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# CLONING AND *AGROBACTERIUM* MEDIATED TRANSFORMATION OF THE PUTATIVE PROMOTER REGION OF *ORYZA SATIVA* $C_3H_{52}$ GENE INTO LOCAL RICE VARIETY

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**Abstract**

A number of zinc finger proteins are known to function in biotic and abiotic stress responses. The putative $OsC_3H_{52}$ gene promoter region, a member of CCCH zinc finger proteins family was isolated and characterized in this study. The *in silico* analysis of 1742bp promoter region of $OsC_3H_{52}$ gene using Plant care and Plant pan promoter analysis tools revealed the presence of ARE, TCA, G-BOX, TC rich repeats and MYBGAHV, GATABOX, WRKY71OS and Circadian *cis*-acting elements, respectively, which are involved in plant growth and development and stress responses. To clone the putative $OsC_3H_{52}$ gene promoter region, a promoter fragment of 1742 bp was amplified using indica rice (cv.Swat-1) genomic DNA. The putative $OsC_3H_{52}$ gene promoter region was cloned adjacent to *GUS* gene using pBIG binary vector and transformed into *Agrobacterium tumefaciens* (EHA105). For transformation of indica rice, cv. Swat-1, calli was induced from rice seed scutella using N6 medium supplemented with 2 mg/L 2,4-D and 0.5 mg/L kinetin. Six weeks old scutellar callli was used for *Agrobacterium* mediated transformation. The regenerated putative pBI $P_{OsC3H52}$*: GUS* transgenic plantlets were confirmed for transgene integration using PCR. Histochemical activity of the *GUS* gene in $P_{OsC3H52}$*: GUS* transgenic rice revealed its expression in calli and different parts of the plant including crown, leaf sheath, young leaves and emerging roots. The above results revealed that the putative $OsC_3H_{52}$ gene promoter is a functional promoter and it should be analyzed further in response to biotic and abiotic stress stimuli.

**Key words:** Indica rice, Zinc finger protein, Promoter, *Cis*-acting elements, *GUS* gene

## Introduction

Rice (*Oryza sativa* L.) is a model monocot plant. Besides rice is the second best cash crop and export commodity of Pakistan. Rice has adaptability to a wide range of growing conditions and being tropical, it is hydrophilic and favors warm humid climate (Salim *et al*., 2003). There are various stresses both biotic and abiotic which affect rice production and total crop yield (Tayagi *et al*., 2004). In abiotic stresses heat, cold, drought and climatic variability with flooding are the important ones. However, pests, diseases, insects and the attack of alien weed species are important biotic stresses (Hyman *et al*., 2008; Lobell *et al*., 2008; Varshney *et al*., 2011; Wassmann *et al*., 2009). Rice productivity has become at risk in recent years as a consequence of drastic climate change. A large percentage of the rice cultivable areas are located in susceptible regions (Manzanilla *et al*., 2011). Limiting water availability and drought are the major restraining factors for rice production (Bouman *et al*., 2005). With an average yield production of 5.0 tons/ha, a financial decline of approximately USD 353.7 million/year is recorded (Wassmann *et al.,* 2011).

Number of genes involved in various abiotic stress responses were characterized using genomic analysis (Fowler & Thomashow., 2002; Seki *et al*., 2002; Rabbani *et al*., 2003; Bartels & Sunkar 2005; Yamaguchi & Shinozaki, 2006) and proteomics (Pechanova *et al*., 2010; Kosová *et al*., 2011). Zinc finger (Znf) Transcription factor (TF) is one of the largest TF families, which are categorized by the presence of common Znf motifs in protein structure including histidines and/or cysteines which binds zinc metal ions to form a complete functional peptide structures essential for their specific functions (Hall, 2005). Znf motifs are present in numerous TF and play critical roles in their interactions with nucleic acids and proteins or other small molecules (Wang *et al*., 2008). Znf proteins have been found associated with many plant developmental and adaptive processes, including seed germination and development (Kim *et al*., 2008) growth and development of embryo (Grabowskaa *et al*., 2009; Wang *et al*., 2008) morphogenesis of flower, leaf senescence (Li *et al*., 2001) and plant architecture (Kong *et al*., 2006).

