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ORIGINAL ARTICLE

Overexpression of *AtNAC2* (*ANAC092*) in groundnut (*Arachis hypogaea* L.) improves abiotic stress tolerance

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Abstract Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown in semi-arid tropics where it experiences moisture stress at different stages of growth resulting in reduced growth and productivity. In this study, we report that the stress tolerance of groundnut can be improved by overexpression of stress-specific transcription factor through transgenic approach. In silico electronic-northern analysis of *AtNAC2* showed increased expression under different abiotic stresses. The transcript levels of a homolog of *AtNAC2* gene were upregulated under different drought regimes in groundnut. Groundnut transgenics overexpressing *AtNAC2* showed enhanced tolerance to drought and salinity with improved yield under water-limited conditions. The study demonstrates that *AtNAC2* is a potential candidate gene to improve stress tolerance by transgenic approach.

Keywords Transcription factor · Groundnut · Transgenics · Drought · Abiotic stress

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Introduction

In semi-arid tropics, high temperature and reduced soil moisture levels affects crop growth and productivity. The impending climate change characterized by an increase in frequency and severity of drought and elevated temperature has accentuated negative impact on the peanut productivity (Bhatnagar et al. 2008; Akcay et al. 2010). Plants have evolved diverse adaptive strategies to cope with water-deficit conditions like drought avoidance, drought escape, and drought tolerance. Drought tolerance phenomenon is complex and polygenic and is based on several inherent and acquired traits. In this context, developing superior genotype having better adaptation to drought stress has relevance. Under water-deficit conditions, the cellular level of tolerance mechanisms which bring about alteration in cell metabolism for plant adaptation, assumes significance. The molecular mechanism of stress perception and signal transduction has been fairly well understood in recent years in plants like *Arabidopsis*, rice, and maize (Shinozaki and Dennis 2003). Several stress-related proteins including transcription factors (TFs) have been identified, including those encoding key enzymes in abscisic acid biosynthesis (Nambara and Marion-Poll 2005), proteins involved in cellular dehydration (Yao et al. 2011), cellular protective enzymes (Puckette et al. 2007), and proteins involved in cell signaling (Zhu 2002).

Transcription factors control large number of genes by up- or down-regulating the target genes by binding to the respective *cis* motifs present in the promoter sequences of the genes (Babitha et al. 2013). TFs such as AP2-ERF, DREBs, CBF, bZIP, zinc-finger, MYB, and NAC [NAM (No Apical Meristem), ATAF1, 2 (*Arabidopsis thaliana* Transcription Activation Factor 1, 2), CUC2 (Cup-Shaped Cotyledon 2)] are directly or indirectly involved in the regulation of plant defense and stress response (Mukhopadhyay et al. 2004; Yanhui et al. 2006).

NAC proteins constitute one of the largest families of plant-specific TFs, and the family is present in a wide range of land plants. NAC TFs were first described a decade ago (Riechmann et al. 2002). Proteins of this family are characterized by a highly conserved DNA-binding domain, known as the NAC domain, in the N-terminal region. The C-terminal region of NAC proteins, which usually contains a transcriptional activation domain, is highly diversified both in length and sequence (Ooka et al. 2003). About 135 and 163 members of this family have been identified in *Arabidopsis* and rice, respectively (Fang et al. 2008). NAC proteins play an important role in diverse processes, including plant developmental processes such as pattern formation in embryos and flowers, formation of secondary walls, leaf senescence (Mitsuda and Ohme-Takagi 2008), and lateral root development (He et al. 2005). NAC TFs are also involved in plant response to various biotic and abiotic stresses such as pathogens, drought, cold, salinity, low-oxygen, and extreme temperature (Puranik et al. 2012). In *Arabidopsis*, three NAC members, ANAC019, ANAC055 and ANAC072 bind to the promoter region of *ERD1* and improved the tolerance to drought stress (Tran et al. 2004). *AtNAC2* is involved in plant responses to hormone signals such as ABA, 1-aminocyclopropane-1-carboxylic acid, and α-naphthalene acetic acid (He et al. 2005). Another *NAC* gene identified from *Hordeum vulgare* L. i.e. *NAC6* showed enhanced pathogen resistance in barley and *Arabidopsis* (Jenson et al. 2007). In addition, many NACs have been characterized in rice, and *SNAC1* is involved in drought stress tolerance at anthesis stage (Hu et al. 2006). Overexpression of root-specific *NAC*, *OsNAC10* improves drought tolerance and yield in rice (Jeong et al. 2010). *SNAC2/OsNAC6*, *OsNAC045*, and *OsNAC063* were shown to enhance tolerance to multiple abiotic stresses (Zheng et al. 2009). Transcription factors *TaNAC4* and *TaNAC8* are found to be involved in both biotic and abiotic stress responses in wheat (Xia et al. 2010a, b). Tobacco transgenics overexpressing *NAC1* from *Eleusine coracana* L. showed enhanced tolerance to several abiotic stresses (Ramegowda et al. 2012).

