Scientia Horticulturae 158 (2013) 22–32

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Cloning and characterization of a carbohydrate metabolism-associated gene *IbSnRK*1 from sweetpotato

Tao Jiang [1], Hong Zhai [1], Feibing Wang, Naike Yang, Bing Wang, Shaozhen He, Qingchang Liu *

Beijing Key Laboratory of Crop Genetic Improvement/Laboratory of Crop Heterosis and Utilization, Ministry of Education, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing 100193, China

ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 22 April 2013
Accepted 24 April 2013

Keywords:
ADP-glucose pyrophosphorylase
*IbSnRK*1 gene
Soluble sugar
Starch
Sucrose synthase
Sweetpotato

ABSTRACT

Sucrose non-fermenting-1-related protein kinase-1 (SnRK1) plays an important role in plant carbohydrate metabolism and starch biosynthesis. In the present study, a *SnRK*1 gene, named *IbSnRK*1, was isolated from sweetpotato (cv. Lushu No. 3) by rapid amplification of cDNA ends (RACE). The open reading frame (ORF) contained 1515 nucleotides encoding 504 amino acids. The deduced amino acid sequence showed high identities with SnRK1 of other plants. Real-time quantitative PCR analysis revealed that the expression level of *IbSnRK*1 gene was significantly higher in leaves of Lushu No. 3 than in its stems and roots. The leaf photosynthetic rate of transgenic tobacco (cv. Wisconsin 38) plants over-expressing *IbSnRK*1 gene was significantly increased comparing with that of the wild-type. The activity of sucrose synthase (SS) and ADP-glucose pyrophosphorylase (AGPase) was increased by 11–28% and 30–92%, respectively, whereas the activity of sucrose phosphate synthase (SPS) was decreased by 27–42%. Sucrose, glucose, fructose and starch were found to be significantly more accumulated in transgenic tobacco plants than in the wild-type. These results suggest that *IbSnRK*1 gene plays important roles in carbohydrate metabolism and starch biosynthesis and may be applied for increasing soluble sugar and starch levels of sweetpotato in the future.

1. Introduction

SnRKs are sucrose non-fermenting-1 (SNF1)-related protein kinases in higher plants (Halford and Hardie, 1998), which are divided into three subfamilies: SnRK1, SnRK2 and SnRK3 (Hrabak et al., 2003). SnRK1 plays an important role in plant carbohydrate metabolism and starch biosynthesis (Polge and Thomas, 2007; Halford and Hey, 2009; Wang et al., 2012). It is required for redox modulation of ADP-glucose pyrophosphorylase (AGPase) activity in response to sucrose (Tiessen et al., 2003) and is involved in regulating the expression of genes encoding sucrose synthase (SS) and AGPase which are two key enzymes involved in the biosynthetic pathway from sucrose to starch (Purcell et al., 1998; McKibbin et al., 2006).

The first *SnRK*1 gene to be characterized was cloned from rye (Alderson et al., 1991), and homologues have since been identified in several plant species such as *Arabidopsis* (Kleinow et al., 2000), maize (Lumbreras et al., 2001), potato (Lakatos et al., 1999) and apple (Li et al., 2010). Zhang et al. (2001) found that antisense inhibition of SnRK1 in developing pollen grains resulted in an almost complete loss of starch accumulation and viability. Kanegae et al. (2005) provided evidence that SnRK1 had a role in starch accumulation in rice. McKibbin et al. (2006) reported that the gene expression and activity of SS and AGPase were increased in the transgenic potato plants over-expressing *SnRK*1, in which starch levels in the tubers were increased by up to 30%. Jain et al. (2008) showed that the expression of a SnRK1 gene coincided with the onset of starch accumulation in sorghum endosperm and microspores, and Wang et al. (2012) demonstrated that the activity of SnRK1, SS and AGPase and the content of soluble sugar and starch were increased in transgenic tomato over-expressing a heterologous *SnRK*1 gene from apple. It is proposed that SnRK1 channels carbon through the storage pathway to starch (McKibbin et al., 2006; Halford and Hey, 2009). Furthermore, it is known that SnRK1 can regulate carbohydrate metabolism by inactivating sucrose phosphate synthase (SPS) via phosphorylation (Sugden et al., 1999; Halford and Hey, 2009). SPS activity was decreased by approximately 20% in transgenic tomato over-expressing SnRK1 gene from apple compared with the wild-type plants (Wang et al., 2012). In addition, Nunes-Nesi et al. (2010) thought that SnRK1 could increase the expression level of SPS.