Based on the spacing between cysteine and histidine residues in zinc finger motifs such as (C-$X_{6-14}$ -C-$X_{4-5}$ -C-$X_3$-H) including the number of zinc finger motifs, *Arabidopsis* CCCH proteins were classified into eleven subfamilies, whereas the rice CCCH proteins containing Znf domain were categorized into eight subfamilies (Lee *et al*., 2012; Wang *et al*., 2008). Zinc finger proteins containing a tandem zinc finger (TZF) domain are characterized by the presence of two CCCH zinc finger domains separated by 18 amino acids (Al-Souhibani *et al*., 2010; Jeong *et al*., 2010). The tandem CCCH zinc finger proteins (TZFs) are also found in other organisms with conserved regions including yeast, worms, humans and plants (Wang *et al*., 2008). In this unique subfamily, the functional characteristics of most of the members are unknown, with the exception of *AtTZF4* gene that is involved in photo-dependent seed germination (Kim *et al*., 2008) and *AtSZF1-AtSZF2* (*AtTZF11-AtTZF10*) involved in salt stress tolerance (Sun *et al*., 2007). *OsTZF1* functions under environmental stress conditions and is a homolog of AtTZF1 sharing similar characteristics in rice (Pomeranz *et al*., 2010; Jan *et al*., 2013). OsTZF1 acts as a repressor of leaf senescence in rice under biotic and abiotic stress conditions. (Jan *et al*., 2013).

Promoter region of a gene plays important role in the regulation of gene expression. Different *cis*-acting elements have major role in the proper expression of a gene. A wide range of promoters related to various stress responses have been characterized however, in the rice CCCH-tandem zinc finger protein family, most of the genes and their promoters have not been characterized (Jan *et al*., 2013). This study aimed to analyze the putative promoter region of $OsC_3H_{52}$ gene for different *cis*-acting elements using bioinformatics tools. The putative promoter of $OsC_3H_{52}$ gene was cloned from local indica rice (cv. Swat-1) into plant expression vector in conjunction with *GUS* reporter gene and transformed rice in order to analyze its histochemical *GUS* gene activity.

## Materials and Methods

All the experimental work was carried out in the Genomics and Bioinformatics labs of The Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan.

***In silico* analysis of the putative promoter region of $OsC_3H_{52}$ gene:** For the *in silico* analysis of the putative promoter region of $OsC_3H_{52}$ gene, Plant Care (Lescot *et al*., 2002) and Plant Pan (Chang *et al*., 2008) bioinformatics tools were used. Sequence 1742 bp in length upstream of start codon (ATG) of $OsC_3H_{52}$ gene was considered as putative promoter region.

**Plant material and growth conditions:** Seeds of local indica rice cultivar Swat-1 were obtained from Agricultural Research Station Mingora, Pakistan. The seeds of rice were incubated at 42°C before germination/callus induction. Seeds were dehusked and used for culturing under sterile conditions in laminar air flow unit.

**Bacterial strain and vectors used for transformation:** *Agrobacterium tumefaciens* strain EHA105 (Hood *et al*., 1986) was used for transformation rice. Binary pBIG (12.7kb) vector was used as backbone for cloning the desired promoter region adjacent to *GUS* gene.

**Amplification of $OsC_3H_{52}$ promoter region and cloning:** The promoter region of $OsC_3H_{52}$ gene, 1742 bp upstream of ATG (P*OsTZF1*), was amplified from the rice genome by PCR using KOD DNA polymerase (Toyobo, http://www.toyobo.co.jp/bio). The primers used are listed in Table 1. The $OsC_3H_{52}$ promoter fragment was inserted into the *Sma*I site of the *GUS* expression vector pBIG (Becker, 1990), generating P$OsC_3H_{52}$*: GUS*.