The emphasis of this study was to assess the functional role of *AtNAC2* (AT5G39610) in groundnut for abiotic stresses. The transgenics developed using this gene showed improved phenotypic characters, besides showing tolerance to moisture stress, salinity with improved yield under water-limited conditions.

Materials and methods

Plant material and stress imposition

The expression pattern of *AhNAC2-2* a groundnut homolog of *AtNAC2* was studied in leaves of groundnut plants exposed to different soil moisture status. Moisture stress treatments were imposed to 4-week-old groundnut seedlings (cv. K-134) by maintaining soil water status at 100, 60, and 40 % field capacity (FC) in pot by gravimetric approach (Govind et al. 2009). The leaf tissues were collected and stored in −70 °C for expression analysis.

Construction of plant transformation vector with AtNAC2 and maintenance of plants

The *AtNAC2* gene also known as *ANAC092* was obtained from the RIKEN Centre Japan (AT5G39610). The 1.2-kb cDNA fragment of *AtNAC2* was subcloned into pTZ57R/T and subsequently the fragment was released using *Kpn*I and *Sal*I (MBI Fermentas) and ligated into p*BinAR* binary vector. The p*BinAR:AtNAC2* plasmid was mobilized to *Agrobacterium* strain *LBA4404* by thr electroporation method and the positive clones were identified using colony PCR analysis with gene-specific primers. *Agrobacterium* harboring the recombinant binary vector (p*BinAR*:*AtNAC2*) was grown in LB medium containing 50 μg/ml kanamycin overnight at 28 °C. The bacterial cells were later resuspended in Winan's AB medium (pH 5.2) and grown for 18 h. Wounded tobacco leaf extract was added to the suspension before infection. Groundnut cv. K-134 was used to develop *AtNAC2* transgenic groundnut plants by *Agrobacterium*-mediated in planta transformation method (Rohini and Sankara Rao 2000).

Isolation of genomic DNA and total RNA for molecular analysis

Genomic DNA from composite samples was isolated by CTAB method (Doyle and Doyle 1990). The PCR analysis was carried out with gene specific and *npt II* primers using 94 °C for 5 min initial denaturation, 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min of 30 cycles, and final extension at 72 °C for 5 min PCR conditions using the following primers: FP-TAGGTACCCTCTTCTCAAAAACCCTTC and RP-GCGTCGACTTTAACTTCGTGATATCTTTAGAC.

Further to carrying out RT-PCR analysis, total RNA was extracted from a few plants of the T_3 generation by using the protocol described by Datta et al. (1989). All care was taken to use RNase-free materials for RNA work according to the instructions (Sambrook et al. 1989). cDNA was prepared using 5 μg of total RNA and reverse-transcribed to make single-stranded cDNA in a 20-μl reaction mix consisting of 25 units M-MuLV reverse transcriptase (MBI Fermentas), 1× MMuLV-RT buffer, 40 pmol oligo dT primer, and 10 μM dNTP mix. Reverse transcription was performed at 42 °C for 1 h, and RNA was reverse-transcribed to cDNA immediately after isolation and stored at

−20 °C. Then, 1 µl of the cDNA mix was used as template for PCR amplification. To study the expression of *AtNAC2* homolog gene in groundnut, the plants were exposed to 60 and 40 % FC conditions. The RT-PCR analysis was carried out using FP-CGGCGAGAAAGAGTGGTATT and RP-GTTTTCACTCCCTTGGG.

Physiological analysis of transgenic groundnut plants

Rate of water loss, specific leaf area (SLA) and relative water content

The rate of water loss was estimated using method explained by Mao et al. (2012).

SLA of leaves was calculated by measuring leaf area (LA) using an area meter, and subsequently dry weight (DW) was recorded. Then SLA was calculated,

$$SLA = (LA/DW).$$

Relative water content (RWC) of leaf discs was measured according to Barrs and Weatherly (1962).

