Oryza Vol. 52 No.4, 2015 (238-244)

Phenotypic characteristics of *OsBAT1* transgenic rice plants in T_3 generation

Ranjan Kumar Sahoo[1], Durga Madhab Swain[1] and Narendra Tuteja[*1, 2]

[1]International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India

[2]Amity Institute of Microbial Technology, Amity University, Noida - 201313, India

**Email: narendra@icgeb.res.in; ntuteja@amity.edu*

ABSTRACT

OsBAT1 is homologous gene of human leukocyte antigen-B associated transcript 1 (BAT1) isolated from rice. It is also called as UAP56, which is a DExD/H-box protein involved in messenger RNA (mRNA) splicing. The function of plant homologue of BAT1, isolated from rice (OsBAT1) especially its involvement in stress tolerance, has been reported in T_1 and T_2 generation. Here, we demonstrate the novel function of OsBAT1 in salinity stress tolerance in rice (Oryza sativa L. cv. IR64) in T_3 generation. Rice over expressing OsBAT1 (T_3 generation) showed tolerance to high salinity (200 mM NaCl) stress. The T_3 transgenics exhibited higher levels of biochemical parameters such as water and chlorophyll contents, net photosynthetic rate, stomatal conductance and intercellular CO_2 content as compared to wild type (WT) plants. Agronomic parameters were also higher in transgenics as compared to WT plants. Our results provide the first direct evidence for a promising function of OsBAT1 in mediating salinity stress response/tolerance in rice and its stability up to T_3 generation.

Key words: *Transgenic rice, salinity stress, photosynthetic characters, ROS*

Rice (*Oryza sativa*) is an important cereal crop that provides a staple diet for almost half of the world's population and is the major food crop cultivated in Asia. The quality and yield of rice is greatly affected by environmental stresses such as salinity, drought, heat and cold. It has been estimated that by 2025, the global population would require about 800 million tonnes (mt) of rice. The growth and productivity of crops decreases due to abiotic stresses. It acts by decreasing photosynthesis, germination percentage and biomass as well as increasing the generation of reactive oxygen species (ROS) (Hadiarto and Tran 2011). Among different abiotic stresses, salinity is a widespread soil problem that limits crop productivity worldwide. This salinity causes severe deterioration of agricultural lands especially in the tropical countries (Mahajan and Tuteja 2005; Munns and Tester, 2008). Different studies done by researchers suggest that the introduction of specific foreign genes into crop plants provides resistance against biotic and abiotic stresses (Xiong *et al.*, 2006; Mazzucotelli *et al.*, 2008; Chen *et al.* 2013). Earlier studies had revealed that some RNA/DNA helicases also play an important role in the abiotic stress management/ resistance (Liu *et al.*, 2002; Vashisht and Tuteja 2006; Kant *et al.*, 2007; Li *et al.* 2008). In rice, the *OsBIRH1* encodes a functional DEAD-box RNA helicase that functions in defence responses against biotic and abiotic stresses when over expressed (Li *et al.*, 2008).

The role of RNA helicases have been reported to perform defined roles during environmental stresses, and their expression and/ or activity is frequently altered during cellular response to abiotic stress (Owttrim, 2013). Recently, the over expression of RNA helicases *OsSUV3* and *OsBAT1* (T_1 and T_2 generation) has been reported to impart salinity stress tolerance in rice plants without yield loss (Tuteja *et al.*, 2013, 2015). However, the role of BAT1 rice homologue (here termed as *OsBAT1*) in salinity stress tolerance has been reported in T_1 and T_2 generation. To check the stability and

function of the gene, we have developed T_3 generation transgenic rice plants (*Oryza sativa* L., cv. IR64) over expressing *OsBAT1* and studied its function under salinity stress.

