Identification and Characterization of OsWRKY72 Variant in Indica Genotypes

Narasimha ASHWINI, Radha Sivarajan SAJEEVAN, Makarla UDAYAKUMAR, Karaba Nalkur NATARAJA

(Department of Crop Physiology, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bangalore, Karnataka 560065, India)

Abstract: Plant WRKY transcription factors (TFs) constitute one of the largest families of proteins involved in biotic and abiotic stress responses. These TFs have a conserved 60 amino acid WRKY domain at the N-terminal and a zinc finger motif at the C-terminal. To examine the relevance of OsWRKY72 in imparting salinity stress tolerance, two indica rice genotypes, Rasi (tolerant genotype) and Tellahamsa (susceptible genotype), were used. In Rasi seedlings at 12 h under 100 mmol/L NaCl stress, OsWRKY72 expression was up-regulated, whereas in Tellahamsa, it was highly up-regulated at lethal stress. Full-length OsWRKY72 cDNA was cloned from these two rice genotypes for further analysis. We identified a variant, termed as OsWRKY72b that carries an additional sequence of 111 bp within the WRKY domain. Expression of OsWRKY72b was higher under salinity stress in Rasi than in Tellahamsa. Disorder prediction of OsWRKY72b showed that the additional sequence in the WRKY domain is ordered thereby maintaining the tertiary structure that might interact with the major groove of DNA. Prediction of phosphorylation sites in OsWRKY72b indicated that a few serine residues could be the potential phosphorylation sites. In this study, we firstly reported a OsWRKY72 variant that could have a role in abiotic stress responses.

Key words: Oryza sativa; indica genotype; OsWRKY72 gene; salinity; transcription factor

Transcription factors (TFs) are regulatory proteins that bind to specific sequences of DNA upstream of a gene, leading to activation or repression of any other gene. In plants, these TFs are classified according to the conserved amino acid sequences in their DNA-binding domains, such as AP2/ERF, WRKY and NAC (Yamasaki et al, 2008). WRKY is one of the largest families of TFs with 75 members in Arabidopsis thaliana, 102 in Oryza sativa subsp. indica, and 103 in Oryza sativa subsp. japonica (Song et al, 2010a). WRKY TFs contain a 60 amino acid WRKY domain, which has a conserved motif WRKYGQK at N-terminal, and a zinc finger motif at the C-terminal (Eulgem et al, 2000; Chen et al, 2012). Both the WRKY and zinc finger motifs are required for the binding of TF to DNA (Mao et al, 2001). All the WRKY proteins have a binding preference to a consensus cis-acting element called the W-box (T/CTGACC/T), while a neighboring sequence to this element partly determines which WRKY interacts with it. For example, if G nucleotide is directly adjacent to W-box at the 5'-end, AtWRKY6 and AtWRKY11 bind to the element, whereas if T, C or A is adjacent to the element, AtWRKY26, AtWRKY38 and AtWRKY43 bind to the element (Ciolkowski et al, 2008). Based on the WRKY and zinc finger motifs, WRKY members are classified into three groups: Group I having two WRKY domains and Cys2- His2 motif, group II having one WRKY domain with Cys2- His2 zinc finger motif, and group III with one WRKY domain containing different zinc finger motifs, Cys2-His/Cys or Cys2-His2 (Eulgem et al, 2000; Chen et al, 2012). WRKY proteins regulate developmental processes such as trichome development, seed development (Johnson et al, 2002) and leaf senescence (Hinderhofer and Zentgraf, 2001), although their major roles are in biotic stress.
response and abiotic stress acclimation (Pandey and Somssich, 2009). Expression profiling of 103 OsWRKY genes in young and mature leaves, panicles and roots indicated that 65 of them are expressed in at least one of the tissues, of which 6 genes show root-specific expression while 4 are panicle-specific (Ramamoorthy et al, 2008). Various studies have demonstrated that WRKYs in rice are rapidly induced under abiotic stress such as drought, salinity (Ramamoorthy et al, 2008; Song et al, 2009), heat (Song et al, 2009; Wu et al, 2009) and osmotic stress (Song et al, 2009). A few WRKY genes are also up-regulated upon phytohormone treatment, such as abscisic acid, gibberellic acid, indole acetic acid, methyl jasmonate, and salicylic acid (Ramamoorthy et al, 2008). For example, OsWRKY72 is induced upon abscisic acid treatment in rice aleurone cells (Xie et al, 2005; Li et al, 2015).