Chlorophyll stability index

One set of leaf discs from wild-type and transgenic plants were incubated in acetone:DMSO (1:1) solution (10 ml) for 72 h as control. Another set of leaf discs were incubated in water for 30 min at 60 °C to give heat stress and were then transferred to acetone:DMSO (1:1) solution (10 ml) for 72 h. Absorbance was recorded at 645, 653, and 663 nm, using UV–Vis spectrophotometer (UV 2450; Shimadzu). Chlorophyll content was estimated by substituting the absorbance values in the following formulae:

Chlorophyll a: $12.7\ (A_{663}) - 2.69(A_{645})V/\text{weight} \times 1{,}000$

Chlorophyll b: $22.9\ (A_{645}) - 4.68(A_{663})V/\text{weight} \times 1{,}000$

Total chlorophyll (mg g FW^{-1}) = (chlorophyll a + chlorophyll b)

Percentage of reduction over control (R) = (control-stressed)/control × 100

Chlorophyll stability index (X) = 100 – R.

Membrane integrity

Percent leakage of solutes reflecting loss of membrane integrity was quantified in both control and stressed leaf discs. The leaflets from plants grown under contained field conditions were collected and allowed for live wilting in laboratory conditions. One set allowed for 4 h (mild stress) live wilting and another set for 12 h (severe stress). After that, respective leaf discs were incubated in 25 ml of water with continuous shaking for 2 h and initial electrical conductivity (EC) was taken using EC-TDS analyzer (ELICO-CM183). Then, the leaf discs were boiled for 30 min and final EC was taken (Hoekstra et al. 2001). The cell leakage was computed by using following formula,

$$\text{Percent leakage} = \text{initial EC}/\text{final EC} \times 100.$$

Yield analysis

The plants were harvested 120 days after sowing (DAS) and several yield parameters such as plant height, pod weight, and total dry matter (TDM) were recorded. The obtained values were subjected to statistical analysis using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using MSTATC software.

Results

In silico analysis of *AtNAC2*

Stress inducibility of *AtNAC2* expression was analyzed by electronic-northern (e-northern) analysis using *Arabidopsis* Botany Array Tool (http://bbc.botany.utoronto.ca). The microarray dataset showed that *AtNAC2* was up-regulated under drought (twofold), oxidative stress and salt stress (Fig. 1a–c). Further, *AtNAC2* showed a homology with NAC family genes from different crop species, to an extent of 49 % in *Brachypodium distachyon*, 54 % in *Brassica napus*, 55 % in *Arachis hypogaea*, 48 % in *Medicago sativa*, 54 % in *Nicotiana tabacum*, 50 % in *Hordeum vulgare*, 55 % in *Gossypium hirsutum*, 50 % in *Triticum aestivum*, 51 % in *Saccharum officinarum*, 58 % in *Oryza sativa*, and 69 % in *Glycine max* (supplemental Fig. 1). There are two groundnut genes homologous to *AtNAC2* gene, namely *AhNAC2* (AC142833; Liu et al. 2011) and a gene identified as ACG49995. The nearest neighbor analysis of phylogenetic view shows that *AtNAC2* has 55 % identity with ACG49995 and 54 % with *AhNAC2*. From this result, we refer to this homologous gene (ACG49995) as *AhNAC2-2* and *AhNAC2* (Liu et al. 2011) was cited here as *AhNAC2-1*.

Expression analysis of *AtNAC2* in groundnuts

To study the expression pattern of *AtNAC2* homolog, i.e. *AhNAC2-2* in groundnut leaves to moisture stress, 4-week-old plants were exposed to gradual soil moisture stress of 60 and 40 % FC by gravimetric approach (Govind et al. 2009). The transcript levels were significantly increased at 60 % FC moisture levels (Fig. 1d). This confirms the stress-inducible nature of *AhNAC2-2*.

Further to study the functional relevance of *AtNAC2*, an overexpression cassette was developed by subcloning

