Journal of Plant Biochemistry and Biotechnology (October–December 2022) 31(4):915–924
https://doi.org/10.1007/s13562-022-00771-8

ORIGINAL ARTICLE

Differential transcriptional activation of *copia* family of different plant retrotransposons

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Received: 20 December 2021 / Accepted: 20 January 2022 / Published online: 18 February 2022
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Abstract

Plant genomes contain a sizeable fraction, ranging from 14 to 75% of retrotransposons (class I elements), predominantly comprising LTR (Long Terminal Repeat) elements. Movement of these elements is mediated via an RNA intermediate by copy-and-paste mechanism. The transposition of the elements is tightly regulated, however, under certain conditions such as stress; they are transcriptionally and possibly transpositionally activated. The 5'-LTRs of retrotransposons contain regulatory sequences required for their transcriptional activation. Each element is usually present in multiple copies and not all copies of an element may be functional possibly due to alterations in its internal sequence domains, without affecting the functionality of their 5'-LTRs. We analyzed the transcriptional activation of six *Ty1-copia* family of retrotransposons selected from six different plants (two monocots and four dicots) by monitoring pattern of GUS expression directed by 5'-LTRs in transgenic tobacco plants in response to a variety of stress factors (cold, UV, H_2O_2, $HgCl_2$, $CdCl_2$, $CuCl_2$, 2,4-D, SA, ABA) and tissue-specific (callus, root, leaf, stem and flower) cues. We show that different 5'-LTRs show differential activation under various conditions. Two retroelements which were considered non-functional apparently have functional 5'-LTRs. No apparent correlation between the presence of sequence elements in the 5-LTRs and transcriptional activation of the retroelements in response to stress and tissue-specific signals could be established. The results suggest that the transcriptional activation and possibly silencing of different retrotransposons is a complex process and may be mediated by multiple interconnected pathways.

Keywords Retrotransposon · Transcriptional activation · Heavy metals · Stress response · 5'-LTRs

Abbreviations

LTR	Long Terminal Repeat
LINE	Long Interspersed Nuclear Element
SINE	Short Interspersed Nuclear Element
ABA	Absissic acid
SA	Salicylic acid

Introduction

Transposable elements are ubiquitous components of all eukaryotes. On the basis of their structure and mode of transposition, they are classified into two main categories: retrotransposons (class I elements) and transposons (class II elements). Transposons contain inverted repeats at their termini and transpose via a DNA intermediate by so-called "cut-and-paste" mechanism. The class I elements (retrotransposons) possibly derived from retroviruses transpose via an RNA intermediate by "copy-and-paste" mechanism. Since a copy of the element moves to a new location during transposition, retroelements have been implicated in genome expansion (Kalendar et al. 2000; Schnable et al. 2009; El Baidouri and Panand 2013). On the basis of presence or absence of long terminal repeats (LTR), retrotransposons are classified into two categories: LTR and non-LTR elements. The LTR elements contain long terminal repeats at their termini. On the basis of their internal domain organization, they are further sub-divided into two families: (i) *Ty1–copia* and (ii) *Ty3–gypsy*. The non-LTR retroelements consist of LINEs (Long Interspersed Nuclear Elements) and SINEs (Short Interspersed Nuclear Elements). The SINEs are non-autonomous elements, defective in transposition functions. There appears to be an

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evolutionary partitioning of retroelements between plant and animal kingdoms. The plant genomes predominantly contain LTR retroelements whereas the animal genomes especially those of mammalians contain preponderance of non-LTR elements (Kumar and Bennetzen 1999; Cordaux and Batzer 2009).

Plants contain 14% to 73% of their genomes as retrotransposons, equally of both *copia* and *gypsy* families (Kumar and Bennetzen 1999; Schnable et al. 2009). The 5’-LTRs of the LTR elements contain the regulatory sequences, necessary for their transcription by RNA polymerase II. Transcription of the element followed by reverse transcription into DNA is an essential prerequisite for retrotransposition. The elements have been shown to be transcriptionally activated in response to (i) abiotic stresses (Zeller et al. 2009; Aprile et al. 2009), (ii) tissue culture conditions (Madsen et al. 2005; Vicient 2010), (iii) tissue specific signals (Grandbastien 2015), and (iv) possibly due to metabolic perturbations (Liu et al. 2004). During normal growth and development, their transcription/retrotransposition is tightly regulated by various mechanisms including epigenetic processes including miRNA pathways (Lisch 2009). In spite of these controls, a tiny fraction of retroelements may remain active possibly due to compromise in their epigenetic controls.

