Plant Physiology and Biochemistry 135 (2019) 460–468

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

# Transcriptional activation of long terminal repeat retrotransposon sequences in the genome of pitaya under abiotic stress

Qiong Nie[a,b], Guang Qiao[a], Lei Peng[a], Xiaopeng Wen[a,*]

[a] Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Institute of Agro-bioengineering/College of Life Sciences, Guizhou University, Guiyang, 550025, PR China

[b] College of Agriculture, Guizhou University, Guiyang, 550025, Guizhou Province, PR China

ARTICLE INFO

*Keywords:*
Abiotic stress
Gene expression
LTR retrotransposon
Pitaya
Somatic variation

ABSTRACT

Frequent somatic variations exist in pitaya (*Hylocereus undatus*) plants grown under abiotic stress conditions. Long terminal repeat (LTR) retrotransposons can be activated under stressful conditions and play key roles in plant genetic variation and evolution. However, whether LTR retrotransposons promotes pitaya somatic variations by regulating abiotic stress responses is still uncertain. In this study, transcriptionally active LTR retrotransposons were identified in pitaya after exposure to a number of stress factors, including in vitro culturing, osmotic changes, extreme temperatures and hormone treatments. In total, 26 LTR retrotransposon reverse transcriptase (RT) cDNA sequences were isolated and identified as belonging to 9 Ty1-*copia* and 4 Ty3-*gypsy* families. Several *RT* cDNA sequences had differing similarity levels with *RTs* from pitaya genomic DNA and other plant species, and were differentially expressed in pitaya under various stress conditions. LTR retrotransposons accounted for at least 13.07% of the pitaya genome. HuTy1P4 had a high copy number and low expression level in young stems of pitaya, and its expression level increased after exposure to hormones and abiotic stresses, including in vitro culturing, osmotic changes, cold and heat. HuTy1P4 may have been subjected to diverse transposon events in 13 pitaya plantlets successively subcultured for four cycles. Thus, the expression levels of these retrotransposons in pitaya were associated with stress responses and may be involved in the occurrence of the somaclonal variation in pitaya.

## 1. Introduction

Retrotransposons are widely distributed in plant genomes and play an important role in the size, structure, function and evolution of these genomes through self-replication and insertion into multiple genomic sites (Amyotte et al., 2012; Jiang and Ramachandran, 2013; Yamada et al., 2014; Li et. al., 2014). Long terminal repeat (LTR) retrotransposons are a dominant type of transposable elements, and have had a great effect on the organization and evolution of plant genomes (Du et al., 2010). LTR retrotransposons can be subdivided into the Ty1-*copia* and the Ty3-*gypsy* superfamilies based on retroviral structural homology and the domain order in the *pol* gene (Kapitonov and Jurka, 2008). Both Ty1-*copia* and Ty3-*gypsy* retrotransposons exist as high-copy-number sequences in plant genomes (Nystedt et al., 2013). They display a high degree of heterogeneity and insertional polymorphism (Carrier et al., 2012). To understand the changes in the genome, it is important to identify and characterize the reverse transcriptase (RT) sequences of LTR retrotransposons.

Retrotransposons are usually silent during normal growth and development. The host gene-silencing machinery appears to play an important role in controlling retrotransposon expression (Ito et al., 2011). However, some retrotransposons show persistent expression in plant somatic tissues (Rico-Cabanas and Martínez-Izquierdo, 2007; Gao et al., 2015). In addition, accumulating evidence also indicates that retrotransposons can escape silencing and become activated transcriptionally and even transpositionally by a variety of biotic and abiotic stresses, including wounding, hormone treatments, tissue culturing, environmental stresses and interspecies hybridization (Kashkush et al., 2003; Liu et al., 2004; He et al., 2012; Voronova et al., 2014). Strawberry (*Fragaria* × *ananassa*) *FaRE1* is induced by abscisic acid (ABA), naphthalene acetic acid and 2,4-dichlorophenoxyacetic acid (2,4-D), as well as by cold stress (He et al., 2010). *ONSEN-1* in *Arabidopsis thaliana* is transcriptionally activated by heat (Ito et al., 2013). *Tto1* in tobacco and *Tos17* in rice are activated when they are tissue cultured (Liu et al., 2004), as well as by wounding and exposure to jasmonate (JA) and fungal elicitors (Takeda et al., 1999). In addition,

* Corresponding author.
*E-mail address:* xpwensc@hotmail.com (X. Wen).

https://doi.org/10.1016/j.plaphy.2018.11.014
Received 7 September 2018; Received in revised form 10 November 2018; Accepted 13 November 2018
Available online 14 November 2018

expression levels of Ty1-*copia* and Ty3-*gypsy* retrotransposons increase with exposure to JA, gibberellin (GA) and salicylic acid (SA) (Hawkins et al., 2008; Fan et al., 2014). Recently, the expression of *GBRE-1* was detected in *Gossypium*, and its expression level was augmented by heat stress (Gao et al., 2015). The transcriptional activation of LTR retrotransposons might considerably alter the expression of adjacent genes (Gaubert et al., 2017). If the hypothesis holds, then retrotransposons may participate in transcriptional responses to stress, resulting in a potentially evolutionary advantage for plants (Cao et al., 2015; Finatto et al., 2015).