MATERIALS AND METHODS

Analysis of T_3generation OsBAT1 plant through PCR, and histochemical assay for â-Glucuronidase (GUS) activity

The integration of *OsBAT1* gene was confirmed through PCR, using genomic DNA extracted from the healthy leaves (0.5 g) of T_3 generation transgenic plants. The PCR amplification was performed with 0.15-0.20 ìg of genomic DNA, using promoter specific forward and gene (*OsBAT1*) specific reverse primers. GUS activity was assayed histochemically by a procedure described by Jefferson *et al.*, (1987) using the indigogenic substrate X-gluc (5-bromo-4-chloro-3-indolyl b-D-glucuronide).

RNA Isolation and quantitative real-time PCR (qRT-PCR)

21 days-old T_3 *OsBAT1* over expressing transgenic and WT rice seedlings were grown on hydroponic system and then treated with 200 mM NaCl for 24 h. The total RNA was isolated using the Trizol reagent (Invitrogen Life Technologies, USA) as per the manufacturer's instructions. cDNA was prepared from total RNA by using RevertAid H minus first strand cDNA synthesis kit (Fermentas, Life Sciences). Expression analysis of *OsBAT1* gene was performed by qRT-PCR as described earlier (Jayaraman *et al.*, 2008).

Leaf disk senescence assay for salinity tolerance

Healthy and fully matured rice leaf squares of 1cm×1cm dimension were taken from similar age transgenic lines (L5, L8, L12 and L17) of T_3 generation along with WT plants. The leaf disks were floated in 100 and 200 mM solution of NaCl for 72 hours. Then the total chlorophyll content was measured as described earlier (Tuteja *et al.*, 2015).

Measurement of photosynthetic characteristics

An infra-red gas analyzer (IRGA, LiCor, Lincoln, NE, USA) was used on a sunny day between 10:00 and 12:00 h to estimate net photosynthetic rate (Pn), stomatal conductance (gs) and intercellular CO_2 concentration (Ci) on the fourth and fifth fully expanded leaves of transgenic lines (L5, L8, L12 and L17) and the WT plants. The atmospheric conditions during the measurement were photosynthetically active radiation (PAR), 1,050 ± 7lmol m-2 s-1, relative humidity 66 ± 4%, atmospheric temperature 24 ± 2^0C and atmospheric CO_2, 350 µmol mol^{-1}.

Agronomic characteristics of T_3 *OsBAT1* over expressing transgenic plants

Several agronomic parameters like plant height, number of tillers/plant, number of panicles/plant, number of filled grains/panicle, number of chaffy grains/panicle, straw dry weight, 100 grain weight, root length, root dry weight, leaf area and plant dry weight were measured at 3rd weeks after initiating the 200 mM NaCl treatment in T_1 transgenic, and water grown WT plants. Growth and yield performance of the transgenic lines survived after salinity stress was compared with WT plant as described earlier (Tuteja *et al.* 2015).

Estimation of endogenous ion content

Endogenous ion content like nitrogen, phosphorus, potassium and sodium were estimated from leaves of T_3 transgenic plants grown on 200 mM NaCl grown and WT plants grown on water. All the experiments were conducted according to the methods described earlier (Tuteja *et al.* 2015).

Statistical analyses

Data were obtained from at least three independent experiments. The mean values obtained are presented as means standard errors of the mean, with a minimum of three replicates. Analysis of one-way variance (ANOVA) was performed on the data using SPSS (12.0 Inc., USA) to determine the least significant difference (LSD) for the significant data to identify the differences in the mean among the treatments. The means were separated by Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

Molecular analysis of the *OsBAT1* over expressing T_3transgenic plants

The construct pCAMBIA1301-*OsBAT1* was used for development of transgenic IR64 rice plants (Figure 1a). Phenotypically there were no significant differences between the transgenic lines (L5, L8, L12 and L17) as compared to the WT plants (Figure 1b). The amplification of transgene (*OsBAT1*) was confirmed

by PCR using promoter (CaMV35S) specific forward and gene specific reverse primers and the fragment of expected size (~1.4 kb) was obtained (Figure 1c). The quantitative real-time PCR (qRT-PCR) showed ~9 fold induction in the transcript level of sense transgenic lines (L5, L8, L12 and L17) as compared to WT plants under normal (unstressed) condition (Figure 1d). The Gus activity was visualized (blue colour) in leaf tissues of all the transgenic lines (L5, L8, L12 and L17) and no blue colour was observed in WT plants (Figure 1eHHH).