One of the major goals of crop biotechnology is to prospect candidate TFs, which activate a cascade of downstream signaling events in response to specific stress leading to overall cellular tolerance. In an earlier study, two-day-old seedlings of Rasi showed tolerant to high temperature and salinity stress (Jayaprakash et al, 1998), while Tellahamsa displayed susceptible to high temperature. In this study, we made an attempt to examine the relevance of the upstream regulatory genes OsWRKY72, and analyzed the expression pattern of OsWRKY72 under salinity stress in two rice genotypes Rasi and Tellahamsa. We cloned full-length OsWRKY72 cDNA from these two rice genotypes and discovered a transcript variant for the first time. The relevance and significance of OsWRKY72a and OsWRKY72b was analyzed and discussed.

MATERIALS AND METHODS

Plant material and stress imposition

Salinity stress was imposed to two-day-old germinated rice seedlings (Jayaprakash et al, 1998). The seedlings were grouped into three sets: the first set was transferred to petri plates (15 cm × 15 cm) containing NaCl solution (100 mmol/L) for induction stress, the second set was exposed directly to lethal (shock) stress (250 mmol/L NaCl), and the third set was maintained as control (in distilled water). The seedlings were grown on blotting paper moistened with distilled water (control) or salt solution (stress), and incubated at 28 °C with a relative humidity of 70%–80%. Three replications were maintained for each treatment. After 12 h of induction stress, the seedlings were transferred to lethal stress, whereas the other sets were continued in lethal stress and control until 24 h (Uma et al, 1993; Jayaprakash et al, 1998). Seedling growth was recorded by measuring root and shoot length (cm) at 12 h and 24 h under the treatments. Data were represented as percent reduction in growth over control and the tissue samples were collected for gene cloning and expression studies.

Isolation of total RNA and cDNA synthesis

Total RNA was isolated from 100 mg tissues using the phenol-chloroform method (Sajeevan et al, 2014). All RNA samples were treated with 1 U of DNase I enzyme (MBI Fermentas, Hanover, USA) at 37 °C for 45 min to remove the presence of genomic DNA before cDNA synthesis. The first strand cDNA was synthesized using 5 μg total RNA in a 20 μL reaction mixture containing 40 pmol oligo (dT) primer, 1X reaction buffer with 10 mmol/L dNTP mix and 200 U of Moloney Murine Leukemia virus reverse transcriptase enzyme (MBI Fermentas, Hanover, USA). The reaction mixture was incubated at 42 °C for 1 h, and the cDNA generated was used as the template for PCR-based cloning (Sambrook et al, 1989) and gene expression.

Gene expression

To examine the expression of OsWRKY72, semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using the gene-specific forward (OsWRKY72a-F2 and OsWRKY72b-F3) and reverse (OsWRKY72a-R2 and OsWRKY72b-R3) primers (Table 1). The PCR was performed in a 20 μL reaction mixture at an annealing temperature of 54 °C with 35 cycles for OsWRKY72b, and 58 °C with 30 cycles for OsWRKY72a. The house-keeping gene, OsActin, was used as the loading control, and OsLEA14 was used to confirm stress effect in plant tissue. The amplified products were separated by agarose gel electrophoresis, and product intensities were quantified using ImageJ 1.45s software (http://imagej.nih.gov/ij). The ratio of band intensities of the target gene to house-keeping gene, OsActin was calculated to assess the quantitative differences in expression. Three replicates were used to calculate the relative expression ratio.

Cloning and sequence analysis of OsWRKY72

The full length OsWRKY72 sequence information from NCBI database (http://www.ncbi.nlm.gov/) was used for designing the forward (OsWRKY72-F1) and reverse (OsWRKY72-R1) primers (Table 1). The stressed and control first strand cDNA were pooled
respectively and used as templates for PCR in a total of 20 μL reaction volume containing Phusion DNA polymerase (Finnzymes, Thermo Fisher Scientific, USA) with an annealing temperature of 54 °C. The genomic DNA was isolated from the 10-day-old seedlings using the DNeasy Plant Mini Kit (QIAGEN, USA) according to the manufacturer’s protocol. PCR was carried out using the gene-specific forward and reverse primers with an annealing temperature of 60 °C and an initial denaturation for 8 min. The PCR amplified products were cloned into T/A cloning vector using InstA/T/A Clone PCR Product Cloning Kit (Fermentas, Hanover, USA), and sequenced (ABI 3730XL sequencer). The identity of the cloned gene was confirmed by BLASTn and BLASTx analyses, and the conserved domain was identified in NCBI database.

