyacetophenone and 4-hydroxyacetophenone, that help with spruce aphid resistance (Delvas et al., 2011; Parent et al., 2017). In the leaves of sensitive trees, these two acetophenones were only detected in the form of corresponding glycosides, indicating that these two compounds were inactive against spruce aphids. In contrast, the leaves of resistant trees also contain the biologically active saponin compounds 3,4-dihydroxyacetophenone and 4-hydroxyacetophenone. The formation of acetophenone glycosides is of great significance for the rapid isolation of potentially more biologically active acetophenone aglycones. This aglycon only appears in the resistant white spruce genotype that expresses the PgbGLU1 gene, and the product of the PgbGLU1 gene acts as a glycosidase to cleave acetophenone glycosides (Parent et al., 2017).

2.1.2.2Responding to abiotic stress

Plants, as sessile organisms, have to face various challenges from the external environment throughout their lives and have evolved mechanisms for self-regulation and protection. The plant hormone ABA is essential for the adaptive growth of plants under various stresses. The ABA glucosyltransferase UGT71B6 and its two highly homologous proteins UGT71B7 and UGT71B8 play a vital role in maintaining ABA homeostasis, dehydration adaptability, osmotic pressure regulation and salt stress tolerance. During the germination process, RNAi plants were sensitive to the exogenous addition of ABA and high salt treatment and showed plant growth defects (Dong et al., 2014). UGT71C5 has been shown to play an important role in ABA homeostasis by catalysing the formation of ABA-glucose ester (GE). The UGT71C5 mutant in *Arabidopsis thaliana* significantly enhances drought tolerance (Liu et al., 2015). Flavonoids are important metabolites in plants and can be used as ultraviolet protection agents, plant antitoxins, signalling molecules and IAA transport regulators (Gould, 2004). Anthocyanins are the main category of flavonoids. They color flowers, fruits and other plant tissues in higher plants, and they can also remove reactive oxygen species (ROS) as a natural antioxidant. UGT79B2/B3 has been proven to be a UDP-rhamnosyltransferase of anthocyanin and anthocyanin 3-O-glucoside. The overexpression of UGT79B2/B3 significantly increases the accumulation of plant anthocyanins, and enhances the ability of plants to resist abiotic stress (cold, high salt, drought) and produce antioxidants (Li et al., 2018). In addition, transgenic plants that overexpress glycosylated quercetin UGT76E can also enhance abiotic stress tolerance by improving antioxidant capacity and upregulating the expression of stress tolerance genes. Glycosylation plays an important role in regulating flavonoid metabolism and enhancing the adaptability of plants to environmental abiotic stresses.

2.2 Coordinating defence functions and autotoxicity

Plants produce a large number of special metabolites to help them solve ecological challenges, such as attacks from herbivores and pathogens, and optimize their Darwinian adaptability under harsh natural conditions (Chae et al., 2014; A. C. Huang et al., 2019; Kessler & Baldwin, 2001). These small defence

molecules usually target specific tissues of the attacker, that is, tissues lacking in plants, such as the nervous system and cardiovascular system. By targeting these specialized tissues, plants avoid the self-poisoning problem of using powerful chemicals for defence. Nicotine and nicotinic acetylcholine receptors produce toxins that target neuromuscular junctions, which are well tolerated by a variety of plants (Baldwin & Callahan, 1993). However, the basic cellular processes of many small molecule defences are common to all organisms (Parker, 2009). When plants are under environmental stress, they will quickly redistribute their energy, implement sacrificial strategies, adjust the balance between growth and defence, and improve their stress resistance by moderately reducing growth levels; these processes prevent medicinal plants from utilizing and accumulating active ingredients needed for growth (Heiling et al., 2020). These active ingredients are usually phytoalexins. Studies have pointed out that although these phytoalexins can reduce the adverse effects of external environmental stress on plants, they will have toxic effects on the plants themselves (Guo et al., 2012). Storing toxic defensive compounds in the form of inactive conjugates is a common strategy to protect plants from their toxicity (Li et al., 2021) because glycosylation modifications can make certain molecules inactive (or toxic) and enhance the water solubility of many lipophilic compounds. Therefore, glycosylation is very important in the process of soil remediation and plant detoxification (Bowles et al., 2006; Vogt & Jones, 2000). Many plant defence compounds are stored in the general glycosylated form and are released as toxic aglycones in the attack reaction (Morant et al., 2008).

Under normal circumstances, geranyllinalool will be oxidized by CYP736A, glycosylated by UGT74P, and then further modified to produce 17-hydroxygeraniol diterpene glycosides (17-HGL-DTGs). In this case, the additional aglycon modification step is blocked. When ingested by herbivores, these 17-HGL-DTGs will be transformed into a class of toxic compounds, namely, hydroxylated-17 HGL-DTGs, which will inhibit the biosynthesis of the basic structural components of the cell membrane (sphingolipids) of herbivores to achieve defence functions. However, if part of the biosynthetic pathway is blocked, such as by silencing NaCYP736As or naut74p in plants, the accumulated geranyllinalool or 17-HGL will flow into nonspecific hydroxylated derivatives, which will also inhibit the plant's sphingolipid biosynthesis and cause toxicity. Through this common mechanism, self-toxic and defensive properties are derived from natural product pathways. Tobacco retains the defence function of its 17-HGL-DTGs and simultaneously avoids toxicity through precise adjustment of terpenoid aglycone modification. Fusarium is a common fungus that infects cereal crops and releases the toxin deoxynivalenol (DON) during the infection process. This toxin not only affects the growth of plant cells but also poses a serious threat to human health. UGT73C5, a glycosyltransferase in *Arabidopsis thaliana* , can glycosylate this toxin and reduce its toxicity. Transgenic plants overexpressing UGT73C5 show enhanced resistance to this toxin (Brigitte Poppenberger et al., 2003). Overexpression of the sorghum glycosyltransferase gene sbHMNGT (UGT85B1) in *Arabidopsis thaliana* enables plants to accumulate dhurrin in the body. Moreover, in this transgenic line, the expression of two cytochrome P450 genes was also upregulated (Tattersall et al., 2001). Kristensen et al. (2005) further confirmed that the transfer of the UGT85B1 gene can restore the various effects caused by the CYP79A1 and CYP71E1 genes being singly expressed in transgenic lines. They speculated that the glycosyltransferase UGT85B1 participates in the formation of a multienzyme complex that relieves the toxicity to plants of some intermediate metabolites. Two glycosyltransferases, BX8 and BX9, were also isolated and purified from maize seedlings. They both use UDPG as the glycosyl donor, and 2,4-dihydroxy-7-methoxy-1,4-benzoxazine- 3-ketone (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) are used as acceptors. Overexpression of BX8 or BX9 in*Arabidopsis thaliana* can reduce the toxic effects of adding exogenous DIBOA and DIMBOA to transgenic plants (Von Rad, Hüttl, Lottspeich, Gierl, & Frey, 2001). Since this type of aglycone exists in the soil around plant roots, it is speculated that plant GTs can glycosylate the secreted compounds of neighbouring plants to reduce the self-toxicity of these secretions.