**Table 1. List of primers used.**

| Primer Type | Primer Name | Primer Sequence |
|---|---|---|
| Primers for amplification of $OsC_3H_{52}$ promoter fragment | | |
| Forward primer | $OsC_3H_{52}$-Pro-F | 5′- GGGAGTGTATCAGGAGTTCTCCT- 3′ |
| Reverse primer | $OsC_3H_{52}$-Pro-R | 5′- GGGCCAAAAAATGTATATATG CA- 3′ |
| Primers for screening pBI $P_{OsC3H52}$*:GUS* transformed colonies and transgenic rice | | |
| Forward primer | pBI-F | 5′- TTCTGCGGACTGGCTTTCTACGTGT- 3′ |
| Reverse primer | $OsC_3H_{52}$-Pr-R150 | 5′-TATTACTGCAACATGGCATTCGTGCAT- 3′ |

**Plant tissue culture:** Mature and healthy seeds were selected, dehusked and rinsed with double distilled water (d.d$H_2O$) for one minute and then washed three times with 0.2% mercuric chloride solution for surface sterilization. Finally seeds were washed five times with d.d$H_2O$. For callus induction N6 basal media was used (Chu, 1975). Hormone 2,4-D with concentration of 2 mg/L and kinetin 1 mg/L concentration was added to N6 media. Agar at the rate of 0.7% was used. The pH of media was adjusted to 5.6-5.8 with the help of pH meter. Around 10 seeds / petri plate containing N6 media were cultured for callus induction and incubated at 25±3 ºC for 16 hours photoperiod and 8 hours dark. For regeneration, MS (Murashige & Skoog, 1962) based modified medium was used.

***Agrobacterium* mediated Transformation:** The procedure of Toki, (1997) was followed for rice transformation. Rice calli were incubated in prepared *Agrobacterium* suspension. After infection with *Agrobacterium*, calli were incubated on co-cultivation medium plates for three days. N6 selection medium supplemented with 30 mg/L hygromycin and 250mg/L carbencillin was used for calli selection. For regeneration, growing calli from selection media were transferred to regeneration medium supplemented with 30 mg/L hygromycin. Till the generation of shoots, calli were cultured on fresh regeneration media plates after every 10 days.

**Molecular analysis:** PCR analysis was carried out to confirm transformation using genomic DNA of transformed rice. Primers used are listed in Table 1.

*GUS* assays were conducted according to Jefferson *et al*., 1987. *GUS* solution was prepared by mixing 50mM sodium phosphate buffer having pH 7.0, Triton X-100 0.1%, 10mM EDTA (pH 8.0), 0.5mg/ml X-Gluc A and 5% methanol and final volume was raised to 10mL with autoclaved distilled water. Putative transformed calli and putative transgenic plantlets were incubated in 200µL GUS solution for 1-12 hours at 37°C.

## Results

***In silico* analysis of the putative promoter region of $OsC_3H_{52}$ gene using Plant Care database:** Plant care database (Lescot *et al*., 2002) was used to identify *cis*-

acting regulatory elements. Important *cis*-acting regulatory elements were identified using the $OsC_3H_{52}$ gene promoter sequence (Fig. 1). The sequence of promoter analyzed was 1742 bp in length. The predicted data was obtained on the basis of homology of query sequence with that of template. Functions of the predicted regulatory elements were also identified. *Cis*-acting regulatory elements identified were; TCA at position -142-151bp, circadian (-286-295), ATGCAAT motif (-314-322), TC rich repeats (-486-495), ARE region (-708-713), G-BOX (-884-890), Skn-1_ motif (-1036-1040). These *cis*-acting elements were present at different positions in sense or antisense positions (Fig. 1; Table 2). G-BOX and skn-1 motif sequences were present in sense direction. However other *cis*-acting elements were observed in antisense orientation (Table 2).

