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ORIGINAL PAPER

Molecular characterization of *StNAC2* in potato and its overexpression confers drought and salt tolerance

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Abstract Potato is one of the most important food crops in the world. Many plant transcription factors (TFs) have been demonstrated to be essential for improvement of plant stress tolerance traits. However, very few TFs were used for improving potato stress tolerance. In this study, we presented the characterization of a new potato *StNAC2* gene. The StNAC2 protein contains five subdomains of NAC proteins and belongs to NAP subfamily. *StNAC2* is constitutively expressed in potato leaves, stems, tubers, flowers and roots. Transcripts of *StNAC2* were significantly induced by *Phytophthora infestans*, the causal agent pathogen of potato late blight. *StNAC2* also could be induced by wounding, salt, drought as well as signal molecules such as salicylic acid and abscisic acid, suggesting that StNAC2 transcription factor involved in the signal transduction cascades in responses to abiotic and biotic stresses in potato. Overexpression of *StNAC2* in transgenic potato significantly enhanced salt tolerance in vitro and drought tolerance in pot growing condition. Thus, the functional analysis of the new *StNAC2* gene in this study will enrich knowledge for understanding the function of the *NAC* genes in potato stress tolerance.

Keywords Potato · *StNAC2* · Transcription factor · Drought tolerance · Salt tolerance

Abbreviations

TFs	Transcription factors
RACE	Rapid amplification of cDNA end
NAC proteins	NAM/ATAF/CUC proteins
SA	Salicylic acid
ABA	Abscisic acid

Introduction

Osmotic stresses, such as drought or salinity, are major abiotic environmental stressors exacerbated by global climate change that limits plant growth and development, and thus causing important economic losses of agronomical yield. Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop (FAO 2008). Compared to other crops, the potato is generally considered to be drought sensitive (van Loon 1981). Drought stress influences the development and growth of potato leaves, stem, shoots, roots and tubers (Ojala et al. 1990). Potato production in many areas of the world is increasingly effected by limited water supplies. The identification of drought tolerance traits and genes in potato would facilitate breeding for yield stability under water-limiting conditions (Evers et al. 2010).

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Plants have developed a diversity of mechanisms to deal with different abiotic stresses, such as drought, salt, heat and cold (Vinocur and Altman 2005). Recent advances in understanding the genetic control of drought tolerance offered new opportunities to develop crops that are less damaged by limited water supplies through genetic manipulation (Hussain et al. 2011; Cominelli and Tonelli 2010; Umezawa et al. 2006). Among them, transcription factors (TFs) play essential roles in plant stress responses by regulating their target genes through binding to the cognate cis-acting elements (Umezawa et al. 2006; Yamaguchi-Shinozaki and Shinozaki 2006). Many plant TFs, have been implicated in abiotic stress tolerance, belong to different families including AP2/EREBP (Dietz et al. 2010), DREB/CBF (Agarwal et al. 2006), bZIP (Kobayashi et al. 2008), MYB/MYC (Dubos et al. 2010), NAC (Puranik et al. 2012), C2H2-ZFP (Huang et al. 2007) and WRKY (Chen et al. 2012).

Efforts have been undertaken to generate drought and salinity tolerant potato by manipulating the expression of stress-responsive genes. Those genes include *TPS* and *TPP* genes from yeast or bacteria (Kondrák et al. 2011; Goddijn et al. 1997; Yeo et al. 2000); *GDP* from *Pleurotus sajor-caju* (Jeong et al. 2001); *coda* from *Arthrobacter globiformis* (Ahmad et al. 2008); *oxo* from barley (Turhan 2005); *BADH* from spinach (Zhang et al. 2012); *GalUR* from the strawberry (Hemavathi et al. 2012); *AtNDPK2*; *DREB1A* and *P5CS* from *Arabidopsis* (Tang et al. 2007; Behnam et al. 2006; Hmida-Sayari et al. 2005); *StMYB1R-1*, *SOD* and *APX* from potato (Shin et al. 2011; Tang et al. 2006). Most of those genes come from other species rather than potato. Although a larger number of plants TFs have shown center role in manipulating plant stress tolerance, very few were used for improving potato stress tolerance.

NAC proteins are plant-specific TFs which have been shown to play important roles in abiotic and biotic stress responses (Puranik et al. 2012). The NAC domain was identified based on the conserved sequences from Petunia NAM and *Arabidopsis* ATAF1/2 and CUC2 proteins (Aida et al. 1997). Many NAC candidate genes have been used to improve stress tolerance ability including drought, cold, salt and dehydration in wide-range plants species covering *Arabidopsis thaliana* (Park et al. 2011; Liu et al. 2011a, b), *Oryza sativa* (Song et al. 2011; Jeong et al. 2010; Hu et al. 2008), *Triticum aestivum* (Tang et al. 2012; Xue et al. 2011), *Nicotiana tabacum* (Liu et al. 2011a, b) and *Glycine max* (Hao et al. 2011) and *Gossypium hirsutum* (Meng et al. 2009).