Some UGTs can glycosylate a large number of metabolites and participate in redox homeostasis. Together with glucosidase, UGTs rapidly form the glycosylation state of a wide range of special metabolites, supporting plant responses in various challenging environments. The precise regulation of the balance between glycosylation and deglycosylation applied to antioxidant molecules and plant hormones allows plants to respond to the environment (Verma et al., 2016). For various substrates, UGT activity may play an important

role in the homeostasis of their respective metabolic pathways. When the aglycon/glycosylated form of a specific molecule participates in this delicate balance, this regulation is essential to maintain redox stability. These results indicate that UGT may participate in redox homeostasis through a series of biochemical mechanisms to support the response of plants to oxidative stress related to pathogen infection. TOGT-activated scopolamine accumulation can not only inhibit virus proliferation, but also buffer the redox state of infected cells, while UGT73B3 and UGT73B5 may detoxify molecules produced by infected cells.

Many studies have also shown that glycosylation not only detoxifies toxins from biological sources but also detoxifies toxins from xenobiotic sources, such as herbicides, pesticides, and various pollutants (Loutre et al., 2003). In vitro experiments have confirmed that Arabidopsis glycosyltransferases UGT72E1, UGT75D1, UGT84A1, UGT84A2, UGT84B1, and UGT75B1 can all act on exogenous 2,4,5-trichlorophenol (TCP) (Mener et al., 2003). Another study has shown that Arabidopsis root culture can quickly detoxify the pollutant 3,4-dichloroaniline (DCA) through glycosylation to generate N-glycosylation-DCA, which is then transported outside the root (Loutre et al., 2003). UGT72E1 and UG72E8 can eliminate the toxicity of the industrial pollutant 2,4,5-trichlorophenol (TCP), which is significant for environmental remediation (Meßner et al., 2003). Taken together, we propose a working model that incorporates secondary metabolite glycosylation during plant growth and defence responses (Figure 1). Growth inhibition under stress conditions is related to the redirection of plant resources such as energy and metabolic precursors from their primary metabolisms and growth for the activation of defense mechanisms.

3GLYCOSYLTRANSFERASE AND THE DIVERSITY OF SECONDARY METABOLITES

GTs are a class of enzymes that can catalyse the transfer of glycosyl groups from activated donors to acceptor molecules. They have stereoselectivity and high efficiency and are widely present in plants. GTs participate in maintaining the homeostasis of cellular metabolism through glycosylation. Glycosyltransferases can recognize a variety of acceptors, catalyse the transfer of activated glycosyl groups from donor molecules to acceptor molecules, change the chemical stability, water solubility, transport capacity and biological activity of acceptor molecules, and then help to improve their bioavailability and biological activity. GTs are a highly differentiated superenzyme family. Glycosyl donor molecules include disaccharides, polysaccharides, nucleoside-2-phosphate sugars, uridine diphosphate glucuronic acid, and others (Mackenzie et al., 1997). The most common glycosyl donors in plants are UDP-glucose, UDP-rhamnose, UDP-galactose, UDP-xylose and UDP-glucuronic acid (Sawada et al., 2005). Common receptors include carbohydrates, proteins, lipids, sterols, antibiotics, terpenes, plant hormones, cyanohydrins, alkaloids, phenols, plant toxins and exogenous substances (Bowles et al., 2005). The glycosylation sites are on the C (C—C), N (—NH2), O (—OH,—COOH) and S (—SH) atoms of acceptor molecules (Vogt & Jones, 2000), which can produce two different kinds of α and β glycosidic bonds (Ross et al., 2001), where the product is the corresponding glycoside or sugar ester (Lim, 2005).

With the further study of GTs, an increasing number of GTs have been identified. To date, the carbohydrate-active enzyme, CAZy (http://www.cazy.org/GlycosylTransferases.html), database includes 793,456 modules in existing families and 19,967 unclassified modules. The CAZy database contains protein families that synthesize, degrade and modify carbohydrates and sugar complexes—glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs) and auxiliary module enzymes (auxiliary activities, AAs). According to the similarity of the GT sequence, the specificity of the catalytic substrate and the stereochemical structure of the catalytic product, the CAZy database divides glycosyltransferases into 114 families (GT1-GT114), of which GT1 is the largest glycosyltransferase family. To date, GTs have been found in the simplest single-celled algae, as well as in mosses, ferns, gymnosperms, and angiosperms, indicating that this enzyme is indispensable in the growth and development of organisms in the plant kingdom. With the evolution of plants, the members of the family are constantly expanding. The enzymes mainly responsible for the glycosylation of plant natural products belong to GT1. GT1 enzyme family members in plants use UDP-activated sugar donors to transfer sugar units to small molecules, so they are also called UDP-dependent glycosyltransferases (UGTs). The GT1 enzyme has made an important contribution to the diversification of plant-specific metabolites and sugars

(Bowles et al., 2006). These enzymes are involved in the production of important defence compounds, such as terpenoid glycosides, glucosides, cyanoside glycosides and flavonoid glycosides (Gleadow & Møller, 2014; Sønderby et al., 2010). These enzymes are involved in the production of important defence compounds, such as terpenoid glycosides, glucosides, cyanoside glycosides and flavonoid glycosides (Noda, 2018). The GT1 enzyme also participates in regulating plant growth and development by regulating plant hormone homeostasis (Piotrowska & Bajguz, 2011). This enzyme further achieves xenobiotic detoxification through glycocoupling, which is a step before the transfer and subsequent storage of modified xenobiotics in vacuoles (Brazier-Hicks et al., 2018).

