Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 11(6):27-32

Research Article

ISSN: 0975-7384

CODEN(USA): JCPRC5

Strictosidine β-*D*-glucosidase (SGD) Gene Study Case in the three Egyptian Cultivars of *Catharanthus Roseus*

Amal M Zweil*, Ibrahim A Ibrahim and M Bekhit

Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt

---

ABSTRACT

Strictosidineβ-D-glucosidase(SGD)(SGD EC:3.2.1.105) as the key enzyme, allows plants to synthesize the enormous variety of 2000 monoterpenoid indole alkaloids particularly in the three plant families Apocynaceae, Rubiaceae, and Loganiaceae SGD is believed to play a role in the divergence of the biosynthesis toward the various alkaloid structures found in different plant cultivars. The size and intensity of the amplified fragment of the gene were compared among the three cultivars (Albus, Roseus and Ocellatus). Depending on gene expression levels in the three cultivars, the content of strictosidine in each cultivar was predicted.

Keywords: Catharanthus roseus; Strictosidine β-D-glucosidase; Egyptian cultivars

---

INTRODUCTION

*Catharanthus roseus (*Madagascar periwinkle), which is a member of Apocynaceae (dogbane) family, produces various monoterpenoid indole alkaloids (MIAs), some of which are valuable for their medical applications. The bisindole MIAs, vinblastine and vincristine that are derived by the oxidative coupling of vindoline and catharanthine, have been used for cancer chemotherapy. This subtropical plant is also known for its drought tolerance, many horticultural varieties with various petal colors have been developed. The estimated haploid genome size of *Catharanthus* varies between 696 Mbp and 2377 Mbp, depending on the reference DNA used for analyses [1-3], this is typical among other Apocynaceae plants (average 1633 Mbp among 8 species) (also see Plant DNA C-values Database)

Strictosidine β-*D*-glucosidase (SGD)

Strictosidine β-*D*-glucosidase (SGD) (SGD - EC:3.2.1.105), as the key enzyme, allows plants to synthesize the enormous variety of 2000 monoterpenoid indole alkaloids particularly in the three plant families Apocynaceae, Rubiaceae, and Loganiaceae [4-6] Glucoside strictosidine and its de glucosylation product(s) formed by strictosidine

β-*D*-glucosidase (figure 1) in *C. roseus* cells have a direct role in plant defense against several microorganisms through a plant protecting antimicrobial action [7]. In addition of this role, SGDs have a more direct role in plant defense related glucosidase as a damage-inducible biochemical defense system [8,9].

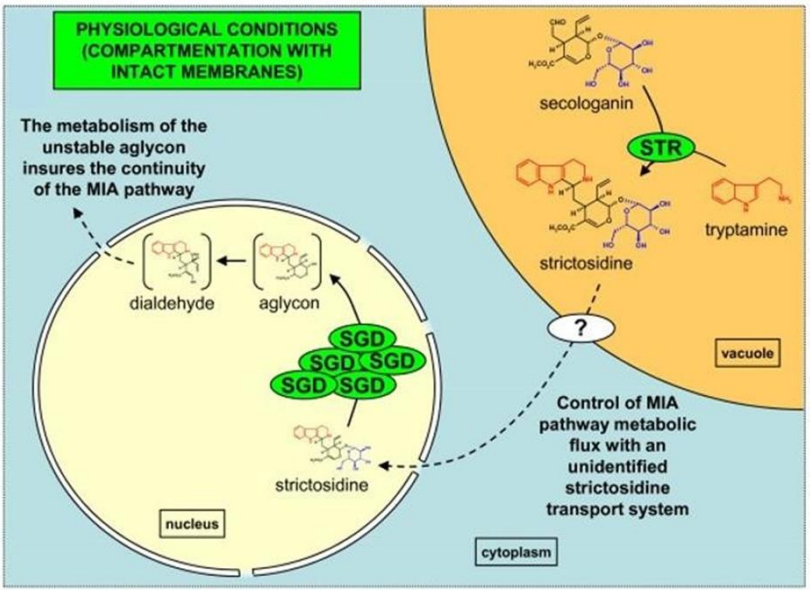

Figure 1. SGD working model illustrating that an unknown transportation system of strictosidine biosynthetic pathway

In *C. roseus*, this part of the pathway is specifically localized to the epidermis of aerial organs. This potential rate limiting step appears as a mean for the cells to control the rate of formation of toxic dialdehyde following the deglucosylation of strictosidine by SGD in the nucleus in relation with the metabolic capacity of the next MIA biosynthetic enzymes.

The Aim of this Study

This study aims to identify the difference between the Egyptian cultivars of Catharanthus roseus content of Strictosidine β-*D*-glucosidase (SGD) gene on transcription level.

