# Functional Characterization of Sterol Glycosyltransferase

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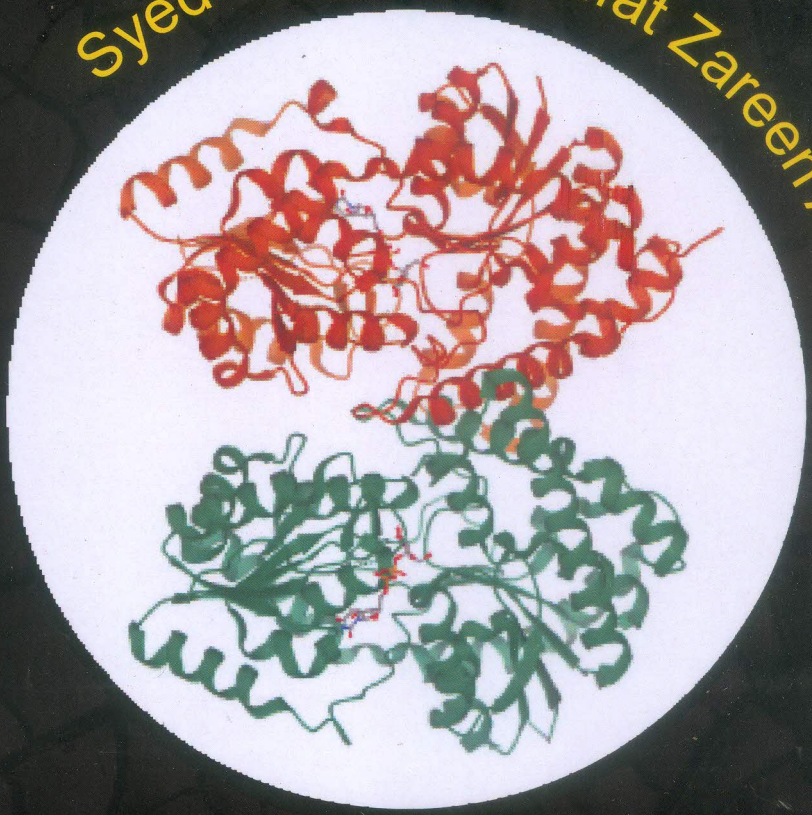

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**Syed Saema**
**Iffat Zareen Ahmad**
Assistant Professor
Integral University, Lucknow (U.P) INDIA

**AARGON PRESS**
New Delhi

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# 1. Introduction

Medicinal plants have been used to flavor and conserve food, in ailment of health disorders and to prevent diseases including epidemics since ancient times. Medicinal plants are plants containing inherent active ingredients to cure diseases or relieves from pain (Okigbo et al. 2008). All over the globe, the use of medicinal plants has significantly supported the primary healthcare (Maciel et al. 2002). From 250 to 500 thousand plant species are estimated to be present on the planet, and only 1-10% are used as food by humans and other animals (Cowan et al. 1999). Plants synthesize a diverse array of chemical compounds which are used to perform important biological functions including the defense mechanism. In 2001, researchers identified 122 compounds used in modern medicine which were derived from "ethnomedical" plant sources; 80% of them had an ethnomedical use or related to the current use of an active elements of the plant (Fabricant et al. 2001). About 12,000 such compounds have been isolated till date which is only 10% of the total (Lai et al. 2004; Tapsell et al. 2006). There is increasing in demand of medicinal plants day by day because of the active molecules in medicinal plants are difficult to prepare synthetically (Thomas 1995). Due to emergence of antibiotic resistant strains and new aliments, it is also logical to exploit the therapeutic potential of plants to get new, less toxic, less expensive and more effective drugs. Though plant based natural compounds called secondary metabolites are very important, it remains unclear how and why they are synthesized in plants. Therefore the major challenge is to elucidate and recognize the biosynthetic steps directing the formation of these valued products from simple building blocks. It is an urgent requirement to know about the pathways, genes and enzymes catalyzing the biosynthetic processes which will help in utilizing the wealth of natural compounds from plants. This investigation is an attempt to