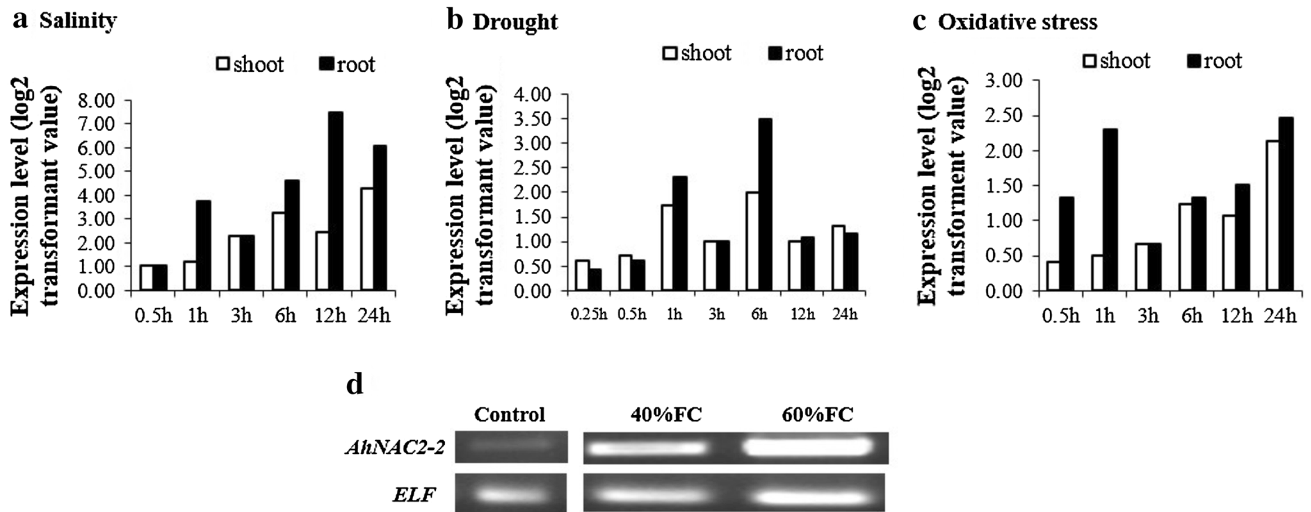

Fig. 1 In silico analysis of *AtNAC2* and expression analysis of *AhNAC2-2*. e-Northern expression data of *AtNAC2* under different stresses like salinity (**a**), drought (**b**), and oxidative stress (**c**). RT-PCR analysis of *AhNAC2-2* (*AtNAC2* homolog) in groundnut at different field capacity (FC) levels (60 and 40 %) (**d**)

Fig. 2 Analysis of T_1 transgenics, binary vector with *AtNAC2* gene cassette (**a**), phenotypic variation in *AtNAC2* transformants (**b**), electrolyte leakage in groundnut transgenics (**c**)

AtNAC2 into p*BinAR* binary vector under CaMV 35S promoter having neomycin-phosphotransferase gene (*npt II*) as a plant selection marker (Fig. 2a). The binary vector was mobilized into *Agrobacterium* strain *LBA4404* and subsequently the gene construct was transferred to groundnut cv. K-134 using in planta transformation method.

Analysis of T_1 transformants

In planta transformation results in the production of many chimeras in the T_0 generation and, hence, T_1 generation seeds were screened using GRID PCR analysis (Ramu et al. 2012). PCR analysis using 35S CaMV promoter forward primer and gene-specific reverse primer indicated that a few plants were positive. The PCR-positive plants were advanced to next generation to study the stress response. Under normal growth conditions, the transgenics showed round and thicker leaves compared to wild-type (Fig. 2b). Transgenic and wild-type plants raised in pots were subjected to moderate drought stress by withholding irrigation for 72 h to reduce the soil water status to 60 % FC and analyzed for membrane integrity in leaf tissue. The transgenic plants showed relatively less membrane damage compared to wild-type (Fig. 2c). Based on the molecular and physiological data, 29 superior plants were advanced to the next generation.

Analysis of T_2 transformants

Seeds from 29 T_1 transgenic lines were raised in containers with 10 kg soil + farmyard manure (FYM) mixture at 3:1 ratio and grown into T_2 plants. After 120 days from sowing, selected plants were harvested, and growth and yield parameters like plant height, branch number per plant, filled pods, unfilled pods, and dry pod weight per plant were recorded. The transgenic lines showed improved growth and productivity (Table 1). Out of 29 T_2 transgenics, 8 lines showed higher pod weight and pod number. The PCR analyses of lines further confirmed the integration of *AtNAC2* gene in groundnut genome (Fig. 3a, b).

AtNAC2 transgenic lines showed improved tolerance to abiotic stress

To assess the stress resistance capacity of *AtNAC2* transgenics and wild-type plants, excised leaves from 35-day-old plants were collected and subjected to a few drought-screening assays in laboratory conditions like chlorophyll stability index (CSI), SLA, RWC, and relative water loss (RWL). The transgenic lines showed significantly higher CSI, RWC, and reduced RWL than wild-type plants. Whereas, in the vcase of SLA, there was not much difference between wild-type and transgenic lines (Fig. 4a–d).

