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Relationship between Neutral Invertase Activity and Sugar Contents in Tomato Fruit and Its Functional Prediction Analysis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CN, YJY and WHR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WHR, ZTT and HS managed the analyses of the study. Authors ZR and XSQ managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: Neutral invertase (NI) probably plays an important role in sucrose metabolism of tomato, but main function and mechanism are unclear. In this study, contents of soluble sugar and NI activity were measured at different stages in tomatoes of *Solanum chmielewskii and Solanum lycopersicumc*, meanwhile analyzed the correlation between contents of soluble sugar and NI activity, also cloned *NI* gene and predicted by bioinformatics for further making clear the function of NI in tomato sugar metabolism.
Study Design: In order to analyze the function and action mechanism of NI, we used the tomato as materials, measured the contents of soluble sugars, activity of NI, and cloned the gene of NI. We also analyzed the NI with bioinformatics, and predicted the molecular function of NI for further making clear the function of NI in tomato sugar metabolism.

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Place and Duration of Study: College of Biological Science and Technology, between February 2016 and March 2017.
Methodology: The contents of soluble sugar in tomato fruits were measured with HPLC (High performance liquid chromatography). The function structure and interacting proteins with NI family were predicted by bioinformatics.
Results: The contents of glucose and fructose were significantly higher in *Solanum lycopersicum* than those in *Solanum chmielewskii*, and greatly increased along with the process of fruit mature period. Nevertheless, the content of sucrose was raised obviously in the mature period of wild type fruit. In normal cultivated tomato, NI activity was positively correlated with fructose and glucose contents, and negatively correlated with sucrose content. In order to further study the function of neutral invertase, the *NI* gene was cloned and analyzed by bioinformatics. The analysis results showed that the NI was glycosyl hydrolases with Glyco_hydro_100 conserved domain.
Conclusion: The contents of glucose and fructose were significantly higher in *Solanum lycopersicum* than those in *Solanum chmielewskii*, and greatly increased along with the process of fruit mature period. The content of sucrose was raised obviously in the mature period of wild type fruit. NI activity in normal cultivated tomato was positively correlated with fructose and glucose contents, and negatively correlated with sucrose content. The NI was glycosyl hydrolases with Glyco_hydro_100 conserved domain, and might play an important role in the regulation of soluble sugar contents in tomato fruit.

Keywords: Tomato; neutral invertase; soluble sugar; bioinformatics.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is a kind of vegetable, which is comprehensive, well-adapted, good for nutrition [1]. So cultivation of high quality and breeding become the hot topic [2]. Along with the improvement of cultivation measure, tomato quantity can be mainly guaranteed, but the quality becomes the prominent question, especially in the horticulture production.

The edible quality is the most important in tomato fruit quality. And the key to decide the edible quality is the content of soluble sugar [3]. The important process of sugar accumulation is related to the sucrose metabolism in tomato fruits [4]. And related enzymes to sucrose metabolism contain acid invertase (AI), neutral invertase (NI), sucrose synthetase (SS) and sucrose phosphate synthetase (SPS). In many researches, the key enzymes in tomato sucrose metabolism are AI and SS, but the function of NI has been neglected. Nevertheless, our research showed that the NI activity was high with the developing stages of tomato fruits, but it is unclear for the mechanism of function and acting in tomato sucrose metabolism. Some present researches show that NI has an important function in sucrose breakdown into glucose and fructose [5]. Other researches suggest that the activity of NI is related to crop resistance [6], but it is unclear of regulating pathway.

In order to analyze the function and action mechanism of NI, we used the tomato as materials, measured the contents of soluble sugars, activity of NI, and cloned the gene of NI. We also analyzed the NI with bioinformatics, and predicted the molecular function of NI for further making clear the function of NI in tomato sugar metabolism.

2. MATERIALS AND METHODS

2.1 Materials

The materials were the wild type tomato (*Solanum chmielewskii* L.) (Sink of sucrose accumulation) and cultivation tomato (*Solanum lycopersicum* Riok, Kesicki, Fobes and Holle) (Sink of hexose accumulation). The second fruit of the first cluster was sampled at 15d, 20d, 25d, 30d, 35d, 40d and 45d after anthesis, respectively. The weight of single fruit was about 1 g used to measure the sugar contents, activity of NI. Three replicate samples were collected.

2.2 Methods

The contents of soluble sugar in tomato fruits were measured with HPLC (High performance liquid chromatography).

The function structure and interacting proteins with NI family were predicted by bioinformatics. The main softwares were ProtParam, ProtScale, ORFFINDER, SOPMA, Plant-mPLoc, SignalP 4.1, TMHMM 、 MEME, QuickGO, SWISS-MODEL, STRING, MEGA7 and SMART.

3. RESULTS

3.1 Changes of Soluble Sugar Contents in Tomato Fruits at Different Developmental Stages

The changes of soluble sugar contents were showed in Fig. 1 at different developmental stages in tomato fruits. The contents of fructose and glucose in the fruits of wild type were obviously lower than those in cultivation tomato at different developmental stages. And that the contents of fructose and glucose increased significantly in mature fruits of cultivation tomato, but sucrose content in the fruits of wild type tomato was higher than that in cultivation tomato.