OsBAT1 over expressing transgenic plants show tolerance to salinity

To test the salinity tolerance, leaf pieces (~1cm×1cm) from T_1 sense transgenic lines (L5, L8, L12 and L17) and WT plants were floated separately on 100 and 200 mM NaCl for 72h. The loss of chlorophyll was lesser in transgenic lines as compared to WT plants (Figure 1f). More chlorophyll content also found in leaf of transgenic lines compared to WT (Figure 1g). It is evident that transgenic plants have more tolerance to salinity stress.

Agronomic attributes and photosynthetic characteristics of *OsBAT1* T_3 transgenic plants

OsBAT1 over expressing transgenic rice plants showed better performance in growth parameters like plant height, number of tillers/plant, number of panicle/plant, number of filled grain/panicle, number of chaffy grains/ panicle, straw dry weight, 100 grain weight, root length, root dry weight, leaf area, root and shoot lengths as compared to WT plants under salinity stress (200 mM NaCl) (Table 1). The photosynthetic characteristic like net photosynthesis rate (Pn), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were higher in transgenic plants as compared to the WT plants under salinity stress (200 mM NaCl). Overall, WT plants showed more damage as compared to transgenic plants (Table 1).

Endogenous ion and chlorophyll content in transgenic plants

Under salinity stress (200 mM NaCl) more accumulation of nitrogen, phosphorus, potassium ions and less accumulation of sodium was found in transgenic lines as compared to the WT plants (Table 1). More chlorophyll content was found in transgenic lines as compared with WT plants under salinity stress (Table 1). Several yield attributes such as days required for flowering, number of tillers/plant, panicles/plant, filled grain/panicle, chaffy grain/panicle, 100 grain weight at 200 mM NaCl were recorded in transgenic lines and found to be almost similar to the WT plants grown in water (0 mM NaCl) (Table 2). However, the WT plants did not survive till flowering stage at 200 mM NaCl stress (Table 2).

The present study was conducted in order to study the stability and salinity tolerance property of OsBAT1 in T_3 generation. Among multiple abiotic stresses, salinity severely affects the whole plant machinery and productivity. The new emerging role of helicases in plant abiotic stress tolerance such as salinity and drought has been reported in earlier studies (Sanan-Mishra *et al.*, 2005; Owttrim, 2006, 2013; Vashisht and Tuteja, 2006; Umate *et al.*, 2010; Sahoo *et al.*, 2012, Gill *et al.*, 2013; Tuteja *et al.*, 2013, 2015). The understanding of mechanism, ion homeostasis and detoxification of ROS are important steps for tolerance against salinity stress (Munns and Tester, 2008).

OsBAT1 over expressing transgenic lines of T_3 generation were raised and four transgenic lines (L5, L8, L12 and L17) were carried forward for study its function under stress. The expression of transgene and the visualization of GUS activity confirm the integration of transgene in the overexpressing lines. Significant tolerance against salinity stress in transgenic lines was observed by leaf disk senescence assay. The transgenic lines exhibited normal growth as WT plants under control conditions and much better growth than WT plants in salinity stress conditions indicating positive influence of the transgene on the plant. The transgenic lines also maintained higher endogenous nutrient contents as compared to WT plants under salinity stress, which revealed the salinity tolerance potential of the transgenic lines. Similar findings were reported earlier in different varieties of rice (Amin *et al.*, 2012; Sahoo *et al.*, 2012; Tuteja *et al.*, 2013) and tobacco by over expression of different genes for e.g. glyoxalase, *PDH45*, *PsMCM6* and *OsCBSX4* (Singla-Pareek *et al.*, 2003; Sanan-Mishra *et al.*, 2005; Dang *et al.*, 2011; Singh *et al.*, 2012). Higher concentration of potassium and lower concentration of sodium were found in leaves of *OsBAT1* over expressing transgenic lines than WT plants under salinity stress. It indicates that the over

