J. Plant Biol. (2010) 53:107–112
DOI 10.1007/s12374-009-9091-1

ORIGINAL RESEARCH

Characterization of a Stress-induced NADP-isocitrate Dehydrogenase Gene in Maize Confers Salt Tolerance in *Arabidopsis*

Yinghui Liu · Yunsu Shi · Yanchun Song · Tianyu Wang · Yu Li

Received: 15 June 2009 / Revised: 19 November 2009 / Accepted: 3 December 2009 / Published online: 12 February 2010

Abstract In this paper, a full-length cDNA-encoding cytosolic nicotinamide adenine dinucleotide phosphate-dependent isocitrate dehydrogenase was isolated from maize and named *ZmICDH*. Analysis of the deduced protein sequence revealed that this gene had considerably high homologies with other plant *ICDH* genes such as those from rice, *Arabidopsis* and poplar. The gene was transcribed in all tissues tested, with the highest amount of transcript in root. Subcellular localization results indicated that ZmICDH was localized in cytosol. In an attempt to further understand the role of *ZmICDH* in maize, the functional characterization of the gene was conducted. The results indicated that the expression of *ZmICDH* was induced by drought and salt stresses. *ZmICDH* enhanced salt tolerance in transgenic *Arabidopsis*.

Keywords *ZmICDH* · Salt · Drought · Stress · Transgenic *Arabidopsis* · Maize

Introduction

Nicotinamide adenine dinucleotide phosphate-dependent isocitrate dehydrogenase (ICDH; EC 1.1.1.42) catalyzes the reversible conversion of isocitrate to 2-oxoglutarate (2-OG) and links C and N metabolism (Hurley et al. 1991; Gallardo et al. 1995). ICDH activity is mainly localized in the cytosol, although minor activity has been found in mitochondria, peroxisomes, and chloroplasts in higher plants (Henson et al. 1986). By supplying the cytosol with 2-oxoglutarate as a primary acceptor for NH_3 assimilation, ICDH is hypothesized to have a key function in the biosynthesis and export of amino acids (Henson et al. 1986; Fieuw et al. 1995). In addition, ICDH has been proposed to have diverse functions, such as nitrogen metabolism, ammonium assimilation, and degradation of lipids and fatty acids.

Since ICDH is a source of 2-OG for amino acid synthesis (Chen and Gadal 1990), NADP-ICDH has a role in the supply of NADPH to the cytosol (Gallardo et al. 1995; Fieuw et al. 1995). Because ICDH requires either NAD^+- or $NADP^+$-producing NADH and NADPH, several authors have suggested that ICDH is one of the most important NADPH generating mechanisms. This role would be crucial in reactive oxygen species (ROS) overproduction. NADPH is also an essential reducing equivalent for the regeneration of GSH by glutathione reductase and for the activity of NADPH-dependent thioredoxin system (Kirsch and Groot 2001; Nakamura 2005), both are important in the protection of cells from oxidative damage. Therefore, ICDH may play an antioxidant role during oxidative stress, and cytosolic ICDH may involve in the supply of NADPH needed for plants against oxidative damage. Several studies have also suggested the potential ICDH regenerating function. Plants exposed to oxidative, abiotic stresses, and heavy metals, such as cadmium-treated or nickel-treated *Silene italica*, showed higher ICDH activity (Daniel et al. 2007; Gálvez et al. 2005). These results suggest that ICDH has a protective antioxidant role against certain environmental stresses in plants.

Y. Liu
Hebei North University,
Zhangjiakou 075000 Hebei Province, China
e-mail: leely519@126.com

Y. Shi · Y. Song · T. Wang (✉) · Y. Li (✉)
Chinese Academy of Agricultural Sciences,
The National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Institute of Crop Science,
12 Zhongguancun South Street,
Beijing 100081, China
e-mail: wangtianyu@263.net
e-mail: yuli@mail.caas.net.cn

To our knowledge, no direct evidences for the *ICDH* function under stress situations have been reported in maize. The aim of the present study was to understand the role of maize *ICDH* in response to high salinity and drought stress.

