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The Expression Profiles of Progesterone 5β-Reductase Gene

Nurşen Çördük and Cüneyt Akı

Plant Tissue Culture and Genetics, Çanakkale Onsekiz Mart University, Faculty of Science and Arts, Department of Biology, Molecular Biology Subdivision, Çanakkale, TURKEY

ABSTRACT

Various species of the genus Digitalis have been used as a source of cardiac glycosides which are important for their use in medicine. Progesterone 5β-reductase (P5βR) plays a key role in the biosynthesis of β-cardenolides of Digitalis. In this research, progesterone 5β-reductase gene expression was determinated in Digitalis trojana Ivanina, an endemic plant of Kazdagi Mountain (Mt. Ida), Turkey. The expression of progesterone 5β-reductase gene was showed variations in the plants which were collected in different seasons and altitudes. The minimum and maximum gene expression profiles were calculated respectively in 836 m and 1191 m altitudes in May. Minimum and maximum gene expression levels were calculated respectively in 1191 m and 1047 m altitudes in July.

Keywords: *Digitalis trojana*, cardiac glycosides, Kazdagi, progesterone 5β-reductase.

INTRODUCTION

Cardenolides or cardiac glycosides (CGs) are a class of natural products and a pharmacologically important group of plant secondary metabolites. They have been used as the most effective heart drugs for treating congestive heart failure, cardiac arrhythmias, and atrial fibrillation [1]. Although CGs are found as secondary metabolites in a diverse group of plants including *Digitalis*, *Strophanthus*, *Scilla maritime* and *Nerium oleander*, several *Digitalis* species (e.g. *D. purpurea*, *D. lanata*) are still potential sources of CGs as applied in human medicine and they have been used therapeutically for the treatment of cardiac insufficiency for more than 1500 years [1,2]. Progesterone 5β-reductase enzyme (P5βR; EC1.3.1.3) is one of the enzymes involved in the biosynthesis of cardenolide progesterone and catalyzes the 5β reduction of progesterone to 5β-prenane-3,20-dione and thus it gives rise to division the road into two phases catalyze biosynthetic pathway that lead to the 5β-type of the cardenolide structure typically for all *Digitalis* plants [3,4]. In this research, expression profiles of progesterone 5β-reductase were determinated in *Digitalis trojana* Ivanina, in the family Plantaginaceae, is an endemic species of Kazdagi, Turkey. The expression patterns of the *P5βR* gene were compared in *D. trojana* which collected at four different altitudes in May and July 2011.

D. trojana plants were collected at different altitudes (836m, 961m, 1041m and 1191 m) in different vegetation phases from the Kazdagı National Park, Balıkesir, Turkey in May 2011 and July 2011. Plant samples were identified by Dr. Ersin Karabacak, Department of the Biology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey. A voucher of the studied plant was deposited in the Çanakkale Onsekiz Mart University Herbarium (Çanakkale, Turkey) under number 938 (May), 939 (July).

Total RNA isolation was carried out from plant's leaves using an Invitrogen Purelink RNA Mini Kit (Kat. No.12183018A) following the manufacturer's instructions. Total RNA concentrations were determined on a 1% agarose gel and estimated spectrophotometrically with WPA Biowave DNA spectrophotometer (18WDC). The synthesis of progesterone 5β-reductase cDNA was performed using total RNA with Fermentas RevertAid^{TM} First Strand cDNA Synthesis Kit (#K1621). Levels of specific mRNAs were assayed using reverse transcription (RT)

followed by PCR. Amplification of the progesterone 5β-reductase was performed from cDNA using the primers as described earlier by Herl et al.[3]. PCR reaction mixture contained 1X High Fidelity PCR buffer with 15 mM $MgCl_2$, 0.4 pmol/µl of each primer, 2.5 µl cDNA and 1.25 U High Fidelity PCR Enzyme Mix. PCR reactions were carried out in a Bio-Rad thermal cycler. PCR was performed using the following program: an initial denaturation at 94°C for 5 min, 30 cycles of a denaturation period at 94°C for 20 s, annealing 50°C for 1 min, extension 72°C for 2 min and finally incubated at 72°C 10 min. PCR products were analyzed by 1% agarose gel electrophoresis in TAE buffer system. Gels were stained with ethidium bromide and visualized by illumination at UV365. The results were normalized to the reference gene Actin using Gel-Pro Analyzer Software v3.0 (Media Cybernetics, USA) and were always expressed as expression ratios relative to a control value. Tree replicates were analyzed per experimental sample.

According to our results, the expressions of the *P5βR* gene were showed differences in plants samples which collected in May and July depending on the season and altitudes (Fig 1).