Sequence comparison of retroelements from different plant species, indicates that their LTR sequences are not conserved and do not cross hybridize possibly indicating their divergent evolutionary lineages (Wicker and Keller 2007; Neumann et al. 2019). A sequence analysis of 5’-LTR sequences of different retroelements of the *copia* family reveals the presence of a multitude of sequence elements known to confer inducibility in response to a variety of factors. We selected six retrotransposons of the *copia* family from six different plant species; four of them have been shown to be active and two inactive in retrotransposition. Non-functionality of an element could be due to mutations in its 5’-LTRs or defect in its internal domain structures. However, it is known that the non-functional elements may have functional regulatory regions i.e. 5’-LTRs (Lall et al. 2002). In order to analyze the regulatory controls involved in transcriptional activation of the elements we analyzed the expression pattern of GUS conferred by 5’-LTRs in transgenic tobacco plants by generating 5’-LTR-GUS constructs and transferring them to tobacco plants. The pattern of GUS expression in transgenic plants indicates that different 5’-LTRs show different pattern of transcriptional activation and some of the non-functional elements have functional 5’-LTRs. The results also demonstrate that the factors responsible for 5’-LTRs activation are present in heterologous plant systems.

Materials and methods

Seeds of plant materials, namely, mung bean (*Vigna radiata*), oat (*Avena sativa*), maize (*Zea mays*), bean (*Phaseolus vulgaris*) and sweat potato (*Ipomoea batatas*) were procured from Indian Agricultural Research Institute, New Delhi and of *Arabidopsis thaliana* and tobacco (*Nicotiana tabacum*) were available in the laboratory.

Retrotransposons and isolation of 5′-LTRs

Six retrotransposons of the *copia* family from six different plant species were selected for studying their 5’-LTR mediated transcriptional activation (Table 1). DNA from different plants was isolated as per the method described by Murray and Thompson (1980) with minor modifications. To amplify the 5’-LTR sequences from the genomic DNA, PCR reactions were set up using primer-sets specific to each of the 5’-LTRs (Table 2). The amplified bands were separated on 1.2% agarose gel, excised and DNA extracted. They were cloned into pGEMT easy vector. Appropriate restriction sites were included in the primers for directional cloning of the 5’-LTR upstream to GUS region in the pCAMBIA1391Z plasmid vector.

Generation of tobacco transgenic plants

The pGEMTeasy plasmids containing 5’-LTR inserts were excised by digestion with appropriate restriction enzymes for directional cloning. The 5’-LTRs were then cloned into the plant binary plasmid vector, pCAMBIA1391Z. The binary plasmid vector containing the LTR construct was transferred to *Agrobacterium* by the freeze–thaw method (Horsch and Klee 1986). The constructs were then transferred to tobacco leaf discs by *Agrobacterium*-mediated transformation (Horsch and Klee 1986). After co-cultivation on the basal medium, leaf discs were transferred onto the plates with MS medium supplemented with 1.0 mg/l BAP, 0.1 mg/l NAA, 250 mg/ml cefotaxime, and 300 mg/ml kanamycin. After 18–25 days, well developed shoots were excised and placed for rooting. The plantlets were grown in flasks and/or transferred to pots and grown in the greenhouse. Integration and structural integrity of the gene construct in the individual transformants were checked by Southern blotting as well as PCR amplification using GUS (forward: 5’-GCGGTAACAAGAAAGGGATCTT-3’ and reverse: 5’-GAACTGATCGTTAAAACTGCCTG-3’) and 5’- LTR specific primers.

Table 1 Characteristics of *copia* family of LTR retrotransposons used in the study

Retroelement	Source plant	Accession no	Size (bp)	Size of 5'-LTR (bp)	Expression under normal conditions	Expression under stress conditions	References
OARE-1	*Avena sativa*	AB061327	7412	1714	No	Wounding, UV, jasmonic acid, salicylic acid	Kimura et al. (2001)
RTvr1	*Vigna radiata*	AY900121	5594	680	No	No	Xiao et al. (2007)
Tpv2-6	*Phaseolus vulgaris*	AJ005762	5846	297	No	UV, salicylic acid	Garber et al. (1999)
Rtsp-1	*Ipomoea batatas*	AB162659	4968	472	Callus	Callus	Tahara et al. (2004)
Stonor	*Zea mays*	AF082134	4542	560	No	No	Marillonnet and Wessler (1998)
Ta1-2	*Arabidopsis thaliana*	X53976	5262	315	No	No	Voytas et al. (1990)