Efforts to explore new genes in potato and utilize them to strengthen stress tolerance are of importance to potato production. *NAC* genes have emerged as important players in plant stress response. Up to now, only one characterized potato *NAC* gene *StNAC* which responds to *Phytophthora infestans* infection and wounding was reported by Collinge and Boller (2001). By analyzing the microarray data, we found that an EST similar to *NAC* gene was highly induced by biotic stimulus (Tian et al. 2006). However, the biological function of this NAC transcription factor (designated as StNAC2) in potato is currently unknown. In this study, we have focused on functional characterization of the potato *StNAC2* gene using transgenic approach to evaluate its impact on drought and salt stress adaptation in potato. Transgenic potato plants overexpressing *StNAC2* showed enhanced salt tolerance in vitro and drought tolerance in pot growing condition. Our results indicate that *StNAC2* plays an important role in biotic and abiotic stress and may serve as a potential candidate gene for improving potato stress tolerance.

Materials and methods

Plant materials

E-potato-3 (*Solanum tuberosum* L.), a potato cultivar of China, was used as transgenic material. Plantlets were propagated in vitro on MS medium supplemented with 3 % sucrose and 0.8 % agar in transparent plastic boxes in a culture room with the 16 h light and 8 h dark growing period at 22 ± 2 °C.

Cloning of *StNAC2* gene

Total RNA was isolated from E-potato-3 leaves using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA synthesis was performed using a SMART RACE cDNA amplification kit with SMART IV™ Oligonucleotide and CDSIII RACE Primers (Clontech, Palo Alto, CA, USA). Two gene-specific primers, GSP5 (5′-CCTAAAACTCCCTTTTCTCT CA-3′) and GSP3 (5′-TTAGCTCTGATCTTCCTCCT G-5′), were designed for rapid amplification of 3′ and 5′ cDNA ends according to the EST fragment sequence (GenBank accession No. CO267919) (Tian et al. 2006). RACE was performed according to protocol supplied by SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA, USA) under the following PCR conditions: an initial 2 min at 94 °C and a final extension step of 72 °C for 5 min, each cycle consisted of 30 s at 94 °C, followed by 1 min at 60 °C and 1 min at 72 °C. The PCR reaction was performed for 32 cycles using *Pyrobest*™ DNA polymerase (Takara BIO INC, Japan). Nucleotide sequences were compared using bioinformatics software from NCBI (http://www.ncbi.nlm.nih.gov) and predicted amino acid sequences were aligned using the GeneDoc (http://www.psc.edu/biomed/genedoc). Phylogenetic analyses based on the resulting alignments were then constructed

using the neighbor-joining method with the MEGA4 programme (http://www.megasoftware.net/mega4).

Analysis of gene expression

To investigate the organ-specific expression of the *StNAC2*, total RNAs were extracted from various tissues including leaves, stems, roots, flowers and tubers from 7-week-old greenhouse-grown potato plants. Total RNAs were isolated from samples using RNeasy plant mini kit (Mycomebio, Beijing, China) combined with a DNase digestion procedure (Takara, Dalian, China) to ensure DNA-free RNA preparations. DNase-treated RNA samples were used for reverse transcription (RT) of the first-strand cDNA using the ReverTra Ace Kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. A ten-fold dilution of the reverse transcript cDNA was used as template for each RT-PCR reaction. The *StNAC2* specific forward primer 1: 5′-TTATCAGTGCCAGTAGCCTTC-3′ and *StNAC2* specific reverse primer 2: 5′-TTAGCTCTGATCTTCCTCCTG-3′ were used to perform RT-PCR amplification (expected length is 277 bp). The constitutively expressed *β-tubulin* gene was used as internal standard for each RT-PCR. *β-tubulin* forward primer: 5′-TTGGACAGTCTGGTGCTG GGAATA-3′, *β-tubulin* reverse primer: 5′-TGGCCAGG GAATCTCAAACAGCAAG-3′ (the amplified length is 481 bp). PCR was performed in a total volume of 20 µl containing: 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 mM of each forward and reveres primers, 2 µl reaction buffers, 1 U Taq DNA polymerase (Takara, Dalian, China) and 1 µl template. The cycling parameters of PCR amplification were as follows: the reaction was performed for 35 cycles with an initial denaturation step at 94 °C for 3 min and a final extension at 72 °C for 5 min, each cycle consisted of 30 s at 94 °C followed by 30 s at 55 °C and 1 min at 72 °C. The products were separated by electrophoresis on 1 % agarose gels.

To determine whether *StNAC2* expression is induced by abiotic stresses and/or exogenous plant regulators, we collected cDNA microarray data from our unpublished data to investigate *StNAC2* expression profile under various treatments. The details of cDNA microarray procedures were described in previous reports by Wang et al. (2005).

Vector construction and transformation of potato

For construction of over expression vector, the *StNAC*2 coding region was PCR amplified from cDNA with the primers NAC2EXF: 5′-CCGGATCCCCTAAAACTCCC TTTTCTCTCA-3′ and NAC2EXR: 5′-CCGAGCTCGTTG TCATAAACTTCTGATGTCACC-3′ which add *Bam*HI and *Sac*I restriction sites separately. The PCR fragments were cloned into a pMD-18 cloning vector (Takara, Dalian, China) to obtain pMD-18-*StNAC2*. After confirmation by sequencing, double digested *StNAC2* coding region was inserted into *Bam*HI and *Sac*I sites of the pBI121 vector by replacing the *GUS* gene. The *StNAC2* expression vector under the control of the CaMV 35S promoter was introduced into *A. tumefaciens* strain LB4404 by the freeze–thaw method and then to transform potato microtuber slices according to the protocol reported by Si et al. (2003).