MATERIALS AND METHOD

This work had been done in Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat city. Catharanthus cultivars Albus, Ocellatus and Roseus were collected from GEBRI's greenhouse (obtained kindly from the farm of faculty of pharmacy Cairo university, plants identified and confirmed kindly by Prof. Dr. Kassem Fouad Kassem El-Sahhar Prof., Department of Agricultural Botany, Faculty of Agriculture, Cairo University; and Prof. Dr. M. Nabil El-Hadidi, Faculty of Science Cairo University, Egypt.). Samples were collected in the spring blooming season as it the highest period of production.

Three Egyptian Catharanthus cultivars (Albus, Ocellatus and Roseus) were used to study the SGD gene expression effect [Figure 2].

Figure 2. Catharanthus cultivars (Albus, Ocellatus and Roseus)

Total plant RNA Isolation:

Total RNA was isolated from shoot tips of the three Egyptian *Catharanthus* cultivars (Albus, Ocellatus and Roseus). The total RNA was isolated using the Trizol reagent (Ambion, USA Cat. no.15596-026) and according to their instructions.

Removal of the genomic DNA contamination

Genomic DNA contamination of the total RNA samples was removed by a treatment of 50 µg RNA with 10 U of DNase I purchased from (Promega Corporation, Madison, USA).

Determining of both purity and integrity of RNA:

RNA quantity was estimated as µg /µl using Nano Drop 2000C spectrophotometer to determine sample concentration. The integrity of RNA samples was assessed by visualization of discrete separated 18S and 25S ribosomal RNAs on formaldehyde-agarose (Fa) gel electrophoresis, according to this protocol, a 100 µl sample buffer was prepared.

RNA samples (4 µl) were mixed with 16 µl of sample buffer and 5 µl of loading buffer (composed of saturated bromophenol blue in 50% glycerol) then the samples were incubated at 65°C for 3-5 min (to disrupt the secondary structures). The RNA tubes were chilled on ice, and then RNA was loaded onto 1.5% agrose gel stained with ethidium bromide to be tested and photographed under UV light (Figure 3).

***SGD* detection using PCR**

Specific primer pair for SGD gene was designed from the conserved sequence and was constructed at (Bioresearch technologies, USA).

The sequence of the primer pair is shown in (table 1).

Table 1. Primer used to detect SGD gene with molecular weight 1434 bp in Catharanthus cultivars

Primer	Primer sequence (5̀-3′)	Annealing Temp.
FSGD	F: ATGGGAT CTAAAGATGATCAGTCC	52 °C
RSGD	R: CACACCATCATCAATAGCATCTCG	

cDNA Synthesis:

The isolated RNA was used to synthesize the first strand cDNA through Reverse Transcription (RT) reactions. The reactions of each RNA sample contained 0.2 µg of freshly diluted RNA, 1 µM of polynucleotide $(dT)_{18}$ primer. The tubes containing the above-mentioned components were incubated at 65°C for 5 minutes and then used for cDNA synthesis according to the H¯Revert aid first strand cDNA synthesis kit (Fermentas INC**.**, Maryland, and USA**)**. RT

reaction samples were incubated in a PCR thermocycler programmed for cycles at 45°C for 60 minutes, 70°C for 5 minutes and then the first strand cDNA solution was stored at 4°C. The PCR device model belonged to Biometra Ltd. Company (TPersonal Thermocycler, Biometra, and Gottingen, Germany). The size and intensity of the amplified fragment of the gene were compared among the three cultivars (Albus, Roseus and Ocellatus).

Depending on gene expression in the three cultivars, the content of strictosidine in each cultivar was predicted.

PCR amplification condition

The 25 µl PCR volume containing 2.0 µl (DNA product),1.0 µl dNTPs (10 mM), 2.5 µl Mg Cl_2 (25 mM), 2.0 µl 10X buffer (10 mM tris, pH 8.0, 50mM KCl and 50 mM ammonium sulphate), 2.0 µl of each primer (0.5 M Table1), 0.25 µl *taq* polymerase (5u/µl). The volume was brought up to 25 µl by nuclease-free water.

The PCR cycling condition involved initial denaturation at 94°C for 4 min. followed by 35 cycles, template denaturation at 94°C for 1 min., primer annealing at 52°C for 1 min. and primer extension at 72°C for 1 min., final extension at 72°C for 5 min., followed by storage at 4°C. PCR thermocycler machines from Biometra (T-Gradient Thermoblock) were used. PCR products were separated on 1.5% agarose gel electrophoresis.