By analyzing retrotransposon sequences, we can determine the compositions of plant genomes and study their evolution and expression. To date, many plant LTR retrotransposons have been isolated and identified, and their effects on the host genome composition, system evolution and gene-expression regulation have been studied (Biswas et al., 2010; Du et al., 2010; Fan et al., 2013). Felice et al. (2009) found that retrotransposons are a leading cause of citrus genome mutations and evolution, with the *RT* sequences in affected buds having deletions and frameshift mutations. Additionally, retrotransposons can influence gene activity directly by inserting inside or close to coding regions, leading to changes in host gene regulation and ultimately plant phenotypes (Butelli et al., 2012; Rebollo et al., 2012; Kim et al., 2015). Therefore, the regulation of retrotransposon activity is an important source of plant genetic variation (Ito et al., 2011).

Pitaya is an exotic non-climacteric fruit with high nutritional and economic values (Lim, 2012; Zhuang et al., 2012). As a member of the family Cactaceae, it demonstrates intensive drought tolerance and is, therefore, recognized as a promising crop in drought-stricken areas (Viñas et al., 2012). Recently, a large number of somatic variations of pitaya were found in high-temperature and drought-stricken areas, as well as in tissue culture (unpublished). Previously, we amplified 23 Ty1-*copia* and 86 Ty3-*gypsy RT* sequences from the genome of pitaya using degenerate oligonucleotide primers based on the conserved *RT* domains of Ty1-*copia* and Ty3-*gypsy* retrotransposons. These sequences were highly heterogeneous and displayed several mutations, including deletions, frameshifts and stop codons (Fan et al., 2012; Peng et al., 2017). The activation of retrotransposons might substantially contribute to genetic variation in plants and could confer an adaptive advantage for plant survival (Finatto et al., 2015). To assess the involvement of LTR retrotransposons and the high occurrence of genetic variations in plant survival, the objectives of the present study were to: 1) detect retrotransposon activity; 2) analyze the phylogenetic relationships among activated fragments; 3) characterize the activation of pitaya LTR retrotransposons in response to abiotic stresses; and 4) detect variation, to lay the foundation for the further genetic improvement of this crop.

## 2. Materials and methods

### 2.1. Plant materials and stress treatments

Pitaya stem buds from the same clone *(Hylocereus undatus* 'Zihonglong') were rapidly micropropagated as previously described (Nie et al., 2015). The rooted plantlets (with age-matched shoots, 5–6 cm in height) were transplanted to pots and grown in a greenhouse for 3 months, and then, they were subjected to various stress conditions. For hormone treatments, the shoots were sprayed with 100 μM of 2,4-D, ABA, SA, $GA_3$ or water (control). For osmosis and salt stresses, the plants were independently soil-drench treated with 20% polyethylene glycol (PEG, MW 6000) and 200 mM NaCl, respectively. Soil drenching with water served as the control. For heat and cold treatments, the plants were placed in climate cabinets at 45 °C and 0 °C, respectively. For UV stress, the plants were placed in a UV box with UV-A at 315–400 nm and irradiance at 0.68–0.8 W $m^{-2}$. There were 30 plants in each treatment group. The shoots from treated and control plants were collected at 2, 6, 12 and 24 h after treatment, immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

### 2.2. Identification of retrotransposon activation

Total RNA from the control and stressed samples were isolated using TransZol Plant RNA Extraction Kit (Tiangen, China), and subsequently treated with DNaseI (TaKaRa, Japan) at 37 °C for 2 h. The integrity of the RNA was determined on a 1% agarose gel. First-strand cDNA synthesis was carried out using the PrimeScript™ RT Reagent Kit (TaKaRa) following the manufacturer's instructions. To detect the transcriptional activation of LTR retrotransposons, RT-PCR was performed based on the *RT* domains of Ty1-*copia*-type and Ty3-*gypsy*-type retrotransposons using degenerate primers. The primer sequences, and the PCR mixture and amplification, were as previously described (Fan et al., 2013). Control reactions with 50 ng genomic DNA (gDNA) and 1 μL $ddH_2O$ were routinely included in the PCR amplification. Amplification products were detected by electrophoresis at 120 V for 40 min in a 1% agarose gel.