According to the classification of glycosylation sites of receptor molecules, GTs catalyse the formation of O–C glycosidic bonds, N–C glycosidic bonds, C–C glycosidic bonds and S–C glycosidic bonds. The corresponding glycosyltransferases are O-GTs, N-GTs, C-GTs and S-GTs, and most of the commonly studied plant glycosyltransferases are O-GTs, such as *Medicago truncatula* UGT71G1 (Shao et al., 2005) and UGT85H2 (Li et al., 2007), and *Vitis vinifera* VvGT1(Offen et al., 2006). The three-dimensional structure and catalytic mechanism of O-GTs, such as *Arabidopsis thaliana* UGT89C1 (Zong et al., 2019), have been studied in depth. In addition, *Arabidopsis thaliana*UGT76C1 and UGT76C2 can catalyse the formation of N-C glycosidic bonds and belong to the N-GT group (Hou et al., 2004). Buckwheat UGT708C1/UGT708C2 belongs to C-GT, which can catalyse 2-hydroxyflavonoids to produce C-C glycosidic bonds (Hou et al., 2004). In addition, GT genes with dual catalytic functions have gradually been identified. For example, rice OsCGT can catalyse the formation of O–C glycosidic bonds and C–C glycosidic bonds (Hao et al., 2016). Maize O–C glycosidic bonds and C–C glycosidic bonds show catalytic activity for the O-glycosylation of naringenin, while they also show catalytic activity for the C-glycosylation of 2-hydroxynaringenin (Ferreyra et al., 2013). *Arabidopsis thaliana* UGT72B1 catalyses the formation of N–C glycosidic bonds and O–C glycosidic bonds (Ferreyra et al., 2013). *Bacillus thuringiensis* ThuS can catalyse the formation of O–C glycosidic bonds and S–C glycosidic bonds at the same time, and ThuS-catalysed S-glycosylation is more effective than O-glycosylation (Wang et al., 2014). The soil bacterium *Streptomyces freundii*UrdGT2 catalyses the formation of C–C glycosidic bonds and can also catalyse the formation of O–C glycosidic bonds (Wang et al., 2014). Glycosyltransferases with multiple catalytic functions have also been identified. For example, in 2008, a glycosyltransferase from*Streptomyces* antibiotics, OleD, was reported; it was the first receptor known to catalyse O–C, S–C and N– glycosyltransferases generated by C glycosidic bonds (Gantt et al., 2008). The new glycosyltransferase UGT73AE1 identified from safflower reported in 2014 can recognize and accept many receptors with different structures, catalyse the formation of O–C, S–C and N–C glycosidic bonds, and catalyse their reverse reaction activity (Xie et al., 2014). CpGT1, the recently identified *Cymbidium acuminata* glycosyltransferase, can also catalyse the formation of O–C, S–C, and N–C glycosidic bonds (Li et al., 2019).

The advent of GTs colors plants secondary metabolites. Glycosides are also active components in many medicinal plants (Table 1). In nature, various types of natural ingredients can be combined with sugars such as aglycones to form glycosides. Therefore, there are many glycoside compounds that are widely distributed. These glycosides are ubiquitous natural products, especially in higher plants. Glycosidic compounds can be distributed in various organs of plants. For example, the roots, rhizomes, stems, leaves, flowers, fruits and seeds of ginseng all contain triterpene saponins. However, different organs in different plants have different distribution points. For example, *Panax notoginseng* has the highest content of saponins in its roots and rhizomes, while *Nerium oleander* has the highest content of cardiac glycosides in its seeds. Most glycoside compounds have a wide range of biological activities and are the effective ingredients of many medicinal plants.

4 CATALYTIC MECHANISM AND STRUCTURE OF PLANT GT

4.1 GT catalytic mechanisms

According to the stereochemical isomerism of glycosylation substrates and products, the catalytic mechanism of GTs can be divided into two categories, namely retention and inversion (Lairson et al., 2008). At present, the reaction mechanism of overturned GTs is relatively well known and is a single exchange SN2 reaction

(bimolecular nucleophilic substitution reaction) (Albesa-Jove & Guerin, 2016). The amino acid residues in the active centre of GTs act as generalized bases (glutamic acid, aspartic acid, histidine, etc.) to deprotonate the hydroxyl groups of the receptor molecule. Then, the deprotonated hydroxyl groups of the receptor serve as nucleophilic groups that attach the phosphate group from the back of the anomeric carbon on the glycosyl donor to form an ion-like transition state of the oxygen complex carbocation, which eventually causes the configuration of the anomeric carbon to be reversed and the phosphate group to leave to complete the glycosylation reaction. The catalytic process is similar to the process in which a flipped glycoside hydrolase catalyses the cleavage of glycosidic bonds. During this reaction, the divalent metal ion generally stabilizes the negatively charged phosphoric acid group.

The catalytic mechanism of the configuration-maintaining type is very controversial (Lairson et al., 2008), and there are two main types: the double substitution reaction mechanism and the SNi-like (self-nucleophilic substitution) reaction mechanism. The double substitution reaction process requires two steps of the SN2 reaction. First, the catalytic amino acid residues in the active centre of the glycosyltransferase attack the heterocephalic carbon of the glycosyl donor to form the glycosyl enzyme covalent binding intermediate, and then, the hydroxyl group of the acceptor molecule acts as a nucleophilic group to attack the heterocephalic carbon of the glycosyl enzyme intermediate; however, but the configuration of the heterocephalic carbon remains unchanged, and the glycosylation reaction is completed. To support this catalytic model, Monegal and Planas (Monegal & Planas, 2006) reported the chemical remediation of a mutant form of α-3-galactosyltransferase (α-3-GalT) by sodium azide. The product of this chemical rescue is the flipped form of an azide sugar, which is consistent with the first step of the double substitution mechanism. Due to the lack of structural information for glycosyl-enzyme covalent binding intermediates, this mechanism has not yet been confirmed. Another catalytic mechanism involving SNi "internal return" has been proposed. This mechanism proposes that the nucleophilic attack and detachment of the leaving group occur on the same side of the glycosyl group, forming a short oxocarbenium-like transition state. The formation of the internal C–O glycosidic bond and the cleavage of the C–O bond between the sugar group and the phosphate group finally complete the catalytic process of configuration retention (Gomez et al., 2012). In short, whether configuration-maintained GTs can be produced through different catalytic mechanisms requires further experimentation.