Table 2 Primer sequences used for cloning 5'-LTRs' in this study

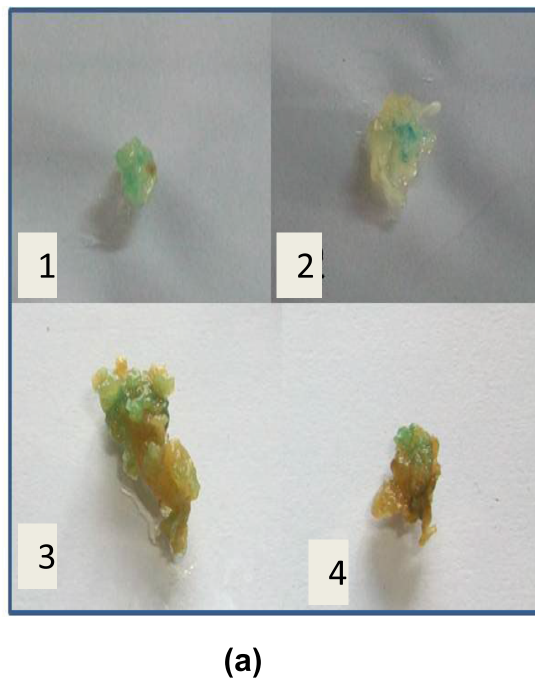
Name of Retroelement	5'-LTR primer sequence
OARE-1	Forward 5'-GTCGACTGGAATTATGCCCTAGAGGA-3'
(*Avena sativa*)	Reverse 5'-TCTAGAACGCATAGATCTAGCTCTGA-3'
RTvr1	Forward 5'-AAGCTTGTTAAGCAGTGAGGTCAACC-3'
(*Vigna radiata*)	Reverse 5'-GGATCCGTGGCATCAGAGCTCTTAGG-3'
Tpv2-6	Forward 5'-GGATCCACCATTGGCTCTTGATACCA-3'
(*Phaseolus vulgaris*)	Reverse 5'-AAGCTTCTACCCAATGATGTCACCAC-3'
Stonor	Forward 5'-AAGCTTTGTTAGGATGTGTCCTCTAC-3'
(*Zea mays*)	Reverse 5'-GGATCCTGTTATGAAATTACTGGTGC-3'
Rtsp-1	Forward 5'-AAGCTTTGTCGGCAAATGGAAGTTTG-3'
(*Ipomoea batatas*)	Reverse 5'-GGATCCAATCTCAAATTTCGCTGCCA-3'
Ta1-2	Forward 5'-GCATGCTGATCCAATTCCTAAGTTGC-3'
(*Arabidopsis thaliana*)	Reverse 5'-GGATCCACCAATGGCTCTGATACCAC-3'

Stress and hormone treatments

Transgenic tobacco plants were exposed to different salts, metals or phytohormones in MS basal medium in dark for 12 h. The transgenic plants were treated as follows: salinity stress (10, 20, 50 and 250 mM of NaCl); heavy metals: $HgCl_2$ (1, 10 and 20 µm), $CdCl_2$ (1 and 2 µm), $CuCl_2$ (100 mM); phytohormones: ABA (50 mM), 2,4-D (50 mM), salicylic acid (50 mM); cold stress (4 °C for 12 h); wounding (stabbed with sharp forceps and kept in MS basal medium in dark for 12 h); UV irradiation (UV exposure for 2 min followed by 12 h in MS basal medium in dark); and H_2O_2 (1% v/v) for 12 h.

Histochemical and spectrofluorometric assays

X-gluc was used as a substrate for histochemical localization of GUS activity (Jefferson et al. 1987). Ten mg of X-gluc was dissolved in 1 ml of dimethylformamide and subsequently diluted in 50 mM phosphate buffer pH 7.0 to give final concentration of 1.0 mg/ml. The tissues were incubated in the X-gluc solution at 37 °C in dark for 10–12 h. The tissues were kept in 70% ethanol at 4 °C to remove chlorophyll. For spectrometric assay the plant tissue frozen in liquid N_2 was ground in 1 ml of extraction buffer (50 mM phosphate buffer pH 7.0, 5 mM DTT, 1 mM EDTA, 0.1% sarcosyl, 0.1% Triton X-100) at 4 °C, and centrifuged at 10,000 g for 5 min. The supernatant (50 ml) was added to 450 ml of the assay buffer (1 mM MUG in the extraction buffer) and incubated at 37 °C for 30 min. The GUS activity was determined by fluorometric assay (Jefferson et al. 1987). The total protein was estimated by the Bradford method (Bradford 1976). The specific activity of GUS was recorded as nanomoles of 4-MU formed per mg protein per hr from the initial velocity of the reaction (Jefferson et al. 1987).



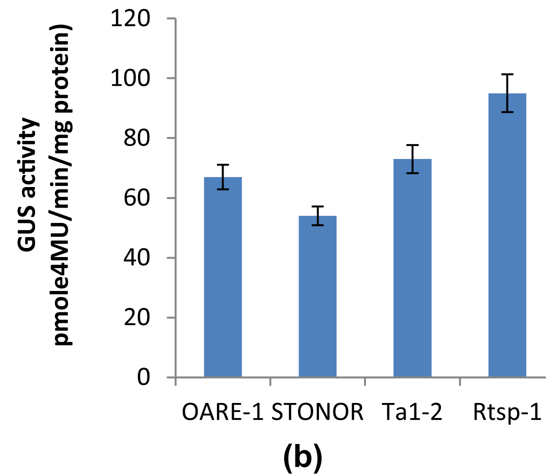


Fig. 1 **a** Histochemical analysis of GUS expression in callus of transgenic tobacco plants harboring 1) *OARE-1, 2) Ta1-2, 3) Rtsp-1*, and 4) *Stonor 5'*-LTR. **b** Histogram depicting variation in GUS expression in callus tissues of different the 5'-LTR-GUS transgenic tobacco plants. Bars represent mean ± SE