Results: RNA was loaded onto agarose gel with ethidium bromide and photographrd under UV light (figure 3)

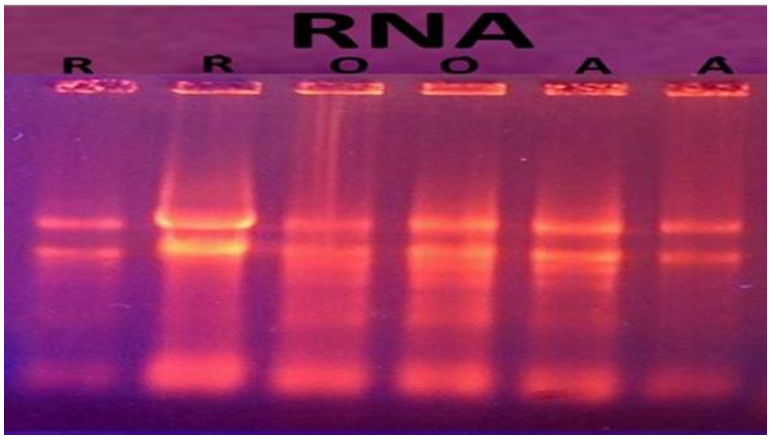

Figure 3. RNA isolated from the three cultivars (Roseus, Ocellatus and Albus respectively)

RESULTS

The quantification of mRNA concerns the same gene in the three cultivars showed that the band density were different: the highest was in Ocellatus cultivar, the lowest was in Albus cultivar and the intermediate was found in Roseus cultivar. These differences could indicate that the strictosidine synthesis was over expressed in the ocellatus cultivar.

At the molecular level of DNA, the regulation sites of gene expression may have different sequences interoperating the different expression sites. Our results indicate that SGD is encoded by a single-copy gene in *C. roseus* and that SGD is most likely located in the ER in three plant cultivars with different TIA structural profiles. Thus, the biosynthetic divergence is unlikely to be due to a difference in cellular compartmentation of SGD between different plant cultivars.

(As shown in Figure 4) The primer pair was designed for real time PCR, so it was amplified just 150 bp of the gene. The amplified fragment was obtained in the three cultivars (Albus, Roseus and Ocellatus). The intensity of the amplified gene was high in Ocellatus cultivar, low in Albus cultivar and medium in Roseus cultivar.

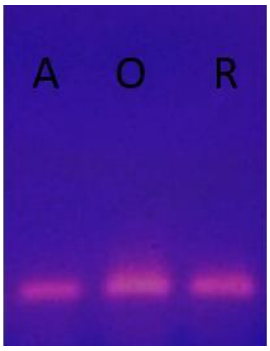

Figure 4. SGD isolated gene presence in the three cultivars

DISCUSSION

SGD is believed to play a role in the divergence of the biosynthesis toward the various alkaloid structures found in different plant cultivars. Strictosidine hydrolysis by SGD leads to an unstable aglycon that converts spontaneously to cathenamine, the main product of the reaction [11,12]. Future research should now focus on the identification of all the intermediates formed after strictosidine hydrolysis and the enzymes that may convert them. These enzymes could well be located close to the Endoplasmic Reticulum ER. This idea is supported by the fact that most of the strictosidine conversion products (e.g. cathenamine) are poorly soluble and thus are difficult to transport to other sites by simple diffusion. Terpenoid Indole Alkaloids TIA biosynthesis involves complex trafficking of intermediate metabolites between different cellular compartments, i.e. chloroplast, cytosol, and vacuole [5, 13].

CONCLUSION

According to gene expression (fragment intensity) we can predict that Ocellatus cultivar has the highest content of strictosidine then Roseus, and the lowest content in Albus cultivar.

REFERENCES

[1] DW Galbraith; KR Harkins; JM Maddox; NM Ayres; DP Sharma; E Firoozabady. *Sci*. **1983,** 220, 1049.1051

[2] BJ Zonneveld; IJ Leitch; MD Bennett. *Ann Bot*. **2005**.

[3] L Barleban; S Panjikar; M Ruppert; J Koepke; J Stöckigt. *Plant Cell*. **2007**, 19, 2886-2897.

[4] J Stoeckigt; MH Zenk. *J Chem Soc Chem Commun*. **1977**, 18, 646-648.

[5] JF Treimer; MH Zenk. *Eur J Biochem*. **1979**, 101, 225-233.

[6] TJC Luijendijk; E van der Meijden; R Verpoorte. *J Chem Ecol*. **1996**, 22, 1355-1366.

[7] A Geerling; MML Ibañez; J Memelink; R Heijden; R Verpoorte. *J Biol Chem*. **2000**, 275, 3051-3056.

[8] R Verpoorte; R van der Heijden; L Memelink. *Alkaloids*. **1998**, 96, 229-244.

[9] LH Stevens. Formation and Conversion of Strictosidine in the Bio-synthesis of Monoterpenoid Indole and Quinoline Alkaloids. Ph.D. Thesis, Leiden University, Leiden, the Netherlands. **1994**.

[10] AH Meijer; R Verpoorte; JHC Hoge. *J Plant Res*. **1993**, 3, 145-164.

[11] Murata; V De Luca; D Bienzle; J Brandle; Ch Sensen. *FEBS Letters*. **2006**, 580, 4501-4507.

[12] A Geerlings; IM Martinez-Lozano; J Memelink; RH Van der; V Robert: *J Biol Chem*. **2000**, 275(4), 3051-3056.

[13] AI Ibrahim; AAl Hemeida; AM Hussain; M Bekhit; AA Zweil. *J Product Dev*. **2013**, 18(1), 77-84.