To compare and verify the expression responses of novel potentially active retrotransposons to various stress treatments, three specific primer pairs, RtCr 5′-ACAACAACAAAATGATGAA-3'/RtCf 5′-TGGAGA ACTTGAAGAAGAG-3′, RtPr 5′-TCGTCCACATACAGTAATAAATA-3'/ RtPf 5′-TCTCTGGGAAGGAACACAT-3′ and RtAr 5′-ATGAAGAACCGCT ACCCTCC-3'/RtAf 5′-ATCCTCAAATGACAGTTGTGCTC-3′, which were designed based on the *RT* sequences using Primer 5.0 software. They were used to detect the expression of the *RT* gene fragments HuTy1C3 (236 bp), HuTy1P4 (196 bp) and HuTy3A3 (221 bp), respectively. The quantitative real-time PCR (qRT-PCR) analysis was performed using SYBR® Fast qPCR Mix (TaKaRa) on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). The PCR consisted of 10 μL SYBR Fast qPCR Mix, 50 ng cDNA, 0.4 μM of each specific primer and 0.4 μL ROX Reference Dye II (50 × ), brought to 20 μL with $ddH_2O$. The PCR amplification was carried out as follows: 95 °C pre-denaturization for 30 s, then 40 cycles of 95 °C for 5 s and 60 °C for 15 s. A comparative threshold cycle (Ct) was used to determine retrotransposons expression, and the Ct value of each sample was normalized to the Ct values of two internal control genes (*HuActin* and *HuUBQ*). The relative expression levels of retrotransposons under different conditions were calculated using the $2^{-\Delta\Delta Ct}$ method.

### 2.3. Cloning and sequence analysis

The RT-PCR products from pitaya shoots induced by PEG, cold and ABA stresses were purified from polyacrylamide gels using a Universal DNA Purification Kit (Tiangen) and individually cloned into the pGEM-T vector (Promega, USA). A total of 64 recombinant colonies were selected using blue–white selection and colony PCR. PCR products were sequenced in both directions using M13 primers and BigDye terminator v3.1 Cycle Sequencing Kit technology (Sangon, China).

Homology queries of cloned sequences were analyzed using BLASTn and BLASTx (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple-sequence alignments were performed with ClustalX version 2. Genetic similarities were calculated at both nucleotide and amino acid levels using Mega Package Version 5.2 (Nie et al., 2015) with p-distances. Phylogenetic analyses were carried out using the NJ and UPGMA methods with 1000 permutations.

### 2.4. Dot-blotting and copy number analysis of retrotransposons

Total DNA was extracted from untreated pitaya using a Plant Genomic DNA Extraction Kit (Tiangen) according to the manufacturer's instructions. The genomic abundance levels of selected *RT* fragments of the LTR retrotransposons were determined using Southern dot-blotting. Serial dilutions of denatured pitaya gDNA (400, 200, 100 and 50 ng) and the *RT* gene's PCR products (400, 200, 100, and 50 pg) were spotted on a Hybond™-N membrane. Subsequently, the DNA was fixed to the membrane at 120 °C for 30 min. The 260- (Ty1-*copia*), 430- (Ty3-*gypsy*), 236- (HuTy1C3) and 196-bp (HuTy1P4) products labeled with digoxigenin (DIG Nick Translation Kit, Roche) were used as probes for detection. Hybridization was performed in the DIG Easy Hybridization Solution at 42 °C overnight in a hybridization oven (Bibby Stuart Scientific, UK). Membranes were washed and visualized by immunological detection according to the instruction manual for the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche). Hybridization signals were quantified using ChemiDocXRS (BioRad, USA). Integrated densities used for the calculation of copy numbers were obtained using ImageJ software (Collins, 2007). A linear-regression equation correlating the natural logarithm of the probe sequence's copy number in the dilution dot-blots and the corresponding densitometric readings was used to estimate the probe's copy number in the gDNA sample: copy number = size of haploid genome × average proportion of nuclear gDNA hybridizing to the probe/probe size (MacRae, 1998). The diploid pitaya genome size was estimated on the basis of the genomic analyses of three *Hylocereus* species and their progeny (Cisneros and Tel-Zur, 2013).

### 2.5. Identification of the LTR retrotransposons' transposition activity levels

The total DNA of 66 plantlets from four cycles of rapid propagation were extracted and evaluated by the 260/280 nm absorption ratio and were then diluted to 50 ng/μl. To detect the transposition activity of *HuTy1P4*, two specific primers, 5r 5′-GCAAGACCCATTCTCATACTG-3′ and 5f 5′-TGGTGTGTTGGTTGAAAAAGT-3′, were designed from the *HuTy1P4* sequences using Primer 5.0 software. The PCR consisted of 50 ng DNA, 5 μM primer and 5 μL Mastermix (containing 0.1 U $\mu L^{-1}$ Taq polymerase, 500 μM dNTP, 20 mM Tris-HCl pH 8.3, 100 mM KCl, 3 mM $MgCl_2$ and other stabilizers and intensifiers), brought to 10 μL with dd$H_2O$. The PCR amplification was carried out as follows: 3 min at 94 °C, 38 cycles of 45 s at 94 °C, 45 s at 55.4 °C, 1 min at 72 °C, and a final extension for 5 min at 72 °C. The amplified products were electrophoresed on a 1.5% agarose gel and viewed under UV light.

### 2.6. Statistical methods

All of the results are expressed as means ± SEs. Each treatment had three biological replicates, and each set of data was analyzed three times. A one-way ANOVA was performed using the software SPSS 21.0. Significant differences between stress treatments and controls were determined by a LSD test. Observations were considered significantly different at $P < 0.05$ (n = 3).