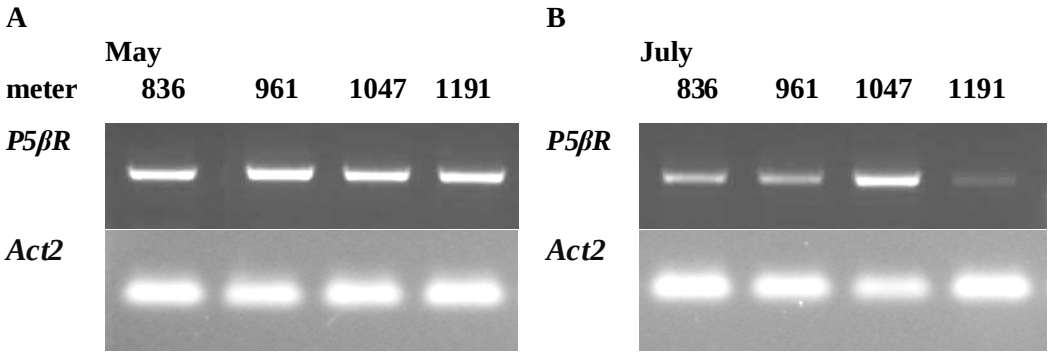

Fig. 1. The expression profiles of *P5βR* gene in the plant samples in May(A) in July (B).

The amounts of *P5βR* gene expression were increased parallel to the altitudes in May. The minimum and maximum expressions of the gene were calculated respectively in 836 m and 1191 m altitudes (Fig 2). The expression profiles of *P5βR* gene were showed much more variability depending to the altitudes in plants which collected in July. The minimum and maximum expressions of the gene were calculated respectively in 1191 m and 1047 m altitudes (Fig 2).

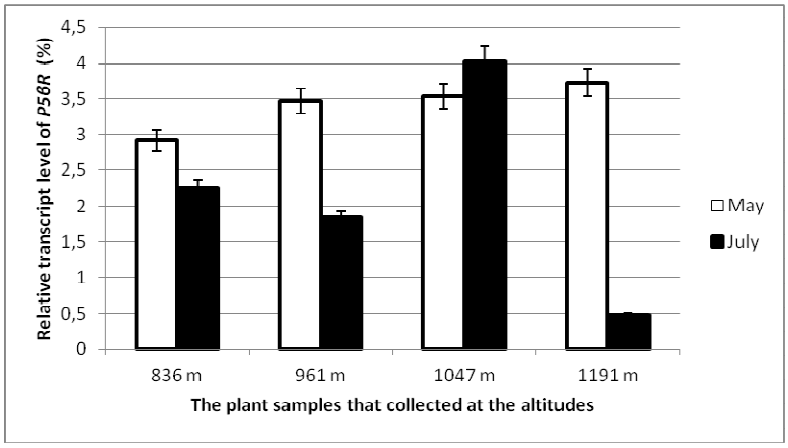

Fig. 2. The relative transcript levels of *P5βR* gene in the plant samples.

Our results are quite similar with the other reports. Roca-Perez et al.[5] reported the expression of *P5βR* in 10 natural populations of *D. obscura* distributed in three bioclimatic belts of Spain. It was determinated that the expression of *Dop5βr* was showed variation at different population according to season. It was found that the profile of the seasonal expression of *Dop5βr* showed increasing levels from February to July and a further reduction in autumn, although harmful climatic conditions seems to induce over expression of *P5βR* gene. It was pointed out that the expression of *P5βR* changed in the time course of the four seasons as a multiple response to distinct plant and/or environmental factors. Another study was reported the expression analysis of *P5βR* in all the organs tested (leaves, roots, stems, mature flowers and vascular bundle) in *D. purpurea*. In this study, RNA transcripts of the gene were present in all the organs but the expression levels of *P5βR* were found more abundant in leaves, mature flowers and the lowest amount was present in the vascular bundle [6] On the contrary, Pérez-Bermúdez et al. [4] carried out a

comparative study into the regulation of *P5βR* and *P5βR2* gene expression in mature flowers, leaves, stem at the base of the rosette, vascular bundle at the basal part of the leaf and roots and their role in cardenolide biosynthesis. In this study, it was point out that the expression of the *P5βR* gene was not showed variation in the all organs. And in this study, the expression of *P5βR* and *P5βR2* were also analyzed in response to wounding, heat shock, cold shock, salt stress and chemical application (salicylic acid, H_2O_2, methyl jasmonate). It has been demonstrated that *P5βR* did not exhibit variation in its expression level under stress conditions.

Different types of climate characteristics are effective in Kazdagı region. As a result of these, wide climatic variations are observed depending to the altitude and season [7,8]. Secondary metabolites play a major role in the adaptation of plants to the changing environment. As a result of biotic and abiotic factors, such as temperature, light intensity, herbivory and microbial attack, plants generate their defense mechanisms by triggering many complex biochemical processes [9]. Changes have been reported at genetic or protein levels that are brought about by environmental conditions and are reflected in a profound alteration of the metabolite pool of the affected plants [10-13]. Many environmental factors like precipitation, mean temperature, soil types, duration of snow-cover and length of the vegetation period and the intensity of radiation have been reported to be different between low and high altitude sites in temperate zones [14-15]. In addition to altitude and seasonal changes, daily temperature changes in the spring and summer seasons are very effective on plant secondary metabolites.

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