Bioinformatic analysis


Statistical analysis

The growth data were analyzed by analysis of variance (ANOVA, SPSS software package for Windows, release 15.0; SPSS Inc., Chicago, USA). Significance differences between means were assessed by the Duncan’s multiple range test (P = 0.05) (Gomez and Gomez, 1976).

RESULTS

Salinity stress response in Rasi and Tellahamsa

Salinity stress caused significant reduction in root and shoot growth of Rasi and Tellahamsa directly exposed to 250 mmol/L NaCl, compared to those of the seedlings induced before being subjected to lethal stress (Fig. 1). The reductions in root and shoot at the end of induction stress in Rasi were 5.44% and 6.99%, whereas those in Tellahamsa were 28.07% and 14.06%, respectively. Similarly, reductions in root and shoot growth at the end of lethal stress were 5.74% and 7.34% in Rasi, whereas 20.87% and 9.36% in Tellahamsa, respectively (Fig. 1-C and -D). The growth data indicated that Tellahamsa is sensitive to salinity stress when compared to Rasi.

Expression of OsLEA14 in indica genotypes under salinity stress

To assess the effect of salinity stress at the cellular level, we analyzed the transcript levels of OsLEA14, a known stress responsive gene, highly expressed under stressful conditions. Expression of OsLEA14 was induced under induction and lethal stress at 12 h in Rasi, while in Tellahamsa the expression was noticed only under lethal stress. At 24 h, the expression of OsLEA14 in Tellahamsa was similar under induction and non-induction stress. However, in Rasi, the expression was relatively more under induction stress (Fig. 2).

Expression of OsWRKY72 under salinity stress

The expression pattern of OsWRKY72 was studied under salinity stress in Rasi and Tellahamsa. The transcript levels were up-regulated at 12 h in Rasi under induction and lethal stress. In Tellahamsa, the increase in expression was observed under lethal stress, however, upon induction there was an increase in expression as compared to control (Fig. 3-A and -C). At 24 h in Rasi, the expression was higher under induction stress, as compared to direct lethal and control (Fig. 3-B and -D). However, the expression in Tellahamsa remained similar under both stress, but the gene was up-regulated compared to control.

Table 1. Primers used in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’-3’)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsWRKY72-F1</td>
<td>TCGATTTGCAGATGGAGAAC</td>
<td>Full length amplification</td>
</tr>
<tr>
<td>OsWRKY72-R1</td>
<td>CAGTGGGCATTGGCATTTGA</td>
<td>Full length amplification</td>
</tr>
<tr>
<td>OsWRKY72a-F2</td>
<td>CCAAAATGACATCTACCTC</td>
<td>Expression of variant-a</td>
</tr>
<tr>
<td>OsWRKY72a-R2</td>
<td>CAGTGGGCATTGGCATTTGA</td>
<td>Expression of variant-a</td>
</tr>
<tr>
<td>OsWRKY72b-F3</td>
<td>CGAGAGGAAACTCTCCCCGATTTTGCC</td>
<td>Expression of variant-b</td>
</tr>
<tr>
<td>OsWRKY72b-R3</td>
<td>GTGAAGGAGTGTCGAGTTTGTGGG</td>
<td>Expression of variant-b</td>
</tr>
<tr>
<td>OsLEA14-F</td>
<td>GCTACTCGTGTAAGAGCCGCGG</td>
<td>Stress confirmation</td>
</tr>
<tr>
<td>OsLEA14-R</td>
<td>GATGGGGAGGTCGACGGTGAGGC</td>
<td>Stress confirmation</td>
</tr>
<tr>
<td>OsActin-F</td>
<td>TCCCATAATGAAGTGACTGATG</td>
<td>Internal control</td>
</tr>
<tr>
<td>OsActin-R</td>
<td>GGACCTGACTGTCATACTC</td>
<td>Internal control</td>
</tr>
</tbody>
</table>
Fig. 1. Responses of Rasi and Tellahamsa seedlings to salinity stress.
A, Phenotypes of Rasi and Tellahamsa seedlings at 12 h of NaCl stress; B, Phenotypes of Rasi and Tellahamsa seedlings at 24 h of NaCl stress; C, Percent reductions in root length (RL) and shoot length (SL) of Rasi and Tellahamsa over its control at 12 h; D, Percent reductions in root length (RL) and shoot length (SL) of Rasi and Tellahamsa over its control at 24 h.
100–250 mmol/L NaCl represents 100 mmol/L NaCl for 12 h followed by 250 mmol/L NaCl for 12 h. Significant differences at the 0.05 level are indicated by lowercase letters.