## 3. Results

### 3.1. Retrotransposon activation by abiotic stresses

Two pairs of degenerate primers were used to amplify the conserved domains of the *RT* genes of Ty1-*copia* and Ty3-*gypsy* retrotransposons. RT-PCR was carried out after subjecting plants to the following stress conditions: in vitro culturing, and exposure to ABA, $GA_3$, 2,4-D, SA, UV, an osmotic agent (PEG), heat, cold and NaCl. PCR products were barely obtained for the untreated samples (controls) but were present in the gDNA (positive control) and stressed samples at various time points after stress induction (Fig. 1), indicating that Ty1-*copia* and Ty3-*gypsy* retrotransposons were usually silent but could be expressed after exposure to abiotic stresses. The greatest expression levels were observed at 12 and 24 h after PEG, NaCl and extreme temperature exposure, and 6 and 12 h after hormone treatments. A cold stress markedly activated both types of retrotransposons. The transcription levels of retrotransposons triggered by heat, ABA, 2,4-D and SA were lower than those triggered by the cold treatment. $GA_3$ did not increase the transcription of Ty3-*gypsy* retrotransposons but weakly stimulated the expression of Ty1-*copia* retrotransposons after 6 and12 h of treatment.

### 3.2. Isolation and characterization of retrotransposon RT sequences

RT-PCR products from PEG-, cold- and ABA-treated plants were cloned and sequenced. In total, 64 plasmid inserts were randomly selected for sequencing, and 43 clones of Ty1-*copia* or Ty3-*gypsy RT* genes with homology to known retroelements (GenBank) were obtained (Table 1). Finally, 26 RT sequences were analyzed after exposure to abiotic stresses. There were 15 Ty1-*copia* sequences, 10 from PEG-stressed samples, named sequentially HuTy1P1 to HuTy1P10, and 5 from cold-stressed samples, named sequentially HuTy1C1 to HuTy1C5. The 11 other sequences were Ty3-*gypsy* type, 1 from PEG-stressed samples, named HuTy3P, 7 from cold-stressed samples, named sequentially HuTy3C1 to HuTy3C7 and 3 from ABA-stressed samples, named sequentially HuTy3A1 to HuTy3A3. All of the sequences were submitted to GenBank (Accession nos. KU984981–KU985006).

These were subsequently used to assess diversity (Table 1). The Ty1-*copia RTs* ranged from 257 bp to 267 bp in length, with the most fragments being 266 bp. The Ty3-*gypsy RTs* ranged from 349 bp to 432 bp in length, with the most variants being 431 and 432 bp. These sequences were AT-rich, at an average of 60% for Ty1-*copia* and 55% for Ty3-*gypsy* types. A high level of nucleotide heterogeneity was observed in the Ty1-*copia RTs,* with the diversity of the nucleotide sequences ranging from 2% to 49%. Compared with the Ty1-*copia RTs*, those of Ty3-*gypsy* showed lower sequence heterogeneity levels, with an average of 22%. Thus, the transcriptionally active *RTs* obtained using the same degenerate primers were not completely uniform, having polymorphisms that resulted in length and base changes, as well as differing stress responses, as a consequence of the heterogeneous LTR retrotransposon transcriptional activity in pitaya.

The putative amino acid sequences were derived by aligning the RT sequences with their corresponding open reading frames (ORFs). The 15 Ty1-*copia* sequences contained the conserved motifs TAFLHG, SLYGLKQ and YVDDM (Fig. 2), and possessed intact ORFs. The isolated Ty3-*gypsy* RTs contained five conserved RT domains, the active amino acid site DD and the highly conserved amino acid sequence MCVDYREL (Fig. 3). Among them, 10 had intact ORFs, while HuTy3P lacked the 3′-region YAKLSKC and the preceding 12 amino acids (Fig. 3). The average amino acid sequence identity levels among individual sequences ranged from 42% to 100% for Ty1-*copia* RTs and 55%–97% for Ty3-*gypsy* RTs, which indicated a somewhat higher heterogeneity levels among the transcriptionally active *RTs* (Table 1).

### 3.3. Phylogenetic analysis of retrotransposon RT sequences

In total, 15 nucleotide sequences of Ty1-*copia RTs* from pitaya cDNA were aligned with 23 RTs reported from pitaya gDNA (GenBank accession nos. JN102305–JN102327, HuRT1–HuRT23). The phylogenetic tree formed 10 branches (Fig. 4a). The 15 Ty1-*copia RTs* from pitaya cDNA were divided into 9 branches, with branch I having the largest family, containing 18 members, 5 RTs from the cDNA and 13 RTs from the gDNA of pitaya. None of these *RTs* contained premature stop codons and/or indels that disrupted the reading frame, and they shared an over 90% nucleotide sequence identity. The nucleotide sequence identity among the groups HuTy1P5, HuTy1P8, HuRT3 and HuRT23, and HuTy1P4, HuRT5, HuRT19 and HuRT21 were 97%–98%. HuTy1P1, HuTy1C2, HuTy1C5 and 5 RTs from the gDNA were located in branch II and shared an 85%–92% nucleotide sequence identity. In this family, all of the *RTs* from the gDNA, except HuRT20, contained a premature stop codon and/or indels. Branch III included HuTy1C1 and 3 RTs from the gDNA and shared an 87%–97% nucleotide sequence identity. HuTy1C3 and HuRT10 were in branch IV and shared a 90% nucleotide identity. HuTy1P2, HuTy1P3, HuTy1P10, HuTy1P7 and HuTy1P6 were unique in branches VI, VII, VIII, IX and X, respectively. Overall, transcriptionally active Ty1-*copia RTs* showed a high level of heterogeneity in pitaya. However, some *RTs* were very similar to each other and to *RTs* from pitaya gDNA.