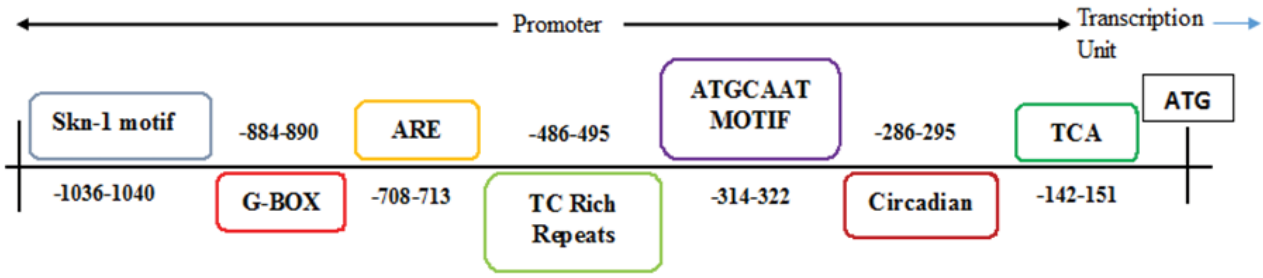


Fig. 1. Pictorial representation of *cis*-acting regulatory elements in the putative promoter region of $OsC_3H_{52}$ gene.

**Table 2. *Cis*-acting regulatory elements in the putative promoter region of $OsC_3H_{52}$ gene analyzed by Plant Care database.**

| Element Name | Sequence | Organism | Matched regions (highlighted in bold) | Function |
|---|---|---|---|---|
| ARE (-) | 708 713<br>AGATCATTTT GGTTCAATTT | *Zea mays* | + AAA CCA<br>- **TTT GGT** | Essential for anaerobic induction |
| ATGCAAAT Motif (-) | 314 322<br>GATTAAACAT AACCATAGGG | *Oryza sativa* | + ATTTGTAT<br>- **TAAACATA** | Conserved in a number of seed storage protein genes |
| G-BOX (+) | 884 890<br>TTACACGTTA | *Pisum sativum* | + **CACGTT**<br>- GTGCAA | Involved in light responsiveness |
| Skn1_motif (+) | 1036 1040<br>ACCTAGTCAT | *Oryza ativa* | + **GTCAT**<br>- CAGTA | Required for endosperm expression |
| TC Rich repeats (-) | 486 495<br>CTTACATCTC TTTTATCTCC | *Nicotiana tabacum* | + TAGAC AAAAT<br>- **ATCTC TTTTA** | Involved in defense and stress responsiveness |
| TCA element (-) | 441 450<br>ATAGGAAGAG<br>142 151<br>AGGGGAAGAC TATAGTTAGG | *Brassica oleraceae* | + TATCCTTCTC<br>- **ATAGGAAGAG**<br>+ CCCCTTCTG<br>- **GGGGAAGAC** | Involved in salicyclic acid responsiveness |
| Circadian (-) | 286 295<br>ACGTAATCTA TAACAACAGT | *Lycopersicum esculentum* | + GAT ATTGTTG<br>- **CTA TAACAAC** | Involved in circadian control |

The name of organisms shows homology to query sequence; the matched regions and the function of each regulatory element have also been given.

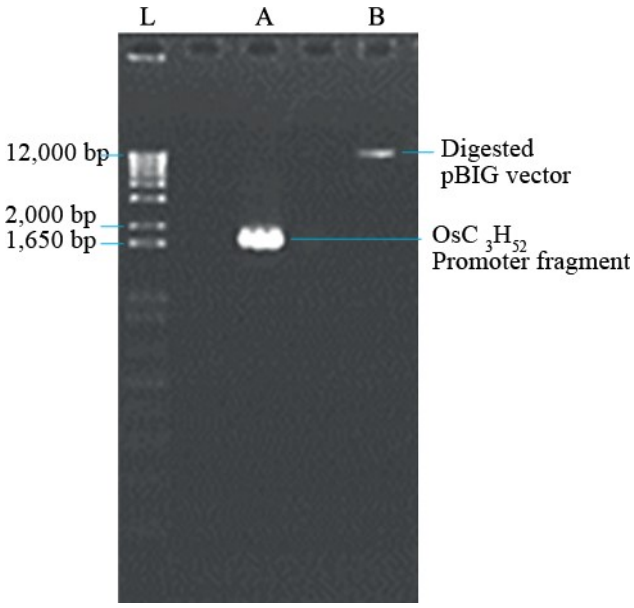


Fig. 2. PCR amplified putative fragment of $OsC_3H_{52}$ gene and digestic pBIG Vector. L represents 1 Kb plus DNA ladder.