Table 1. Phenotypic attributes of wild type (WT) and T_3 generation of *OsBAT1* over expressing transgenic lines (Line 5, Line 8, Line 12 and Line 17) of rice (*Oryza sativa* L. cv. IR64) grown under 0 or 200 mM NaCl

Attributes / NaCl conc (mM)	Wild type plants		T_3 *OsBAT1* transgenic plants							
			Line 5		Line 8		Line 12		Line 17	
	0	200	0	200	0	200	0	200	0	200
Plant height(cm)	72±3.2^{a}	32.6±1.3^{b}	76±3.2^{a}	71±3.5^{a}	76±3.1^{a}	72±3.2^{a}	79±3.0^{a}	73±3.1^{a}	77±3.2^{a}	71±3.1^{a}
Root length (cm)	28±0.8^{ab}	11.6±0.03^{b}	29±1.0^{a}	23±1.0^{ab}	28±1.4^{a}	24±1.4^{ab}	27±1.2^{a}	26±1.0^{a}	28±1.3^{a}	23±1.0^{a}
Root dry weight (g)	2.6±0.12^{b}	1.4±0.02^{c}	2.9±0.14^{a}	2.1±0.13^{a}	3.0±0.12^{a}	2.7±0.15^{a}	2.8±0.2^{a}	2.2±0.16^{a}	2.7±0.1^{a}	2.3±0.15^{a}
Leaf area (cm^2/plant)	91±2.4^{ab}	44.72±2.0^{c}	96±1.0^{a}	83±1.6^{ab}	97±1.0^{a}	85±1.5^{ab}	96±1.6^{a}	84±1.5^{ab}	97±1.5^{a}	86±1.4^{ab}
Total chlorophyll (mg/g f wt)	9.04±0.22^{b}	2.14±0.08^{c}	9.24±0.5^{a}	9.04±0.4^{a}	9.25±0.5^{a}	9.01±0.3^{a}	9.04±0.3^{a}	9.02±0.3^{a}	9.14±0.4^{a}	8.04±0.5^{a}
Total protein (mg/g f wt)	1.77±0.55^{b}	0.64±0.34^{c}	1.92±0.88^{a}	1.81±0.87^{ab}	1.97±0.91^{ab}	1.78±0.85^{ab}	1.92±0.85^{a}	1.7±0.88^{a}	1.94±0.55^{a}	1.76±0.83^{a}
Net photosynthetic rate (P_N, μ mol CO_2 $m^{-2}s^{-1}$)	9.26±0.5^{b}	6.02±0.23^{c}	10.32±0.6^{a}	8.24±0.6^{a}	10.52±0.1^{a}	9.34±0.3^{a}	10.05±0.4^{a}	9.14±0.5^{a}	10.04±0.3^{a}	8.64±0.5^{a}
Stomatal conductance (gs, m mol $m^{-2}s^{-1}$)	231±11.4^{a}	104.3±5.2^{b}	238±10.9^{a}	201±11.5^{a}	246±10.9^{a}	209±10.8^{a}	241±10.2^{a}	212±10.2^{a}	251±11.5^{a}	206±10.1^{a}
Intracellular CO_2 (*Ci*, μ mol mol^{-1})	220±11.2^{a}	101.1±4.3^{b}	222±10.2^{a}	207±10.2^{a}	226±10.6^{a}	201±10.1^{a}	222±11.4^{a}	205±10.1^{a}	228±10.4^{a}	206±10.2^{a}
Nitrogen (%)	0.282±0.011^{b}	0.102±0.004^{c}	0.302±0.012^{a}	0.283±0.012^{a}	0.314±0.011^{a}	0.282±0.011^{a}	0.307±0.012^{a}	0.286±0.011^{a}	0.311±0.011^{a}	0.281±0.012^{a}
Phosphorus (%)	0.242±0.011^{b}	0.121±0.002^{c}	0.242±0.010^{a}	0.233±0.010^{a}	0.271±0.011^{a}	0.242±0.011^{a}	0.272±0.011^{a}	0.251±0.011^{a}	0.252±0.011^{a}	0.241±0.010^{a}
Potassium (%)	0.132±0.003^{b}	0.082±0.002^{c}	0.145±0.002^{a}	0.136±0.002^{a}	0.156±0.002^{a}	0.132±0.002^{a}	0.162±0.001^{a}	0.146±0.005^{a}	0.151±0.001^{a}	0.140±0.005^{a}
Sodium (%)	0.043±0.001^{a}	0.051±0.001^{a}	0.042±0.001^{a}	0.046±0.001^{a}	0.045±0.001^{a}	0.045±0.001^{a}	0.047±0.001^{a}	0.045±0.001^{a}	0.044±0.001^{a}	0.043±0.001^{a}