Confirmation of transgenic potato plants

Regenerated shoots from transformed microtuber slices were cultured on rooting medium with 200 mg/l kanamycin and 250 mg/l carbenicillin. Genomic DNA was isolated from the leaves of well rooted and wild-type potato plants. Isolated DNA was subjected to PCR analysis to amplify the *nptII* gene using *nptII* specific primers. The PCR conditions were the same as those described in gene expression part.

Southern blot analysis was conducted to confirm the stable integration of transgenes into potato genomic DNA. Hybridization probes were prepared from a 520 bp *nptII* gene fragment which was labeled using the DIG DNA labeling kit (Roche Molecular Biochemicals, Germany). DNA isolation and digestion, pre-hybridization, hybridization, washing conditions and hybridization signal detection were same as the procedures described by Tian et al. (2010). *Pst* I enzyme was used to digest genomic DNA.

Real-time RT-PCR was used to detect transgene expression levels. In vitro cultured young leaves collected from transgenic and control plants were used to isolate total RNA for analyses of the *StNAC2* expression level. The q*StNAC2* specific forward primer: 5′-CATGCATGAAT ATCGTTTGAGTG-3′ and q*StNAC2* specific reverse primer 2: 5′-CAACTTTCATCATCTCTATAGTT-3′ were used to perform real-time RT-PCR amplification (expected length is 140 bp) on the Bio-RAD CFX Connect optics module PCR System using SYBR Premix Ex Taq™ (TaKaRa, Dalian, China). The constitutively expressed *ef-1α* was used as internal reference gene for calculating relative transcript levels. Forward primer: 5′-GGAAACGGATAT GCTCCA-3′, reverse primer: 5′-CTTACCTGAACGCC TGTCA-3′ (the amplified length is 101 bp). The PCR thermal cycle conditions were as following: denature at 95 °C for 3 min and 40 cycles for 95 °C, 10 s, 60 °C, 20 s, 72 °C, 15 s. The relative quantification value was calculated by the $2^{-\Delta\Delta CT}$ method.

Assessment of salt tolerance of transgenic potato plants in vitro

The shoot tips of transgenic and control plants were cultured in plastic boxes containing 20 ml MS medium supplemented with 150 mM NaCl and kept in a culture room

with the 16 h light and 8 h dark growing period at 22 ± 2 °C. After 4 weeks of the salt treatment, the plantlets were harvested and the heights, fresh weight, root numbers, root length of plantlets were measured. Each treatment contained 25 plantlets which were cultured in 5 boxes.

Evaluation of transgenic plants for drought stress tolerance

Tubers of transgenic and control potato plants were grown in plastic pots (Φ 20 cm) containing 2.5 kg compost soil mix in a naturally illuminated greenhouse for 5 weeks. Then, the selected 30 uniform plants of each line were moved from greenhouse to a well-sheltered environment. One repeat contains 10 plants per genotype. Same volume of water was irrigated in each pot in each time without outflow. Water was withheld from the plants being drought stressed. Drought tolerance was evaluated by relative wilt percentage of plant leaves 3, 5, 8 days after water deficit stress. Finally, the plants were allowed to recover by re-watering. Three rounds of water withholding were conducted.

Statistical analysis

All data were analyzed by analysis of variance using SAS statistics program. Statistical differences are referred to as significant when $P < 0.05$ or 0.01.

Results

StNAC2 gene cloning and structural features

A 1,257 bp full-length *StNAC2* cDNA was obtained by assembling the overlapping 3′-RACE and 5′-RACE sequences. The ORF comprises 849 nucleotides and encodes a predicted protein of 282 amino acids (Fig. 1a). The ORF is flanked by 81 bp 5′-UTR and a 3′-UTR which is 327 nucleotides long including the poly (A) tail. The *StNAC*2 sequence has been deposited in GenBank under accession No. EF091874. NCBI Blastp results showed that the predicted protein contains no apical meristem (NAM) domain in the N-terminus from 9 to 135 amino acids, indicating it is a NAC family protein (Fig. 1a).

Phylogenetic analysis of several NAC proteins

Based on an amino acids alignment of five plant NAC proteins (Fig. 1b), higher sequence similarities were found in the N-terminus rather than in the C-terminus. Amino acid sequence comparison of five plant NAC proteins showed that StNAC2 shares 38.4, 38.1 and 37.9 % similarity with StNAC (CAC42087), SlNAC (AAR88435) and CaNAC1 (AAW48094), respectively. StNAC2 shares higher 54.6 % similarity with the *Arabidopsis* NAP (CAA10955) rather than another potato NAC protein StNAC.

NAC proteins could be classified into several subfamilies on the basis of similarities in NAC domains (Puranik et al. 2012). We reconstructed a phylogenetic tree from the amino acid sequences of 25 NAC proteins. Phylogenetic analysis showed that the 25 NAC proteins belong to five NAC subfamilies. StNAC2 was clustered into the NAP subfamily, while another StNAC from potato clustered into ATAF group. It is clear that StNAC and StNAC2 belong to different group of NAC family (Fig. 1c), indicating that StNAC2 is a new member of potato NAC proteins.