***In silico* analysis of the putative promoter region of *$OsC_3H_{52}$* gene using Plant Pan database:** Using this database transcription factor binding sites as well as other regulatory elements including tandem repeats region were identified in the putative promoter region of *$OsC_3H_{52}$* gene. Different *cis*-acting elements such as AACACOREOSGLUB1, ANAERO1CONSENSUS, BIHD1OS, GATABOX, MYBGAHV, TATABOXOSPAL and WRKY71OS *cis*-acting regulatory elements were present in the promoter region (Table 3). The *cis*-acting elements in the promoter region of *$OsC_3H_{52}$* gene showed high homology with the *cis*-acting elements found in rice and tomato genes (Table 3). Consensus sequences of these elements were found in the sense and antisense orientation when compared with that of tomato and rice. One tandem repeat region was also identified using Plant Pan Database. This repeat region ranged from -1628 to - 1714 and had a consensus sequence of TTC which repeated 26 times (Table 3).

**Amplification of *$OsC_3H_{52}$* promoter region and Construction pBIG binary vector:** Efforts were made to amplify the promoter region of *$OsC_3H_{52}$* gene from the genomic DNA of indica rice cv. Swat-1. The primers used are listed in Table 1. The size of the amplified fragment was around 1700 bp (Fig. 2). For construction of *$P_{OsC3H52}$:GUS* expression vector, pBIG vector was used. Vector was digested with *Sma*I restriction enzyme. As a result a linearized fragment was obtained (Fig. 3). The colonies obtained as a result of cloning were subjected to colony PCR and positive colonies were identified (data not shown). The primers used for colonies screening are listed in Table 1. Selected colonies were subjected to plasmid isolation and sequencing. After the collection of sequence data it was confirmed that plasmid orientation was correct. Interestingly, it was found that the sequence of promoter region of *$OsC_3H_{52}$* gene isolated from cv. Swat-1 had slightly difference in sequence at 5` side (data not shown). This difference was found mainly in the region of repeat sequence present in the promoter region of *$OsC_3H_{52}$* gene. The vector map of *$P_{OsC3H52}$:GUS* expression vector is shown in Fig. 3.

**Table 3. *Cis*-acting regulatory elements and tandem repeat sequence in the putative promoter region of *$OsC_3H_{52}$* gene analyzed by Plant Pan database.**

| Transcription Factors | Consensus Sequence | Function |
|---|---|---|
| AACACOREOSGLUB1 | AACAAAC | Involved in controlling endosperm specific expression |
| ANAERO1CONSENSUS | AAACAAA | Involved in fermentative pathway |
| BIHD1OS | TGTCA | Involved in disease resistant response |
| GATABOX | GATA | This motif is in CaMV 35S promoter and also involved in tissue specific expression |
| MYBGAHV | TAACAAA | Found in gibberellin n responses |
| TATABOXOSPAL | TATTTAA | Found in promoter of rice pal gene encoding phenylalanine ammonia lyase (salicylic acid synthesis) |
| WRKY71OS | TGAC | Transcriptional repressor of gibberellin signaling pathway |

**Tandem repeat sequence**

5′-TTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC -3′

**Table 4. Summary of the Transformation of indica rice cv. Swat-1 with pBI *$P_{OsC3H52}$: GUS* vector.**

| Genotype | Non responding seeds | Callus formation | | % Callus formation |
|---|---|---|---|---|
| | | Abnormal calli | Normal Calli | |
| Cv. Swat-1 | 20 | 10 | 70 | 80 |

*Total number of rice seeds used were 100.

