3 Biotech (2018) 8:345
https://doi.org/10.1007/s13205-018-1370-4

ORIGINAL ARTICLE

Identification of splice variant of *OsGBF1* in *Oryza sativa* ssp. *indica* genotypes under salinity stress

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Received: 21 October 2017 / Accepted: 24 July 2018 / Published online: 28 July 2018

Abstract

G-box-binding factors are plant transcription factors, involved in a wide range of biological processes including abiotic stress responses. In this study, we analyzed the expression of *OsGBF1* during salt stress in two contrasting *Oryza sativa* spp. *indica* genotypes, *Rasi* and *Tellahamsa*. Two-day-old seedlings were exposed to NaCl stress under two different conditions. One set was exposed to 100 mM NaCl before transferring to 250 mM (induction stress), while another set was transferred directly to 250 mM (shock stress). During early induction stress, *OsGBF1* was up-regulated in *Rasi* when compared to *Tellahamsa*. We cloned full-length *OsGBF1* from these two genotypes, and analyzed the sequences. Our analysis indicated the presence of transcript variants, which are designated as *OsGBF1a* and *1b*. *OsGBF1b* variant retained introns, which lead to the generation of premature termination codon. *OsGBF1b* transcript levels were not significantly different at 12-h of induction stress in *Tellahamsa* and *Rasi*. At 24-h of shock stress, *OsGBF1b* was up-regulated in both genotypes and the transcript was more in *Rasi*. Since, *OsGBF1a* and *1b* are predicted to be splice variants, we examined expression pattern of *OsSKIP*, a splicing factor and component of the spliceosome. In induction stress, *OsSKIP* was up-regulated at 12- and 24-h in *Rasi* when compared to *Tellahamsa*. On the contrary, at 24-h shock stress, *OsSKIP* was down-regulated in *Rasi* when compared to *Tellahamsa*. It is possible that *OsSKIP* expression was increased in *Rasi* during induction stress for accurate splicing that could aid in tolerance. This is the first report on *OsGBF1* splice variant and the variant could have specific functions linked to stress tolerance in rice.

Keywords Induced salt stress · Intron retention · Premature termination codon · *OsSKIP*

Introduction

Transcription factors (TFs) are proteins that bind to DNA-regulatory sequences in the promoter regions of target genes to modulate the process of transcription. The major transcription factors (TFs) belong to the family of basic leucine zipper (bZIP), AP2/ERF, MYB, NAC, WRKY, Zn finger proteins (Golldack et al. 2011) and one of the largest families of plant TFs that specifically interact with the G-box element 5′ CACGTG 3′ are classified as G-box-binding factors (GBFs) (De-Vetten and Ferl 1994). Most of the genes that are responsive to cold, salt, dehydration, hypoxia and abscisic acid (ABA) are known to have G-boxes in their promoters (Shinozaki and Yamaguchi-Shinozaki 1997; Menkens et al. 1995). The G-box-binding factors have the modular structures like leucine zipper at the C terminal, proline rich domain at the N terminal and basic regions (De-Vetten and Ferl 1994). The two families of TFs that are considered as GBFs are, bZIP and basic helix loop helix (bHLH) proteins (Siberil et al. 2001). In *Arabidopsis* bZIP proteins are divided into ten groups (A, B, C, D, E, F, G, H, I, and S) based on similar basic region and additional conserved motifs, among them group G comprises GBFs (Jakoby et al. 2002). In *Arabidopsis*, three cDNA clones encoding GBF proteins, namely, *GBF1, GBF2* and *GBF3* have been isolated. *GBF1* and *GBF2* are expressed in leaves (light and

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-018-1370-4) contains supplementary material, which is available to authorized users.

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dark grown) and roots, whereas *GBF3* is expressed in roots and dark grown leaves (Schindler et al. 1992). In wheat, *GBF1* expression was up-regulated within 24-h of exogenous ABA treatment (Sun et al. 2015). Since, GBFs can be important players in stress response; we attempted to identify prospective TF linked to salinity stress response in rice. We are reporting the discovery of *OsGBF1* splice variant with introns. We hypothesize that this variant could have specific functions linked to stress tolerance in rice.

