Introduction

Plants have evolved an extraordinary capacity to perceive changes in their external environment and adapt rapidly to maximize opportunity and minimize risk. This plasticity depends on the integration of growth, development, metabolism, and the evolution of diverse mechanisms to regulate cellular homeostasis. Increasing evidence suggests that glycosylation is one of these mechanisms, with the identification of large glycosyltransferase enzymes able to recognize hormones, secondary metabolites, biotic, abiotic chemicals and toxins in the environment. Glycosyltransferases, which catalyze the transfer of a sugar residue from an activated donor to an acceptor molecule, are found in all the living organisms. Since, plants in contrast to animals are sessile organisms and cannot move away from adverse environmental conditions, they need to adapt themselves to environmental stresses. Therefore, they have evolved distinct mechanisms by which tolerance against these stresses can be achieved, including a huge range of small molecule compounds active in defense and signaling.

Sterol glycosyltransferase (EC 2.4.1.173) in plants catalyze glycosylation of phytosterols and related compounds to generate their glyco-conjugates. Sterol glycosides are characteristic lipids of plant membranes. The biosynthesis of these lipids is catalyzed by the membrane-bound UDP-glucose: sterol glucosyltransferase (SGT). SGTs play an important regulatory role in the activity of sterols in higher organisms. The glycosyltransferase multigene family is categorized into 94 families based on sequence similarity, signature motifs, stereochemistry of the glucoside linkage formed, and known target specificity (http://afmb.cnrs-mrs.fr/CAZY). Of these 94 families SGTs have been grouped into Family 1 of the classification scheme. Plant secondary metabolite glycosyltransferases (UGTs) play an important role in this adaptation as glycosylation changes the stability, solubility and biological activity of such small molecules and creates a high diversity of different kinds of plant metabolites. They are crucial for the biosynthesis of secondary metabolites and the regulation of the activity of several signaling molecules and defense compounds and also play a significant role in the detoxification and compartmentation of endogenous compounds and xenobiotics (Jones and Vogt 2001). Because of the importance of sterols in membrane fluidity, permeability and the phospholipid dependence of UDP
Glc: sterol glucosyltransferase (Bouvier-Nave et al. 1984), it has been postulated that sterol glucosides may have a role in adaptation to temperature stress (Palta et al. 1993). Transcriptional analysis using microarray comprising a large set of genes including 109 secondary product glycosyltransferases suggested the role of glycosylation in defense response of *Arabidopsis thaliana*. Many mammalian and microbial GTs have been employed for the synthesis of oligosaccharides and antibiotic glycosides. The advanced knowledge in the catalytic activities of these glycosyltransferases in glyco-conjugate synthesis has attracted considerable interest from the biotechnology community in recent years. These enzymes are discussed in terms of their regio and enantio selective substrate recognition, sugar donor selectivity and their utility as biocatalysts in whole-cell systems. A large group of glycosterols called saponins, comprising glycosylated triterpenoids, steroids and steroidal alkaloids, occurs in many plants. These phytochemicals contain sugar chain coupled to C-3 hydroxyl group and possess antifungal activity. Traditionally, saponins (glycosterols) have attracted attention owing to their pharmacological activities. Several pharmaceuticals and food additives are based on plant chemical structures because of the antimicrobial, antioxidative and anticarcinogenic nature of several of these natural compounds. The use of recombinant glycosyltransferases could also have interesting industrial applications, providing a unique toolbox for the specific design of modified natural products. Finally, UGTs are suitable candidates to improve food or crop quality and a better understanding of their *in vivo* function could have interesting prospects for plant metabolic engineering.

The identification and cloning of several sterol glycosyltransferase genes have opened up the use of genetic approaches to investigate the biological functions of sterol glucosides. Indeed, transgenic organisms with manipulated sterol glycoside metabolism are powerful tools to study their functions. Few enzymes of *SGT* gene family have been purified from *W. somnifera* to near homogeneity (Madina et al. 2007a, b) and were identified as the family of *SGT* genes expressed in the leaves of *W. somnifera*. Full length cDNA of one member of *SGT* gene family of *Withania*, i.e., *WsSGT*L1, has been cloned and expressed in *Escherichia coli* (Sharma et al. 2007). Expression and activity of these genes have been shown to be differentially regulated with respect to abiotic stresses such as heat and salicylic acid treatments. Hence, glycosyltransferases represent a magnificent area of research which can be proved beneficial to mankind. The area still needs to be explored, as not much work has been
done on sterol glycosyltransferases. The glycowithanolides found in *W. somnifera* with excellent medicinal properties offer an attractive area to find out their glycosylation pattern and to find the genes which are responsible for glycosylation. This is the main hurdle in understanding the biological function of sterol glycoside at molecular level. Therefore, the present study focuses to understand the function of different members of sterol glycosyltransferase gene family of *W. somnifera* in heterologus system, like, *A. thaliana*, both wild type and knockout mutant. *A. thaliana* contains two genes, UGT80A2 (*At3g07020*) and UGT80B1 (*At1g43620*), (Warnecke *et al.* 1999) which encode UDP-Glc: sterol glycosyltransferases, enzymes that catalyze the synthesis of SGs and have significant similarity (67%) to SGTs of *W.somnifera*. Lines having mutations in UGT80A2 and UGT80B1 were identified and characterized. The UGT80A2 lines were viable and exhibited relatively minor effects on plant growth. Conversely, UGT80B1 mutants displayed an array of phenotypes that were pronounced in the embryo and seed. Most notable was the finding that UGT80B1 was allelic to transparent testa and displayed a transparent testa phenotype and a reduction in seed size (Debolt *et al.* 2009). There is a need to characterize *WsSGT* genes in detail to elucidate their function as well as role in stress response/tolerance at molecular level as well as in plant metabolism, plant defense mechanism, and medicinal properties.