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Evaluation of the TPS gene as an internal control gene in Gossypium genotype for the quantitative detection of cotton transgenic product using Time-Real PCR technique

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Abstract

Nowadays 6 million farmers are planting transgenic plants in 16 various countries around the world. Currently, more than 27% of the cultivated transgenic plants are in 9 developing countries. Having attention to increased production of transgenic plants in the world, monitoring of these transgenic products has important in order to production, exporting and importing. In the current situation, it is important to design and implement the qualification and quantification tests in our country for tracing the transgenic plants due to the production of some of these plants such as rice and cotton. There are various diagnostic methods for monitoring transgenes, but the Real-time PCR is one of the most precise approaches among them. On the other hand, given that the application of this method based on using an internal control gene, the utilized gene is required to have specific characteristics and specifications. In this study, we succeeded in introducing the internal control gene of Trehalose 6- phosphate-synthase (TPS) for evaluating the transgenic cotton plant in the world for the first time.

Keywords: Transgenic Plants; Internal Control Gene; Real-Time PCR; Trehalose 6-Phosphate-Synthase (TPS)

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1. Introduction

Cotton is the most important fiber plant in the world with a lot of genetic changes, and these changes have been performed to improve the agronomic characteristics and quality of its fiber. The need for tracing the transgenic plants is to allow the consumers to choose and consciously consume of these products (2). Therefore, there is a demand for the design and implementation of the tracing tests for these plants, especially in our country. Generally, these tests are classified into three stages of tracing, identification, and assessment of qualitative. At the moment, genetically modified plants are often distinguished by the conventional PCR method (5), but the efficiency of this method is only in tracing and identification of mentioned products. For achieving to aims of the qualitative evaluations, we can introduce the real-time PCR method. Not only is able this method to quality assessment, but also it is a simple method with high specificity and high sensitivity (5). The quality assessment method in the real-time PCR technique is based on the use of an internal control gene which has special properties. This gene should be present in a little number in each genome, as well as, having a low heterogenesis between cultivars and the lower

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hemogenesis among other plants (4). Ding *et al*. (2004) introduced the SPS gene for the detection of transgenic rice plant (1). In the present study, an internal control gene was used for the quantitative assessment of transgenic cotton, as well as for estimating the number of gene copies. Also, in this study, the determination of zygosity status was introduced in T1 generations using relativistic quantitative assessment methods for the first time.

2. Materials and Methods

In the present study, plant seeds were obtained from Karaj Agricultural Research Institute. The seeds of the cotton plant were five different varieties of Gossypium hirsutum including Neishabour, Taghva, ZETA 2, Acala SJ and Coker 312, and one variety from G.barbadense) and seeds of other plants including *Hordeum vulgare*, *Zea mays*, *Oryza sativa* , *Ficus carica*, *Pistacia vera*, *Taxus baccata*, *Triticum aestivum*, *Rosa hybrid* and *Glycine max*. Cotton seeds were cultured in sterilised Petri dishes and between two layers of wet tissue paper after delinting. The obtained seedlings were transferred to small pots after 12 days. In the next step, from the young and healthy leaves of cotton and other plants were used for DNA extraction. The extraction of DNA in cotton was done based on the Hayyan method which is a specific method for DNA extraction in cotton, and for extraction of DNA from other studied plants was used from modified CTAB method. Finally, for determining the amount of DNA, spectrophotometry and electrophoresis methods were used on the agarose gel. In this research, for the design of primers and probes was used from the Beacon Designer (Ver 7) software for use in the real-time PCR (TaqMan) system. The probe sequences and the primers listed in Tables 1 were used for the present study.

Table 1. The sequences of primers and probes used in real- time PCR reaction

PCR system	Name	Direction	Sequence	Length (bp)
Endogenous real-time PCR	*TPS F*	Sense	5′-ACGAACTTTCCCATTTCCTTTCG-3′	23
	TPS R	Anti-Sense	5 -CGAGAAGGAGATACTTACTTGCAG-3′	24
	TPS P	Prob	-BHQ5 -CCCACAAATCCGCCTCTCCTCCGC-3 TET-	24

3. Results and Discussion

One gene can be used specifically as an internal control gene in one species when being available in a little number in each genome. In the other words, there is a minimal number of copies for each haploid genome, and a low hemogenesis among other plants. Kosmas *et al*. (2006) demonstrated by Southern blot technique that there is one copy of TPS gene in each genome A and D of cotton (*Gossypium* genus). This trait is a special privilege for an internal control gene, which exists in both of the cotton genomes (A and D), and the number of its copies is equal to one number. In this test, cotton DNA was affected in serial dilution under real-time PCR reaction, and the duplicated curves have supported the efficiency of primers and probes.

When different varieties of *G.hirsutum* species and one another cultivar (*G.barbadense*) were used in the real-time PCR test, we found that designed primers and probes have the potential of duplication in the DNA samples of the available cultivars. Also, our results showed that there was a minimum allelic variation between the present cultivars. Therefore, it was determined that the heterogenesis rate of the different genus of *Gossypium* cultivars was minimal in real-time PCR. Overall, the duplication of the TPS gene in the coker cultivar of cotton, and as well as not observation of the DNA duplication in other plants indicate the specificity of the TPS gene as an internal control gene and high degree of hemogenesis of the TPS gene among other plants by designed primers and probes in *Gossypium* genus.

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