* Corresponding author. Tel.: +86 10 62733710; fax: +86 10 62733710.
E-mail address: liuqc@cau.edu.cn (Q. Liu).
[1] These authors contributed equally to this work.

0304-4238/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2013.04.027

SnRK1 plays an important role in growth and development of plants by regulating metabolism in response to carbon availability in their respective systems (Halford and Paul, 2003). Repressing the expression of SnRK1 in pea seeds, transgenic seeds exhibited maturation defects, reduced conversion of sucrose into storage products, lower globulin content, and frequently altered cotyledon surface, shape and symmetry, as well as occasional precocious Germination (Radchuk et al., 2006). SnRK1-repressed pea showed that SnRK1 coordinated metabolic and hormonal signals such as auxin, cytokinin (CK) and ABA during early steps of pea cotyledon growth and differentiation (Radchuk et al., 2010).

SnRK1 is involved in plant stress responses. The first indication of an involvement of SnRK1 in plant stress response was the salt hypersensitivity of the antisense StubGAL83 transgenic potato plants, which suggests that SnRK1 might activate protection systems against this stress (Lovas et al., 2003). Furthermore, several lines of evidence indicate that SnRK1 is involved in plant–pathogen interactions (Hao et al., 2003). The expression of an antisense sequence of Arabidopsis SnRK1 in tobacco increased its sensitivity to virus attack whereas over-expression of a sense sequence increased its resistance, suggesting that SnRK1 might be a component of plant antiviral defense (Polge and Thomas, 2007). Cho et al. (2012) found that both rice and Arabidopsis SnRK1 activities critically influenced stress-inducible gene expression and the induction of stress tolerance.

Sweetpotato, *Ipomoea batatas* (L.) Lam., is an important food crop in the world. It is also an alternative source of bio-energy as a raw material for fuel production (Zang et al., 2009; Santa-Maria et al., 2009; Yang et al., 2009; Gao et al., 2011). The potential of this crop as a food and carbohydrate source is widely recognized (Jarret et al., 1992). The improvement of this crop by conventional hybridization is limited because of its high male sterility, incompatibility and hexaploid nature (Dhir et al., 1998). Gene engineering offers great potential for improving sweetpotato.

A number of genes have been isolated from sweetpotato (Lin et al., 1993; Bae and Liu, 1997; Durmus et al., 1999; Kim et al., 1999; Wang et al., 1999; Chen et al., 2002, 2003, 2006; Wang et al., 2003; Yap et al., 2003; Huang et al., 2004, 2005a,b; Hamada et al., 2006; Lalusin et al., 2006; Seo et al., 2008; Chen et al., 2009; Tanaka et al., 2009; Xu et al., 2010; Zhou et al., 2010; Wang et al., 2013). However, there is a little work on cloning of carbohydrate metabolism-associated genes in sweetpotato (Liu, 2011). Hamada et al. (2006) cloned the starch-branching enzyme I gene (*IbSBE*I) from sweetpotato and this gene might work in concert with the AGPase large subunit during the primary phase of starch granule formation. Tanaka et al. (2009) cloned the *SRF*1 gene encoding Dof zinc finger transcription factor preferentially expressed in storage roots of sweetpotato, and it is suggested that *SRF*1 modulates carbohydrate metabolism in storage roots through negative regulation of a vacuolar invertase gene. Here, we report cloning and characterization of a carbohydrate metabolism-associated gene *IbSnRK*1 from sweetpotato.