Table 3 5'-LTR directed GUS expression in different tissues of transgenic plants

Retroelement	Callus	Leaf	Stem	Root	Flower
OARE	+	−	+	+	−
RTvr1	−	+	−	−	+
STONOR	+	−	−	−	−
Ta1-2	+	+	−	−	+
Tpv2-6	−	+	+	−	−
Rtsp-1	+	+	−	−	−

+ GUS staining; − No GUS staining

Results

Regulatory sequences (5′-LTRs) of retrotransposons: computational analysis

The 5'-LTRs of retroelements contain regulatory sequences required for transcriptional activation of the element. The reverse transcriptase domains of the elements are conserved but a high degree of divergence exists among DNA sequence of the 5'-LTRs of different elements. The analysis of presence of various *cis*-acting elements within the 5'-LTR sequences using PLACE database (Higo et al. 1999) indicates the differential presence of sequence elements. In silico analysis of the 5'-LTRs reveals many different putative regulatory sequence elements, which may have a role in transcriptional activation of the elements. Differences among the regulatory sequences of different retroelements imply that they may be differentially regulated. In order to delineate the transcriptional controls specified by 5'-LTRs on retroelements, we selected 6 different retrotransposons for the study. The selected elements shown to be functionally active are: *OARE-1* (oats), *Tpv2-6* (bean), *Rtsp-1* (sweet potato) and *RTvr-1* (mung bean) and the inactive ones are *Stonor* (maize) and *Ta1-2* (*Arabidopsis*). The structural features of the selected retroelements are given in Table 1. The *OARE-1* element is the largest (7412 bp) with over 1700-bp of each LTRs. The other elements vary in size from 4542 bp (*Stonor*) to 5846 bp *(Tpv2-6*) in length. We analyzed the transcriptional activation of the elements by generating transgenic tobacco plants with the 5'-LTR-GUS gene constructs and assayed the GUS expression.

Transcriptional activation of retroelements in response to tissue-specific signals

Transgenic tobacco plants carrying the 5'-LTR-GUS gene constructs were raised through tissue culture. Integration of the construct, number of copies integrated and the structural integrity of the construct were checked by Southern analysis and PCR amplification. The transgenic plants having a single integration of the transgene were selected

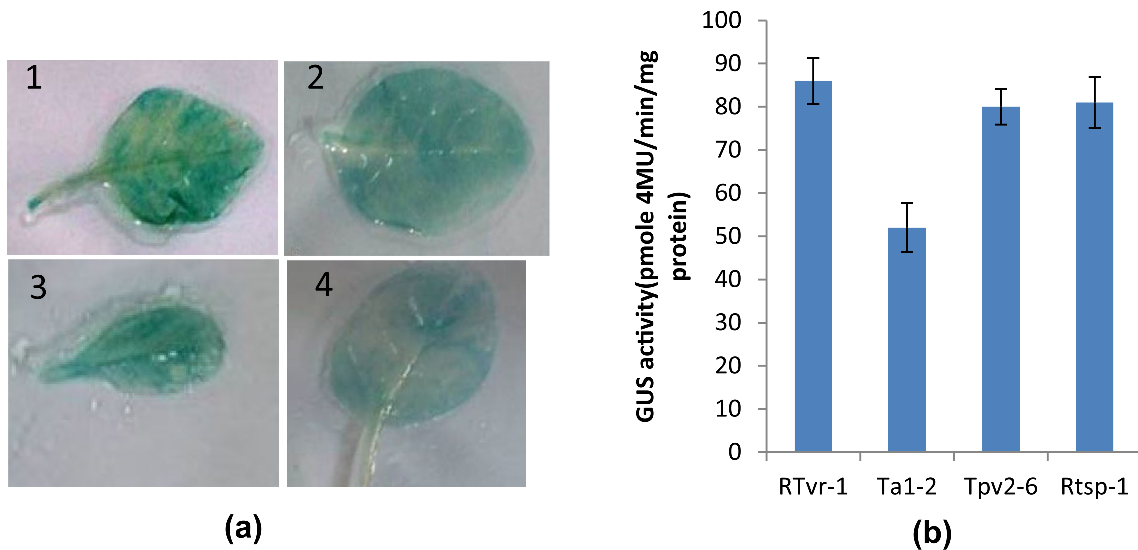


Fig. 2 **a** Histochemical analysis of GUS expression in leaves of transgenic tobacco plants harboring 1) *RTvr-1,2) Ta1-2,* 3) *Tpv2-6, 4) Rtsp-1*. **b** Variation in GUS expression in leaf tissue of different the 5'-LTR-GUS transgenic tobacco plants