Materials and Methods

Isolation of a cDNA Encoding Cytosolic *ZmICDH* from Maize

A drought-induced clone (545 bp) was obtained from a maize SSH library constructed by Li et al. (Li et al. 2007). The sequence was then subjected to BlastX analysis to search for similarity. The Blast result showed that the sequence represented a gene homologous to cytosolic NADP dependent isocitrate dehydrogenase of rice. To obtain the full length of maize *ICDH* gene, we performed database searches for the predicted maize *ICDH* mRNA sequence. At last, a cDNA sequence (accession number: ACF88442), which encoded a predicted protein with the highest similarity to rice cytosolic *ICDH* (AF155334), was chosen for further study and designated as maize *ZmICDH*.

The complete coding region of the *ZmICDH* cDNA was amplified by polymerase chain reaction (PCR) using the specific primer: 5′-CAAGGTCTCCAACCCAATCG-3′ (forward) and 5′-ACGGTGACGAAGCATTCAAAG-3′ (reverse). The condition for amplification was at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 90 s, plus a final extension at 72°C for 10 min. Maize seedling cDNA was used as amplification template. Three independent PCR products were purified and sequenced.

Subcellular Localization of ZmICDH

The ZmICDH was fused with green fluorescent protein (GFP) under the control of the CaMV35S (the cauliflower mosaic virus) promoter. The full-length open reading frames (ORF) of *ZmICDH* without the termination codon were amplified by PCR with the following primers: 5′-AT*ccatgg*TCTCCAACCCAATCGTCGA-3′ containing *Nco*I site (forward), 5′-TTT*actagt*AGGGCACACCA ACACT-3′ containing *Spe*I site (reverse). The fragment was introduced into the same site of the pCAMBIA1302 vector, creating an in-frame fusion between the *ZmICDH* and *GFP* (p35S: ZmICDH-GFP). The fusion construct and control (p35S: GFP) were transformed into onion epidermis cells by particle bombardment. After incubation of transformed onion epidermis cells for 24 h at 28°C, GFP was detected by a confocal fluorescence microscopy (Zeiss, LSM510 Meta).

Analysis of Expression Pattern and Responses to Salt and Drought Stresses

A drought tolerant maize inbred line “CN165” was used in this study. The plants were grown in pots of 30 cm diameter and 50 cm depth with 20 kg soil mix (soil: vermiculite: organic fertilizer = 3:2:1) under a rainout shelter. Before stress treatment, equal volume of water was applied to each pot every other day to keep the plants in all pots under uniform water status. Fourteen-day-old seedlings were subjected to 200 mM NaCl for 48 h and 20% poly(ethylene glycol; PEG) for 48 h, respectively. Unstressed control plants were grown in parallel and harvested at given time points. The sampled tissues were used for RNA and protein extraction.

Total RNA was extracted from 1 g of different maize tissues using the Trizol isolation reagent (Invitrogen). To remove contaminating DNA, RNAs (10 μg) were treated with RNase-free DNase (Promega). DNase-treated RNA samples (1 μg) were reverse-transcribed using M-MLV reverse transcriptase (Invitrogen). The reverse transcription (RT) reactions were performed at 37°C for 1 h. Further, 1 μl of first strand cDNAs were used as templates for quantitative real-time PCR amplification with a pair of gene-specific primers: 5′-GAGCGAAGGAGGCTATGTG-3′ and 5′- ACGGAAATGACGGGTAACCG-3′. A maize *ACTIN* gene fragment, used as an internal control, was amplified with the primers: 5′-CTCCTTCACTACGA CTGCTG-3′and 5′-GGGCACCTAAACCTTTCTG-3′. Quantitative real-time PCR was performed using the Rotor-Gene 3000 Series real-time amplification system under the following conditions: denatured for 3 min at 94°C and then run for 45 cycles of each at 94°C 15 s, 60°C 20 s, and 72°C 30 s. The cDNA was amplified using SYBR Green Supermix (Takara). Amplification experiments were conducted in triplicate. Data analysis was performed with Rotor-Gene software 6.0 (Corbett Research) and the $2^{-\Delta\Delta C}$T method for relative quantity analysis (Kenneth and Thomas 2001).