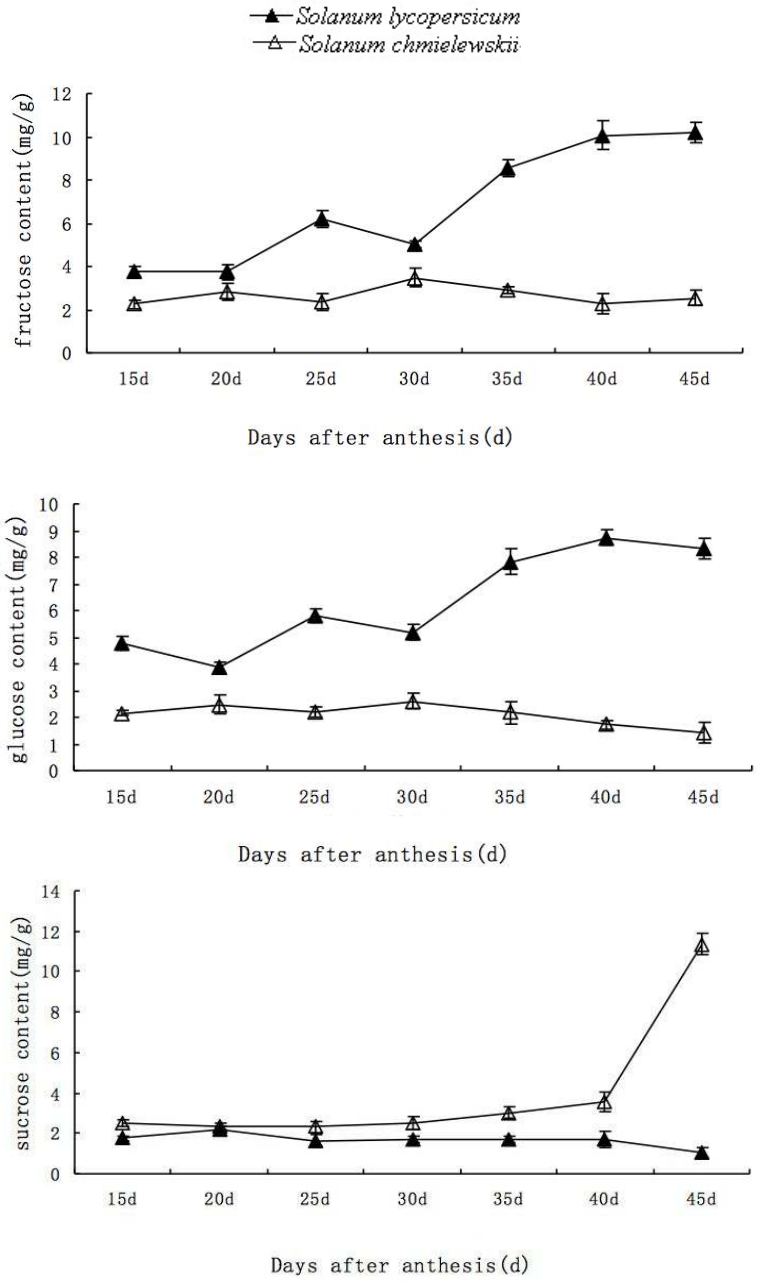

Fig. 1. Dynamic changes of soluble sugar contents in tomato fruits at different developmental stages

3.2 Changes of NI Activity in Tomato Fruits at Different Developmental Stages

The activity of NI in fruits of wild type tomato was no obvious change in the whole developmental stages. But in fruits of cultivation tomato, NI activity was increased significantly and higher than that in wild type tomato at mature stage (Fig. 2).

3.3 Correlation Analysis between Sugar Contents and NI Activity in Tomato Fruits

The activity of NI in cultivated tomato fruits appeared significantly positive correlation with the contents of fructose and glucose, and markedly negative correlation with the content of sucrose. But in the wild type tomato fruits, the correlation was non-significant between NI activity and contents of soluble sugars (Table 1). So we just further analyzed the cloned gene of NI by bioinformatics in cultivated tomato as follows.

3.4 Prediction for the Functional Structure and Interaction Protein of NI in Cultivation Tomato

There were 8 members of NI family in cultivation tomato, named CINV1.1, 1.2, 1.3, 1.4 and CINV2.1, 2.2, 2.3, 2.4. After clone and sequencing, the gene sequences corrected through NCBI blast, then predicted the functional structure and interaction proteins of NI (Table 2 and Fig. 3). The NI belonged to non-secreted protein, with conservative domain of Glyco_hydro_100 (Glycosyl hydrolase family 100).

In this research, we predicted the possible interaction proteins with NI family. The results showed that 9-like phosphatidylinositol 4-phosphate 5-kinase 9-like (pip5k9) and sucrose nonfermenting 4-like protein-like (SNF4) could interact with NI. Some researches show that CINV1 negatively regulates the pip5k9, and followed negatively regulates the elongation of root cell mediated by sugar [5]. So prediction for interaction proteins with NI in tomato would provide reference for the further study on the action pathway and regulation mechanism of NI.