4.2 The structure of plant GTs

GTs have large differences in their primary structures (the homology is usually 25%-45%), but the folding mode of their spatial structures is relatively simple. According to the folding characteristics of the three-dimensional structures of GTs, these enzymes can be divided into four types: GT-A, GT-B, GT-C and GT-D (Liu & Mushegian, 2003). GT-A is a superfamily of nucleoside diphosphate transferases (including nucleotide transferases) that is characterized by two tightly bound β/α/β Rossmann domains, in which the N-terminus is a glycosyl donor binding domain and is responsible for the recognition of specific glycosyl donors: the relatively conserved N-terminal DXD (Asp-X-Asp) motif specifically recognizes and binds to glycosyl donors by interacting with divalent cations. The C-terminus is a receptor-binding domain that can recognize and bind to receptor molecules by forming specific hydrogen bonds with the receptor (Breton, Fournel-Gigleux, & Palcic, 2012). GT-B superfamily members also include other enzymes involved in sugar metabolism, such as sugar epimerase (UDP-GlcNAc-2-epimerase) (Wrabl & Grishin, 2001). GT-B folds are also composed of two Rossmann folds of β/α/β domains. The difference is that the N-terminal domain is involved in binding to glycosyl receptors, and there are multiple rearrangements of secondary structures to form a spatial structure. The diverse receptor binding domains enable members of the GT-B superfamily to have a broader spectrum of substrates. The C-terminal domain is involved in the binding of glycosyl donors, and the connection between them is not too tight. The two domains "face each other", and the binding of the ligand is related to the conformational change of the relative orientation (Coutinho et al., 2003). The C-terminal domain of GT-B folds has a highly conserved PSPG (putative secondary plant glycosyltransferase) structural motif, which plays an important role in glycosyl donor recognition and catalysis. In addition, unlike those of GT-A superfamily, the catalytic effects of GT-B superfamily members do not depend on divalent metal ions (Breton et al., 2006), and there is no evidence to prove the correlation between their catalytic activity and

the binding of metal ions. The GT-C superfamily consists of larger hydrophobins located on the endoplasmic reticulum or plasma membrane; these family members have an N-terminal transmembrane region and a C-terminal loop region, with a modified DXD motif in the first extracellular loop. At present, there is no evidence that the DXD motifs of the GT-C and GT-A superfamilies have a common evolutionary origin (Liu & Mushegian, 2003). Zhang et al. (2014) resolved the crystal structure of the uncharacterized "domain of unknown function" DUF1792 (PDB 4PFX) of *Streptococcus parasanis* dGT1 with a resolution of 1.4 Å. This domain adopts a novel folding method and has glucosyltransferase activity. The catalytic activity of this domain depends on divalent metal ions and is finally named GT-D glycosyltransferase folding. Since this domain is highly conserved in bacteria and does not exist in eukaryotes, it can be used as a new antibacterial target for in-depth research. It has been shown that the stereochemistry of glycosylation is not directly related to the overall folding degree of GTs (Table 2).

There are many types of plant GTs that work on a wide range of catalytic substrates. Glycosylation can directly affect the water solubility, stability and biological activity of compounds (Lepak et al., 2015; Takahashi et al., 2012). In addition, the glycosylation of natural products by GTs can produce new biologically active compounds, which is of great significance for modern drug development. With the identification of new GTs, the three-dimensional structure of GTs has been analysed. The three-dimensional structure reveals the details and catalytic mechanism of the interaction between enzyme and substrate, which provides a basis for further explaining the specificity and sensitivity of the substrate and is helpful for the application of glycosyltransferases in biocatalysis and genetic engineering. Currently ,16 structures of plant GTs have been analysed, including UGT72B1, UGT74F2, and UGT89C1 in *Arabidopsis thaliana* ; UGT71G1, UGT78G1, and UGT85H2 in *Medicago truncatula* ; VvGT1 in *Vitis vinifera* ; UGT78K6 in *Clitoria ternatea* ; Os79 in rice; PtUGT1 in*Polygonum tinctorium* ; and UGT76G1 in *Stevia rebaudiana* , etc. (Table 3). Although the amino acid sequence homology of these enzymes is relatively low, their three-dimensional structures are very similar. The structures of these plant glycosyltransferases belong to the GT-B folding type and contain two Rossmann folded $\beta/\alpha/\beta$ domains and an elongated strip between the N-terminal domain and the C-terminal domain. In the gap, the N-terminal domain is involved in the binding of glycosyl receptors. Due to the diversity of plant glycosyltransferase receptors, the N-terminal domain is less conserved, but there is an important amino acid site involved in the catalytic reaction of glycosylation (His/Asp) that is highly conserved, and the glycosyl donors bind to the relatively conserved PSPG region of the C-terminal domain. The catalytic site is located in the space between the two domains (Figure 2). In plant GT1, two highly conserved residues play a crucial role in the SN2-like mechanism, in which the stereochemistry of the C1 anomeric carbon is reversed during the reaction.

4.2.1 Donor binding region of plant GTs

The glycosyl donor can stably bind to the PSPG region of plant glycosyltransferase through multiple hydrogen bond interactions with the amino acid residues in the PSPG region of C-terminal domain of plant glycosyltransferase. The last two amino acid residues in the PSPG region (such as Glu381 and Gln382 in MtUGT71G1, Asp374 and Gln375 in VvGT1) are considered as the key amino acids for glycosyl recognition (Shao et al., 2005). In 2019, Zong et al. (Zong et al., 2019) reported on the structure and function of rhamnose transferase AtUGT89C1, and found that after Asp356 was mutated to Ala, AtUGT89C1's rhamnose transfer activity was completely lost, while the H357Q mutant showed resistance to mice. The dual transfer activity of plum sugar and glucose further proves the key role of the last two amino acid residues in the PSPG region in the process of glycosyl recognition. In addition, the relatively conserved threonine (Thr143 in MtUGT71G1, Thr141 in VvGT1) located in the glycosyl donor binding center is considered to play a very important role in the recognition of glycosyl donors. Thr141 in VvGT1 is mutated to Ala A certain degree of loss of activity afterwards also proved this conjecture (Wendy Offen et al., 2006). Pro147 and Ile148 in AtUGT89C1 form a hydrophobic interaction with the 6-position methyl group of the rhamnose ring, which is very important for the recognition of the rhamnosyl group (Zong et al., 2019).

