Journal of Integrative Agriculture 2018, 17(2): 296–305

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Overexpression of *IbSnRK1* enhances nitrogen uptake and carbon assimilation in transgenic sweetpotato

REN Zhi-tong, ZHAO Hong-yuan, HE Shao-zhen, ZHAI Hong, ZHAO Ning, LIU Qing-chang

Key Laboratory of Sweetpotato Biology and Biotechnology, Ministry of Agriculture/Beijing Key Laboratory of Crop Genetic Improvement/Laboratory of Crop Heterosis and Utilization, Ministry of Education/China Agricultural University, Beijing 100193, P.R.China

Abstract

Nitrogen is an important nutrient for plant development. Nitrogen and carbon metabolisms are tightly linked to physiological functions in plants. In this study, we found that the *IbSnRK1* gene was induced by $Ca(NO_3)_2$. Its overexpression enhanced nitrogen uptake and carbon assimilation in transgenic sweetpotato. After $Ca(^{15}NO_3)_2$ treatment, the ^{15}N atom excess, ^{15}N and total N content and nitrogen uptake efficiency (NUE) were significantly increased in the roots, stems, and leaves of transgenic plants compared with wild type (WT) and empty vector control (VC). After $Ca(NO_3)_2$ treatment, the increased nitrate N content, nitrate reductase (NR) activity, free amino acid content, and soluble protein content were found in the roots or leaves of transgenic plants. The photosynthesis and carbon assimilation were enhanced. These results suggest that the *IbSnRK1* gene play a important role in nitrogen uptake and carbon assimilation of sweetpotato. This gene has the potential to be used for improving the yield and quality of sweetpotato.

Keywords: carbon assimilation, *IbSnRK1*, nitrogen uptake, sweetpotato

1. Introduction

Nitrogen is one of the most important essential macronutrients for growth and development of plants. It is a vital element of DNA, RNA and proteins, and its transport and metabolism are necessary for the survival of living organisms (Chen *et al*. 2016). The nitrogen uptake efficiency is important for achieving high yield and excellent quality in agricultural production (Zhao *et al*. 2016). Therefore, how to improve the nitrogen absorption is the primary issue which breeders concern.

Plant nitrogen metabolism is a complicated process. Plants uptake nitrogen with the help of nitrogen transport proteins (NRT) (Lezhneva 2014). The first enzyme involved in nitrate assimilation is nitrate reductase (NR) which converts nitrate to nitrite (Davenport *et al*. 2015), and subsequently nitrite is converted to ammonium by the second key enzyme nitrite reductase (NiR), which is redistributed to different tissues of plants (Orsel *et al*. 2002). The nitrogen assimilation is tightly linked to photosynthesis and carbon metabolism (Vincentz *et al*. 1993; Tobin *et al*. 2005).

The sucrose non-fermenting 1 (SNF1) protein kinase, which belongs to serine/threonine protein kinase, is one of the most important energy and stress regulators. In higher plants, the SNF1 protein kinase family is divided into three

Received 17 January, 2017 Accepted 28 February, 2017
Correspondence LIU Qing-chang, Tel/Fax: +86-10-62733710, E-mail: liuqc@cau.edu.cn

doi: 10.1016/S2095-3119(16)61611-8

subfamilies, SnRK1, SnRK2, and SnRK3. SnRK1 regulates carbon and nitrogen metabolisms by inactivating the sucrose phosphate synthase (SPS), 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA) and NR, and activating sucrose synthase (SUS) and α-amylase (Halford *et al*. 2003). Overexpression of *StSnRK1* in potato increased the starch content and decreased the glucose level (Mckibbin *et al*. 2006). *AKIN10* and *AKIN11* from *Arabidopsis* were found to be involved in starch biosynthesis (Fragoso *et al*. 2009). Li *et al*. (2010) cloned the *SnRK1* gene from *Malus hupehensis* and found that its overexpression in tomato resulted in the increased starch content. They further found that overexpression of this gene increased carbon assimilation and nitrogen uptake and modified fruit development (Wang *et al*. 2012).

Sweetpotato, *Ipomoea batatas* (L.) Lam., is an important food crop. Several genes have been cloned from sweetpotato (Liu 2017). However, nitrogen metabolism related genes have not been reported to date in sweetpotato. Jiang *et al*. (2013) cloned the *IbSnRK1* gene from sweetpotato and found that its overexpression increased the starch content in tobacco. In this study, we found that its overexpression enhanced nitrogen uptake and carbon assimilation in sweetpotato.

2. Materials and methods

2.1. Plant materials

In our previous study, the *IbSnRK1* gene was cloned from sweetpotato *cv*. Lushu No. 3 (Jiang *et al*. 2013). The expression vector pCAMBIA3301 with *IbSnRK1* and *bar* genes driven by a CaMV 35S promoter, respectively, was constructed and transformed to sweetpotato *cv*. Lizixiang according to the method of Yu *et al*. (2007). The transgenic plants were produced and identified with PCR analysis as described by Wang *et al*. (2016). The primers were listed in Table 1. The transgenic plants, wild type (WT) and empty vector control (VC) were transferred to soils in a greenhouse and further in a field for subsequent study.

2.2. Expression analysis of *IbSnRK1*

The 4-week-old *in vitro*-grown plants of Lushu No. 3 were treated with 8 mmol L^{-1} $Ca(NO_3)_2$ in liquid Murashige and Skoog (MS) medium, and sampled at 0, 3, 6, 12, 24, and 48 h after treatment to analyze the expression of *IbSnRK1* by real-time quantitative PCR (qRT-PCR) according to the

Table 1 Primers used in this study

Primer name	Primer sequence (5′→3′)
Primers for constructing expression vector	
IbSnRK1-OE-F	CGGGATCCATGGATAGCAGAGGAGGTGG
IbSnRK1-OE-R	CGAGCTCCTAAGAGACTTTGAGATGGACAATA
Primers for identifying transformation	
35S-F	GAGGCTTACGCAGCAGGTC
IbSnRK1-R	CTAAGAGACTTTGAGATGGACAATA
Primers for real-time quantitative PCR	
Actin-F	AGCAGCATGAAGATTAAGGTTGTAGCAC
Actin-R	TGGAAAATTAGAAGCACTTCCTGTGAAC
SnRK1-F	TCTTAGTCCCAAGAGAAGAAAAAT
SnRK1-R	TAAATAAAATCTATTCAAGGCAATG
NRT1.1-F	GGATGATAGCAGCGGCGTTA
NRT1.1-R	CTTCACCGTCACCTTATCCACA
NRT1.3-F	ATATCAGACCTTTCAACAACCCTG
NRT1.3-R	TTTGGAACTTGGCTTCTATCAGG
NRT2.4-F	CCATTTATCCAACTGCCACTGA
NRT2.4-R	GTGGAGACGAAGCAGGTGAAG
NRT3.1-F	ACCATTGCCCTCTTCAACATTC
NRT3.1-R	CGCCATAAGCCTTCTACTCCTCT
NR-F	AGCTTGGGACGAGACTCTTAATAC
NR-R	CCAGGTGCCTTTCCTTTGC
GS-F	GGGTGATTGGAATGGTGCTG
GS-R	GTCGGCGGTCTCGTGTCTC
GOGAT-F	GGGTCTTGAAGTGCTTGGATG
GOGAT-R	GCTGGACAATGAGCAGAAATAGA
SUS-F	AGCAATCTGCAAAGAGGACCA
SUS-R	TCTCACATATTCCCAAACACCAG
*AGPL*I-F	GAGATATCCCACATCCAACGACTT
*AGPL*I-R	TAGGGCCAAGTTAGCGTCGATG