Fig. 2. Expression analyses of OsLEA14 in the seedlings of Rasi and Tellahamsa.
Lanes 1, 2 and 3 correspond to expression in Tellahamsa, whereas lanes 4, 5 and 6 correspond to expression in Rasi. At 12 h, lanes 1 and 4 represent induction stress with 100 mmol/L NaCl; At 24 h, lanes 1 and 4 represent 100 mmol/L NaCl for 12 h followed by 250 mmol/L NaCl for 12 h; Lanes 2 and 5 are in lethal stress with 250 mmol/L NaCl, and lanes 3 and 6 are in control (distilled water) at 12 h and 24 h, respectively.
Identification of OsWRKY72 variant in indica genotype

Using the available rice genome information, we cloned 738 bp and 849 bp cDNAs from Rasi and Tellahamsa, respectively. The sequence analysis using NCBI BLASTn indicated 100% identity with Oryza sativa subsp. indica WRKY72, and hence, these two clones were designated as OsWRKY72a and OsWRKY72b. Sequence analysis revealed that in OsWRKY72b, 111 bp is fragmentally duplicated from different regions of the coding sequence with 45 nucleotides within the domain and 66 nucleotides from the start of the open reading frame (Fig. 4). This sequence is deposited in NCBI GenBank with the accession number KT373801.

The additional region in OsWRKY72b was cloned from genomic DNAs of Rasi and Tellahamsa, and sequenced. Sequence analysis indicated the presence of extra sequence in the genomic DNA of Rasi and Tellahamsa (data not shown). Analysis of the translated product of OsWRKY72b revealed an interrupted WRKY domain due to the presence of the additional sequence.

Expression of OsWRKY72b in salinity stress

We analyzed the expression pattern of OsWRKY72b to understand the relevance of this variant under salinity stress. In Tellahamsa, the transcript level of OsWRKY72b was lower at 12 h as compared to the

---

Fig. 3. Expression analyses of OsWRKY72a by semi-quantitative reverse transcriptase-PCR.

Lanes 1, 2 and 3 correspond to expression in Tellahamsa, whereas lanes 4, 5 and 6 correspond to expression in Rasi. At 12 h, lanes 1 and 4 represent induction stress with 100 mmol/L NaCl; At 24 h, lanes 1 and 4 represent 100 mmol/L NaCl for 12 h followed by 250 mmol/L NaCl for 12 h; Lanes 2 and 5 are in lethal stress with 250 mmol/L NaCl, and lanes 3 and 6 are in control (distilled water) at 12 h and 24 h, respectively.

Fig. 4. Identification of OsWRKY72b in indica rice genotype.

Alignments of OsWRKY72a and OsWRKY72b amino acid sequence. The region within red colored arrow indicates the WRKY domain, and additional sequence present in OsWRKY72b is represented by colored amino acids.
control and was not detected at 24 h of induction and direct lethal stress (Fig. 5). However, in Rasi, increases in expression upon induction stress were noticed at 12 h and 24 h when compared to lethal stress and the control.

**Disorder prediction of OsWRKY72**

The disordered regions of OsWRKY72a and OsWRKY72b were predicted using the predictor of natural disordered regions (PONDR) tool. The overall disorder percent in OsWRKY72a was 60.82, with the longest disordered region of 96 residues, and the shortest of 8 residues. The total number of disordered regions in the protein was five, with majority of them being in the N-terminal. However, the region outside the WRKY domain at the C-terminal end was also

![Fig. 5. Expression of OsWRKY72b in seedlings of Rasi and Tellahamsa under salinity stress.](image)

Lanes 1, 2 and 3 correspond to expression in Tellahamsa, whereas lanes 4, 5 and 6 correspond to expression in Rasi. At 12 h, lanes 1 and 4 represent induction stress with 100 mmol/L NaCl; At 24 h, lanes 1 and 4 represent 100 mmol/L NaCl for 12 h followed by 250 mmol/L NaCl for 12 h; Lanes 2 and 5 are in lethal stress with 250 mmol/L NaCl, and lanes 3 and 6 are in control (distilled water) at 12 h and 24 h, respectively.