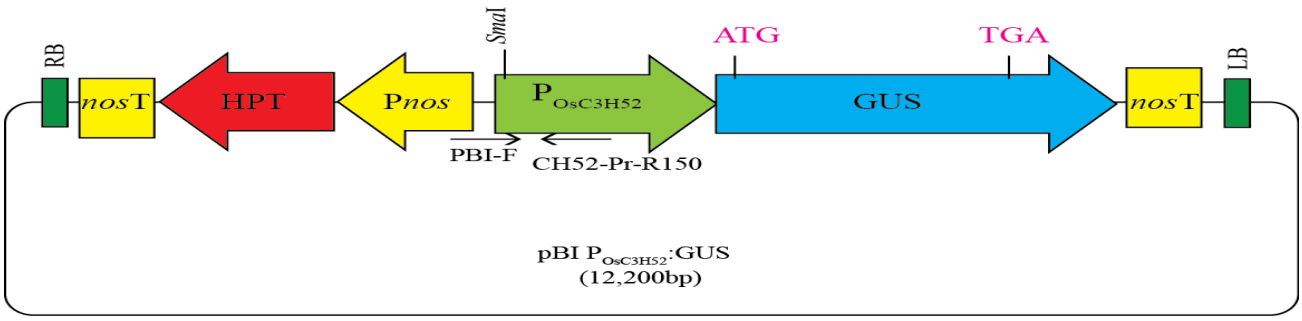


Fig. 3. Schematic map diagram of pBI P $_{OsC3H52}$: GUS vector construct containing putative *OsC$_3$H$_{52}$* promoter fused with GUS reporter gene. HPT, hygromycin resistant gene. nosT, nopaline synthase terminator. Pnos, gene for nopaline synthase promoter. LB and RB, left border and right border of T-DNA region, respectively.


Fig. 4. Transformation of indica rice cv. Sawati-1 with pBI *P$_{OsC3H52}$:GUS* vector construct. A. Selection of *Agrobacterium* mediated Transformed calli on selection media. B. regeneration of transformed calli. C. Plantlet formation during regeneration of transformed calli.

***Agrobacterium* mediated Transformation of Indica rice (cv. Swat-1):** For rice plant transformation, tissue culture is a pre-requisite. Calli were induced on N6 medium. Total 100 seeds were germinated on N6 media plates and only 80 seeds gave rise to callus. Among these 80 seeds calli, majority were normal, healthy and growing and used for transformation (Table 4).

The *Agrobacterium* EHA105 cells were transformed using pBI *P$_{OsC3H52}$:GUS* and colonies were obtained containing hygromycin resistant gene (data not shown). Six weeks old calli (80 in number) were infected with culture of PCR positive pBI *P$_{OsC3H52}$: GUS* transformed *Agrobacterium* culture. During selection browning was observed in calli. Putative transformed calli were resistant to antibiotic (hygromycin) and maintained their growth with no browning and whitish yellow appearance (Fig. 4A). After fifteen days on regeneration media, greening was observed in 10 pBI *P$_{OsC3H52}$:GUS* transformed calli (Fig. 4B). After thirty days on regeneration medium, only 4 calli started regeneration indicating that the regeneration ability of transformed calli was very low (Fig. 4C), when compared to untransformed calli (data not shown).

**Analysis of pBI *P$_{OsC3H52}$: GUS* containing putative transgenic plants:** DNA was extracted from 7 putative transgenic plants and subjected to PCR using primer pair (pBI-F and *C$_3$H$_{52}$*-Pr-R$_{150}$) along with positive control (the plasmid DNA) and negative control (DNA from untransformed plant). Out of 7 putative transgenic plants, 6 plants showed positive results (Fig. 5).

The generated transgenic plants were also analyzed for *GUS* gene expression using histochemical activity of the *GUS* gene. Transient GUS staining was observed in pBI *P$_{OsC3H52}$:GUS* transformed calli (Fig. 6A). However, untransformed calli showed no GUS staining (data not shown). Histochemical activity of the *GUS* gene was also observed in putative transgenic plants (Fig. 6B). GUS staining could be observed in different parts of the plant including crown, leaf sheath, young leaves and emerging roots (Fig. 6B).