Table 1 Plant height, pod weight, and total dry matter (TDM) at harvest of *AtNAC2* transgenics (T_2 generation) and wild-type plants

Line no.	Plant height (cm)	Pod weight (g/plant)	TDM (g/plant)
1452-3	$47.7^{a} \pm 2.3$	$27.2^{bc} \pm 1.7$	$57.2^{a} \pm 1.5$
1747-4	$40.0^{ab} \pm 2.0$	$25.5^{c} \pm 1.6$	$48.2^{ef} \pm 1.4$
1506-8	$45.7^{a} \pm 2.1$	$29.5^{b} \pm 1.0$	$54.3^{b} \pm 2.8$
1285-2	$37.3^{ab} \pm 1.5$	$30.5^{a} \pm 1.4$	$53.7^{bc} \pm 0.4$
1374-5	$45.0^{a} \pm 3.0$	$28.6^{b} \pm 1.3$	$51.6^{cd} \pm 2.5$
1486-1	$39.3^{ab} \pm 1.5$	$28.5^{b} \pm 1.2$	$49.4^{de} \pm 1.1$
1701-3	$44.0^{ab} \pm 2.6$	$28.2^{b} \pm 0.8$	$48.1^{ef} \pm 1.7$
1390-2	$40.3^{ab} \pm 1.5$	$28.6^{b} \pm 1.1$	$49.9^{de} \pm 1.8$
Mean	42.4 ± 2.1	28.3 ± 1.2	51.5 ± 1.5
K-134 (WT)	$32.4^{b} \pm 2.4$	$25.7^{c} \pm 0.8$	$46.2^{f} \pm 0.2$
LSD at α 0.05	10.94	1.720	2.353

Lowercase letters indicate orders obtained by Duncan's multiple range test

Mean mean ± SD, *WT* wild-type

Similarly, excised leaves were used to assess the electrolyte leakage by exposing to desiccation stress in laboratory condition by air drying for 4 h (mild stress) and 12 h (severe stress). Transgenic plants showed an average of 42.47 % leakage under mild stress and 49.35 % under severe stress, whereas wild-type had shown 46.24 % under mild stress and 68.47 % under severe stress condition respectively (Table 2a).

Further, the chlorophyll content in transgenic under mild stress was 1.93 mg g FW^{-1} as against 1.4 mg g FW^{-1} in wild-type (Table 2b).

To study the relative salinity tolerance capacity of transgenics, the leaf discs were floated on 300 mM NaCl solution for 72 h and then chlorophyll content was measured. Transgenic lines showed an average chlorophyll content of 2.20 and 1.35 mg g FW^{-1} under control and NaCl stress conditions, respectively, whereas wild-type had 1.93 and 0.97 mg g FW^{-1}, respectively (Table 2b).

These results indicate improved growth and yield characteristics in the transgenics compared to wild-type. The TDM was significantly improved in transgenics in both T_2 (Table 1) and T_3 generation (Table 3). A positive correlation between TDM and pod weight (Fig. 4e) signifies the importance of the transgene.

Expression analysis of *AtNAC2* in groundnut transgenics

To analyze the transcript levels of *AtNAC2*, four PCR positive lines (Fig. 5a, b) with higher TDM (Table 3) were