Expression of *StNAC2* in different organs and response to biotic and abiotic stimuli

RT-PCR was conducted to determine *StNAC2* expression levels in various potato tissues at the flowering stage. The results showed that *StNAC2* was constitutively expressed in leaves, stems, tubers, flowers and roots (Fig. 2a). The expression levels in leaves and stems were stronger than in tubers, flowers and roots.

The induction patterns of *StNAC2* response to biotic and abiotic stimuli are shown in Fig. 2b. The results indicated that *StNAC2* expression was significantly induced by *P. infestans* and peaked at 8 h after inoculation. After mechanical wounding, accumulation of *StNAC2* transcripts was increased after 8 h and kept until 36 h. Expression profiling showed that *StNAC2* was up-regulated by ABA at an increasing rate from 2 to 12 h and reached peak at 12 h. After that, slight decline was observed at the 24-h time point and then increased slightly. Induction patterns of SA showed a peak at 2–8 h and then declined. ETH and MeJA induced *StNAC2* expression at a low level with little variation in abundance during the time course (data not shown). The results demonstrated that *StNAC2* is responsible for biotic and abiotic stresses.

Confirmation of transformates by PCR and Southern blotting

After *Agrobacterium*-mediated transformation using *CaMV 35S-StNAC2* expression cassettes, twelve independent kanamycin-resistant putatively transformed potato lines were generated and further confirmed by PCR analysis using *nptII* specific primers and Southern blotting. Partial results are shown in Fig. 3a, b. The insertion number of transgene is about 1–2 copies in three selected transgenic overexpression lines OE2, OE3 and OE4 (Fig. 3b). Real-