Detection of ZmICDH Protein Activity in Non-denaturing Polyacrylamide Gels

Approximately 0.5 g of seedlings was used for protein extraction. After grinding on ice with 10% polyvinylpolypyrrolidone, soluble proteins were extracted as described by Chen et al. (Chen et al. 1988). Aliquots of extracts were mixed 3:1 with sample buffer (250 mM Tris-HCl (pH 7.6), 0.4% bromophenol blue, 43% glycerol) and immediately loaded (100 μg protein per lane) onto polyacrylamide gels (stacking gel: 3% acrylamide, 0.08% N, N-methylene bisacrylamide, 250 mM Tris-HCl, pH 8.0; resolving gel: 7.5% acrylamide, 0.2% N, N-methylene bisacrylamide, 250 mM Tris-HCl, pH 8.0). Gels were polymerized by using ammonium persulfate

and N, N, N, N- tetramethylethylenediamine. Electrophoresis was performed at 4°C. The running buffer contained 25 mM Tris-base and 190 mM glycine (pH 8.8). For detection of ZmICDH activity, gels were soaked in staining solution (180 mM Tris-HCl, pH 8.0, 0.1 mM $NADP^+$, 15 mM $MgCl_2$, 8 mM sodium isocitrate, 0.4 mM 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, 0.5 mM phenazine methosulfate) at 37°C for 60 min. Specificity of the reaction was shown by omitting either isocitrate or $NADP^+$, respectively, from the staining solution.

Generation of Transgenic *Arabidopsis* Plants

The full-length *ZmICDH* ORF was amplified with P*fu* DNA polymerase (Takara) using maize leaf cDNA as template and primers corresponding to the 5′ and 3′ ends of the *ZmICDH* gene with added *Nco*I and *Bst*E II restriction sites: 5′-AT*ccatgg*TCTCCAACCCAATCGTCGA-3′ and 5′-CG*ggttacc*GCATGGCGATCCTCGGG-3′. The *ZmICDH* ORF was cloned into the *Nco*I and *Bst*E II sites of the pCAMBIA3301 plasmid. *Agrobacterium* mediated transformation was performed via the floral dipping technique of *Arabidopsis thaliana* (Clough and Bent 1998). Transgenic plants were selected by spraying the herbicide Basta (50 mg l^{-1}) on the seedlings three times. Presence of the transgene was confirmed by PCR analysis and RNA gel blots. Homozygous T4 lines were obtained by self-crossing and used in stress treatment experiments.

Evaluation of Salt Tolerance

Seeds of wild-type and transgenic *A. thaliana* (ecotype: Columbia) were allowed to germinate on Murashige and Skoog (MS) agar medium (with 0 mM, 50 mM, 100 mM, 150 mM NaCl, respectively) under light/dark cycle conditions of 16/8 h at 22°C. Plates were oriented vertically with seedlings kept upside down. Root and shoot length was recorded at the tenth day of germination. Three replicates were performed for each experiment.

In the greenhouse, transgenic *Arabidopsis* plants were grown in pots containing compost soil for 3-4 weeks, with a light/dark cycle of 16/8 h at 22°C and 60-70% of relative humidity. For salt stress treatments, the water solution was supplemented with NaCl to a final concentration of 200 mM and plants were placed in a container with capillarity uptake for 7 days.

Results

Identification and Sequence Analysis of *ZmICDH*

Through database searches and PCR amplication, the sequence of the full-length *ZmICDH* cDNA cloned in the present study was 1,239 bp in length encoding an open reading frame of 412 amino acids. At the amino acid level, ZmICDH shared 96% identity with the cytosolic NADP-ICDH from rice (AF155334), 86% identity with a NADP-ICDH from *Arabidopsis* (NP176768), and 87% identity with a poplar NADP-ICDH (ABA18651).

ZmICDH Protein was Targeted to Cytosolic

The *ZmICDH* was fused with GFP under the control of the CaMV35S promoter. 35 S:GFP-ICDH and 35 S:GFP were introduced into onion epidermal cells by particle bombardment. Observation by confocal microscopy revealed that the free GFP proteins were distributed throughout the cell (Fig. 1a-c), while the ZmICDH-GFP fusion proteins was strongly targeted to cytosolic of onion epidermis cell (Fig. 1d-f). The result supported that the ZmICDH we obtained was a cytosolic NADP-ICDH enzyme.