Materials and methods

Salinity stress imposition, RNA isolation and cDNA synthesis

To study the relevance of *OsGBFs*, 2-day-old seedlings of *Oryza sativa* ssp. *indica* genotypes, *Rasi* and *Tellahamsa* were exposed to salinity stress under laboratory conditions (Jayaprakash et al. 1998). The seedlings were segregated into three sets; one set was exposed to 100 mM NaCl before transferring to 250 mM NaCl (induction stress), second set was transferred directly to 250 mM NaCl (shock stress) and third set was allowed to grow in water (control). After 12-h of induction stress, seedlings from 100 mM were transferred to 250 mM NaCl, whereas other two sets were continued in the same treatments (Supplementary Fig. 1a). Seedlings were harvested at 12- and 24-h post-stress imposition, frozen in liquid nitrogen and used for gene expression studies. Total RNA was isolated from 100 mg tissue by modified phenol–chloroform method (Sajeevan et al. 2014). All RNA samples were treated with 1 U of *DNaseI* enzyme (MBI Fermentas, Hanover, MD, USA) at 37 °C for 45 min and heat inactivated at 75 °C for 10 min. First-strand cDNA was synthesized from total RNA (3 µg) in a final reaction volume of 20 µl using 40 picomol of oligo (dT) primers, 1× reaction buffer with 10 mM dNTPs, and 200 U Moloney Murine Leukemia virus reverse transcriptase (MMLV-RT; MBI Fermentas, Hanover, MD, USA) at 42 °C.

Expression analysis

Expression of *OsGBF1a, OsGBF1b* and *OsSKIP* was studied by semi-quantitative reverse transcriptase (RT)-PCR using specific primers (Table 1). PCR was performed at an annealing temperature of 57 °C (30 cycles) for *OsGBF1a* and 56 °C (33 cycles) for *OsGBF1b. OsActin1* was used as the loading control. The intensity of amplified products separated on agarose gel was quantified using ImageJ 1.45 s software (http://imagej.nih.gov/ij). A quantitative difference in expression was calculated as the ratio of band intensities of target gene to that of housekeeping gene. The relative expression ratio from three independent replicates was calculated and represented. Significant difference between the values was calculated using Student's *t* test.

Cloning of full-length *OsGBF1*

To analyze the features of *OsGBF1*, full-length gene was cloned using cDNA generated from salt stressed tissues of *Rasi* and *Tellahamsa* using gene-specific primers (Table 1). The gene sequence reported in NCBI (http://www.ncbi.nlm.gov/) database (AY606941.1) was used to design specific primers (Table 1). The PCR products were cloned using the InsTAclone PCR cloning kit (MBI Fermentas Hanover, MD, USA), and sequenced (ABI 3730Xl sequencer). The identity of the cloned gene was confirmed by BLASTn analysis in NCBI database.

Results and discussion

The rice genotypes, *Rasi* and *Tellahamsa* used in this study have been found to be tolerant and sensitive to salinity stress, respectively. There was significant difference in growth between *Rasi* and *Tellahamsa* under salinity stress. At the end of shock stress, 18% reduction in growth was observed in *Tellahamsa*, whereas only 5.9% reduction was noticed in *Rasi*, suggesting that *Rasi* is relatively tolerant to salinity

Table 1 List of primers used and their application

OsGBF1 F1	ATGGGAAATGACGAAGCTGTAGTTACTC	Full-length amplification
OsGBF1 R1	TTACCTTGCGGCTACAGCATCAGTC	Full-length amplification
OsGBF1a F2	ATCCAGCCCTTAGTCCA	Expression of variant-a
OsGBF1a R2	CCACCAGTTTCCACGCCTGATT	Expression of variant-a
OsGBF1b F3	GCCCATTGAGCATGGAGCCAG	Expression of variant-b
OsGBF1b R3	CTAAGCTGCAATCTCCACTGCATTG	Expression of variant-b
OsSKIP F4	GGTGCTTCAGAGAGGTCTGG	Expression
OsSKIP R4	CCTCCACCCTCGTAATCATCT	Expression
OsActin F	CCATAATGAAGTGTGATGT	Internal control
OsActin R	GGACCTGACTCGTCATACTC	Internal control

stress (Supplementary Fig. 1b). *OsGBF1* was up-regulated at 12-h of induction stress in *Rasi* compared to *Tellahamsa*. At 24-h post-induction stress, *GBF1* expression was increased in *Tellahamsa*. However, in *Rasi, GBF1* expression was increased in both induction and shock stresses when compared to control (Fig. 1a, b). Similar results were reported in wheat by Sun et al. (2015). *Triticum aestivum GBF1* transcript levels were increased upon exposure to 200 mM NaCl, indicating the stress responsive nature of the gene. Sequence analysis of *OsGBF1* showed identity with salinity stress inducible bZIP protein. In tolerant genotype *Rasi*, the expression level of *GBF1* was more than *Tellahamsa*, suggesting that this regulon might have a role in stress response in rice.