4.2.2 Receptor binding regions of plant GTs

The N-terminal domains of plant GTs are responsible for binding glycosyl receptors. Due to the large

differences between the N-terminal domains, the types of glycosyl receptors of different GTs are diversified. For example, MtUGT71G1 recognized triterpenoids and flavonoids as glycosylation receptors, and mainly produced glucosylation products in 3'-O of flavonoids. The double point mutation Y202A/F148V changed the specificity of MtUGT71G1 to quercetin, which changed the glycosylation site from 3'-OH to 3-OH (Shao et al., 2005). VvGT1 has catalytic activity on many different flavonoids (including quercetin and kaempferol) to produce 3-O glucoside (Offen et al., 2006). MtUGT85H2 can recognize flavonoids and isoflavones as glycosyl acceptors, but has a certain stereoselectivity. When biochanin A and genistein are used as glycosyl receptors, 7-O-glucoside is mainly produced, while kaempferol and quercetin are used as substrates, 3-O-glucoside is mainly produced. The activity test and kinetic analysis results of the enzyme on the substrate showed that the specificity of MtUGT85H2 to flavonols was significantly higher than that of isoflavones. The N-terminal domains of different glycosyltransferases have similarities. Multiple hydrophobic amino acids form a hydrophobic environment, and glycosyl receptors are mainly bound to this hydrophobic region. In the complex structure of VvGT1 and kaempferol, kaempferol binds in a hydrophobic pocket composed of Phe15, Ile87, Phe121, Phe200, Val281 and Phe372 (Offen et al., 2006). In AtUGT72B1 and TCP (2,4,5-In the complex structure of trichlorophenol, TCP mainly binds to the hydrophobic pocket composed of Leu118, Phe119, Phe148, Leu183 and Leu197 through hydrophobic interactions. In the complex structure of AtUGT89C1 and quercetin, quercetin binds to Phe16 , Phe95, Ile91, Leu88, Phe165, Ala355, Phe359 and other hydrophobic pockets (Tintor et al., 2013). Therefore, the interaction between glycosyl receptors and the N-terminal domain of plant GTs is mainly through hydrogen bonding and hydrophobic interaction. Some protein intramolecular interactions will indirectly affect the activity of plant GTs (Li et al., 2007).

5 Conclusion and Prospect

In the evolutionary history of interdependence between plants and the environment, plants will perceive light signals, temperature signals, adversity stress in the surrounding environment, and balance their growth and development through integration with endogenous hormones and other secondary metabolic pathways. The efforts of scientists greatly furthered the research on the core regulatory factors in plants. However, because plants are immobilized organisms, the working modes of various pathways in the body are relatively complex. Many of these metabolites have very low background levels in the body, but each organ is very sensitive to changes in their concentration, so maintaining their homeostasis is essential for the normal growth and development of plants. To further understand the precise regulation of secondary metabolites, bettering the complex plant regulation network is a must. However, how glycosyltransferases and their catalysed glycosylation respond to signals in the environment and regulate metabolic pathways remain uncharted. Herein, we focused on this scientific problem and found that (1) Phytohormone is the main immune signal molecule level, and its dynamic changes enable plants to respond quickly and appropriately to danger. To balance the tradeoff between growth and defense, the biosynthesis, transportation and homeostasis of signal molecules are strictly regulated. Such GTs are used to regulate the dynamic balance of plant hormones to coordinate DvG, as well as other secondary metabolites (NHP, saponins, phenolics, terpenoids, and lignins) through their ability to manipulate pathogen effectors. (2) The detoxification or pollutant removal effect of glycosylation to coordinate defense function and autotoxicity has been recognized to a certain extent. Most toxic defense compounds are stored in the form of inactive conjugates in the defense reaction to release toxic aglycones, which is an efficient and energy-saving stress response strategy. (3) The role of GTs and the evolution of the multi-gene GT family have consolidated the plasticity of development and metabolism. GTs have a variety of functions and biological activities, with a wide range of substrates. Meanwhile, glycosylation can produce a cascade effect. A variety of small molecule compounds produced by glycosylation play an important role in buffering the effects of biotic and abiotic stresses on plants. It is also an important source of active ingredients of medicinal plants, which also fits the adverse effect is beneficial to the accumulation of active components in medicinal plants. (4) The three-dimensional structures of 16 GTs revealed the details of enzyme-substrate interaction and catalytic mechanism, which provided a basis for further elucidation of substrate specificity and specificity, and contributed to the application of glycosyltransferase in biocatalysis and genetic engineering.

More attention should be attached that now, not the to-bes and has-beens. Most of the related studies

focused on the model plant*Arabidopsis thaliana* . However, whether there is a synergistic effect between different phytoalexins produced by the same plant and whether the structurally diverse secondary metabolites maintain conserved functions in immunity, or whether they mediate the highly diverse defence mechanisms of plants remain uncharted. Along with plant innate immunity and SAR, it can be speculated that glycosylation of secondary metabolites may be a relatively energy-saving and efficient storage and release strategy for phytoalexins. All plant GTs atomic resolution protein structures are obtained by X-ray crystallography. The emerging freeze electron microscopy technology recently provides a powerful tool for the study of protein structure and protein-protein interaction in the (near) natural state. Although the elimination of the glycosylation step of phytoalexins often leads to autotoxicity, it is largely unknown whether the defence function and autotoxicity have the same mechanism. To solve this problem, the immediate target of the metabolite must be known. However, a large number of potential targets and the metabolic complexity of herbivores after eating make this task challenging. Understanding these mechanisms can open the way for the application of plant-specific metabolites in human health and agriculture.

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Conflicts of interest

The authors declare that they do not have any conflict of interest.

Author's contributions

Wen-jin Zhang collated documents and wrote the manuscript; Li-kun Chang and Ye Cao helped perform the arrangement of tables and figures; Sheng Wang, Chuan-zhi Kang and Li Zhou contributed significantly to analysis and manuscript preparation; Chao-geng Lyu assistance to the revision of the manuscript; Luqi Huang provided valuable idea; Lan-ping Guo provided financial supports and valuable discussion. All authors read and approved the final manuscript.