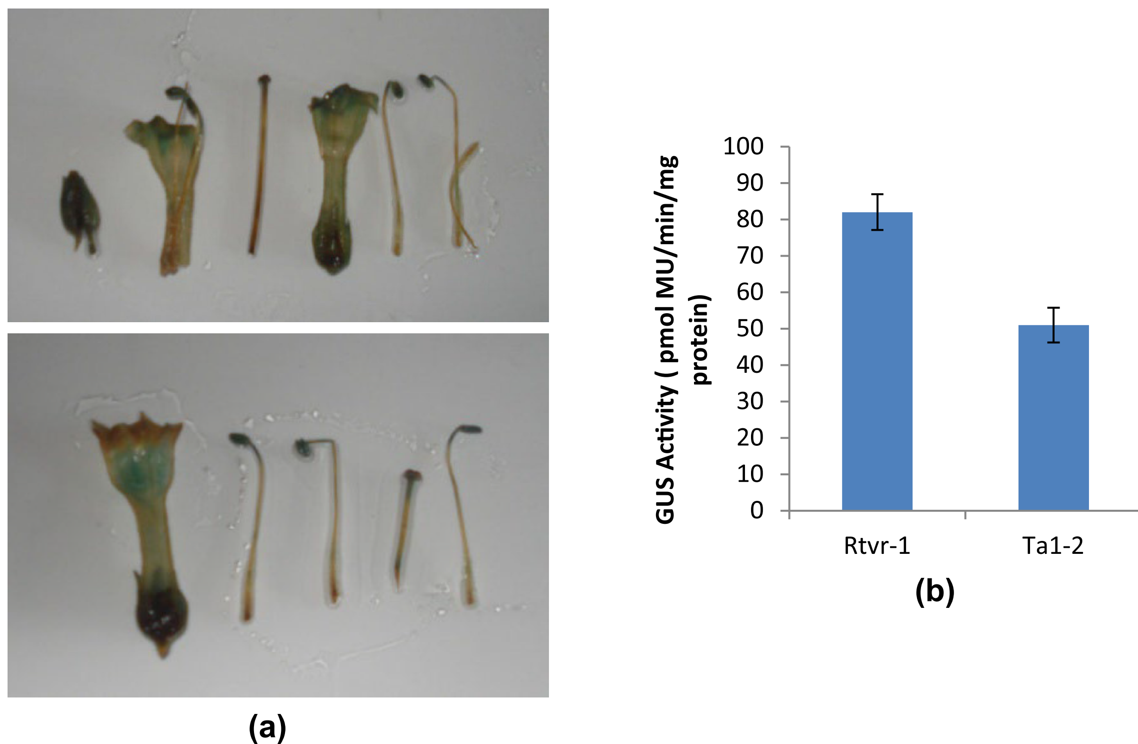


Fig. 3 **a** Histochemical analysis of GUS expression in floral tissues of (A) *RTvr-1* (B) Ta1-2 harboring 5'-LTR GUS transgenic tobacco plants. **b** Histogram depicting variation in GUS expression in floral tissue of different the 5'-LTR-GUS transgenic tobacco plants

for analysis. Multiple different transgenic plants from each construct were generated. At least ten different transgenic plants arising from independent transformation events from each construct were generated. Since the expression is known to be dependent on the site of integration, among the resulting transgenics, the best expressing plants were selected for analysis. If multiple different transgenic plants showed no GUS expression, then it was considered, no expression. GUS activity was assayed in callus as well as in different plant tissues (leaves, stem, roots and flowers). The GUS expression was also monitored spectrofluorometrically in different tissues. The 5'-LTRs of *Stonor* are active

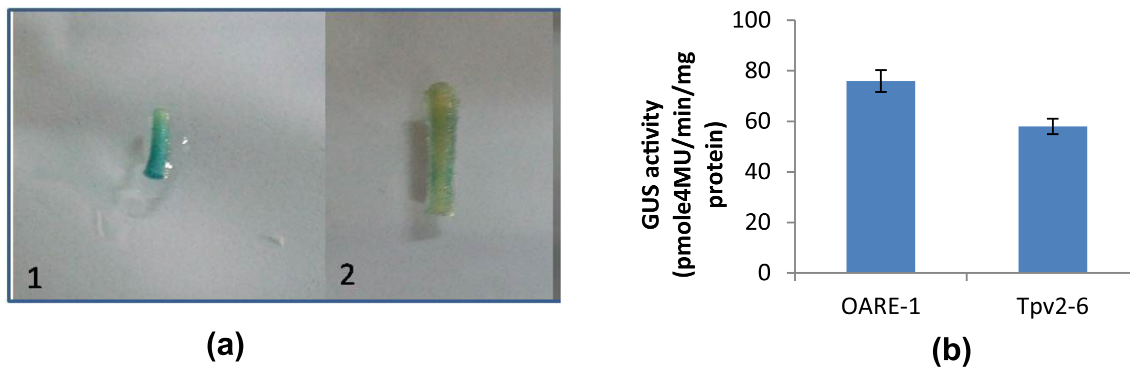


Fig. 4 **a** Histochemical analysis of GUS expression in stem of transgenic tobacco plants 1) *OARE-1,* 2) *Tpv2*-6. **b** Histogram depicting variation in GUS expression in stem tissue of different the 5'-LTR-GUS transgenic tobacco plants