ZmICDH Showed a Constitutive Expression Pattern and Responded to Salt and Drought Stress

To investigate tissue-specificity and stress-induced of *ZmICDH* expression, quantitative real-time PCR (qRT-PCR)

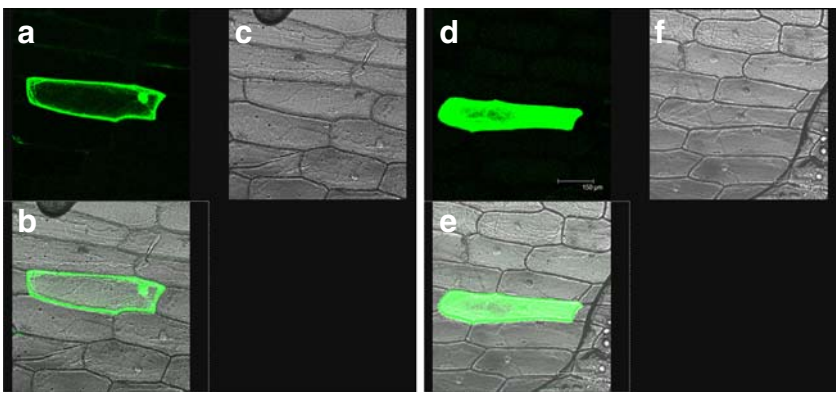

Fig. 1 Subcellular localization of ZmICDH protein. The GFP control plasmid (**a**, **b**, **c**) and the fusion construct for ZmICDH-GFP (**d**, **e**, **f**) were introduced into the onion epidermis cells. The photographs were taken under the bright light for the morphology of the cell and in the dark for green fluorescence. **a**, **d** Fluorescence image; **c**, **f** bright image; **b**, **e** combination of both. *Bar* 150 μm

amplification with gene-specific primers for the coding region of the gene was used. Expression of *ZmICDH* could be detected in all the tissues tested (root, leaf, stem, silk, ear, and embryo). The highest transcript amount was detected in root (Fig. 2a), about threefold than in embryo and fourfold than in leaf and stem. The lowest transcript was detected in seed.

The expression characteristics of *ZmICDH* in maize subjected to drought and salt stresses were also analyzed. Following 6 h of exposure to 20% PEG, the transcript level of *ZmICDH* began to increase. After 24 h and 48 h of treatment (Fig. 2b), the transcript amount increased about threefold than the control. As for salt treatment, Fig. 2c showed *ZmICDH* was strongly induced by salt. The transcript reached a peak at 3 h, with more than tenfold than in the control. All the results showed salt and drought stresses could increase the transcript of *ZmICDH*.

To analyze the stress-induced expression of ZmICDH on the protein level, native polyacrylamide gel electrophoresis stained for ZmICDH activity was performed using crude extracts of proteins from different seedlings at time points of treatments. The results displayed that both salt and drought treatments could also induce the expression of the ZmICDH in protein level (Fig. 2d).

Over-expression of *ZmICDH* Enhanced Salt Tolerance in *Arabidopsis*

To investigate whether *ZmICDH* functions under stress, we generated *Arabidopsis* transgenic plants in which *ZmICDH* was over-expressed under the control of CaMV35S promoter. To check the expression level of the *ZmICDH* in the transgenic plants, five independent transgenic lines were subjected to semi-quantitative PCR analysis (data not shown). Two stable lines showing higher *ZmICDH* transcript were chosen for further studies.

Under normal conditions, these *35 S:ZmICDH* plants displayed similar morphological phenotypes as the wild plants (data not shown). To further understand the role of *ZmICDH* in stress condition, we first studied the salt tolerance of the *ZmICDH* over-expressing plants at early stage of seedling development. Figure 3a showed germination of wild-type (WT) and two individual transgenic T_3 lines on 100 mM NaCl was responded to different growth pattern during 10 days after germination. The *35 S:: ZmICDH* lines grew better than the control. In Fig. 3c, the *ZmICDH* over-expressing transgenic *Arabidopsis* lines and control plants were grown on MS medium (content with 0 mM, 50 mM, 100 mM, 150 mM NaCl, respectively) for 10 days, young seedlings were photographed and the root and shoot length were measured. Seedling development of the wild-type and the transgenic lines were similar when cultured on normal MS and low NaCl (50 mM) medium. However, root and shoot of the wild type was significantly impaired on MS medium supplemented with 100 and 150 mM NaCl, whereas the transgenic