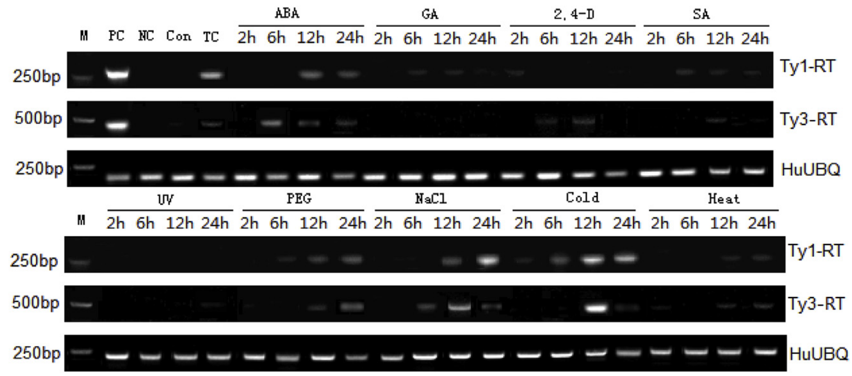


**Fig. 1.** RT-PCR profiles of Ty1-*copia* and Ty3-*gypsy* type retrotransposons. M: DL2000 DNA ladder, PC: positive control (gDNA), NC: negative control (total RNA), Con: controls (untreated samples), TC: in vitro culture.

**Table 1**
Sequence analysis of LTR retrotransposon *RT* genes isolated from pitaya cDNA.

| | Plasmids inserts | RT fragments | Different fragments | Length[a] (bp) | A + T | Nucleotide similarity (mean) | Amino acid similarity (mean) |
|---|---|---|---|---|---|---|---|
| Ty1-*copia* | 29 | 25 | 15 | 257-267/266 (60%) | 60 | 51- 98 (68) | 42-100 (64) |
| PEG | 15 | 13 | 10 | 257-267/266 (40%) | 59 | 52-98 (65) | 45-100 (67) |
| Cold | 14 | 12 | 5 | 263-266/266 (80%) | 61 | 64-92 (72) | 69-88 (76) |
| Ty3-*gypsy* | 35 | 18 | 11 | 349-432/432&432 (91%) | 55 | 59-99 (78) | 57-97 (78) |
| PEG | 10 | 2 | 1 | 349 | 58 | | |
| Cold | 15 | 10 | 7 | 431-432/432 (57%) | 55 | 68-99 (79) | 75-97 (80) |
| ABA | 10 | 6 | 3 | 431-432/431 (67%) | 55 | 92-93 (92) | 83-87 (85) |

[a] Indicates the length range/the most common length (the percentage of the most common fragments in the total).

A phylogenetic tree based on nucleotide sequences of 11 Ty3-*gypsy* *RTs* isolated from pitaya cDNA and 86 RTs reported from pitaya gDNA (GenBank accession nos. KU977005-KU977089, KX090146 , HURT1–HURT86) was also constructed. In total, 97 Ty3-*gypsy* *RTs* were divided into 7 branches (Fig. 4b), and the 11 RTs from pitaya cDNA were grouped into 4 branches. HuTy3C1, HuTy3C3, HuTy3C4, HuTy3C5, HuTy3A1, HuTy3A2, HuTy3A3 and 29 gDNA *RTs* were located in branch I. Among them, the nucleotide sequences of HuTy3A3 and HURT1 were 100% identical, while those of HuTy3C1, HuTy3C3, HuTy3A2 and HURT6, HuTy3C4, HuTy3C5 and HURT33 were 98%–99% identical. HuTy3C2, HuTy3C6 and 17 RTs from the gDNA were located in branch II, and among them, HuTy3C2 and HURT15 were very similar, sharing a 99% nucleotide identity. HuTy3C7 and six gDNA *RTs* were located in branch III, and HuTy3C7 shared 99% and 98% identities with HURT16 and HURT23, respectively. HuTy3P1, HURT38, HURT79 and HURT86 were assigned to branch V. These RTs from the pitaya cDNA were highly heterogeneous. Some *RT* cDNA sequences were very similar to the gDNA sequences, indicating that they may be the corresponding transcripts.