![Fig. 6. In silico analysis of OsWKY72a and OsWKY72b.](image)

A and C, Predictor of natural disordered regions (PONDR) profiles for disorder; B and D, Phosphorylation site prediction using NetPhos 2 serv. The black rectangle on the threshold line indicates the region interacts with its binding partners.
disordered (Fig. 6-A). In OsWRKY72b, the overall disorder percent was 52.84 with the longest disorder region of 96 residues and the shortest being 8 residues. The additional sequence within the WRKY domain was not disordered (Fig. 6-C). Therefore, in OsWRKY72b, the additional sequence was ordered, which is probably essential to maintain the structural integrity of the WRKY domain that interacts with the major groove of DNA.

Phosphorylation of OsWRKY72 and its variant

The N-terminals of OsWRKY72a and OsWRKY72b, stretch of residues S14, S15, S16, S18, S19, S20, S25 S28, S63 S64, S71, S73 and S88 probably are potential phosphorylation sites (Fig. 6-B and -D). In the variant sequence, residues S161, S176, S194 and S197 showed a score of 0.783–0.957, indicating potential phosphorylation targets (Fig. 6-B and -D). A stretch of serine residues were identified at the C-terminal of OsWRKY72a and OsWRKY72b, of which S228 and S265, respectively, were predicted to be phosphorylated.

DISCUSSION

In this study, we have shown that high temperature tolerant indica rice Rasi is also tolerant to salinity stress at the seedling stage (Fig. 1). Gene expression analysis indicated that the stress responsive OsLEA14 is up-regulated upon induction and lethal salinity stress in Rasi. And, Tellahamsa, a genotype sensitive to high temperature and salinity stress, did not show the similar pattern and the expression of OsLEA14 was noticed only at lethal stress (without induction) (Fig. 2). In Arabidopsis, LEA14 is up-regulated under high light, dehydration, cold, and salt stress (Singh et al, 2005). In sweet potato, LEA14 is induced by dehydration, salt and abscisic acid (Park et al, 2011). Since Rasi has shown early induction of OsLEA14, it might be having efficient mechanism to activate early stress response.

In Rasi, OsWRKY72a is up-regulated upon induction stress at 12 h and 24 h when compared to Tellahamsa, which showed high expression only under lethal stress at 12 h (Fig. 3). This suggests that induction stress is sufficient to activate WRKY72a in Rasi that in turn can activate genes with W box. Previously, it has been documented that WRKYs from O. sativa subsp. japonica are up-regulated under different abiotic stress (Ramamoorthy et al, 2008). The expression of nine WRKY genes (OsWRKY8, OsWRKY12, OsWRKY13, OsWRKY14, OsWRKY16, OsWRKY17, OsWRKY23, OsWRKY26 and OsWRKY45) as shown by northern blot analysis, is influenced by high salinity (Qiu et al, 2004). OsWRKY72 in O. sativa subsp. japonica is also up-regulated under salinity stress (Song et al, 2010b).