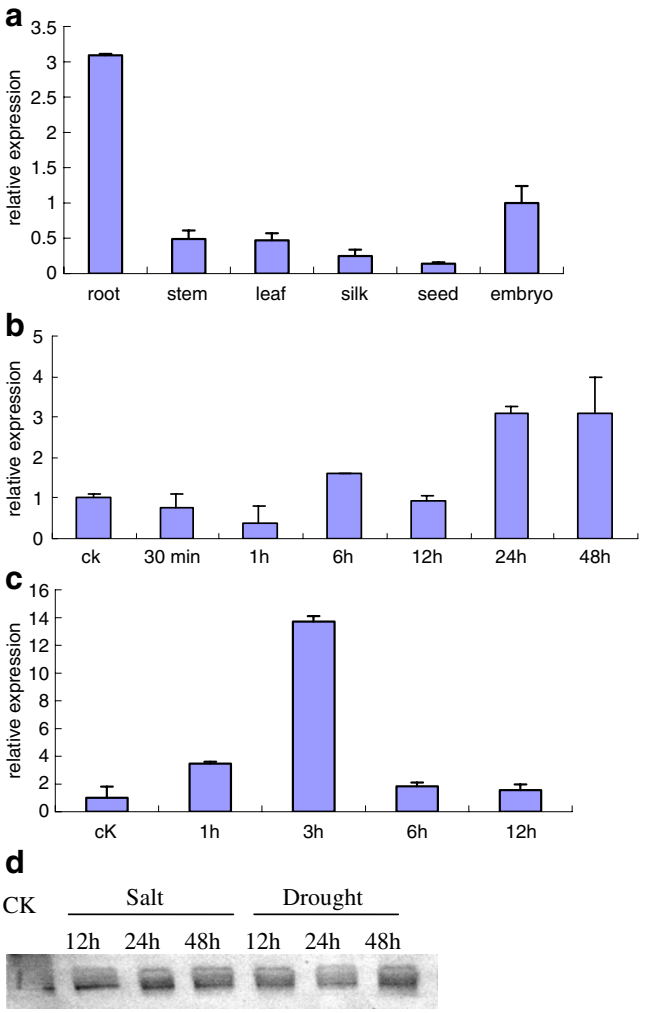

Fig. 2 Specific expression characteristics of *ZmICDH* transcripts and stress response. All the relative expression for *ZmICDH* was obtained with respect to the housekeeping gene *ACTIN*. The $2^{-\Delta\Delta C}$T method was used for analysis of relative gene expression. Each cDNA sample was assayed separately for the *ZmICDH* gene and for *ACTIN* (endogenous control), Values are the means ± SD of at least three measurements. **a** Relative expression of *ZmICDH* in different organs. The expression level of *ZmICDH* was analyzed by qRT-PCR. The organs used in the analysis were root, leaf, stem, silk, ear, and embryo. (**b**) *ZmICDH* response to drought stress. Total seedling RNA was extracted from the seedlings at different time points of PEG treatment (30 min, 1 h, 6 h, 12 h, 24 h, and 48 h) and evaluated by qRT-PCR. Transcripts level of *ACTIN* was measured using the same RNA samples, as a control experiment of the qRT-PCR. **c** *ZmICDH* response to salt stress. Total seedling RNA was extracted from the seedlings at different time points of salt treatment (1 h, 3 h, 6 h, and 12 h) and evaluated by qRT-PCR. **d** The protein levels were determined by the gel dyeing. Native PAGE stained for ZmICDH activity. Crude extracts (10 mU) from different treatment (salt: 12 h, 24 h, and 48 h; drought: 12 h, 24 h, and 48 h) were separated in a native PAGE and analyzed by ZmICDH activity staining

plants were less affected. After 10 days on high salt MS medium (100 mM NaCl), the average wild-type shoot length was 0.5 cm, while the transgenic lines were 0.81 cm and 0.82 cm. Particularly, on 150 mM NaCl, the root length of the wild-type plants decreased a half than that of the transgenic plants (Fig. 3c). To further examine the responses of *ZmICDH* in salt stress, the *ZmICDH* overexpressing transgenic *Arabidopsis* lines and control plants were grown in pots for 3 weeks and then treated with 200 mM NaCl for 7 days. The control plants withered and leaves were whitened, but the transgenic lines still grew well with green leaves (Fig. 3b). All the above results indicated that the over-expression of *ZmICDH* in *Arabidopsis* could enhance the tolerance to salt stress.