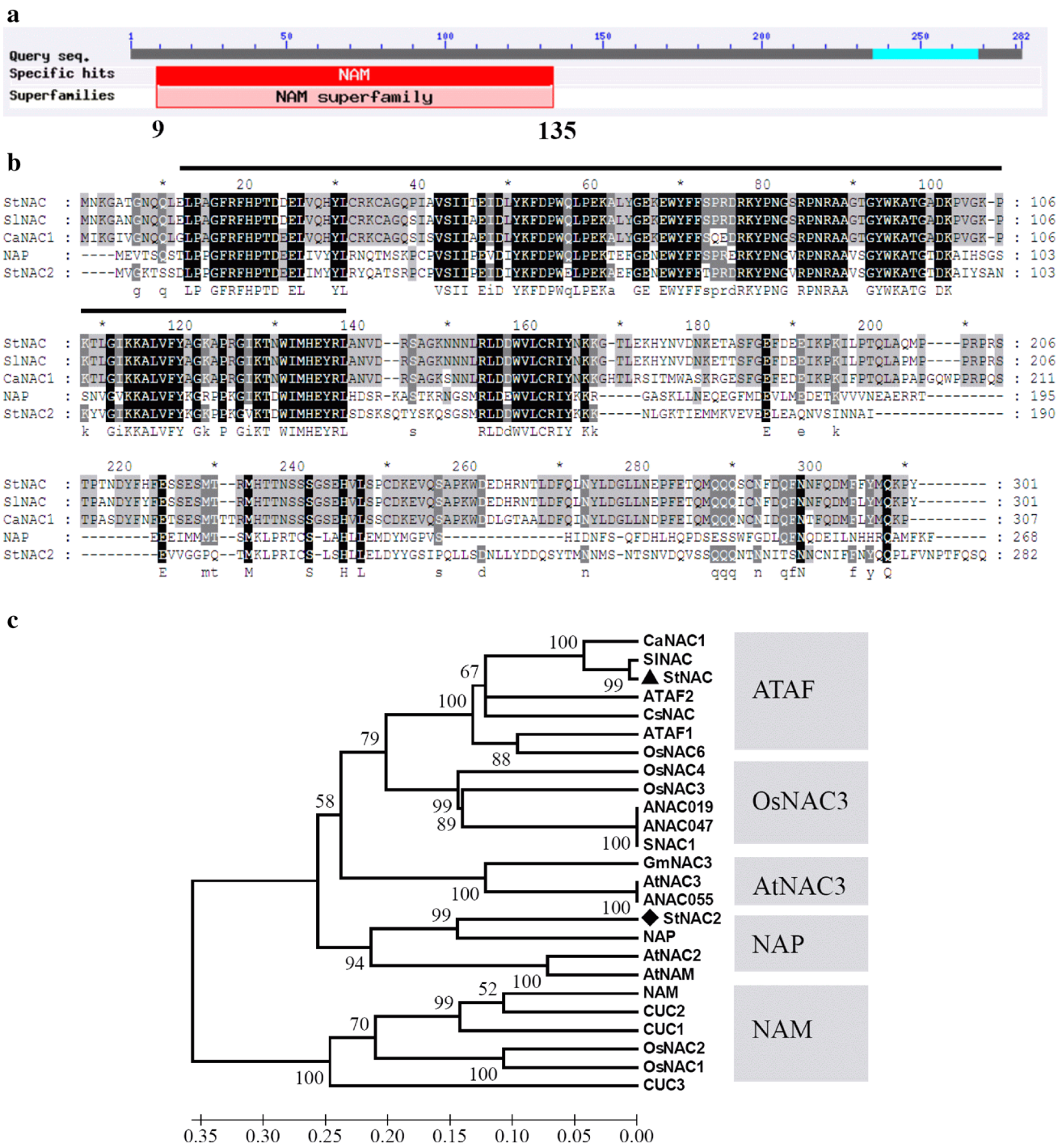

Fig. 1 Amino acid sequences alignment of StNAC2 with four NAC proteins and phylogenetic tree of 25 plant NAC proteins. **a** The NAM domain predicted by Blastp. **b** Amino acid sequence comparison of five plant NAC proteins. The NAM domain is shown by *lines* above the amino acid sequences. Identical amino acids are shaded in *black*, and similar amino acids are shaded in *gray*. Sequences were aligned using Clustal X and GeneDoc. **c** Phylogenetic analyses were conducted in MEGA4 using the neighbor-joining method. The *numbers* beside each node represent bootstrap values (≥50 %) based on 1,000 replications. The *scale bar* indicates the relative amount of change along branches. The five sub-subfamilies distinguished within the NAP, OsNAC3, ATAF, AtNAC3, and NAM subfamilies are shown in *gray rectangles*. The GenBank accession numbers for amino acid sequences are as follows: *A. thaliana* ATNAC2 (NP_188170), AtNAM (AAD17314), CUC1(BAB20598), CUC2 (BAA19529), CUC3 (AAP82630), NAP (CAA10955), ANAC047 (NP_187057), ATAF1 (NP_171677), ANAC055 (AAM61076), ATAF2 (CAC35884), AtNAC2 (BAB20600), AtNAC3 (BAB20599); *Oryza sativa* ANAC019 (AAT02360), SNAC1 (ABD52007), OsNAC1 (BAC53810), OsNAC2 (BAC53811), OsNAC3 (BAA89797), OsNAC4 (BAA89798), OsNAC6 (BAA89800); *Petunia hybrida* NAM (CAA63102); from soybean: GmNAC3 (AAX85980); *Citrus sinensis* CsNAC (ABQ96643); *Solanum lycopersicum* SlNAC (AAR88435); *Solanum tuberosum* StNAC2 (ABK96797.1) and StNAC (CAC42087); *Capsicum annuum* CaNAC1 (AAW48094)

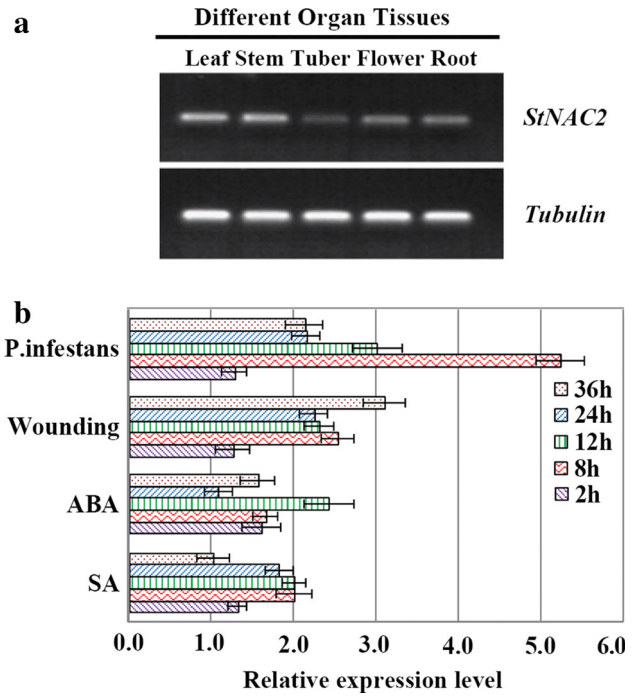

Fig. 2 Expression of *StNAC2* in different organs and response to biotic and abiotic stresses and signal molecules. **a** Expression of *StNAC2* in leaves stems, roots, tubers and flowers in potato. RT-PCR was performed with specific primers of *StNAC2*. **b** Expression of *StNAC2* in response to biotic and abiotic stresses and signal molecules. For *P. infestans* treatments, the third and fourth fully expanded leaves of 6-week-old plants were detached and inoculated with zoospores of *P. infestans* as described previously (Tian et al. 2006). For signal molecules treatments, fully expanded leaves from 6-week-old greenhouse-grown potato plants were detached and sprayed with 10 mM salicylic acid (SA), 100 μM abscisic acid (ABA). Leaves sprayed with distilled water were used as a control. For wounding treatment, detached leaves were incised with a blade several times. Samples were collected and frozen in liquid nitrogen and stored at −70 °C before total RNA isolation at the indicated times. Microarray results from our unpublished data, the experimental design is same as described previously by Wang et al. (2005). *Error bars* show SD from three biological repeats

time RT-PCR revealed that transgenic lines OE2, OE3 and OE4 displayed constitutively elevated expression of *StNAC2* and were used for further investigation (Fig. 3c).