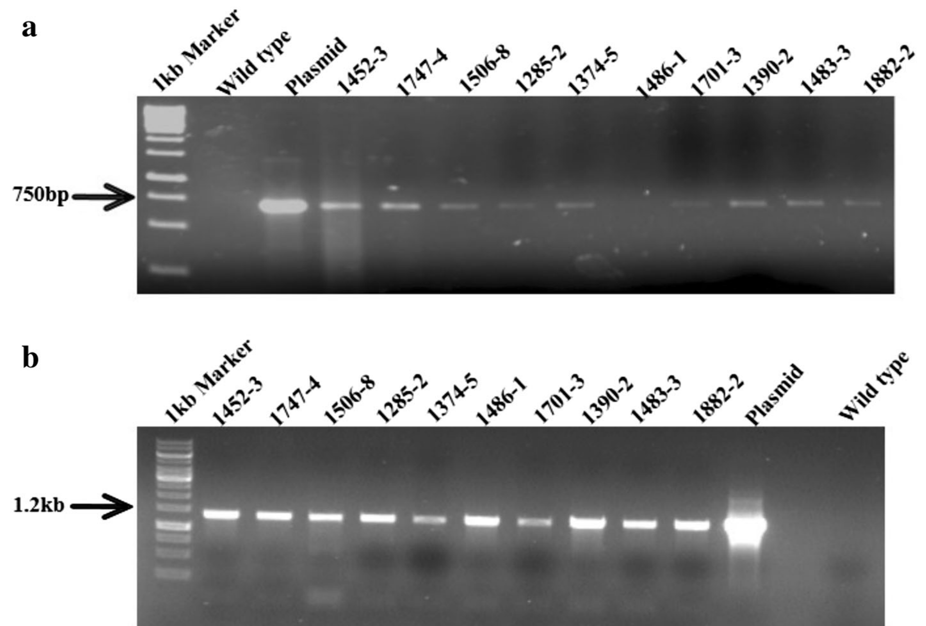

Fig. 3 Molecular confirmation of T_2 transgenics through PCR analysis using *npt II* (**a**) and *AtNAC2* gene-specific primers (**b**)

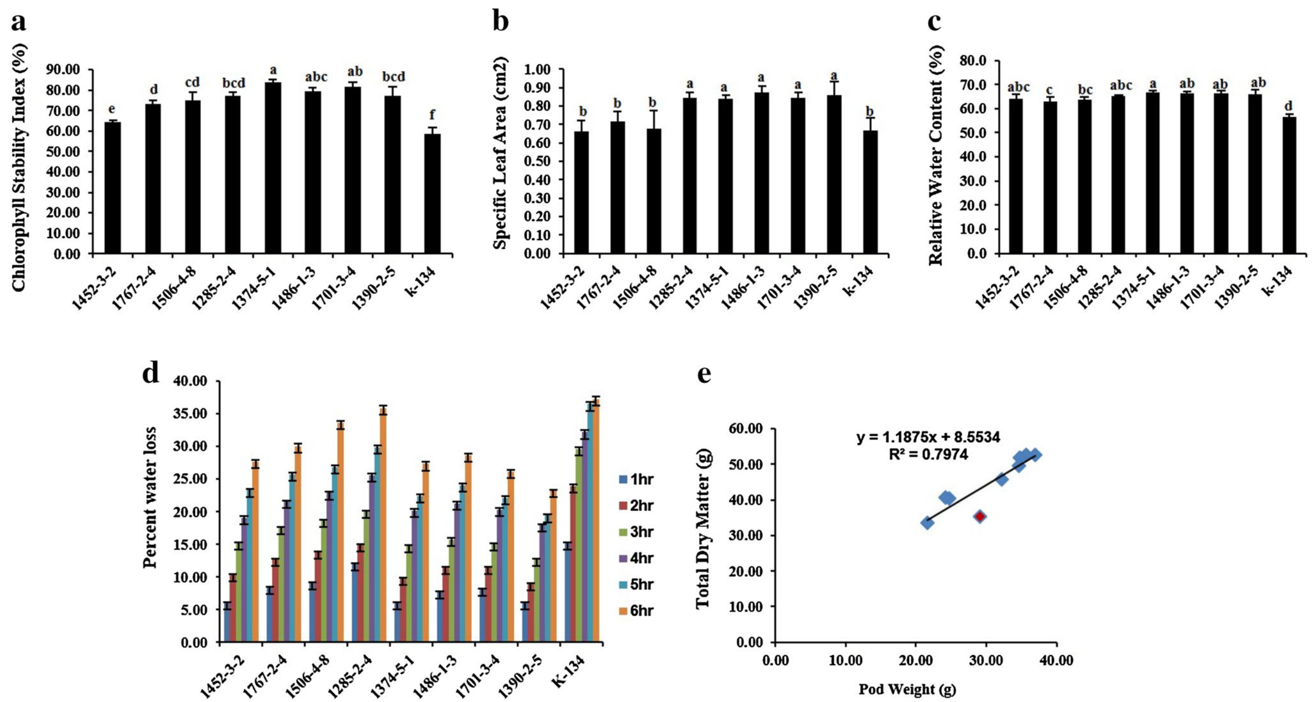

Fig. 4 Physiological analysis of T_3 *AtNAC2* transgenics. Assessment of chlorophyll stability index (**a**), specific leaf area (**b**), relative water content (**c**), and relative water loss (**d**), correlation between pod weight and TDM of T_3 generation *AtNAC2* transgenics and wild-type plants (**e**)

selected for RT-PCR analysis. Total RNA was isolated and cDNA was synthesized and amplified with *npt II* and *At-NAC2* gene specific primers. The transcript levels of these genes were significantly higher in transgenic lines, whereas wild-type plant did not show any expression of either of the genes (Fig. 5c).