Table 1. The correlation between sugar accumulation and neutral invertase activity in tomato fruits

	Sugar	NI
Solanum lycopersicum	Fructose	0.861*
	Glucose	0.827*
	Sucrose	-0.800*
Solanum chmielewskii	Fructose	0.072
	Glucose	-0.069
	Sucrose	0.142

*Note: *Correlation is significant at 0.05 level*
***Correlation is significant at 0.01 level*

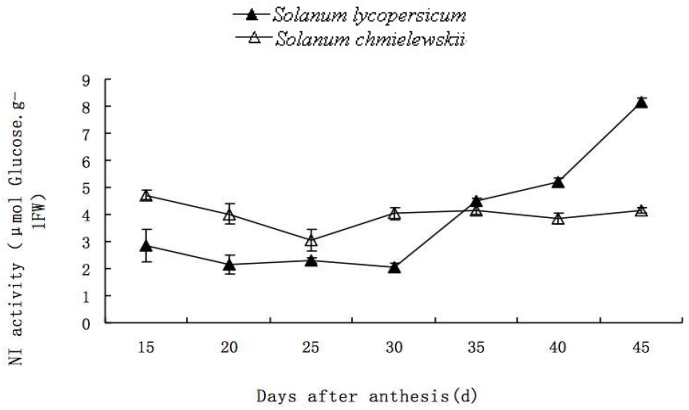

Fig. 2. Changes of neutral invertase activity in tomato fruits at different developmental stages

Fig. 3. Functional structure prediction of CINV1.1 by smart database
Note: Because of no difference in NI family structure, just list the predicted map of CINV1.1

Table 2. Functional structure prediction of neutral invertase in tomato

Protein name	Gene ID	Functional domain	Start position	End position	E-value
CINV1.1	101248735	Glyco_hydro_100	170	613	2.60E-217
CINV1.2	101255835	Low complexity region	156	167	N/A
		Glyco_hydro_100	173	612	1.50E-219
		GDE_C	354	604	1.5E-8
CINV1.3	101251950	Glyco_hydro_100	32	672	N/A
CINV1.4	101245982	Glyco_hydro_100	190	625	N/A
CINV2.1	101253328	Glyco_hydro_100	1	568	N/A
CINV2.2	100134879	Glyco_hydro_100	109	544	2.80E-220
CINV2.3	101254703	Glyco_hydro_100	89	525	3.00E-218
CINV2.4	101252138	Glyco_hydro_100	1	552	N/A

4. DISCUSSION

Invertase (Inv) is the very important enzyme in regulating plant action [7]. Inv expression can regulate plant physiology when plant suffers biotic stress and abiotic stress, besides having an important function in regulation of plant growth and development [8]. Inv plays very important role in sucrose metabolism, but the adjusting and controlling pattens are different with SS. Sucrose synthetase catalyzes sucrose to fructose and UDP-glucose, but reversibly catalyzes to compound sucrose in the suitable condition. However, Inv irreversibly catalyzes the sucrose to resolve glucose and fructose. The energy from that sucrose resolves to fructose and glucose is used to compound polyblend and amino acid [9]. So it's very important to study the Inv function, especially in sugar metabolism.

Inv can be grouped to acid invertase (pH 4.5-5.0) and neutral invertase (pH6.5-6.9, pH7.6-8.0) based on optimal pH value [10]. Acid invertase(AI) mainly distributes in cell wall (CWINV) and vacuole (VINV). But neutral invertase named cell solute invertase (CINV) expresses in plasma membrane, cell nucleus, chloroplast and mitochondria [11-12].

Many studies show that AI and SS play very important roles in sucrose catabolism [13], but the function of NI is neglected. Our research showed that NI activity in cultivation tomato fruit changed obviously at different developmental stages, and appeared significant positive correlation with contents of fructose and glucose, but appeared significant negative correlation with sucrose. The results showed that NI might play an important role in sugar metabolism of tomato fruit. The results of bioinformatics analysis also showed that NI contained domain of Glyco_hydro_100, suggesting that NI belonged to glycosyl hydrolase family.

Our further study will focus on the promoter analysis of tomato neutral invertase family, search stress response element, correlate analysis to sequence motif and function domain. After that, we will construct the overexpression and RNAi vector of tomato NI, and proceed genetic transformation, in order to deeply study the functional mechanism of NI family in tomato growth and development, improvement of fruit quality and stress response, and then serve for improving the tomato quantity, quality and resistance.

5. CONCLUSION

The contents of glucose and fructose were significantly higher in *Solanum lycopersicum* than those in *Solanum chmielewskii*, and greatly increased along with the process of fruit mature period. Nevertheless, the content of sucrose was raised obviously in the mature period of wild type fruit. In normal cultivated tomato, NI activity was positively correlated with fructose and glucose contents, and negatively correlated with sucrose content. In order to further study the function of neutral invertase, the *NI* gene was cloned and analyzed by bioinformatics. The analysis results showed that the NI was glycosyl hydrolases with Glyco_hydro_100 conserved domain, and might play an important role in the regulation of soluble sugar contents in tomato fruit.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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