Transgenic plants confers salt tolerance in vitro

We analyzed the salt tolerance of transgenic potato in vitro. As shown in Fig. 4, control and transgenic potato lines (OE2, OE3 and OE4) grew normally on MS media without NaCl. When they grew on media supplemented with 150 mM NaCl, the growth of both was inhibited, however, growth performance of transgenic potato lines is better than control plants (Fig. 4a). We further tested the gene expression level under the salt stress. Compared to in vitro plants grown on MS media, *StNAC2* gene expression levels were up-regulated both in control and transgenic potato lines, but gene expression level is still significantly high in transgenic potato lines than in control plants (Fig. 4b). As reflected in quantitative estimation (Table 1), the root numbers, height of plants of stressed transgenic potato lines were significantly higher compared with controls. Root length and fresh weight of transgenic potato lines are still higher than control, even if difference is not significant in all three transgenic plants. The results indicated that the transgenic potato plants conferred tolerance to salt stress in vitro.

Overexpression of *StNAC2* in transgenic potato enhances tolerance to drought stress

To determine whether the transgenic lines confer tolerant to drought conditions, control and transgenic plants (OE2, OE3 and OE4) were grown in the same pots and growing conditions. Under normal growth conditions, three transgenic lines showed no obvious abnormal morphological phenotype compared with control. Five weeks later, water was withheld for up to 8 days and the wilt percentages of leaves were measured. Control plants started to show wilted symptoms after 3 days post-treatment (Fig. 5a). Three overexpression lines were slightly wilted, but the wilting percentage of leaves is less than that of control (Fig. 5d). With the development of water deficiency, the degree of wilting of leaves on both control and transgenic plants became severe. But wilting symptom in transgenic lines is less severe compared with control. After 8 days of drought stress, control plants were wilted around 100 % percentage, while the transgenic plants showed about 90 % wilting percentage (Fig. 5d). After plants were totally wilted, the pots were watered fully again, and then the second round water withholding was performed. The transgenic plants recovered faster than control. Similar to the first round of water withholding, the transgenic lines show less wilting symptom than the control plants. After three rounds of water withholding the transgenic lines survived but display chlorotic leaves, while control plants are almost dead (Fig. 5b). Expression pattern of *StNAC2* under drought stress condition was tested by real-time RT-PCR on potato leaves. As shown in Fig. 5c, *StNAC2* expression was gradually increased after water withholding both in the control and transgenic lines. But the transcripts increased significantly in three transgenic plants. The most elevation follows 5 days water deficiency. After that, transcripts of *StNAC2* are still keeping in higher level in transgenic plants. The high level expression *StNAC2* is consistent with low wilting percentage. These results revealed that overexpression of *StNAC2* in transgenic potato enhances tolerance to drought stress.

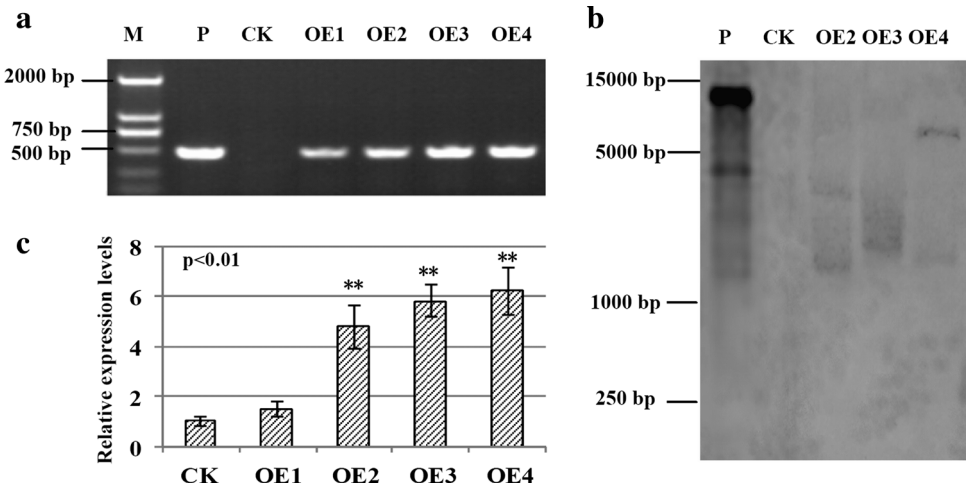

Fig. 3 Molecular characterization of transgenic potato lines. **a** PCR analyses of genomic DNA to detect the presence of *nptII* gene in putative transgenic plants. The 450 bp fragment of the *nptII* gene was amplified by the *nptII* specific primers. *Lane M*: molecular marker (DL2000), *Lane P*: plasmid (positive control), *Lane CK*: negative control and *Lane OE1-OE4*: four selected putative overexpressing transgenic lines. **b** Southern blot analysis of *Pst* I digested genomic DNA isolated from three different transformants and control plants and hybridized with the DIG labeled *nptII* probe. *Lanes P* represent plasmid (positive control), *Lanes CK and OE*: DNA samples isolated from control and three transgenic potato plants. **c** qRT-PCR analysis of relative transcript levels of *StNAC2* in four transgenic lines under normal conditions. Transcript levels of control (CK) were used as reference, which was set at a relative expression level of '1'. *Error bars* indicate the standard error; the experiments were repeated three times alone with at least five independent repetitions of the biological experiments. *Asterisks* above *each column* indicate a significant difference ($P < 0.01$) between control and transgenic lines