We cloned two transcript variants of GBFs, 1082 and 1309 bp from stressed cDNA of *Rasi* and *Tellahamsa*. BLASTn analysis of the 1082 and 1309 bp variants showed 99 and 100% sequence identity with a query coverage of 100 and 83%, respectively, with *O. sativa* (*indica* cultivar group) salt stress inducible bZIP transcript. Since the transcript identified was similar to GBF1, these variants have been designated as *OsGBF1a* and *OsGBF1b*. Genomic DNA sequence analysis of *OsGBF1* revealed the presence of 11 exons and 10 introns. In *OsGBF1a* introns are spliced; however, in *OsGBF1b*, the introns between exons 1 and 2, 5 and 6 were retained (Fig. 2). The lengths of both introns are 112 bp, with the first and second intron at 60 and 520 nucleotides, respectively, from translation start site (TSS). *OsGBF1b* sequence has been deposited in GenBank under the accession number JZ903929. The common alternate splicing events reported include intron retention (IR), exon skipping, alternative 5′ or 3′ site selection, and mutually exclusive exons, of which IR is common in plants (Syed et al. 2012). The first intron which we are reporting starts with AT and ends with AG. However, some rare donor–acceptor sites such as AT-AC, GC-AG, GT-GG have also been reported (Dubrovina et al. 2013). The second intron starts with GT and ends with AG, as reported in plants (Dubrovina et al. 2013). *OsGBF1b* has no open reading frame due to premature termination codon (PTC) at 66 nucleotides from the start site (Fig. 2). In *Arabidopsis*, IR in mRNA results in generation of PTC (Feng et al. 2015). The alternative splicing of *Circadian Clock Associated 1* (*CCA1*) and serine/arginine-rich (SR) pre-mRNA's produced PTC containing transcripts due to IR (Filichkin et al. 2015; Reddy and Shad 2011).

To analyze the expression of *OsGBF1b* under control and salt stress conditions in rice genotypes, we designed variant-specific primer (Table 1; Fig. 2). At 12-h post-induction stress, *OsGBF1b* expression was similar in *Tellahamsa* and *Rasi*. However, in *Rasi* the transcript was down-regulated in induction stress compared to control and shock stress (Fig. 3a). At 24-h, *OsGBF1b* expression was up-regulated in both the genotypes during shock stress,