We identified 738 bp and 849 bp cDNAs for OsWRKY72a and OsWRKY72b, respectively, from two indica genotypes. We noticed an interruption in the WRKY domain of OsWRKY72b (Fig. 4). Using the rice genome automated annotation system and GENSCAN software, alignments of the existing Expressed Sequence Tag’s and WRKY domain, respectively, confirm the presence of an intron in the domain (Wu et al, 2005; Xie et al, 2005). The size and sequence of the intron are variable, but the position of the intron within the group is highly conserved (Eulgem et al, 2000). Amino acid sequence comparison between OsWRKY72a and OsWRKY72b showed that the codon R was followed by the additional sequence, which is in accordance with the previous report that R type intron (splicing of the intron at the codon R) was found in the WRKY domain of genes in the group I, IIc, IId and III (Wu et al, 2005). Previously, variant forms have been identified for OsWRKY1, OsWRKY8, OsWRKY35, OsWRKY39 and OsWRKY57. In OsWRKY35, 54 nucleotides of the fourth intron are retained, whereas OsWRKY39 retains the first intron of the predicted gene (Xie et al, 2005). Here, we report the sequence of OsWRKY72b encompassing an intron of 111 bp in the WRKY domain. The significance of introns has been studied in other cases. For example, rice Tubulin β (OsTub6) in the 5′-UTR has 446 bp leader intron, which is capable of intron mediated enhancement of gene expression as shown in transient assay (Giani et al, 2009). In Arabidopsis MHX, 5′-UTR intron of 416 bp is shown to increase gene expression, without acting as a transcriptional enhancer (Akua et al, 2010). An internal element within the intron has been shown to be essential for expression of a reporter gene, which can enhance translation efficiency, and is dependent on its location in the 5′-UTR (Akua and Shaul, 2013). The intron that we have reported is in the coding region 504 bp from the transcriptional start site. However, significance of this intron in OsWRKY72b is unclear.

The expression levels of OsWRKY72b were high in Rasi under induction stress at 12 h and 24 h when compared to in Tellahamsa (Fig. 5). The difference in the expression of the variant detected could be low primarily due to low transcript levels. The sequences
in the WRKY domain are fragmentally duplicated from regions of the coding sequence. It is reported that group III WRKY genes in rice have evolved by tandem and segmental gene duplication compared to Arabidopsis (Agarwal et al., 2011). In this study, we observed the expression of the variant under salinity stress only. Further studies under other abiotic and biotic stress can give a better understanding on the role of OsWRKY72b in stress response.

By using PONDR profile, we predicted that in OsWRKY72a and OsWRKY72b, the N-terminal region was disordered, whereas the WRKY domain in the C-terminal was ordered. The PONDR score for the WRKY domain is 0.58, which is slightly above the threshold of 0.50. In OsWRKY72b even in the presence of the additional sequence, the WRKY domain was ordered (Fig. 6-C). This can be justified since the domain is required for DNA binding and maintenance of the tertiary structure which is a prerequisite for execution of protein function. The N- and C-terminal regions are disordered which could be involved in protein-protein interactions.

Analysis of protein microarray probed, with different Arabidopsis activated mitogen-activated protein kinases (MPks), indicated that WRKY transcription factors are phosphorylated. The WRKY53, WRKY62 and WRKY64 are phosphorylated by MPK7, MPK6, and MPK10 respectively, and phosphorylation determines the stability of WRKY6 (Popescu et al., 2009). We predicted the phosphorylation sites in OsWRKY72a and OsWRKY72b, and a few serine residues were found to be phosphorylated (Fig. 6-B and -C). Previous study has shown that OsWRKY30 (group I) has multiple SP (serine residue followed by proline residue) sites that are phosphorylated by MPK3, and this confers drought tolerance in transgenic rice, mutation of the SP sites abolishes phosphorylation and does not impart drought tolerance when compared to wild type (Shen et al., 2012). However, in OsWRKY72a and OsWRKY72b, we did not observe multiple SP sites as reported in group I, but serine residues are present at the N-terminal region, and also within the additional sequence which we have predicted to be phosphorylated. Mutational studies of these serine residues and phosphorylation assay will provide insight into the significance of phosphorylation in OsWRKY72a and OsWRKY72b.

In conclusion, our results indicate that OsWRKY72a is differentially expressed in indica genotypes. The identified OsWRKY72b transcript variant retains an intron within the WRKY domain, and is up-regulated under salinity stress in Rasi. The predicted WRKY domain and the potential serine residues of OsWRKY72a and OsWRKY72b were found to be ordered and phosphorylated. Further characterization is needed to identify the diverse roles of the variant in plant development and imparting stress tolerance.

ACKNOWLEDGEMENTS

We thank Niche Area of Excellence-Indian Council for Agriculture Research [Grant No. 10(15)/2012] and Department of Science and Technology-Fund for Improvement of Science and Technology Infrastructure Government of India for providing financial support.

REFERENCES


Akua T, Berezin I, Shaul O. 2010. The leader intron of AtMHX can elicit, in the absence of splicing, low-level intron-mediated enhancement that depends on the internal intron sequence. BMC Plant Biol, 10: 93–102.


(Managing Editor: WANG Caihong)