



SDI Review Form 1.6

Journal Name:	American Journal of Experimental Agriculture
Manuscript Number:	2013_AJEA_7285
Title of the Manuscript:	A Tomato Inducer of CBF Expression 1 (SlICE1) is Involved in Cold Stress Signaling
Type of the Article	

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	1) The introduction didn't write in good style and structure. It is necessary to rewrite step by step. It's a mix of concepts. You can start with one comprehensive topic, environmental stresses, and continue to your main topic, the role of ICE1 in Tomato. This part could be include environmental stresses, cold and salt stresses with more details, network of cold and salt responsive genes, and even the role of Ca²⁺ in cold and salt stresses, the role of CBFs in cold and salt stresses, the role of ICE1 in <i>Arabidopsis th.</i> And then finished by this question that what/how is the role of ICE1 in Tomato. 2) Line 77: edit the word of "Genome sets" 3) Line 166: at first, you talk about salt and cold stresses. It is also necessary to add "salt" to the sentence. 4) Line 171 to 186: add the method and materials were used for those analyses to the method and materials (M&M) section. 5) Line 191: the method should be explained in the section of M&M. 6) Same to No.1, There is same problem in "result and discussion" style and structure. 7) Line 364: the chold stress should change to cold stress.	We deeply appreciated detailed comments and informative suggestions raised by the reviewer #1 on our manuscript. We have carefully revised the manuscript according to suggestions made by reviewers, as follows. Those corrected parts were written with red characters in the revised text. The sentences describing ICE1 homologs in monocots were removed for avoiding diffuseness. 1) We fully agree with the indicated matter by reviewer #1. We thoroughly revised the sections of introduction to describe preceding studies and background more briefly and moved several sentences to Discussion. 2) The part was corrected to "genome sets" 3) The part was corrected to "under cold and salt stresses". 4) A sentence of "To examine effects of calcium antagonists on ICE1 protein profiles, mature leaves of 4 week old-Tomato plants were preincubated in 10 mL of 2 mM CaCl₂ (as control), 10 mM EGTA-Na or 10mM LiCl₃ for 1 hr, and then subjected to cold stress (4°C)." was added into the section of Materials and Methods to explain those analysis. 5) A sentence of "To examine involvement of



SDI Review Form 1.6

		protein kinase and proteasome on ICE1 protein stability, mature leaves of 4 week old-Tomato plants were preincubated in 10 mL of 5 μM K252a (protein kinase inhibitor) or 50 μM MG132 (proteasome specific inhibitor) for 2 hr, and then K-252a-treated leaves were subjected to cold stress (4°C).” was added into the section of Materials and Methods. 6) We fully agree with the indicated matter by reviewer #1. We separated the sections of results and Discussion to describe the data and evaluation precisely. Several sentences referring previous researches were moved to the section of Discussion as follow. “Arabidopsis ICE1 appeared to be upregulated at both the mRNA and protein levels under cold stress (Chinnusamy et al. 2010). Thus, the transcription of ICE homologs could be regulated in different manner between tomato and Arabidopsis.” “Trehalose 6-phosphate synthase (TPS) is a key enzyme for trehalose synthesis pathway to catalyze UDP-galactose and glucose to trehalose 6-phosphate. Then trehalose phosphate phosphatase (TPP) catalyzes dephosphorylation of trehalose 6-phosphate to generate trehalose. It was reported that one of tomato TPS-related genes, SITPS1, is induced in response to both cold and salt stresses (Tomikubo et al. 2007).” 7) The parts was corrected to “to cold stress”.
--	--	---