Discussion

Plants exposed to desiccation stress alter cell metabolism for adaptation by expression of several stress-responsive genes. Many stress-responsive genes are up-regulated and some are down-regulated in many species and several

Table 2 Physiological analysis of T_3 *AtNAC2* transgenics and wild-type plants under both mild stress and severe desiccation stress conditions in laboratory

Lines	Membrane leakage under mild stress (%)	Membrane leakage under severe stress (%)
(a) Assessment of membrane electrolyte leakage (%)		
1452-3-2	46.91	52.13
1767-2-4	45.85	52.20
1506-4-8	45.00	54.82
1285-2-4	37.36	41.28
1374-5-1	43.75	50.89
1486-1-3	39.96	48.15
1701-3-4	38.77	42.64
1390-2-5	42.11	52.73
Mean	42.47	49.35
K-134 (WT)	46.24	68.47

Lines	Control	Mild desiccation stress	NaCl stress (300 mM)
(b) Influence of mild desiccation stress and NaCl-induced stress on total chlorophyll content (mg g FW^{-1})			
1452-3-2	1.73	1.71	1.37
1767-2-4	1.94	1.67	1.38
1506-4-8	1.97	1.84	1.32
1285-2-4	2.43	1.90	1.12
1374-5-1	2.45	2.04	1.42
1486-1-3	2.27	1.87	1.17
1701-3-4	2.40	1.99	1.49
1390-2-5	2.41	2.39	1.52
Mean	2.20	1.93	1.35
K-134 (WT)	1.93	1.40	0.97

Table 3 Plant height, pod weight, and total dry matter at harvest of *AtNAC2* transgenics (T_3 generation) and wild-type plants

Line no.	Plant height (cm)	Pod weight (g/plant)	TDM (g/plant)
1452-3-2	$36.33^{abc} \pm 0.6$	$24.73^{e} \pm 0.9$	$40.47^{c} \pm 1.6$
1767-2-4	$35.33^{bc} \pm .3.2$	$21.67^{f} \pm 0.8$	$33.43^{d} \pm 1.1$
1506-4-8	$35.33^{bc} \pm 0.6$	$24.23^{e} \pm 1.6$	$40.67^{c} \pm 0.7$
1285-2-4	$39.33^{a} \pm 1.5$	$32.20^{c} \pm 0.7$	$45.80^{b} \pm 1.9$
1374-5-1	$38.33^{ab} \pm 1.2$	$36.93^{a} \pm 1.4$	$52.53^{a} \pm 1.1$
1486-1-3	$37.00^{abc} \pm 1.0$	$35.63^{ab} \pm 1.2$	$52.70^{a} \pm 2.0$
1701-3-4	$38.00^{ab} \pm 1.0$	$34.80^{ab} \pm 1.9$	$52.00^{a} \pm 1.8$
1390-2-5	$37.33^{ab} \pm 1.2$	$34.67^{b} \pm 0.4$	$49.70^{a} \pm 2.6$
Mean	37.13 ± 1.42	30.61 ± 6.06	45.91 ± 7.11
K-134 (WT)	$34.00^{c} \pm 1.7$	$22.20^{d} \pm 1.2$	$35.13^{d} \pm 0.6$
LSD at α 0.05	2.752	2.070	2.885

Lowercase letters indicate orders obtained by Duncan's multiple range test

Mean mean ± SD, *WT* wild-type

stress-specific TFs have been well studied (Babitha et al. 2013). The NAC family TFs are a large class of TFs and they play diverse roles in the development and responses to environmental stimuli (Puranik et al. 2012; Ramegowda et al. 2012). Only a few stress-specific NAC proteins have been characterized (Nakashima et al. 2012) for their relevance in imparting tolerance to abiotic stresses. Over 140 putative *NAC* or *NAC-like* genes have been identified in rice and classified into different groups based on trait specificity (Fang et al. 2008). Phylogenetic analysis of deduced amino acid sequences of *AtNAC2* with other closest accessions of NAC proteins showed that this gene belongs to the NAC family TF (supplemental Fig. 1) which are reported to have a stress-responsive nature. AtNAC2 is also stress responsive as confirmed by e-northern analysis (Fig. 1a–c). *AtNAC2* and other NAC proteins have been found to be transcriptional activators (Aida et al. 1997; Fujita et al. 2004; Tran et al. 2004; Xie et al. 2000). The overexpression of *AtNAC2* in *Arabidopsis* showed improved tolerance to salt stress and have a role in lateral root development and involved in ethylene and auxin signaling (He et al. 2005). The *AhNAC2-2* gene (a homolog to *AtNAC2*) also showed the stress inducibility under moisture stress condition in groundnut (Fig. 1d). Previously, it was shown that *AhNAC2*-1 protein binds to specific NAC response elements (NACRE) in vitro and play a role in ABA signaling (Liu et al. 2011).