# 2. Establishment of Direct Regeneration System in *Withania Somnifera*

## 2.1 INTRODUCTION

*Withania somnifera* (L) Dunal belongs to the family of solanaceae that holds medicinal properties.This plant is widely used in traditional Ayurvedic drug preparations. At present, *W. somnifera* is extensively grown and cultivated for medical purposes. All parts of the plant like root, stem, leaves, fruits (berries) are unique in their medicinal properties according to their diverse metabolites (Bharti et al. 2011; Bhatia et al. 2013; Namdeo et al. 2011). Therapeutic properties include anti-stress, anti-inflammatory, antitumour, anti-convulsant, anti-hyperglycaemic, immunomodulatory, neuropharmacological, hepatoprotective, cardioprotective, chemoprotective, musculotropic, radiosensitizing activities also with anti-ageing, macrophage-activating, rejuvenating, aphrodisiac, hypocholesterolemic and hemopoetic effects (Ahlawat et al. 2012; Jain et al. 2012; Singh et al. 2010; Sharma et al. 2011; Uddin et al. 2012). Conventional methods of cultivation (seed) for the production of *W. somnifera* roots is less than its need due to reduced yield, takes a long time, infertility of seeds, and seedlings are liable to plant infections like seedling mortality and blight, leaf blight, seed rotting etc. (Misra et al. 1997). This medicinally vital plant species has been depleted from its natural habitat and is currently introduced in the list of endangered species (Kanungo and Sahoo 2011; Patel and Krishnamurthy 2013) by the International Union for Conservation of Nature and Natural Resources (Kavidra et al. 2000; Supe et al. 2006). Due to its wide application, this plant is high in demand and has attracted various researchers for doing genetic manipulation that will in turn increase its pharmaceutical properties. Therefore, an efficient plant regeneration protocol is a prerequisite for its genetic transformation system.

# 3. Overexpression of WsSGTL1 Gene in W. Somnifera and Characterization of Transgenic Lines

## 3.1 INTRODUCTION

*W. somnifera* is especially attractive for studying the enzymes involved in steroidal transformations like glycosylation because it is a rich source of a variety of pharmacologically important withanolides and their derivatives such as withanosides, sitoindosides, withanomides etc. (Chatterjee et al. 2010; Chaturvedi et al. 2012; Chen et al. 2011; Jayaprakasam et al. 2003; Sharma et al. 2011). Withanolides are a group of naturally occurring steroids based on ergostane nucleus and characterized by a lactone-containing side chain (Abouzid et al. 2010). Involvement of steroid nucleus, side chain and additional ring formation are known for their structural diversity. The withanosides (saponins) are mainly comprised of withanolides with one or more glucose units attached to C-3 or C-27 positions (Bhattacharya et al. 2006; Matsuda et al. 2001). Withanolide biogenesis and accumulation is limited to specific genera of Solanaceae family, among them *Withania* shows maximum production of withanolide in more than 200 diversified forms, with or without functional groups (Chaurasiya et al. 2012; Chen et al. 2011; Misra et al. 2006). Glycosylation of sterols and their derivatives involves a glycosidic bond formation between sugar residue and a 3β-hydroxy group of sterols (Christie 2012; Shimamura 2012). Glycosylation reaction involves the transfer of sugar moieties to a wide range of acceptor molecules, mainly plant secondary metabolites. The reaction is catalyzed by glycosyltransferases (GTs) (EC 2.4.x.y) grouped in family 1 out of total the 94 families (http://www.cazy.org/Glycos ylTransferases.html).

# 4. RNAi Mediated Silencing of *WsSGTL1* Gene in *W. Somnifera* and Characterization of Transgenic Lines

## 4.1 INTRODUCTION

RNAi mediated gene silencing is referred as cosuppression or posttranscriptional gene silencing in plants; quelling in fungi and gene silencing in animals (Cogoni and Maciano, 1997; Dehio and Schell, 1994; Elmayan et al., 1998; Nakayashiki and Nguyen, 2008; Price and Gatehouse, 2008). The term "RNA interference (RNAi)" was given by Fire et al. (1998). The first proof that dsRNA could attain efficient gene silencing through RNAi came from studies on the nematode *Caenorhabditis elegans*. Researches in the fruit fly *Drosophila melanogaster* have contributed greatly towards understanding the biochemical nature of the RNAi pathway (Elbashir et al. 2001). SiRNAs and their role in post-transcriptional gene silencing (PTGS) in plants were first discovered by David Baulcombe's group at the Sainsbury Laboratory in Norwich, England and reported in Science in 1999 (Hamilton et al.1999). Currently, SiRNA is widely used for in vitro knockdown/suppression of gene expression to assess the function of gene. The mechanism of silencing of gene proceedes with the entry of a double-stranded RNA (dsRNA) molecule into the cell that triggers the RNAi pathway. Dicer is an enzyme that cleaves the long double-stranded molecule into small double-stranded fragments with 5' phosphorylated ends and 2-nt unpaired and unphosphorylated 3' ends. These small fragments that embody siRNA are approximately 21-23 nucleotides in length (Wilson et al. 2013). Each siRNA unwind into two single-stranded RNAs (ssRNAs), one is the passenger strand and the other one is guide strand. The passenger strand is degraded and the guide strand binds into the RNA-induced silencing complex (RISC). RISC uses the guide strand to find the mRNA that has a complementary sequence

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