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Figure and Table legends

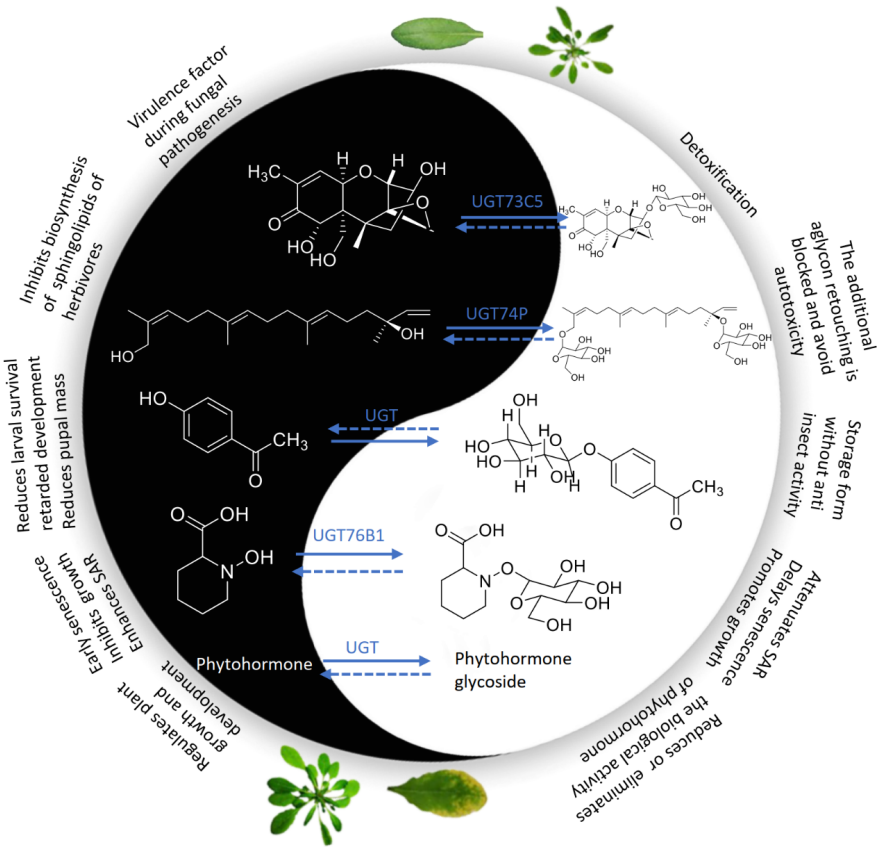

Figure 1 The Taiji model of glycosylation to maintain the steady state of plant secondary metabolites

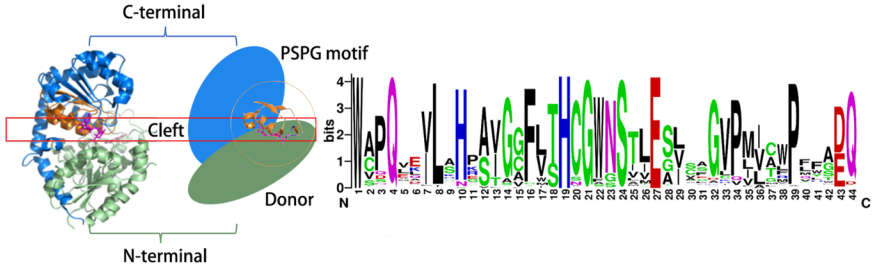

Figure 2 The structure of GT-B and the PSPG domain

Table 1 Common medicinal plants containing active glycosides

The Latin name for medicinal plants	Active ingredients	Biological activity
Xanthium sibiricum Patrin ex Widder	Xanthostrumarin	Anti-hyperglycemic
Arctium lappa L.	Arctiin	Anti-inflammatory, immunomodulatory, anti-viral, anti-tumor

The Latin name for medicinal plants	Active ingredients	Biological activity
Bupleurum chinense DC.; *Bupleurum scorzonerifolium* Willd.	Bupleurum saikosaponin	Antipyretic, sedative, anti-inflammatory, immunomodulatory, anti-viral, anti-tumor, liver protection
Pueraria lobata (Willd.) Ohwi	Puerarin, daidzin, daidzein-7,4'-diglucoside	Antiarrhythmic, immunomodulatory, hypotensive, antioxidation
Anemarrhena asphodeloides Bunge.	Timosaponin	Treatment of Alzheimer's disease, protection of cerebral ischemia injury, anticoagulant, Antioxidation, anti-tumor, anti-osteoporosis, anti-inflammatory, antihypertensive, antihyperglycemic, hypolipidemic
Commelina communis L.	Anthocyanins	Antioxidation, anti-inflammatory, anti-tumor,anti-hyperglycemic,hypolipidemic
Gardenia jasminoides Ellis	Geniposide, gardenoside, dehydroxy geniposide, shanzhiside, geniposide, crocin	Anti-inflammatory, antioxidation, antipyretic, analgesic, Hepatoprotective and cholagogic, anti-hyperglycemic
Prunella vulgaris L.	Triterpen saponins, rutin, hyperoside	Anti-tumor, anti-inflammatory, antibacterial, antitussive, hypotensive, hypolipidemic
Buddleja officinalis Maxim.	Robinin, mimengoside A, B	Anti-inflammatory, diuretic, antispasmodic
Scutellaria baicalensis Georgi	Baicalin, baicalein	Anti-inflammatory, antibacterial, antiviral, antioxidation
Phellodendron chinense Schneid.; *Phellodendron amurense* Rupr.	Berberine-7-O-glucoside	Hypotensive and antitumor
Gentiana scabra Bunge	Gentiopicroside, swertiamarin, trilobatin, amarogentin, amaroswerin	Liver protection, anti-inflammatory, antioxidation, analgesic, anti-tumor, strengthening the stomach
Forsythia suspensa (Thunb.)Vahl	Forsythiaside A, forsythin, triterpen saponins	Anti-inflammatory, antioxidation, anti-endotoxin, antipyretic, immunomodulatory
Dendranthema indicum (L.) Des Moul.	Robinia pseudoacacia-7-rhamnoside glucoside	Anti-inflammatory, bacteriostatic
Paris polyphylla	Pariphyllin, dioscin, steroidal saponins	Sedation, pain relief, hemostasis, antibacterial, anti-tumor
Smilax glabra Roxb.	Astilbin	Diuresis and analgesia