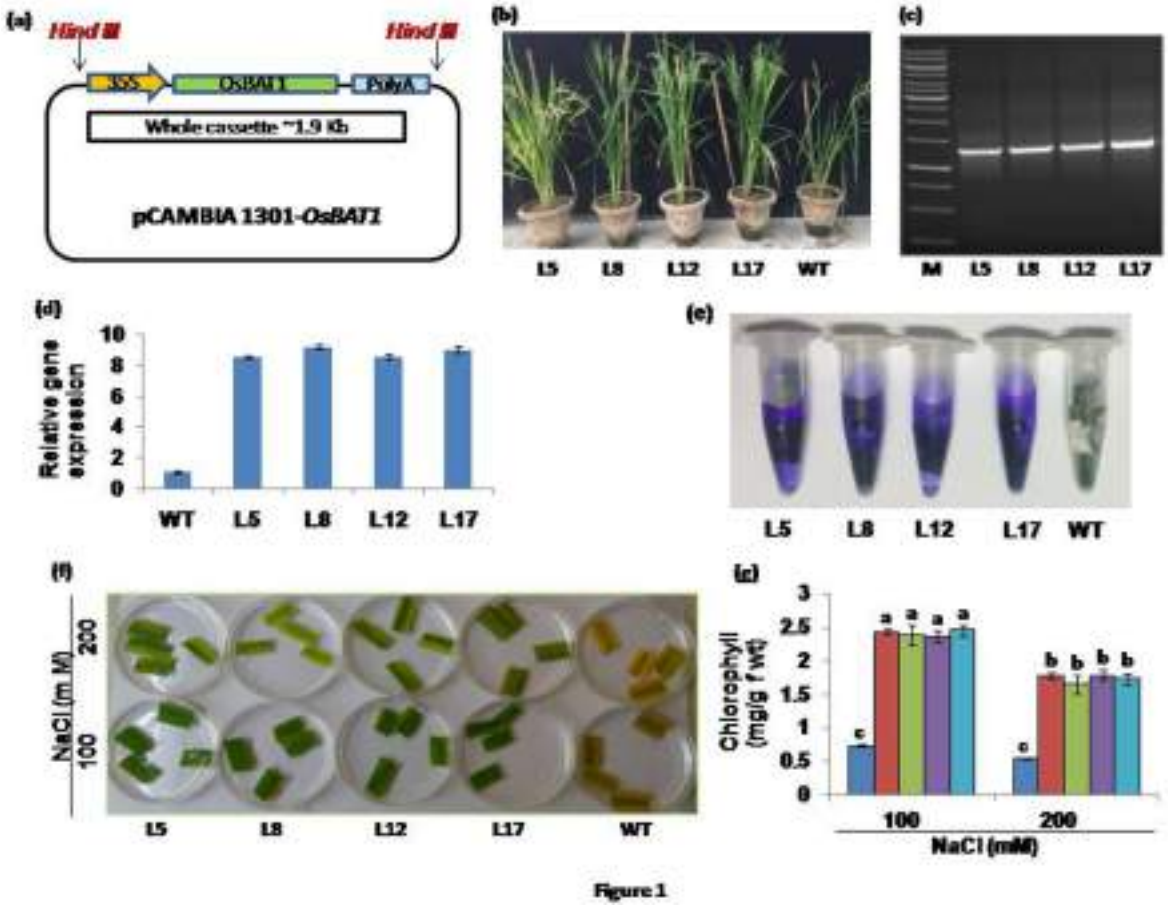

Figure 1

LEGENDS TO FIGURES

Fig. 1. Analysis of *OsBAT1* overexpressing T_3 transgenic IR64 rice plants. (a) Schematic diagram of pCAMBIA1301 containing *OsBAT1* gene (1.3 kb) in *HindIII* restriction enzyme site. (b) Overexpressing *OsBAT1* T_3 transgenic lines with WT plants were used for analysis. (c) Confirmation of transgenic (T_3) lines through PCR analysis of the *OsBAT1* gene by the use of CaMV35S promoter specific forward and *OsBAT1*gene specific reverse primers showing the required amplification of 1.4 kb in 4 transgenic lines (L5, L8, L12 and L17). (d) Relative expression of *OsBAT1* gene in WT and transgenic lines under control (unstressed) condition. (e) Visualization of GUS activity in the leaf tissue of transgenic lines. (f) Leaf disk senescence assay for salt tolerance in transgenic rice lines. (g) Chlorophyll content (mg/g f wt) in transgenic lines under 100 and 200 mM NaCl. Each value represents mean of three replicates ± SE. Means were compared using ANOVA. Data followed by the same letters are not significantly different at P<0.05 as determined by least significant difference (LSD) test. [a,b,c] indicate significant differences at P<0.05 level as determined by Duncan's multiple range test (DMRT).

expression of *OsBAT1* may restrict the entry of sodium ions in the leaves of transgenic lines thereby contributing towards protection of photosynthetic machinery from salinity stress.

The *OsBAT1* over expressing transgenic lines retained more chlorophyll than WT under salinity stress, which is in agreement with the earlier reports (Sanan-Mishra *et al.*, 2005; Moradi and Ismail, 2007; Dang *et al.*, 2011; Singh *et al.*, 2012; Sahoo *et al.*, 2012). The photosynthetic activities like net photosynthesis rate (Pn), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were decreased by salinity stress but a lesser reduction was observed in *OsBAT1* transgenic lines as compared to WT plants. The better control over photosynthesis apparatus under salinity stress may be due to retention of chlorophyll content in these transgenic lines. It has been reported earlier also that tolerance in *PDH45* and *SUV3* over expressing rice plants in stress results due to maintenance of the photosynthetic apparatus (Gill and Tuteja, 2010; Tuteja

Table 2. Comparison of various yield parameters of WT and T_3 generation of *OsBAT1* over expressing transgenic lines (Line 5, Line 8, Line 12 and Line 17) of rice (*Oryza sativa* L. cv. IR64) grown under 0 or 200 mM NaCl.