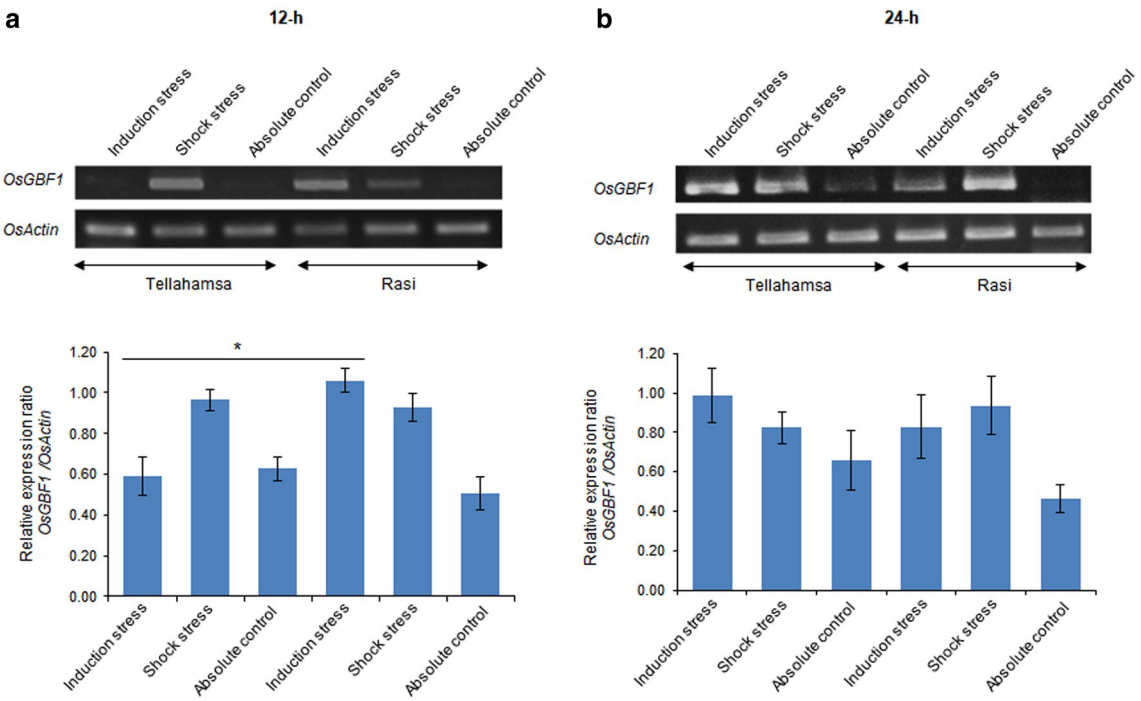

Fig. 1 Expression of *OsGBF1* in *Rasi* and *Tellahamsa* under salinity stress. **a** *OsGBF1a* expression 12-h of stress. **b** *OsGBF1a* expression 24-h of stress. The bar diagram indicates relative expression of the gene at 12- and 24-h of stress. Asterisk (*) and line drawn above the bars indicate significant difference in similar stresses and control treatments (*t* test; $P < 0.05$)

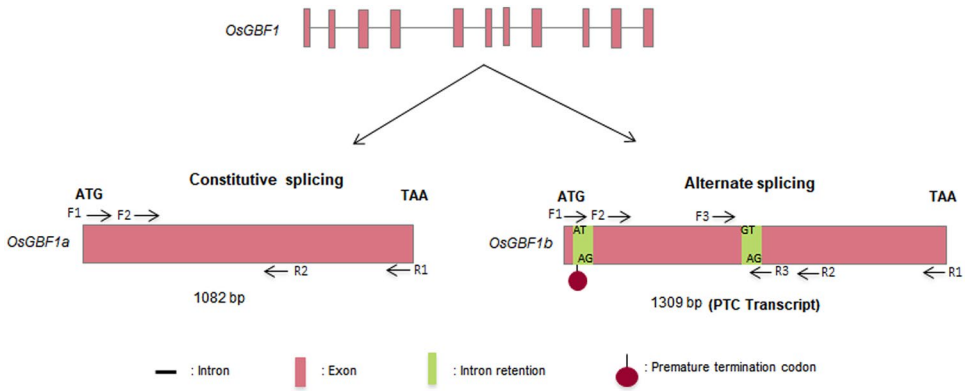

Fig. 2 Schematic representation of *OsGBF1* splice variants, *OsGBF1a* and *OsGBF1b*. Arrows indicate position of the primers used in this study, F- and R-represents forward and reverse primer, respectively. For description of F1-R1, F2-R2, and F3-R3 refer Table 1. AT-AG and GT-AG correspond to donor–acceptor splice sites in intron 1 and 2, respectively