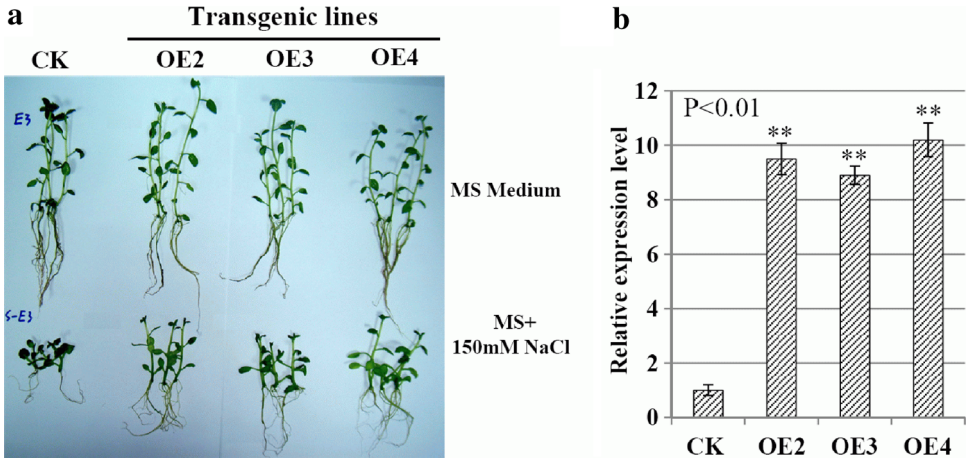

Fig. 4 Overexpression of *StNAC2* in potato plants confers salt tolerance in vitro. **a** Appearance of transgenic and control plants after salt stress. Plants were grown on MS media supplemented with or without 150 mM NaCl for 4 weeks. OE2, OE3 and OE4 represent overexpressing transgenic lines. **b** qRT-PCR analysis of relative transcript levels of *StNAC2* in transgenic lines under salt stress. *Bar* represents the standard error of three biological repeats. *Asterisks* above *each column* indicate a significant difference ($P < 0.01$) between control and transgenic lines

Discussion

Recently, 110 *NAC* genes were identified in potato and several *NAC* genes were highly induced by abiotic stress (Singh et al. 2013). Up to date, only one potato *NAC* gene *StNAC* was characterized in detail. *StNAC* was rapidly and strongly induced by wounding and *P. infestans* (Collinge and Boller 2001). In this study, we have cloned and characterized another potato *StNAC2* gene. *StNAC2* is a NAP family gene rather than ATAF family to which *StNAC* belongs. In this regard, *StNAC2* is a new *NAC* gene cloned from potato in the present research. Since the identification of first NAC domain gene NAM from *Petunia hybrid* which involved in shoot apical meristem development (Souer et al. 1996), many NAC proteins have been reported to be involved in processes of plant

development, for example, lateral root formation (Xie et al. 2000), cell expansion of specific flower organs (Sablowski and Meyerowitz 1998) and secondary wall thickening in woody tissues (Mitsuda et al. 2007). Besides their roles in plant development, NAC domain genes are also involved in various plant stress response including water, salt, cold, wounding, insect feeding and pathogen infection (Collinge and Boller 2001; Hegedus et al. 2003; Nogueira et al. 2005; Oh et al. 2005; Lin et al. 2007). In this study, we found that *StNAC2* expression was significantly induced by potato late blight agent pathogen *P. infestans*. Besides, *StNAC2* could be induced by wounding, salt and drought as well as signal molecules including ABA and SA (Figs. 2, 4, 5). Recent studies suggest a crosstalk between plant responses to pathogens and abiotic stresses (Abuqamar et al. 2009; Mauch-Mani and Flors 2009). Thus, *StNAC2* may play important role in the signal transduction network responses to abiotic and biotic stresses in potato. ABA signal pathway is well documented involving in abiotic stresses such as drought, low temperature and osmotic stress (Danquah et al. 2014). Expression analysis revealed *StNAC2* transcription was induced by ABA treatment (Fig. 3b), so we presume that *StNAC2* might play roles in potato responses to abiotic stress, probably through ABA-dependent pathway.

Over expression of many *NAC* genes has been shown to improve drought, salt and cold tolerance in transgenic plants (Puranik et al. 2012), which promotes us to investigate function of *StNAC2* in potato drought and salt stresses. Our results showed that overexpression of *StNAC2* in transgenic potato significantly enhanced salt tolerance in vitro and drought tolerance in pot growing condition.