Discussion

In plants, the major NADP-ICDH activity presented in the cytosol, usually representing more than 90% of the total NADP-dependent activity, whereas only low levels of NADP-ICDH activities occur in chloroplasts, peroxisomes, and mitochondria (Henson et al. 1986). Chen and Gadal (1990) demonstrated that the cytosolic enzyme was responsible for 95% of the total ICDH activity in green tobacco leaves (Chen and Gadal 1990). Our results of the subcellular localization showed the ZmICDH-GFP fusion proteins was targeted to cytosolic of onion epidermis cell supporting that the ZmICDH was a cytosolic NADP-ICDH enzyme in maize.

Our results also showed that the maize NADP-ICDH was regulated constitutively since the presence of the *ZmICDH* transcript could be detected in all the tissues such as root, stem, leaf, silk, seed, and embryo with the highest expression in root. Such a regulation of gene expression had been reported previously in soybean, pea, and other plants (Chen et al. 1989; Udvardi et al. 1993; Hernandez et al. 2001). NADP-specific isocitrate dehydrogenase was a key cytosolic enzyme that supplies carbon skeletons for primary nitrogen assimilation in plants, which may be correlated with the higher ICDH-specific activity found in root.

Abiotic stresses including salt and drought stress induce accumulation of ROS that are detrimental to cells when ROS is at high concentration because they cause oxidative damage to membrane lipids, proteins, and nucleic acids (Jo et al. 2001). NADPH is heavily used for anabolic reactions and is a proton donor at the beginning of ROS-scavenging pathways (Jo et al. 2001; Jo et al. 2002). As key enzymes generating NADPH, ICDHs have received much less attention until recently. Among the eukaryotic ICDH isoenzymes, IDH has been known to play a major role in the oxidative decarboxylation of isocitrate in the tricarboxylic acid cycle (Lee et al. 2002). Jo et al. showed the potential role of NADP-ICDH in protection against radiation induced oxidative damage and demonstrated that regulation of mitochondrial redox balance and defense against oxidative damage are some of the primary functions of mitochondrial NADP-dependent isocitrate dehydrogenase (Jo et al. 2002). Lee suggested that NADP-ICDH also had an important role in protecting cells against ionizing radiation-induced oxidative damage (Lee et al. 2002). Thus, protecting cells against oxidative damage regardless of the origin was probably a primary biological function of ICDH (Jo et al. 2002; Lee et al. 2002; León et al. 2002). To our knowledge, no direct evidences for the ICDH function under stress situations have been reported in maize. Our results showed *ZmICDH* was induced by salinity and drought stresses and the *ZmICDH* transgenic *Arabidopsis* led to enhance salt tolerance. All the above results might indicate a new function of *ICDH* which need further study.

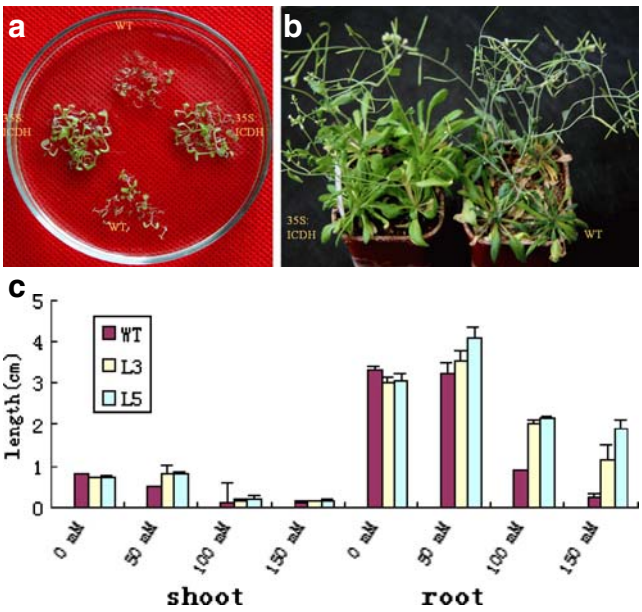

Fig. 3 Constitutive expression of maize *ZmICDH* under salt stress in *Arabidopsis.* **a** Germination of seeds from WT and two individual T3 lines with constitutive *35 S::ZmICDH* expression on 100 mM NaCl at the tenth day after germination. **b** Improved salt tolerance of *ZmICDH* over-expression *Arabidopsis* on 200 mM NaCl treatment after 7 day treatment. **c** Shoot and root length of WT and 2 individual transgenic lines on NaCl (0 mM, 50 mM, 100 mM, and 150 mM) for 10 days. Standard deviations (*error bars*) were calculated from results of three independent experiments (n=15 for each experiment)