The Latin name for medicinal plants	Active ingredients	Biological activity
Patrinia scabiosaefolia Fisch. ex Link.	Scabioside, daucosterol	Sedative, anti-inflammatory, anti-tumor, hepatoprotective and cholagogic
Belamcanda chinensis (L.) DC.	Tectorigenin, tectoridin	Anti-inflammatory, analgesic, anti-allergic
Sophora tonkinensis Gapnep.	Trifolirhizin	Anti-tumor
Pulsatilla chinensis (Bge.) Regel	Saponin	Resistance to amoeba
Scrophularia ningpoensis Hemsl.	Harpagide, aucubin	Liver protection, immunomodulatory
Paeonia suffruticosa Andr.	Paeonol, paeonolide, apiopaeonoside, paeoniflorin, oxypaeoniflorin, benzoyl paeoniflorin, benzoyloxypaeoniflorin, galloyl paeoniflorin	Antibacterial, analgesic and sedative
Paeonia lactiflora Pall.; *Paeonia veitchii* Lynch.	Paeoniflorin, albiflorin, oxypaeoniflorin, benzoyl paeoniflorin	Anti-platelet aggregation, dilate blood vessels, improving microcirculation, antioxidation, anti-convulsions
Cynanchum atratum Bge, Cynanchum versicolor Bge	Cardiac glycosides	Cardiotonic
Rheum palmatum L.; *Rheum tanguticum* Maxim.ex Balf.; *Rheum officinale* Baill.	Anthraquinones	Cause diarrhea
Cassia angustifolia Vahl.; *Cassia acutifolia* Delile.	Sennoside, aloe-emodin-8-O-β-D-glucoside, rhein-8-glucoside	Cause diarrhea
Aloe barbadensis Miller.; *Aloe ferox* Miller.	Aloin	Cause diarrhea
Prunus humilis Bge.; *Prunus japonica* Thunb.; *Prunus pedunculata* Maxim.	Amygdalin	Antitussive
Euphorbia pekinensis Rupr.	Euphornin	Cause diarrhea
Pharbitis nil (L.) Choisy	Pharbitin	Cause diarrhea
Croton tiglium L.	Crotonoside	Cause diarrhea
Dioscorea nipponica Mak.	Dioscin, gracillin	Anti-inflammatory, anti-tumor, antiviral, hypolipidemic, hypotensive
Acanthopanax gracilistylus W.W. Smith	Syringin, acanthopanax B1	Anti-inflammatory and immunomodulatory
Periploca sepium Bge.	Cardiac glycosides (periplocin)	Cardiotonic
Dianthus superbus L.	Anthocyanidins	Antioxidation
Kochia scoparia (L.) Schrad.	Triterpen saponins	Anti-inflammatory, anti-allergic, anti-hyperglycemic
Pyrrosia lingua (Thunb.) Farwell; *Pyrrosia sheareri* (Bak.) Ching; *Pyrrosia petiolosa* (Christ.) Ching	Mangiferin,isomangiferin	Anti-inflammatory, anti-allergic, antibacterial, antiviral, expectorant, antitussive

The Latin name for medicinal plants	Active ingredients	Biological activity
Polygonum cuspidatum Sieb. et Zucc.	Polydatin	Antioxidation, hypolipidemic, antibacterial, antiviral, hepatoprotective, antitussive, antiasthmatic
Citrus reticulata Blanco	Hesperidin,neohesperidin	Anti-inflammatory, antioxidation, anti-bacterial, anti-tumor, immunomodulatory
Citrus aurantium L.; *Citrus sinensis* (L.) Osbeck	Flavonoid glycosides (hesperidin, neohesperidin, naringin, rhoifolin, lonicerin)	Anti-inflammatory, antioxidation, anti-hyperglycemic, antibacterial, anti-tumor, immunomodulatory
Citrus medica L.	Hesperidin	Anti-inflammatory, antioxidation, anti-bacterial, anti-tumor, immunomodulatory
Allium macrostemon Bge.; *Allium chinense* G. Don	Steroidal saponins	Antiplatelet aggregation, antioxidation, hypolipidemia
Crataegus pinnatifida Bge.	Hyperoside, rhamnosylvitexin	Anti-tumor, antihypertensive, anti-inflammatory, antispasmodic
Paederia scandens (Lour.) Merr.	Paederoside, scandoside, astragalin, kaempferol-3-O-rutinoside, kaempferol 3-O-rutinoside 7-O-glucoside, isoquercitrin, quercetin 3-O-glucoside-7-O-rhamnoside, quercitrin, linarin, asperuloside	Analgesia
Cirsium setosum (Willd.) MB.	Robinia pseudoacaciain-7-rhamnoside, rutin	Hemostasis
Sanguisorba officinalis L.	Ziyu-glycoside-I,II,A,B,E	Hemostasis, antioxidation, anti-inflammatory, anti-tumor
Sophora japonica L.	Rutin	Antioxidation, antiviral, vasodilator, anti acute pancreatitis
Panax notoginseng (Burk.) F. H.Chen	Saponin, flavonoid glycosides	Promoting blood circulation and removing blood stasis
Carthamus tinctorius L.	Carthamone, crocin	Anti-inflammatory, antioxidation
Prunus persica (L.) Batsch; *Prunus davidiana* (Carr.) Franch.	Amygdalin	Relieving cough and asthma
Achyranthes bidentata Bl.	Triterpen saponins	Protect liver, Hypolipidemia, cardiotonic
Vaccaria segetalis (Neck.) Garcke	Vac-segoside, vaccarin, isosaponarin	Antioxidation, anti-tumor
Drynaria fortunei (Kunze) J. Sm.	Naringin, bavachin	Promoting bone injury and bone growth, preventing osteoporosis, hypolipidemia

The Latin name for medicinal plants	Active ingredients	Biological activity
Arisaema heterophyllum Blume.	Triterpen saponins	Analgesic, sedative, anti-tumor, expectorant
Sinapis alba L.; *Brassica juncea* (L.) Czern. et Coss.	Glucosinolates, glucosinalbin	Antitussive, expectorant, antiasthmatic
Gleditsia sinensis Lam.	Saponins (triterpen saponins)	Anti-inflammatory, anti-tumor
Trichosanthes kirilowii Harms.	Triterpen saponins	Anti-inflammatory, anti-tumor
Peucedanum decursivum (Miq.) Maxim.	Nodakenin	Antiasthmatic, antiplatelet aggregation, analgesia
Platycodon grandiflorum (Jacq.) A. DC.	Platycodin	Expectorant
Prunus armeniaca L.	Amygdalin	Relieving cough and asthma
Aster tataricus L. f.	Astersaponin, chrysanthemin	Antibacterial, antitussive, expectorant
Descurainia sophia (L.) Webb ex Prantl	Cardiac glycosides (evomonoside, helveticoside, evobioside)	Protecting myocardium and improving cardiovascular function
Lepidium apetalum Willd.	Glucosinolates	Antitussive, expectorant, antiasthmatic
Siraitia grosvenorii (Swingle) C. Jeffrey ex Lu et Z. Y. Zhang	MogrosideIV, V; siamenosideI	Clearing away heat and relieving cough
Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou	Jujuboside A, B	Tranquilizing the mind
Polygala tenuifolia Willd.	Tenuigenin	Expectorant, antitussive, hypotensive
Apocynum venetum L.	Flavonoid glycosides	Antihypertensive, antidepressant, antioxidation
Gastrodia elata Bl.	Gastrodin	Tranquilizing the mind
Panax ginseng C. A. Mey.	Ginsenoside	Anti-tumor, immunomodulatory, analgesia, neuroprotection
Panax quinquefolium L.	Ginsenoside	Anti-tumor, immunomodulatory, analgesia, neuroprotection
Codonopsis pilosula (Franch.) Nannf.	Tangshenoside	Antioxidation
Astragalus membranaceus (Fisch.) Bge.	Astragalus saponin, acetytastragaloside, astragaloside, daidzein	Immunomodulatory, antithrombotic, antioxidation
Glycyrrhiza uralensis Fisch.	Glycyrrhizin(glycyrrhizic acid)	Liver protection, antiviral and antibacterial
Acanthopanax senticosus (Rupr. et Maxim.) Harms	Quercetin, hyperoside, daidzein	Antioxidation, anti-tumor, anti-hyperglycemic
Gynostemma pentaphyllum (Thunb.) Makino	Gypenoside	Anti-fatigue and anti hypoxia
Rhodiola crenulata (Hook. f. et Thoms.) H. Ohba	Salidroside	Anti-fatigue, anti hypoxia