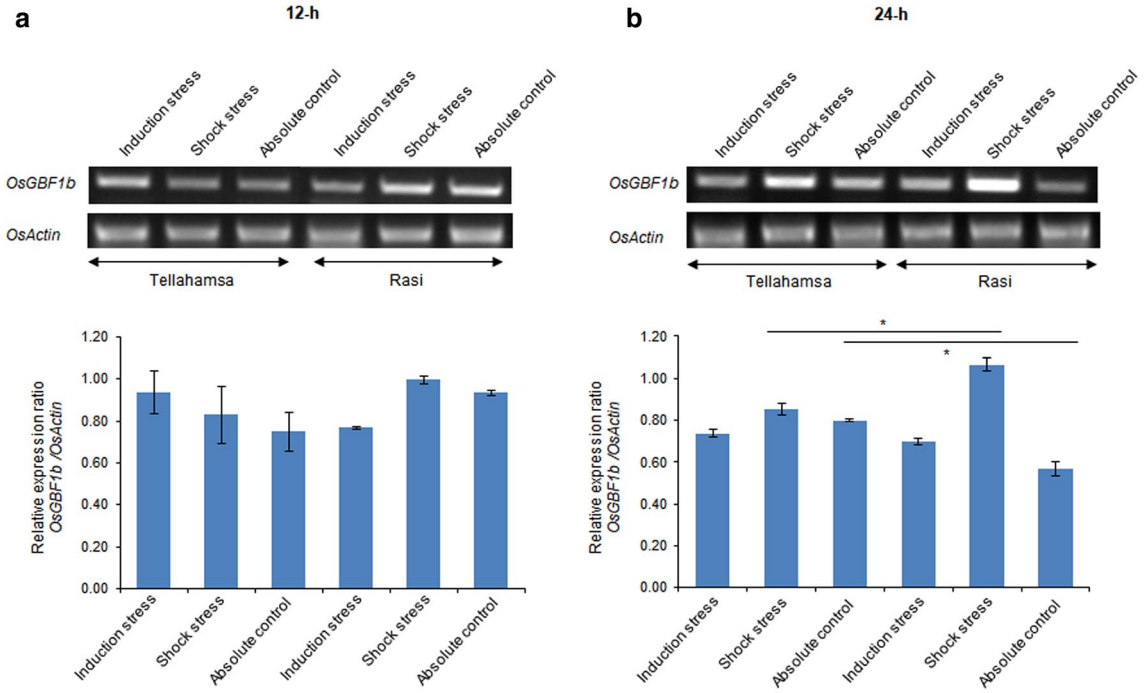

Fig. 3 Expression of *OsGBF1b* in *Rasi* and *Tellahamsa* under salinity stress. **a** *OsGBF1b* expression 12-h of stress. **b** *OsGBF1b* expression 24-h of stress. The bar diagram indicates relative expression of the gene at 12- and 24-h of stress. Asterisk (*) and line drawn above the bars indicate significant difference in similar stresses and control treatments (*t* test; $P < 0.05$)

and the expression levels were high in *Rasi* as compared to *Tellahamsa* (Fig. 3b). It was documented that alternate splice forms are high in salt stress-induced *Arabidopsis* as compared to control, with IR being prevalent event under salt stress generating a PTC (Ding et al. 2014; Feng et al. 2015). The alternate spliced genes include those that have a role in abiotic stress response, hormone signaling, transcriptional regulation, and RNA processing (Ding et al. 2014). It is intriguing to speculate as to what could be the purpose of this transcript in stress conditions. Transcripts with PTC and/or long 3′ untranslated regions (UTRs), splice junction downstream of stop codon, PTC with downstream splice junction, and open reading frames in 5′ UTR are substrates for nonsense mediated decay (NMD) (Kalyna et al. 2012). In *Arabidopsis*, analysis of At5g37055 (*SERRATED LEAVES AND EARLY FLOWERING—SEF*) revealed three different IR transcripts (involving intron-1 and -2) containing PTC, but not targeted to NMD pathway, on the contrary transcript generated by alternative 3′ splice site (involving exon-3) containing PTC is targeted to NMD pathway (Kalyna et al. 2012). In our study *OsGBF1b* was up-regulated in shock stress at 24-h, which makes us to predict that this variant is probably not a substrate of NMD. At 24-h post-shock stress, both genotypes showed an increase in *OsGBF1b* expression. Our observation is in accordance with the documentation that spliced variants increase with the salt concentration