Table 4 5'-LTR directed GUS expression in response to various stress factors

Retroelement	$HgCl_2$	$CdCl_2$	$CuCl_2$	UV	Cold	H_2O_2	2,4-D	SA	ABA	Wounding
OARE	+	+	−	+	−	−	+	+	−	+
RTvr1	−	−	+	+	−	+	+	+	+	+
STONOR	−	−	−	−	−	−	−	−	−	−
Ta1-2	−	−	−	+	−	+	−	−	−	+
Tpv2-6	+	+	+	+	+	−	+	+	+	+
Rtsp-1	+	+	+	−	+	−	+	+	−	−

+ GUS expression; − No GUS expression

only in callus and show no expression in any other tissues assayed. Normally retroelements are active in tissue culture conditions but the 5'-LTRs of *RTvr1* and Tpv2-6 did not show any activity in callus (Fig. 1a, b; Table 3). Though they are active in leaves and stems, RTvr1 is also active in flowers. *Ta1-2* has been classified as a nonfunctional element (Voytas et al. 1990) but it's 5'-LTR is active in leaf and floral tissues besides callus (Figs. 1a, b, 2a, b, 3a, b; Table 3). Only the *OARE1* element shows activation in roots. Besides callus OARE-1 is also active in stems (Figs. 1a, b, Fig. 4a, b; Table 3). Fluorometric analysis of GUS activity suggests that 5'-LTR from Rtsp-1 is a more efficient promoter in callus tissue (Fig. 1a, b; Table 3). The activation of the retroelements as explained earlier refers to functionality of 5'-LTRs under normal growth conditions except in the callus tissue. The differential transcriptional activation of 5'-LTR in response to tissue-specific cues would possibly depend on the presence of sequence elements. The results demonstrate that retrotransposons of the *copia* family isolated from different plants are differentially regulated in response to tissue-specific expression.

Transcriptional activation of retroelements in response to abiotic stresses, phytohormones and heavy metals

Retrotransposons are known to be transcriptionally activated in response to stresses and metabolic perturbation in both plants and animal systems. We analyzed the expression of retroelements from six different plant species in response to salt stress (NaCl), UV, wounding, free radicals (H_2O_2), cold, and phytohormones (2,4-D, SA, ABA). The transgenic plants carrying different 5'-LTR-GUS constructs were exposed to different treatments and GUS expression was assayed spectrofluorimetrically. The salinity stress (NaCl) has no effect on activation of any of the retroelements analyzed. The *Stonor* element of maize, which is considered to be inactive (Marillonnet and Wessler 1998), shows no activity in response to any of the effectors including heavy metals. The results are presented in Table 4. The *Phaseolus* element, *Tpv2-6* is activated by all the factors except free radicals (H_2O_2). The 5'-LTRs of the *Arabidopsis* element *Ta1-2* is active in response to UV, wounding and H_2O_2 but shows no activation under mercury, cadmium, copper, cold, phyohormones (2,4-D, SA and ABA). The *Rtsp1* element of sweet potato is activated in response to 2,4-D, SA, UV and cold stress but not in response to ABA, wounding and H_2O_2. Except *Stonor*, all

the other five elements are activated by UV irradiation. The results conclusively demonstrate the differential regulation of the six retroelements in response to 2,4-D, SA, ABA, UV, cold stress and free radicals (H_2O_2) (Table 4).

Effect on transcriptional activation of the 5'-LTRs of eight retroelements by heavy metals i.e. mercury ($HgCl_2$), cadmium ($CdCl_2$), and copper ($CuCl_2$) was monitored by spectrofluorimetric assays. The *Tpv2-6* and *Rtsp-1* retroelements are expressed in response to all the three heavy metals (mercury, cadmium and copper) tested (Table 4). The *OARE* element from oats is active only under $HgCl_2$ treatment whereas *Rtvr* from *Vigna radiata* shows activation only in response to $CuCl_2$. The *Stonor* and *Tal-2* elements are not activated in response to any of the heavy metals tested.

Discussion

A large proportion of genome expansion during the long evolutionary process has been ascribed to retrotranspositional activity of transposable elements. The maize genome containing over 75% retroelements appear to testify to this proposition (Schnable et al. 2009). Transposition of retroelements is, by and large, stabilized and they appear to be under tight regulatory controls. However, such controls are relaxed under certain conditions including in response to tissue-specific cues and stresses (Grandbastien 2015) Normally a high degree of sequence and size similarities exist between 5' and 3'-LTRs of an element. An element could be non-functional due to defects in its internal domains or in its 5'-LTR. Since the elements normally present in multiple copies, not all the copies may be functional. However, a non-functional element may have functional 5'-LTRs (Lall et al. 2002).