Acknowledgments The research was supported by the 973 project (grant no. 2006CB101700), the 863 project (grant no. 2006AA10Z188), National Natural Science Foundation (grant no. 30730063)

References

Chen RD, Gadal P (1990) Structure, functions and regulation of NAD- and NADP-dependent isocitrate dehydrogenases in higher plants and in other organisms. Plant Physiol Biochem 28:411–427

Chen R, Le Marechal P, Vida1 J, Jacquot JP, Gadal P (1988) Purification and comparative properties of the cytosolic isocitrate dehydrogenases (NADP) from pea (*Pisum sativum*) roots and green leaves. Eur J Biochem 175:565–572

Chen RD, Bismuth E, Champigny ML, Gadal P (1989) Chromatographic and immunological evidence that chloroplastic and cytosolic pea (*Pisum sativum* L.) NADP-isocitrate dehydrogenases are distinct isoenzymes. Planta 178:157–163

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Daniel M, González EM, Frendo P, Puppo A, Arrese-Igor C (2007) NADPH recycling systems in oxidative stressed pea nodules: a key role for the $NADP^+$-dependent isocitrate dehydrogenase. Planta 225:413–421

Fieuw S, Muller-Rober B, Salvez S, Willmitzer L (1995) Cloning and expression analysis of the cytosolic $NADP^+$-dependent isocitrate dehydrogenase from potato. Plant Physiol 107:905–913

Gallardo F, Gálvez S, Gadal P, Canovas FM (1995) Changes in NADP-linked isocitrate dehydrogenase during tomato fruit ripening. Planta 196:148–154

Gálvez L, González EM, Arrese-Igor C (2005) Evidence for carbon shortage and strong carbon/nitrogen interactions in pea nodules at early stages of water stress. J Exp Bot 56:2551–2561

Henson CA, Duke SH, Collins M (1986) Characterization of $NADP^+$-isocitrate dehydrogenase from the host plant cytosol of lucerne (*Medicago sativa*) root nodules. Physiol Plant 67:538–544

Hernandez JA, Ferrer MA, Jimenez A, Barcelo AR, Sevilla F (2001) Antioxidant systems and $O_2^{\cdot-}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127:817–831

Hurley JH, Dean AM, Koshland DEJ, Stroud RM (1991) Catalytic mechanism of $NADP^+$-dependent isocitrate dehydrogenase: implications from the structures of magnesium- isocitrate and $NADP^+$ complexes. Biochemistry 30:8671–8678

Jo SH, Son MK, Koh HJ, Lee SM, Song IH, Kim YO, Lee YS, Jeong KS, Kim WB, Park JW, Song BJ, Huhe TL (2001) Control of mitochondrial redox balance and cellulardefense against oxidative damage by mitochondrial NADP-dependent isocitrate dehydrogenase (IDPm). J Biol Chem 276:16168–16176

Jo SH, Lee SH, Chun HS, Lee SM, Koh HJ, Lee SE, Chun JS, Park JW, Hun TL (2002) Cellular defence against UVB-induced phototoxicity by cytosolic NADP-dependent isocitrate dehydrogenase. Biochem Biophys Res Commun 292:542–549

Kenneth JL, Thomas DS (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2-^{\Delta\Delta C}$T method. Methods 25:402–408

Kirsch M, Groot HDE (2001) NAD(P)H, a directly operating antioxidant? FASEB J 15:1569–1574

Lee SM, Koh HJ, Park DC, Song BJ, Huh TL, Park JW (2002) Cytosolic NADP-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. Free Radic Biol Med 32:1185–1196

León AM, Palma JM, Corpas FJ, Gómez M, Romero-Puertas MC (2002) Antioxidative enzymes in cultivars of pepper plants with divergent sensitivity to cadmium. Plant Physiol Biochem 40:813–820

Li HY, Wang TY, Shi YS, Fu JJ, Song YC, Wang GY, Li Y (2007) Isolation and characterization of induced genes under drought stress at the flowering stage in maize (*Zea mays*). DNA Seq 18:445–460

Nakamura H (2005) Thioredoxin and its related molecules. Antioxidative Redox Signal 7:823–828

Udvardi MK, McDermott TR, Kahn ML (1993) Isolation and characterization of a cDNA encoding $NADP^+$-specific isocitrate dehydrogenase from soybean (*Glycine max*). Plant Mol Biol 21:739–752