Yield attributes / NaCl conc (mM)	Wild type plants		T_4 *OsBAT1* transgenic plants							
			Line 5		Line 8		Line 12		Line 17	
	0	200	0	200	0	200	0	200	0	200
Time required for flowering (days)	91±2.1^{a}	ND*	92±3.5^{a}	91±2.1^{a}	92±3.0^{a}	91±2.5^{a}	92±2.8^{a}	91±2.8^{a}	91±2.5^{a}	91±2.5^{a}
No. of tillers/plant	23±1.0^{c}	ND	27±0.12^{ab}	24±1.2^{ab}	23±0.14^{ab}	22±1.1^{ab}	28±0.12^{a}	25±1.0^{a}	26±0.12^{a}	23±1.0^{a}
No. of panicle/plant	22±0.5^{c}	ND	25±0.11^{ab}	21±1.0^{ab}	25±0.12^{ab}	21±1.0^{ab}	28±0.15^{a}	23±1.1^{a}	28±0.11^{a}	21±1.1^{a}
No. of filled grain/ panicle	84±3.0^{b}	ND	96±3.2^{a}	81±3.1^{a}	97±3.23^{a}	86±4.0^{a}	98±3.27^{a}	86±3.1^{a}	98±2.87^{a}	85±2.1^{a}
No. of chaffy grains/panicle	11±0.21^{a}	ND	06±0.12^{b}	07±0.11^{b}	04±0.06^{b}	04±0.21^{b}	07±0.21^{b}	07±0.12^{b}	04±0.11^{b}	06±0.13^{b}
Straw dry weight (g)	56±1.2^{b}	ND	56±3.05^{a}	46±2.1^{a}	62±2.1^{a}	62±1.4^{a}	64±1.8^{b}	56±1.3^{a}	62±1.4^{b}	57±1.2^{a}
100 grain weight	2.63±0.1^{a}	ND	2.65±0.12^{b}	2.62±0.12^{a}	2.66±0.12^{b}	2.62±0.10^{a}	2.63±0.14^{b}	2.62±0.10^{a}	2.63±0.12^{b}	2.62±0.11^{a}

ND-No data, * control plants did not survive till harvesting under 200 mM NaCl. Each value represents mean of three replicates ± SE. Means were compared using ANOVA. Data followed by the same letters in a row are not significantly different at $P > 0.05$ as determined by least significant difference (LSD) test. a, b, c indicate significant differences at $P > 0.05$ level as determined by Duncan's multiple range test (DMRT).

et al., 2013).

The exact mechanism of salinity stress tolerance in plants is still not well known. This study offers unique function of *OsBAT1* in providing salinity stress tolerance in transgenic plants without affecting yield. This can also provide a good example of the exploitation of components of nucleic acid metabolism pathways including splicing factor for enhanced agricultural production that withstand the extreme climatic conditions and ensure food security.

ACKNOWLEDGEMENTS

N. T. thanks to Dr. Pawan Umate and Mrs. Taranjot Kaur for their help in initial stages of the work and Dr. Sarvajeet Singh Gill for statistical analysis. The work on helicases in N. T.'s laboratory is partially supported by Department of Biotechnology, Government of India. We do not have any conflict of interest to declare.

REFERENCES

Amin M, Elias SM, Hossain A, Ferdousi A, Rahman MS, Tuteja N and Seraj ZI 2012. Overexpression of a DEAD box helicase, PDH45, confers both seedling and reproductive stage salinity tolerance to rice (*Oryza sativa* L.). Mol. Breed. 30: 345–354.

Chen Y, Li F, Ma Y, Chong K and Xu Y 2013 Overexpression of OrbHLH001, a putative helix-loop-helix transcription factor, causes increased expression of AKT1 and maintains ionic balance under salt stress in rice. J. Plant Physiol. 170: 93–100.

Dang HQ, Tran NQ, Gill SS, Tuteja R and Tuteja N 2011. A single subunit MCM6 from pea promotes salinity stress tolerance without affecting yield. Plant Mol. Biol. 76: 19–34.

Gill SS and Tuteja N 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48: 909–930.

Gill SS, Tajrishi M, Madan M and Tuteja N 2013. A DESD-box helicase functions in salinity stress tolerance by improving photosynthesis and antioxidant machinery in rice (*Oryza sativa* L. cv. PB1). Plant Mol. Biol. 82: 1–22.

Hadiarto T and Tran LS 2011. Progress studies of drought-responsive genes in rice. Plant Cell Rep. 30: 297–310.

Jayaraman A, Puranik S, Rai NK, Vidapu S, Sahu PP, Lata C and Prasad M 2008. cDNA-AFLP analysis reveals

differential gene expression in response to salt stress in foxtail millet (*Setaria italica* L.). Mol Biotechnol. 40: 241–251.