Activation of retroelements is thought to be brought about by multiple pathways which may involve intricate interplay of a variety of factors:— LTR regulatory sequences, specific transcription factors, epigenetic factors and also miRNA pathways (Grandbastien 2015). Whatever factors contribute to activation or silencing of the elements, the absolute requirement must be the presence of regulatory sequences within the elements capable to respond to these factors. Our study shows that retroelements are differentially regulated in response to tissue culture signals, stress-mediated signals, developmental and tissue-specific signals. In addition we show that 5'-LTRs of certain retro elements are expressed in response to heavy metals (Hg^{2+}, Cd^{2+} and Cu^{2+} salts).

Numerous citations of transcriptional activation of retroelements have been reported earlier under tissue-culture conditions (Hirochika et al. 1996; Lall et al. 2002; Liu et al. 2004). Cellular differentiation during tissue culture possibly leads to partial relaxation of epigenetic controls, leading to transcriptional activation (Koukalova et al. 2005). Retroelements studied in our experiment show different pattern of expression in heterologous plant tissues. Callus induction cause genomic rearrangement and epigenetic changes different from that of natural conditions, resulting in altered gene expression (Miguel and Marum 2011). Callus tissues derived from *OARE-1, Sonor, Ta1-2* and *Rtsp-1* transgenics show 5'-LTR activation, while retroelements considered active, RTvr1 (*Vigna radiata*) and Tpv2-6 (*Phaseolus vulgaris*) are not active in differentiating tobacco calli.

Retroelements from cereals (monocots) *OARE1* (oats), *Stonor* (maize) are inactive in transgenic tobacco leaves. *OARE1* is active in stem and root tissues. It contains no specific element. Such differences in expression cannot be explained on the basis of dicot/monocot divisions. In case of DNA transposons, there is considerable evidence of horizontal gene transfer of elements from monocots to dicots exemplified by the existence of common transposons families in both dicots and monocots. It is not clear whether such scenario exists with respect to retrotransposons.

It is considered that retroelements are active in floral and embryonic cells (Lall et al. 2002; Jaaskelainen et al. 2013). However, we observed that several elements are not active in floral tissue. The results points to interplay of complete set of events in transcriptional activation of retroelements in tissue specific manner. Jaaskelainen et al. (2013) analyzed the expression pattern of BARE elements in barley using Gag and RT specific antibodies. The Gag protein is localized in provascular tissues, developing floral spikes and pre-fertilization ovaries. In silico analysis has revealed that 5'-LTR of *OARE-1* has multiple copies (six copies) of ROOTMOTIFTAPOX1 element (Elmayan and Tepfer 1995), which might be playing a role in root-specific expression. ROOTMOTIFTAPOX1 has also been identified in the 5'-LTRs of *Rtsp-1, Tvp2-6* but none of these elements are activated in the roots. A possible reason may be the absence of auxiliary factors necessary for expression in roots or presence of a negative regulatory motif that prevents expression of these elements in root tissue.

Expression of LTR retroelement in heterologous system in floral tissues has been earlier reported in *Panzee* element from pigeon pea (Lall et al. 2002). *Ogre,* a *copia* type LTR retroelement from peas, also reported to be expressed in floral tissues in its host system (Neumann et al. 2003). In our study transgenic plants carrying 5'-LTR of *RTvr-1, Ta1-2* expressed GUS in the floral organs. It has been shown that retroelements are unexpectedly reactivated and transpose in the pollen vegetative nucleus, which accompanies the sperm cells but does not provide DNA to the fertilized zygote in *Arabidopsis* (Slotkin et al. 2009). In silico analysis of 5'- LTR sequences did not identify any

floral tissue specific element except POLLEN1LELAT52 responsible for pollen specific activation of tomato (Bate and Twell 1998).

Activation in response to stress factors

The selected retrotransposons show differential activation with respect to different stresses tested i.e. UV, Cold, H_2O_2 and wounding. Stress induced activation of transposons was proposed in the genome shock hypothesis by Barbara McClintock (1984). Activation of the retroelements in response to stress has been extensively reviewed by Gransdbastien (2015).

In spite of the presence of putative sequence elements, 5'-LTR from *Stonor* is completely inactive. Since this elements are active in callus, the inactivity may not be attributed to mutational defects in the 5'LTR sequences. In contrast to *Stonor* 5'-LTRs of *Tpv2-6* and *Rtsp-1* are highly active under stress. In silico analysis of 5'-LTR of both *Tpv2-6* and *Rtsp-1* identified LTRECOREATCOR15, a core element of Low Temperature Responsive Element (LTRE) from *cor15a* gene found in *Arabidopsis* (Baker et al. 1994). This element is also involved in cold induced expression of BN115 gene from *Brassica napus* (Jiang et al. 1996). Activation of 5'-LTRs' of *Rtsp-1* and *Tpv2-6* may be attributed to presence of LTRE sequences. In addition the 5'-LTR region of *Rtsp-1* contains CBFHV element present in the promoter of genes expressed under dehydration and low temperature conditions in barley (Xue, 2002; Svensson et al. 2006). The CBHFV element might have a role in activation of 5'-LTR from *Rtsp-1* retroelement in response to low temperature induced stress.