To unravel the phylogenetic relationships with other species, the putative amino acid sequences of 15 Ty1-*copia* RTs from pitaya cDNA and 10 Ty1-*copia* RTs derived from the GenBank database were used for alignment. The phylogenetic tree showed that the 15 RTs fell into 9 families based on branching patterns (Fig. 5a), which was consistent with Fig. 4a. There were great differences (< 80% similarity) between the RT families from pitaya and the active retrotransposons *Tntl, Tto1, Tosl 7, FaRE1, ONSEN* and *GBRE-1* from other species. However, HuTy1C3 in family IV, having a strongly supported clade (100% bootstrap value), showed the greatest homology (91% identity) with *MERE1* from *Medicago truncatula*. This indicates that they belong to the same retrotransposon family and possessed a common progenitor.

The phylogenetic tree based on the 11 new putative amino acid sequences of pitaya Ty3-*gypsy* RTs and 12 Ty3-*gypsy* RTs from other species had 8 branches, and the new obtained RTs were assigned to 5 branches (Fig. 5b). Only HuTy3C7 and six Ty3-*gypsy* RTs from other species were located in the same branch (branch IV). HuTy3C7 was most closely related to CAD45567.1, followed by ABD43143.1 and AAL79340.1, with each sharing an 80% amino acid sequence similarity.

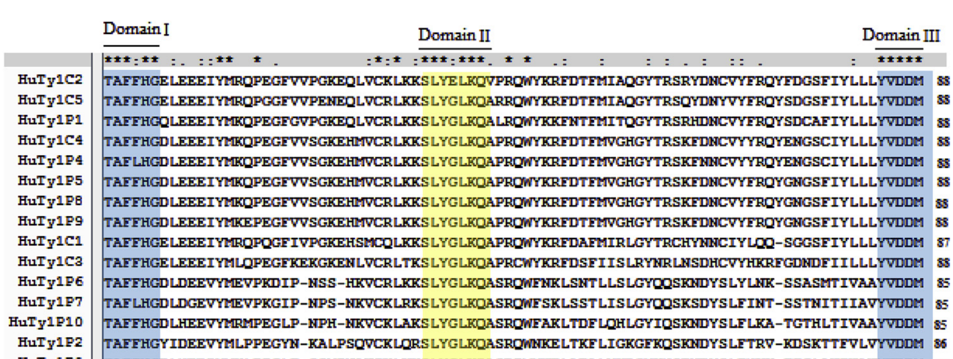


**Fig. 2.** Alignment of inferred amino acid sequences of Ty1-*copia* RTs isolated from pitaya by ClustalX. "-" , "*" , ":" and "." indicate gaps introduced for the optimal alignment, and identical, conserved and semi-conserved amino acid residues in all of the sequences, respectively.


**Fig. 3.** Alignment of inferred amino acid sequences of Ty3-*gypsy* RTs isolated from pitaya by ClustalX. "-", "*", ":" and "." indicated gaps introduced for the optimal alignment, and identical, conserved and semi-conserved amino acid residues in all of the sequences, respectively.

The other four families showed large genetic differences with the RTs of other species. Based on our analysis, the Ty3-*gypsy* RTs based on pitaya cDNA showed a high level of divergence from the RTs other species.

(a) The phylogenetic tree of Ty1-*copia* RTs. The *RTs* from other species are transcriptionally active *Tntl* (X13777.1, *Nicotiana tabacum*), *Tto1* (D83003.1, *N. tabacum*), *Tosl 7* (EU155081.1, *Oryza sativa*), *FaRE1* (FJ871121.1, strawberry), *ONSEN* (JX035973.1, *Arabidopsis*), GBRE-1 (KF740825.1, *Gossypium*), *MERE1* (ACL97386.1, *Medicago truncatula*) and AKM12491.1 (*Coffea ebracteolata*), JQ975219.1 (*Pinus massoniana*) and CAD43247.1 (*Patellifolia procumbens*). (b) Phylogenetic tree of Ty3-*gypsy* RTs. The RTs from the other species are CAD45567.1 (*Elaeis guineensis*), AFR43611.1 (*Ziziphus jujuba*), CAN66348.1 (*Vitis vinifera*), AAL79340.1 (*O. sativa*), XP_007049933.1 (*Theobroma cacao*), ABD43143.1 (*Orobanche ramosa*), BAB40830.1 (*Morus bombycis*), XP_006602532.1 (*Glycine max*), ACU42183.1 (*Prunus mume*) and AGX45501.1 (*Prunus salicina*), AM901562.1 (*Musa acuminata*) and ADF46007.1 (*Eleocharis cellulosa*). The branch lengths are proportional to the genetic distances as estimated by the UPGMA method with 1000 bootstrap replications.

### 3.4. Expression levels of HuTy1C3, HuTy1P4 and HuTy3A3 under abiotic stress conditions

HuTy1C3, HuTy1P4 and HuTy3A3 were investigated to understand the responses of different retrotransposons to different stress conditions (Fig. 6). HuTy1C3 was not constitutively expressed in the control but could be induced by $GA_3$, SA, cold, heat and in vitro culturing. The expression levels of the HuTy1C3 fragments were elevated to a maximum 340-fold increase during in vitro culturing and a 214-fold increase was observed after 24 h of cold treatment. Low expression levels were observed when the samples were subjected to PEG or NaCl stress. The expression levels of HuTy1P4 was very low under stress-free conditions but high under in vitro culturing conditions, as well as when exposed to PEG, NaCl, cold, ABA, $GA_3$ and 2,4-D treatments. The relative expression level of HuTy1P4 was greatest in in vitro cultured samples at 179-fold that of the control. Similarly, its expression was increased by SA and heat stresses. HuTy3A3 was expressed after exposure to SA, UV, NaCl, cold and heat treatments, as well as during in vitro culturing. The greatest expression of HuTy3A3 was also found in the in vitro-cultured shoots. HuTy3A3 expression was not detected in the $GA_3$-treated samples but was expressed at a low level in ABA- and 2,4-D-treated samples. Thus, the different RTs were differentially