The Latin name for medicinal plants	Active ingredients	Biological activity
Eucommia ulmoides Oliv.	Aucubin, eucommioside, geniposide	Antihypertensive, hypolipidemic, antineoplastic, antiviral, anti-inflammatory
Dipsacus asperoides C. Y. Cheng et T. M. Ai	Triterpen saponins	Preventing liver damage, preventing osteoporosis and antioxidation
Allium tuberosum Rottl.	Saponin	Expectorant
Paeonia lactiflora Pall.	Paeoniflorin, paeonol	Spasmolysis
Broussonetia papyrifera (L.) Vent.	Saponin, vitamin B	Antioxidation
Adenophora tetraphylla (Thunb.) Fisch.	Triterpen saponins	Antioxidation
Ophiopogon japonicus (Thunb.) Ker-Gawl.	Ophiopogonin(steroidal saponins)	Anti-inflammatory, anti-tumor, antioxidation, immunomodulatory
Asparagus cochinchinensis (Lour.) Merr.	Steroidal saponins	Anti-tumor, antioxidation, immunomodulatory
Polygonatum odoratum (Mill.) Druce	Steroidal saponins	Anti-hyperglycemic, antioxidation
Cornus officinalis Sieb. et Zucc.	Verbenalinp	Anticoagulant, antioxidation, immunomodulatory
Celosia cristata L.	Kaempferitrin, amaranthin	Anti osteoporosis, anti-tumor
Momordica cochinchinensis (Lour.) Spreng.	Momordica saponin	Anti-inflammatory, Antihypertensive

Table 2 Classification of GTs

GT family members	Inverting	Retaining	Unknown
GT-A	**2,7,**12,**13,14,**16,21,25,**29,31,**	**62,64,78,81,88**	
GT-B	**1,**9,**10,**17,19,**23,**26,**28,30,**33,**	**52,112**	
GT-C	22,39,48,50,**53,**57,58,59,**66,**83,85,87		
others	**51** (Lysozyme-type)		
Unknown	11,18,37,38,54,61,67,73,74,75,**76,**	**89,**	0,102,103,**104,**
Unknown	91,**101**,110	91,**101**,110	91,**101**,110

Note: Bold means that the GT family has at least one resolved three-dimensional structure. The structures of other GT-A/GT-B/GT-C families are predicted by Liu and Mushegian (Jing & Mushegian, 2003) or CAZy database.

Table 3 Crystal structure information of plant GTs

UGT	Resolution (Å)	Crystal complex	PDB number	Year	Ref.
MtUGT71G1	2.0 2.6	UDP UDP-glucose	2ACV 2ACW	2005	(Shao & H., 2005)

UGT	Resolution (Å)	Crystal complex	PDB number	Year	Ref.
VvGT1	1.9 1.9 2.1	UDP UDP-2FGlc, kaempferol UDP and quercetin	2C1X 2C1Z 2C9Z	2006	(Offen, Martinez-Fleites, Min, Kiat-Lim, & Davies, 2006)
MtUGT85H2	2.1	Apo	2PQ6	2007	(L. Li et al., 2007)
AtUGT72B1	1.45 1.75 1.9	UDP UDP and Tris UDP-2FGlc, 2,4,5-trichlorophenol	2VCH 2VG8 2VCE	2007	(Brazier-Hicks et al., 2008)
MtUGT78G1	2.1 2.1	UDP UDP and myricetin	3HBJ 3HBF	2009	(Modolo et al., 2009)
CtUGT78K6	1.85 1.85 2.55 2.7 1.75	Apo UDP Delphinidin Petunidin Kaempferol	3WC4 4WHM 4REM 4REM 4REL	2015	(Hiromoto et al., 2015)
Os79	1.8 2.3 2.4	UDP-open UDP-closed UDP-2FGlc, trichothecene	5TME 5TMB 5TMD	2016	(Wetterhorn et al., 2016)
Os79Q202A Os79H122A/L123A Os79T291V Os79	1.47 1.29 1.58 2.17	UDP UDP UDP UDP, deoxynivalenol-3-glucose	6BKO 6BK1 6BK2 6BK3	2017	(Wetterhorn et al., 2017)
AtUGT74F2	2.56 2.0	UDP, salicylic acid (BA) UDP, 2-bromobenzoic acid (2BA)	5U6M 5U6S	2017	(Thompson, Iancu, Neet, Dean, & Choe, 2017)
AtUGT74F2T15S AtUGT74F2T15A AtUGT74F2T15S	2.0 2.0 1.8	UDP, salicylic acid UDP,2-bromobenzoic acid UDP,2-bromobenzoic acid	5U6N 5V2K 5V2J		
PtUGT1	2.14	Indoxyl sulfate	5NLM	2018	(Hsu et al., 2018)
AtUGT89C1	2.7 3.0 3.21 3.2	Apo UDP UDP-β-L-rhamnose Quercetin	6IJ7 6IJ9 6IJA 6IJD	2019	(G. Zong et al., 2019)
UGT76G1	1.8 1.75 1.99	(SeMet) UDP UDP UDP, rebaudioside A	6O86 6O87 6O88	2019	(Lee, Salomon, Wu, & Jez, 2019)
UGT76G1 UGT76G1H25A UGT76G1 UGT76G1	1.69 1.7 2.1 1.7	UDP UDP, GOL AQ9, GOL, UDP AQ9, AUO, GOL, UDP	6INF 6ING 6INH 6INI	2019	(Yang, Zhang, Ke, Yang, & Z Hu 2019)

UGT	Resolution (Å)	Crystal complex	PDB number	Year	Ref.
GgCGT	2.6 1.9 1.8	UDP-Glc UDP/phloretin UDP/nothofagin	6L5P 6L5S 6L7H	2020	(M. Zhang et al., 2020)
UGT74AC1	2.02 2.10	SgUGT74AC1 UDP-glucose	6L8Z 6L8Z	2020	(Jiao Li et al., 2020)
Bs-YjiC	2.44	UDP	2IYA	2021	(Dai et al., 2021)