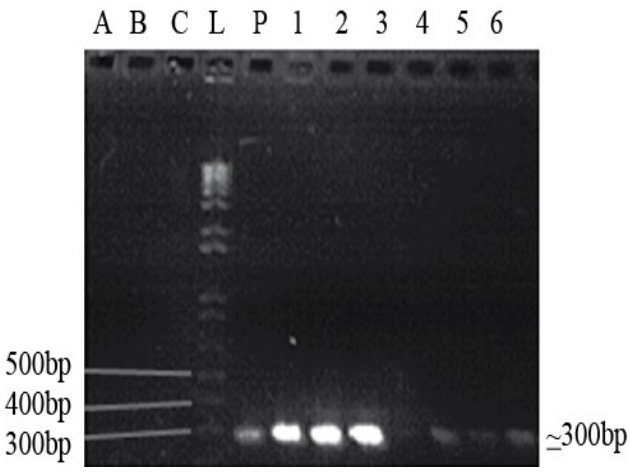


Fig. 5. PCR analysis of the putative pBI *P$_{OsC3H52}$:GUS* transformed transgenic rice. P is positive control (plasmid DNA) and A, B and C are negative controls. L.1 kb plus ladder.

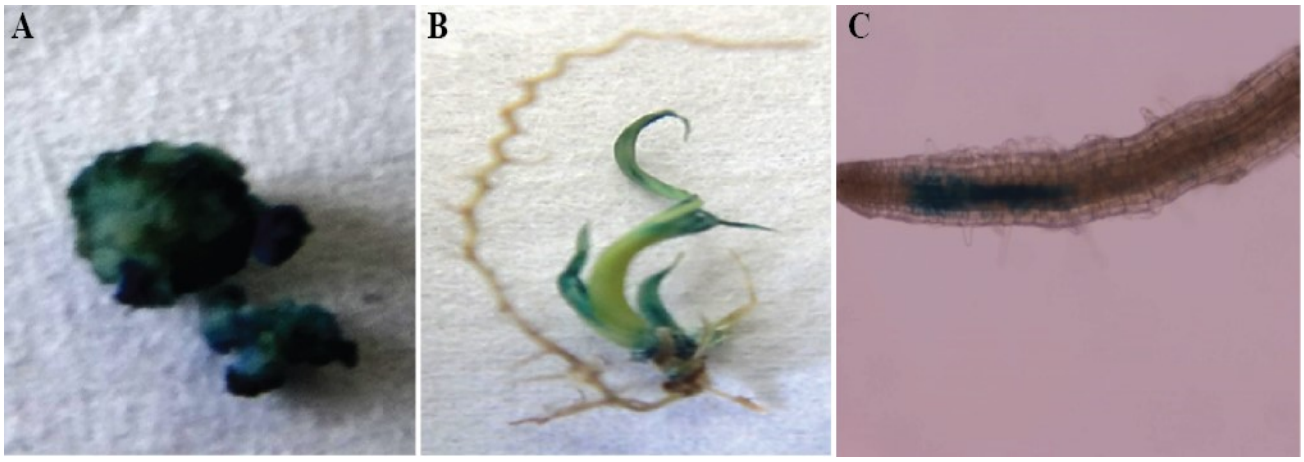


Fig. 6. Histochemical activity of the *GUS* gene in $P_{OsC3H52}$:*GUS* transformed rice transgenic rice (cv. Swat-1). A) Callus showing *GUS* Staining. B) Transgenic rice plantlet showing *GUS* staining. C) Close up view of root expressing *GUS* gene.

**Discussion**

In the recent era of climate change, scientists are struggling hard to improve the production of crops that are adapted to biotic and abiotic stresses. Genetically modified crops are gaining importance for sustainable food production (Donde *et al*., 2014). There are still number of stress inducible genes in rice that are yet to be characterized (Jan *et al*., 2013). $OsC_3H_{52}$ gene belongs to CCCH zinc finger proteins family and was considered for further characterization. Several stress responsive genes have been characterized in transcriptomic analysis of rice (Chao *et al*., 2014) that play important roles in plant stress resistance responses. *OsGZF1* (Chen *et al*., 2014) plays role in the regulation of nitrogen concentration, *GhTZF1* (Zhuo *et al*., 2014) is expressed in different vegetative and reproductive tissues and *OsTZF1* (Jan *et al*., 2013) is induced in abiotic stresses.