H_2O_2 plays important role in stress signaling. Earlier reports of H_2O_2 mediated expression of retrotransposons can be seen in TLC1.1 form *Solanum chilense* in transgenic tobacco (Salazar et al. 2007). Activation in response to H_2O_2 could be mediated by the defense related *cis* acting elements like WBOXNTCHN48 involved in elicitor responsive transcription of various defense genes in tobacco (Yamamoto et al. 2004) found in 5'-LTR of *Ta1-2*. WBOXNTERF3 was identified in the 5'-LTRs of *RTvr-1, Ta1-2, Tpv2-6 and T12-C14* retroelements. It is possible that the motif WBOXNTERF3 is involved in wounding mediated expression of the elements. The wounding activated retroelements contain additional stress activated and hormone regulated elements which could activate their expression in response to wounding. The 5'-LTR of *RTvr-1* contains CURECORECR element, the core sequence of a CuRE (copper-response element) found in Cyc6 and Cpx1 genes in *Chlamydomonas* (Quinn et al. 2000). CURECORECR motif might be responsible for activation of RTvr-1 in response to Cu^{2+} ions.

Heavy metals toxicity leads to overproduction of ROS in living systems. Under normal conditions, ROS controls processes like programmed cell death, pathogen defense and development. Enhanced production of these species as a consequence of heavy metal toxicity impacts the intrinsic antioxidant defense system of cells and causes oxidative damage (Mittler 2002). Plants produce various thiols, peptides, metallothioneins like proteins in response to heavy metal treatment. These products sequester the metal into vacuoles and lead to detoxification (Cobbett and Goldsbrough 2002). Additionally, HSPs are also activated in response to heavy metals. This activation of HSP is mediated by HSE (Heat Shock Elements) (Hall 2002). Mercuric chloride induced GUS expression in *OARE-1, Tpv2-6 and Rtsp-1* 5'-LTR constructs. Cadmium chloride activates promoter elements in the 5'-LTRs of *Tpv2-6* and *Rtsp-1*. In silico analysis of 5'-LTRs of *OARE-1*, *Tpv2-6, Rtsp-1* and *Rtvr-1* could not identify any specific motif for heavy metal-mediated activation. However, the 5'LTRs of *OARE-1* and *RTvr-1* contains CCAATBOX1 found in the promoter of heat shock protein genes and act cooperatively with HSEs to increase the heat shock promoter activity (Rieping and Schöffl 1992). Since heat shock proteins are activated in plants on treatment with heavy metals, it is possible that CCAATBOX1 may have played a role in transcriptional activation of 5'-LTR-GUS in response to heavy metals.

Activation in response to phytohormones

Phytohormones play essential role in various physiological processes in plant. *Tal-2* (*Arabidopsis*) beside *Stonor* are not activated in response to any of the phytohormones (2,4-D, SA and ABA). Phytohormones play essential role in various physiological processes in plant. TLC1 element has shown to be induced in response to salicylate in heterologous system (Salazar et al. 2007). Similarly Tnt1 is activated in tobacco, and in heterologous system of tomato in response to SA (Mhiri et al. 1997). TLC1.1 retrotransposon is activated by synthetic auxin, 2, 4-D (Salazar et al. 2007). ABA is shown to activate TLC1.1, a member of the TLC family of retroelements in heterologous condition, though ABA did not activate TLC1.1 in its natural host, *Solanum chilense* (Salazar et al. 2007). In silico analysis of 5'-LTR sequences of the selected retroelements reveals the presence of hormone responsive elements such as DPBFCOREDCDC3, ASF1MOTIFCAMV, WBOXATNPR1, TCA1MOTIF. Presence of these elements may be attributed to activation of retroelements in response to hormones.

It is difficult to explain the functionality of these retrotransposons, only thing could be stated that transcriptional activation/non activation must be a consequence of

interplay of multitude of factors known and some yet to be uncovered. Since the study is based on heterologous system, the possible differences in terms of variations between natural host and heterologous systems cannot be ruled out. The ability of 5'-LTR sequences to respond to a wide variety of stress conditions in a heterologous system, as exhibited by *OARE-1, RTvr, Tpv2-6, and Rtsp-1*, is not common among plant retrotransposons. Besides differential expression, another important observation in our present study is variation in expression pattern in natural host and heterologous system. This variation has been cited in numerous reports earlier, but the mechanism behind this phenomenon is still not clear and requires detailed investigation.

Acknowledgements Financial assistance of University Grants Commission (UGC) is gratefully acknowledged. SM acknowledges the CSIR for fellowship.

Author's contribution SM designed and performed the experiments. KCU conceptualized the study and designed the experiments. DS contributed the lab support and some of the supplies. KCU and SM wrote the manuscript.

Declaration

Conflict of interest Authors report no conflict of interest.

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