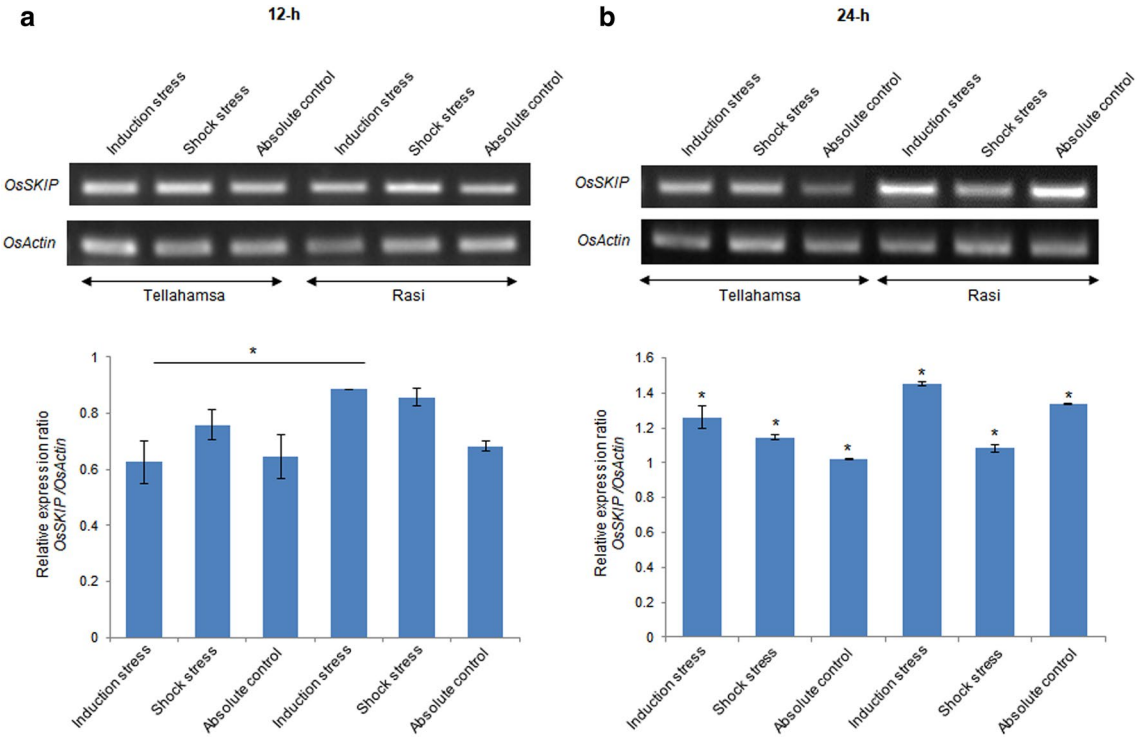

Fig. 4 Expression of *OsSKIP* under salinity stress. **a** 12-h stress. **b** 24-h stress. The bar diagram indicates relative expression of the gene at 12- and 24-h of stress. Asterisk (*) and line drawn above the bars indicates significant difference in similar stresses and control treatments (*t* test; $P < 0.05$)

(Feng et al. 2015). Analysis of differential alternate spliced genes under salt stress included those with role in abiotic stress, suggesting that alternative splicing is not a random process, but is responsive to stress (Ding et al. 2014). *OsGBF1b* noticed in our study seems to be generated not by aberrant splicing, and the increase in expression that we have observed indicates that this variant probably has a role in salinity stress tolerance.

Accurate splicing of transcripts is essential for protein expression. Since, *OsGBF1b* variant showed differential expression, we studied the expression profile of *OsSKIP* under salt stress. *OsSKIP* (a homolog of pre-mRNA splicing factor 45 in yeast), part of spliceosome complex closely associated with SR45 (Wang et al. 2012). At 12- and 24-h of induction stress, *OsSKIP* expression was up-regulated in *Rasi*, when compared to *Tellahamsa*. However, at 24-h shock stress, *OsSKIP* expression was down-regulated in *Rasi* when compared to *Tellahamsa* (Fig. 4a, b). *OsSKIP* is constitutively expressed in leaf, root, stem, and is up-regulated during salinity stress in rice (Hou et al. 2009). It has been reported that *SKIP* under salt stress is required for splicing of pre-mRNA encoding salt responsive genes such as *NHX1, RD29A* and *P5CS1* (Feng et al. 2015). Hence, *OsSKIP* expression was probably increased in *Rasi* during induction of salt stress for accurate splicing of stress responsive genes that could aid in tolerance. This is the first report on the discovery of *OsGBF1* splice variant, which shows intron retention resulting in generation of premature termination codon. We hypothesize that this variant could have specific functions linked to stress tolerance.

Acknowledgements We thank Niche Area of Excellence-Indian Council for Agriculture Research (NAE-ICAR) Grant File. No. 10 (15)/2012 and DST-FIST Government of India, New Delhi, India, and Dow AgroSciences, Indianapolis, USA (No./S-8/C-3/5055/2011-12) for providing financial support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The research has been conducted with the consent of the authorities and that the research does not involve human participants and/or animals.

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