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Research article

Promoter regions of potato vacuolar invertase gene in response to sugars and hormones

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ABSTRACT

Potato vacuolar acid invertase (StvacINV1) (β-fructofuranosidase; EC 3.2.1.26) has been confirmed to play an important role in cold-induced sweetening of potato tubers. However, the transcriptional regulation mechanisms of *StvacINV1* are largely unknown. In this study, the 5′-flanking sequence of *StvacINV1* was cloned and the *cis*-acting elements were predicted. Histochemical assay showed that the *StvacINV1* promoter governed *β-glucuronidase* (*GUS*) expression in potato leaves, stems, roots and tubers. Quantitative analysis of GUS expression suggested that the activity of *StvacINV1* promoter was suppressed by sucrose, glucose, fructose, and cold, while enhanced by indole-3-acetic acid (IAA), and gibberellic acid (GA_3). Further deletion analysis clarified that the promoter regions from −118 to −551, −551 to −1021, and −1021 to −1521 were required for responding to sucrose/glucose, GA_3, and IAA, respectively. These findings provide essential information regarding transcriptional regulation mechanisms of *StvacINV1*.

1. Introduction

Potato (*Solanum tuberosum* L.) tubers are often stored at low temperature to prevent sprouting and minimize disease losses. However, low temperature storage leads to the accumulation of reducing sugars in potato tubers, a process called cold-induced sweetening. Reducing sugars, such as glucose and fructose, react with free amino acids during frying for potato chips or French fries, resulting in unacceptable color change and acrylamide formation, which are major economic and healthy concerns [1,2].

Potato vacuolar acid invertase (StvacINV1) (β-fructofuranosidase; EC 3.2.1.26), which hydrolyzes sucrose into glucose and fructose, has been confirmed to play an important role in cold-induced sweetening. Invertase activity was first detected in sprouted potato tubers in 1903 [3]. Later, a large number of reports have described the physiology and enzymology characteristics of potato invertase. The accumulation of reducing sugars in low temperature-stored potato tubers occurs concomitantly with the formation of invertase [4]. Six acid invertase genes, including four cell wall invertases and two vacuolar invertases, have been identified by searching the existing databases [5]. The vacuolar invertase gene, *StvacINV1*, was strongly induced by low temperature [5]. Suppression of *StvacINV1* prevents the reducing sugar accumulation in cold-stored tubers, as a consequence, the processing quality can be improved effectively [5–11]. Transcripts of *StvacINV1* were detectable in all potato organs, with a higher level in senescence leaves comparing to other organs [5]. Vacuolar invertase was suggested to have a wide range of regulatory functions in plant growth and development in addition to its major role in carbohydrate metabolism [12].

Differential expression of a gene upon exposure to environmental stimuli is essential for the gene to function in specific biological processes. The *cis*-acting elements residing in the promoter of individual gene contribute to the gene expression regulation [13]. A large group of plant transcription factors have been characterized to bind to the *cis*-acting elements in the promoter to active or suppress the gene expression [14]. Promoter sequences are as important as the coding sequences for gene function. The promoters of potato cell wall invertase genes, *invCD111*, *invCD141*, *invGE*, and *invGF* have been cloned and analyzed, according to which the association of cell wall invertase genes expression with sexual and vegetative growth cycles in potato was indicated [15,16]. However, the promoters of potato vacuolar acid invertase genes were rarely reported previously. In the present research, the

Abbreviations: ABA, abscisic acid; BAP, 6-benzylaminopurine; GA, gibberellic acid; GARE, GA-response element; GUS, β-glucuronidase; hiTAIL-PCR, high-efficient thermal asymmetric interlaced polymerase chain reaction; IAA, indole-3-acetic acid; qRT-PCR, quantitative RT-PCR; SRE, sugar-repress element; StvacINV1, *Solanum tuberosum* vacuolar acid invertase 1.

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promoter of *StvacINV1* gene was cloned and its responses to sugars, hormones, and cold were dissected in potato plantlets and post-harvest tubers.