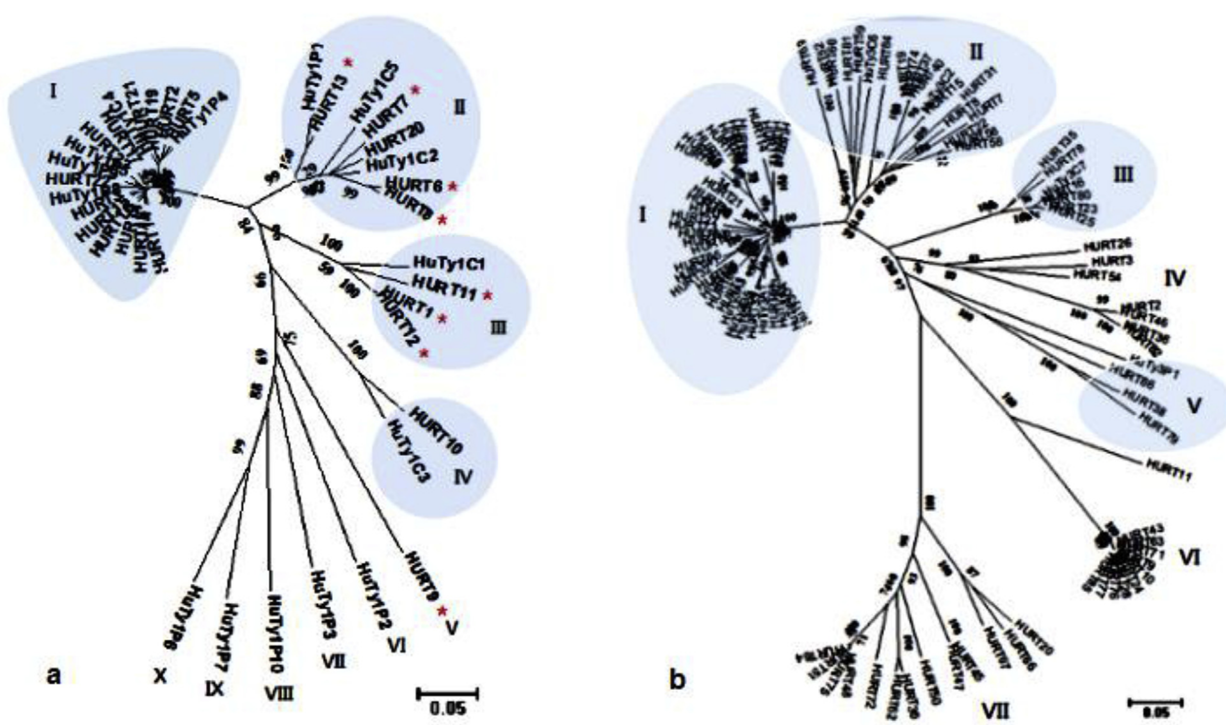


**Fig. 4.** Neighbor-joining tree of nucleotide sequences constructed based on the alignments of the Ty1-*copia* (a) and Ty3-*gypsy* (b) *RTs* from pitaya cDNA and gDNA. "*" indicates stop codons and/or frameshifts and deletion mutations.