Table 1 Quantitative estimation of phenotypic characters of *StNAC2* transgenic potato plants under salt stress condition in vitro

Lines	Root numbers (≥5 mm)	Root length (cm)	Height of plantlets (cm)	Fresh weight (g)
OE2	8.6 ± 1.6 b	5.3 ± 0.6 a	4.5 ± 0.9 a	0.24 ± 0.09 b
OE3	5.9 ± 1.1 c	4.7 ± 0.3 ab	3.3 ± 0.7 b	0.27 ± 0.08 ab
OE4	11.4 ± 1.9 a	4.5 ± 0.5 ab	4.6 ± 0.5 a	0.33 ± 0.08 a
CK	1.7 ± 0.5 d	3.4 ± 0.4 b	2.2 ± 0.4 c	0.19 ± 0.04 b

Data represent the average mean ± SD. Values with significant differences according to *t* tests were indicated by low case letters ($P < 0.05$), ($n = 25$)

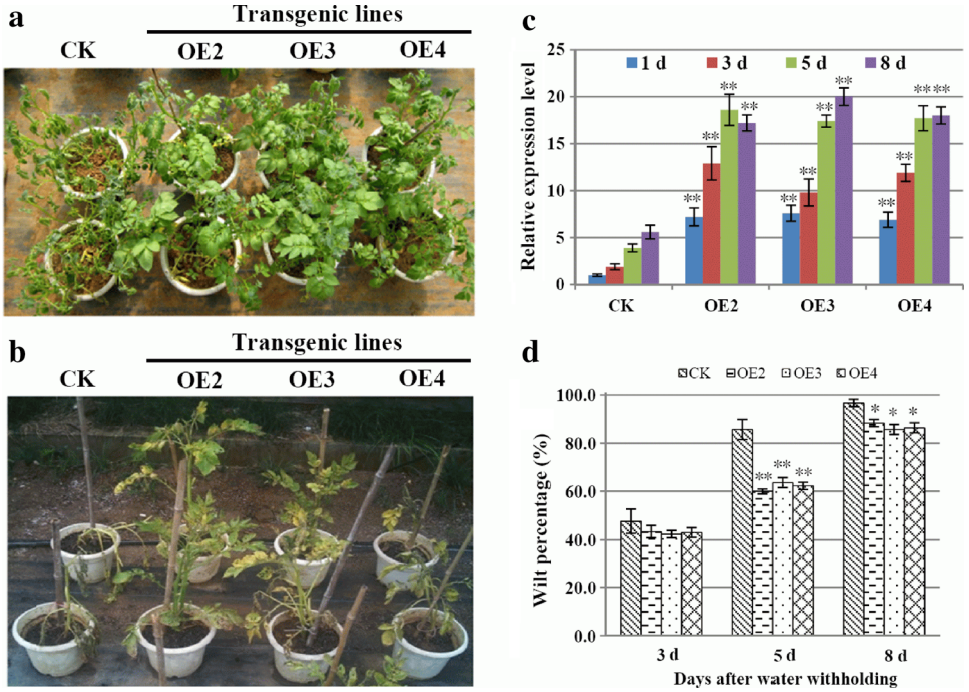

Fig. 5 Drought tolerance assays of *StNAC2*-overexpressing transgenic potato. A water withholding assay was performed with 5-week-old plants for three rounds (8 × 3 days) up to 24 days. **a** Plants performance after 3 days at first round water withholding. **b** Performance of plants recovered after the third round water withholding. **c** qRT-PCR analysis of relative transcript levels of *StNAC2* in three transgenic lines under drought stress condition. **d** Wilt percentage of transgenic potato plants after 3, 5 and 8 days when water was withheld at first round. In (**a**) and (**b**), OE2, OE3 and OE4 represent three *StNAC2*-overexpressing transgenic lines. CK is non-transgenic control potato. In (**c**) *error bars* indicate the standard error; *Asterisks* above *each column* indicate a significant difference ($P < 0.01$) between control and transgenic lines. In (**d**), *each bar* represents the standard error of three independent experiments ($n = 10$). *Asterisks* above *each column* indicate a significant difference ($P < 0.05$) between control and transgenic lines

One of the problems is the constitutive overexpression of stress-related genes including *NAC* genes that often cause abnormal development or yield penalty (Priyanka et al. 2010; Kasuga et al. 1999; Nakashima et al. 2007). We did not find obvious developmental differences between the transgenic plants and the wild-type plants under normal growth condition. However, we still cannot rule out the possibility that *StNAC2* plays a role in potato development.

Although the molecular bases of the improved stress tolerance of the *StNAC2* transgenic potato have not been completely resolved in this study, our data suggest that the biological role of StNAC2 is mainly associated with plant adaptation to abiotic and biotic stresses. Overexpression of this gene could enhance drought and salt tolerance in potato, making it a potential candidate for engineering stress tolerant potato. The growth and productivity of potato are often threatened by environmental factors, such as drought, salt, and cold. Further study of the role of the *StNAC2* gene in responding to different stresses may contribute to a deeper understanding of the cross-reactions of potato plants to multiple abiotic stresses. Especially it is worth to test the ability of the late blight disease resistance on overexpression plants, since *StNAC2* could be significantly induced by *P. infestans*. Whether the overexpression of *StNAC2* could achieve the ultimate goal of improved yield in potato in drought- and salt-prone environments awaits field trials in the future.

Author contribution Qinfen Xu: Gene cloning, transformation. Evaluation of salt tolerance in in vitro transgenic plants. Qin He: Gene expression analysis, qRT-PCR. Southern blotting. Shuai Li: Evaluation of drought tolerance of transgenic plants. Zhendong Tian: Design experiments. Phylogenetic analysis. Writing MS.

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