2. Results

2.1. Isolation of the 5′-flanking sequence of StvacINV1

The 5′-flanking sequence of *StvacINV1* gene was isolated from the genomic DNA of potato cv. E-potato 3 using high-efficient thermal asymmetric interlaced polymerase chain reaction technique (hiTAIL-PCR) [17]. Twenty clones of hiTAIL-PCR products were sequenced which led to the isolation of four highly similar sequences, possibly the 5′-flanking sequences of *StvacINV1* alleles residing in the tetraploid genome. The result showed that the putative transcriptional start site (TSS, +1) was located 18 bp upstream of the translation start codon. A TATA-box and a CAAT-box were located at the −28th and the −71st position, respectively, characterizing a promoter structure of the cloned sequence (Fig. 1). Five E-box elements (EBOX, CANNTG) [18], nine sugar-repress elements (SRE, TTATCC) [19], and eight GA-response elements (GARE, TAACAAA) [20] were found spreading all over the 1.5 kb sequence, suggesting the transcription of *StvacINV1* may be regulated by various factors. We also isolated the 5′-flanking sequences of *StvacINV1* from other potato genotypes, however, they shared 94.1%–99.9% identity and no informative differences in structure among the sequences were observed (Supplementary Fig. 1).

2.2. Promoter activity of the 5′-flanking sequence of StvacINV1

To determine the promoter activity of the 5′-flanking sequence of *StvacINV1*, a 1.5 kb sequence isolated from E-potato 3 was fused to the coding sequence of *β-glucuronidase* (*GUS*) to construct a vector denoted as pE1521::GUS, and transformed to E-potato 3. Twenty-six transgenic plants were obtained. The four-week-old plantlets of the transgenic lines were stained blue by X-Gluc solution (Fig. 2), demonstrating that the 5′-flanking sequence of *StvacINV1* had promoter activity that can govern the expression of the *GUS* reporter gene in plant. The *GUS* expressed mainly in the leaves and nodes of plantlets, and expressed at a low level in roots. The GUS signal was also detected in almost all tissues of tubers except for periderm and cortex. Based on the GUS staining assay, three transgenic lines (pE1521::GUS-5, pE1521::GUS-6, and pE1521::GUS-10) with relatively higher GUS activity were selected for further investigation.

2.3. The response of StvacINV1 promoter to sugars

StvacINV1 catalyzes a hydrolytic reaction in which sucrose is the substrate, and glucose and fructose are the products. Nine SREs were predicted in the promoter of *StvacINV1*, among which seven were packed in the −360 to −524 region (Fig. 1), suggesting that the substrate or the products of StvacINV1 may regulate the expression of *StvacINV1*. The response of *StvacINV1* promoter to sugars was determined by GUS staining and quantitative RT-PCR (qRT-PCR). Histochemical assay exhibited a reduced GUS staining by sugars, especially glucose and fructose (Fig. 3a). The qRT-PCR analysis revealed that the relative expression level of *GUS* was significantly decreased in sugar-fed plantlets (Fig. 3b), which was in accord with the histochemical assay, suggesting that the activity of *StvacINV1* promoter was suppressed by feeding of sucrose, glucose, and fructose. This was reinforced by detecting the relative expression level of *StvacINV1* governed by the natural *StvacINV1* promoter. The mRNA abundance of the *StvacINV1* gene was significantly lower in any sugar-fed plantlets than that of non-sugar control (Fig. 3c).

2.4. The response of StvacINV1 promoter to hormones

Eight GAREs were predicted in the promoter of *StvacINV1* (Fig. 1), implying the activity of *StvacINV1* promoter might be

Fig. 1. The 5′-flanking sequence of *StvacINV1*. The start codon, transcription start site (TSS), TATA-box, CAAT-box, E-box, sugar-repressive element (SRE), and GA-responsive element (GARE) are underlined. The primer sites are italicized. Numbers indicate the position relative to the TSS.