SDI Review Form 1.6

Minor REVISION comments	1) It is better to increase the time of treatment to 24 h (0, 1, 5, 10, and 24). It would show the different of gene expression and protein production with more resolution (for Fig 2 and 3). 2) If it is possible, compare the SIICE1 sequence with the known ICE1 sequence of another member of solanum genus. You may find new subfamily of ICE1 in the solanaceae. 3) Line 214: this sentence isn't complete. Explain the role of TPP1 in the trehalose turn over. 4) Line 235 to 243: can be added in brief to the data that achieved by expression profile of TPP1 and TPS1. 5) Fig 2B: It is better to use one gel photo that contains all samples. You have used several individual gel fragments together. 6) Fig 3: it is better to run new gel for RT-PCR products of CBF2 and CBF3.	1) We fully agree with the indicated matter by reviewer #1. We would like to examine those detailed time course on gene expression analysis which is in progress for our forthcoming research paper. 2) Miura et al., our collaborator, reported about phenotype of SIICE1-overexpressing tomato at Plant Biotechnology as following. Miura K, Shiba H, Ohta M, Kang SW, Yuasa T, Iwaya-Inoue M, Kamada H, Ezura H (2012) SIICE1 encoding a MYC-type transcription factor controls cold tolerance in tomato, <i>Solanum lycopersicum</i>. <i>Plant Biotechnol</i> 29: 253-260. Accession number (AK24172) of SIICE1 was corrected to AK247172, identical to that of SIICE1 reported by Miura et al. The report was added into references of the revised text. We focused on the endogenous protein profiles of SIICE1 and involvement of it in SITPS1 regulation in our manuscript. 3) About RT-PCR of SITPS1 and LeCOR413, the sentence was added to the part of Results and discussion as following. "RT-PCR analysis indicated that the expression of SITPS1 and LeCOR413 increased significantly at 5 hr after cold stress following upregulation of SICBF1 at 3 hr after cold stress (Fig. 5)." To explain the role of rice TPP1 in trehalose synthesis, the sentence is added as following. "Trehalose 6-phosphate synthase (TPS) is a key enzyme for trehalose synthesis pathway to catalyze UDP-galactose and glucose to trehalose 6-phosphate. Then trehalose phosphate
---------------------------------------	---	---



SDI Review Form 1.6

		phosphatase (TPP) catalyzes dephosphorylation of trehalose 6-phosphate to generate trehalose. It was reported that one of tomato TPS-related genes, SITPS1, is induced in response to both cold and salt stresses (Tomikubo et al. 2007).” 4) To explain our previous studies of rice TPP1 and tomato TPS1, the sentence is added as following. “Our previous studies demonstrated that the expression of OsTPP1 is regulated at 3 hr after the treatment and the maximum expression is 24 hr after cold stress and that various stresses including cold, salt and heat stress upregulated the expression of SITPS1 (Nakamura et al. 2011, Tomikubo et al. 2007).” 5) We fully agree with Reviewer #1’ comment about the separated panels of immunoblot for detection of the ICE1-related proteins. If possible, we would like to repeat the same experiments to show a unified picture of the immunoblot. However, unfortunately I have moved from Kyushu University to University of Miyazaki this year. Now, I am trying to setup the experimental system for the immunoblot here, but it is necessary to wait for a while before carrying out the same experiment. Therefore, we believe that detail data about the protein profiles of whole sets of SLICE1 protein in the presence of various Calcium antagonists should be waited for further analysis which is going to be described in the next report. 6) We fully agree with Reviewer #1’ comment
--	--	--



SDI Review Form 1.6

		about the weak signals of CBF2 and CBF3. If possible, we would like to repeat the same experiments to show the different signals of CBF1/2/3 by performing more number of PCR cycles. However, unfortunately I have moved from Kyushu University to University of Miyazaki this year. Now, I am trying to setup the experimental system for the RT-PCR here, but it is necessary to wait for a while before carrying out the same experiment. Furthermore, Zhang et al reported that cold stress on tomato induced CBF1 expression significantly but not CBF2 nor CBF3 as mentioned in the section of Results. The observation is consistent to our present result on cold stress-induced CBF1. In the manuscript, we would like to concentrate on the significant induction of LeCBF1 in response to cold stress to avoid diffuseness. Thus, the sentences describing the expression of CBF1/2/3 was changed as follows. "In the contrast, cold stress enhanced the expression of SlCBF1 significantly at 1 and 5 hr (Fig. 3). At the same PCR cycles as that for LeCBF1, increase in PCR signals of LeCBF2 nor LeCBF3 was not observed."
<u>Optional/General</u> comments	The data of the article is enough and seems complete. But all parts of the article were written in bad style and structure. This data could be rewrite and then published.	