Jefferson RA, Kavanagh TA and Bevan MW 1987. GUS fusions: b glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6: 3901–3907.

Kant P, Kant S, Gordon M, Shaked R and Barak S 2007. STRESS RESPONSE SUPPRESSOR1 and STRESS RESPONSE SUPPRESSOR2, two DEAD-box RNA helicases that attenuate Arabidopsis responses to multiple abiotic stresses. Plant Physiol. 145: 814–830.

Li D, Liu H, Zhang H, Wang X and Song F 2008. OsBIRH1, a DEAD-box RNA helicase with functions in modulating defence responses against pathogen infection and oxidative stress. J. Exp. Bot. 59: 2133–2146.

Liu HY, Nefsky BS and Walworth NC 2002. The Ded1 DEAD-box helicase interacts with Chk1 and Cdc2. J. Biol. Chem. 277: 2637–2643.

Mahajan S and Tuteja N 2005. Cold, salinity and drought stresses: an overview. Arch. Biochem. Biophys. 444: 139–158.

Mazzucotelli E, Mastrangelo AM, Crosatti C, Guerra D and Stanca AM 2008. Abiotic stress response in plants: when posttranscriptional and posttranslational regulations control transcription. Plant Sci. 174: 420–431.

Moradi F and Ismail AM 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann. Bot. 99: 1161–1173.

Munns R and Tester M 2008. Mechanisms of salinity tolerance. *Ann*. Rev. Plant Biol. 59: 651–681.

Owttrim GW 2006. RNA helicases and abiotic stress. Nucleic Acids Res. 34: 3220–3230.

Owttrim GW 2013. RNA helicases: Diverse roles in prokaryotic response to abiotic stress. Plant Sig. Behav. 10: 95–109.

Sahoo RK, Gill SS and Tuteja N 2012. Pea DNA helicase 45 promotes salinity stress tolerance in IR64 rice with improved yield. Plant Signal. Behav 7: 1037–1041.

Sanan-Mishra N, Pham XH, Sopory SK and Tuteja N 2005. Pea DNA helicase 45 overexpression in tobacco confers high salinity tolerance without affecting yield. Proc. Natl. Acad. Sci. USA 102: 509–514.

Singh AK, Kumar R, Pareek A, Sopory SK and Singla-Pareek SL 2012. Overexpression of rice CBS domain containing protein improves salinity, oxidative, and heavy metal tolerance in transgenic tobacco. Mol. Biotechnol. 52: 205-16.

Singla-Pareek SL, Reddy MK and Sopory SK 2003. Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. Proc. Natl. Acad. Sci. USA 100: 14672–14677.

Tuteja N, Sahoo RK, Garg B and Tuteja R 2013. OsSUV3 dual helicase functions in salinity stress tolerance by maintaining photosynthesis and antioxidant machinery in rice (*Oryza sativa* L. cv. IR64). The plant J. 76, 115-127. DOI: 10.1111/tpj.12277.

Tuteja N, Sahoo RK, Huda KMK, Tula S and Tuteja R 2015. OsBAT1 augments salinity stress tolerance by enhancing detoxification of ros and expression of stress-responsive genes in transgenic rice. Plant Mol. Biol. Rep. 10.1007/s, 11105-014-0827-9.

Umate P, Tuteja R and Tuteja N 2010. Genome-wide analysis of helicase gene family from rice and Arabidopsis: a comparison with yeast and human. *Plant Mol. Biol.* 73: 449–465.

Vashisht AA and Tuteja N 2006. Stress responsive DEAD-box helicases: a new pathway to engineer plant stress tolerance. J. Photochem. Photobiol. *B.* 84: 150–160.

Xiong LZ, Hu HH, Dai MQ, Yao JL, Xiao BZ 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc. Natl. Acad. Sci. USA. 103: 12987–12992.