2. Materials and methods

2.1. Plant materials

Sweetpotato (*Ipomoea batatas* (L.) Lam.) cv. Lushu No. 3 was employed in this study. This cultivar has high starch content and is resistant to stem nematodes. A suppression subtractive hybridization (SSH) cDNA library was constructed using Lushu No. 3 (Zhai et al., 2010). One expressed sequence tag (EST) encoding a basic SnRK1 domain was selected from the SSH cDNA library for cloning the gene. Tobacco (*Nicotiana tabacum* L.) cv. Wisconsin 38 was used for identifying the function of the cloned gene.

2.2. Cloning of IbSnRK1 gene

Total RNA was extracted from 0.5 g of fresh leaves of 4-week-old in vitro-grown plants of Lushu No. 3 with the RNAprep Pure Plant Kit (Tiangen Biotech, Beijing, China). RNA samples were reverse-transcribed according to the instructions of Quantscript Reverse Transcriptase Kit (Tiangen Biotech, Beijing, China). A rapid amplification of cDNA ends (RACE) procedure was employed to amplify the 5′ and 3′ ends of the coding region using GeneRacer™ Kit (Invitrogen, Carlsbad, CA, USA). Based on the sequence of EST (SHLL.seq.screen_100.Contig19), primers were designed using the Primer 3 program (http://frodo.wi.mit.edu/primer3/). The primer sequences were as follows: 5′ RACE: 5′-AGCATTCGTGGGATCAAGTC-3′; 5′-GGTGGAGGAACTGCCAAATA-3′; and 3′ RACE: 5′-GTGGCTATCTGGGAGCTGAG-3′; 5′-GTCTGTTGGCCATGGACTTT-3′.

PCR amplifications were performed with an initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and final extension at 72 °C for 10 min. PCR products were separated on a 1.0% (w/v) agarose gel. Target DNA bands were recovered by gel extraction, then cloned into PMD19-T (TaKaRa, Beijing, China), and finally transformed into competent cells of *Escherichia coli* strain DH5α. White colonies were checked by PCR and the positive colonies were sequenced (Invitrogen, Beijing, China).

2.3. Genomic sequence of IbSnKR1 gene

Sweetpotato genomic DNA was extracted from fresh leaves of 4-week-old in vitro-grown plants of Lushu No. 3 using the EasyPure Plant Genomic DNA Kit (Transgen, Beijing, China). The corresponding fragment was amplified using primers: 5′-ATGGATAGCAGAGGAGGTGG-3′ and 5′-CTAAGAGACTTTGAGATGGACAAT-3′. PCR amplifications were conducted with an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 5 min and final extension at 72 °C for 10 min. Cloning and sequencing of the corresponding product was done as described above.

2.4. Sequence analysis of IbSnRK1 gene

The full-length cDNA of *IbSnRK*1 gene was analyzed by an online BLAST at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). For the multiple sequence alignment analysis, the amino acid sequences of IbSnRK1 and other SnRK1 homologs from different plant species retrieved from NCBI were aligned using the DNAMAN software (Lynnon Biosoft, Quebec, Canada). The phylogenetic analysis was conducted with the MEGA4 software (http://www.megasoftware.net/). Theoretical molecular weight and isoelectronic point (p*I*) were calculated using ProtParam tool (http://web.expasy.org/protparam/). Exon/intron analysis of *IbSnRK*1 gene was carried out using Spidey program (http://www.ncbi.nlm.nih.gov/spidey/). The conserved domain of IbSnRK1 protein was scanned by InterProScan program (http://www.ebi.ac.uk/Tools/pfa/iprscan/).

2.5. Expression analysis of IbSnRK1 gene in Lushu No. 3

Total RNA was isolated from the roots, stems and leaves of Lushu No. 3, respectively, following the instructions of RNAprep Pure Plant Kit (Tiangen Biotech, Beijing, China). RNA samples were reverse-transcribed using Quantscript Reverse Transcriptase Kit (Tiangen Biotech, Beijing, China). The cDNA solution was used as templates for PCR amplification with a pair of gene-specific primers of the *IbSnRK*1 gene: 5′-GGCCAGCTACTAGCATCTCTT-3′ and 5′-TTGACGACAATGGTCTTGAACT-3′, which are expected