Overexpression of *Triticum aestivum NAC2* in *Arabidopsis* was shown to enhance primary root growth, early flowering, reduced water loss, higher leaf water status, higher cell membrane stability, better osmotic adjustment, and increased biomass under stress (Mao et al. 2012). Similarly, *AtNAC2* groundnut transgenics showed reduced rate of water loss and maintained higher RWC as compared to wild-type (Fig. 4c, d). This clearly indicates that transgenics have higher ability to retain water and maintain higher leaf water status. Maintenance of higher chlorophyll content which reflects the stay-green nature of *AtNAC2* transgenics (Fig. 4a) (Table 2b) under stressful condition is one of the important parameters which result in maintenance of higher canopy photosynthetic rate during stress. The transgenics also showed reduced membrane damage under stress (Table 2a). Maintaining better membrane integrity will help in functional stability of macromolecules (Levitt 1980). Similar stress responses were noticed in transgenics overexpressing *SNAC2* and *AhNAC2-1* in rice and *Arabidopsis*, respectively (Hu et al. 2008; Liu et al. 2011).

Several transgenic plants with relevant regulatory genes have been developed in different crop species for abiotic stress conditions (Karaba et al. 2007; Bhatnagar et al. 2008; Anami et al. 2009; Yoshida et al. 2010; Jeong et al. 2010). In this context, our data on overexpression of *AtNAC2* in

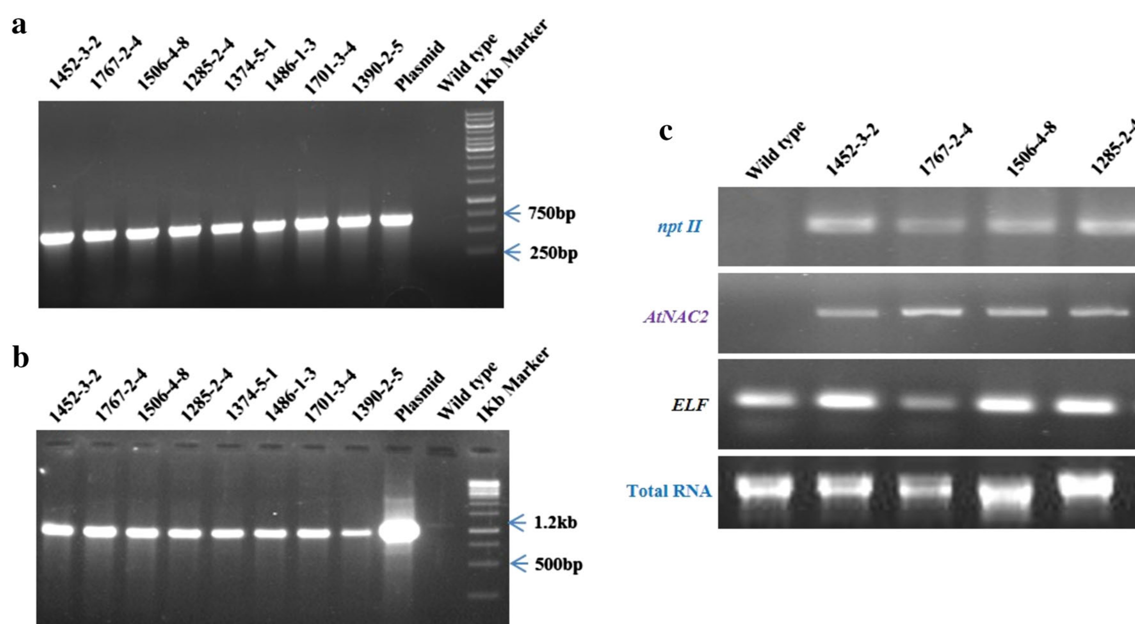

Fig. 5 Molecular confirmation of T_3 generation transgenics by PCR analysis with *npt II* (**a**) and *AtNAC2* gene specific primers (**b**) using genomic DNA as template and RT-PCR analysis with *npt II* and *AtNAC2* gene specific primers using cDNA as template (**c**). Elongation factor (*ELF*) was used as loading control

groundnut strengthens the idea of using regulatory genes as candidate genes to improve stress tolerance of crops.

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