Promoters are present upstream of transcription initiation site approx. 35-40 bp upstream. Promoter site includes regions that control many stress responses and biological processes (Butler & Kadonaga, 2002). In this study the putative $OsC_3H_{52}$ gene promoter of 1742 bp was analyzed for *cis*-acting regulatory elements found at different locations using Plant pan and Plant care analysis tools. These included ARE, TCA, G-BOX, TC Rich Repeats, MYBGAHV, GATABOX, BIHD1OS and WRKY71OS etc. and their functions were predicted using bioinformatic approach (Fig. 1; Table 2 & 3). GATA motif is responsible for regulating light responses in CaMV 35S promoter (Lam & Chua., 2000), MYB is a transcription factor in gibberellin pathway needed for alpha amylase gene transcription activation (Gubler *et al*., 1995), BIHD1OS is binding site for *OsBIHD1* and involved in disease resistance responses (Luo *et al*., 2005), TC Rich repeats region is involved in defense and stress mechanisms (Xue *et al*., 2008) and WRKY is found to be a transcriptional repressor of gibberellin signaling pathway in *Amy32b* promoter (Zhang *et al*., 2004; Zhang & Wang, 2005). It is proposed that the *cis*-acting regulatory elements of $OsC_3H_{52}$ gene promoter's might possibly be involved in biotic and abiotic stress tolerance responses.

By using plant pan promoter analysis tool, a tandem repeat region was also identified having consensus sequence of TTC from -1628 bp to -1714 bp. Tandem repeats are more prone to mutation than other parts of genome (Gemayl *et al*., 2010). Comparison of $OsC_3H_{52}$ promoter region sequences of iaponica rice cv. Nipponbare and indica rice cv. Swat-1 revealed a 15 base deletion in the tandem repeat sequence in cv.Swat-1 (data not shown). Mostly the mutations in tandem repeats are due to alteration in number of repeating units (Verstrepen *et al*., 2005; Al souhibani *et al*., 2010; Gemayl *et al*., 2010 and 2012). On the basis of these findings it is speculated that changing the number of repeating units of tandem repeats in putative $OsC_3H_{52}$ gene promoter region might have affected its transcriptional activity.

Indica rice was not considered suitable for tissue culture and transformation procedures (Lin & Zhang, 2005; Nishimura *et al*, 2005). Different strategies have been implemented so for the transformation of indica rice but very few showed successful results with optimum rate of transformation. However, *Agrobacterium*-mediated transformation in indica rice variety has yet an unresolved matter and a huge hurdle in transformation procedures. The reason behind this is poor tissue culture response and genotype incompatibility (Nishimura *et al*, 2005). We made efforts to optimize conditions for local indica rice cv. Swat-I via *Agrobacterium*-mediated transformation. Callus induction media for co-cultivation procedure of transformation, containing 200µM acetosyringone increased frequency of transformation (Kumari *et al*., 2007). Similar findings were obtained in this study by using same concentration of acetosyringone for three days in co-cultivation medium that improved the frequency of transformation (data not shown).

The *GUS* gene reporter system is one of the most effective techniques in the characterization of gene regulation and analysis of promoters in plant molecular biology (Fior & Gerola., 2009). Histochemical analysis was carried out in order to confirm the functional activity of transformed putative $OsC_3H_{52}$ gene promoter (Fig. 6). *GUS* protein activity was observed in pBIG $P_{OsC3H52}$:*GUS* transgenic plants which confirmed the presence of transgene in putative transformants.

Based on the data of *cis*-acting elements, it is suggested that putative $OsC_3H_{52}$ gene promoter might have roles in biotic and abiotic stress responses in rice plants. Transgenic rice plants expressing stress inducible genes under the putative $OsC_3H_{52}$ gene promoter are expected to have enhanced biotic and abiotic stress tolerance.

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