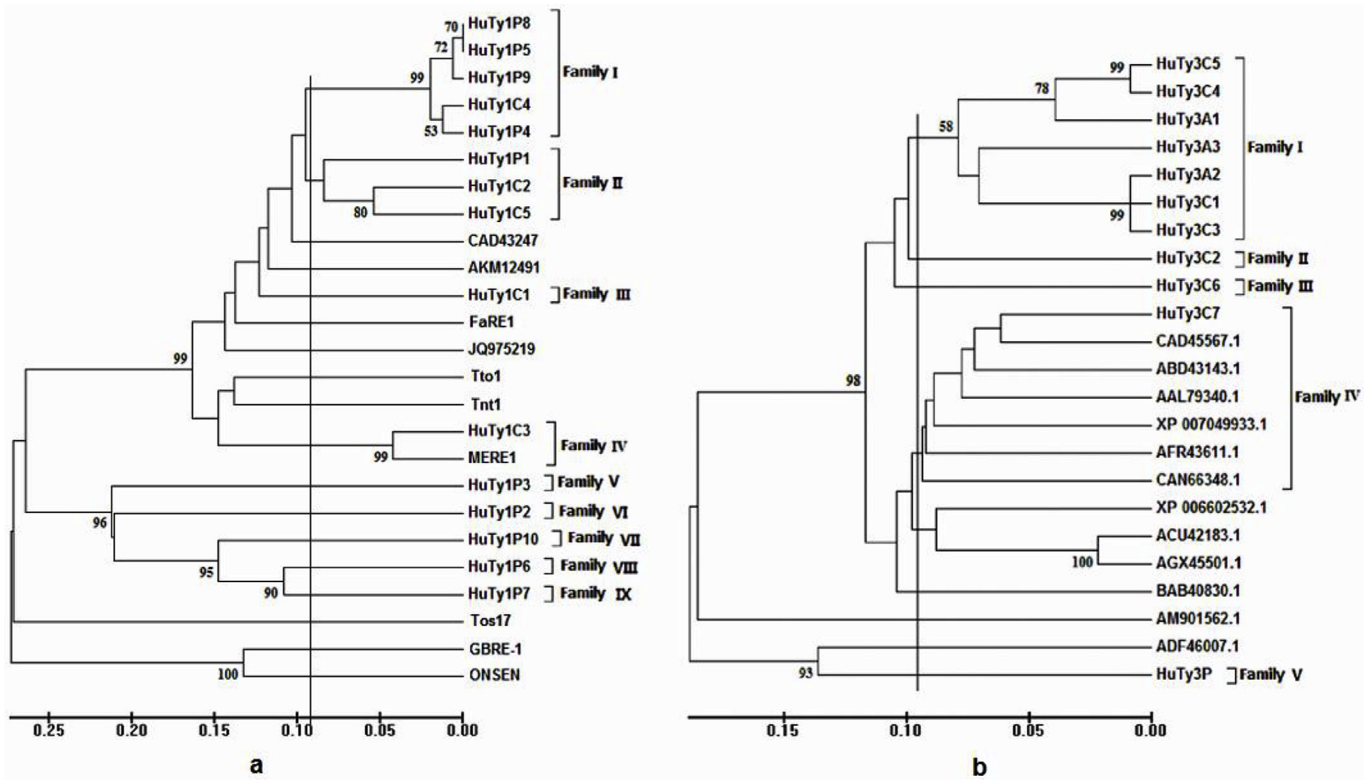


**Fig. 5.** Phylogenetic tree of the predicted amino acid sequences for the transcriptionally active *RTs* of pitaya and those of other plants obtained from GenBank.

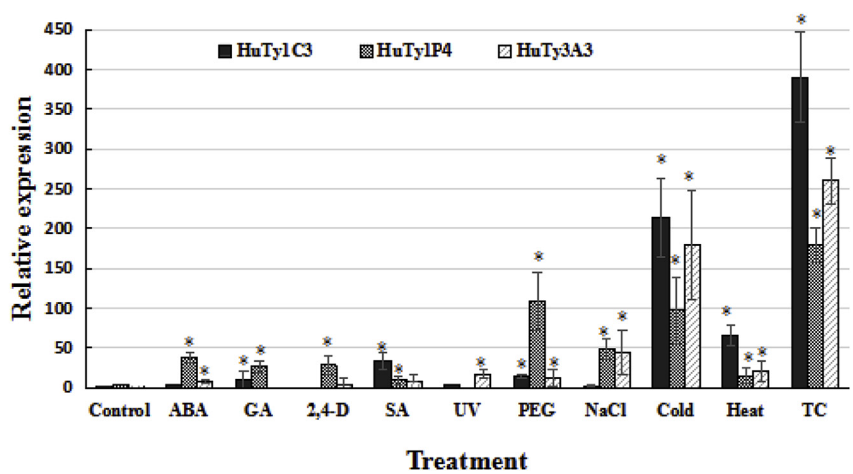


**Fig. 6.** Expression profiles of HuTy1C3, HuTy1P4 and HuTy3A3 under abiotic stress conditions and after hormone treatments as assessed by qRT-PCR.

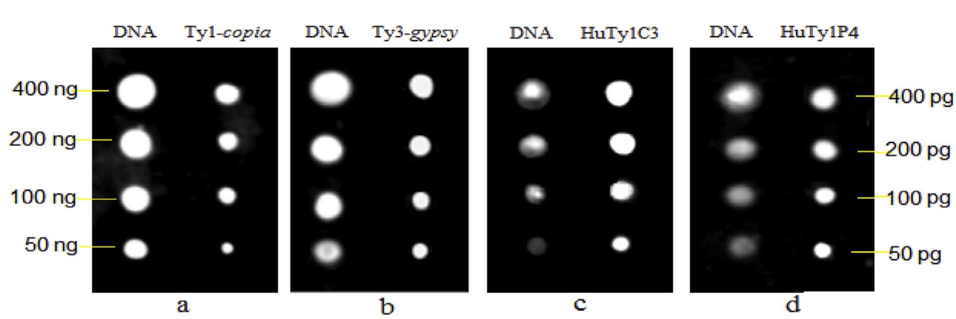


**Fig. 7.** Dot-blot analysis to estimate the retrotransposon copy numbers in the pitaya genome. (a) Ty1-*copia* retrotransposons, (b) Ty3-*gypsy* retrotransposons, (c) HuTy1C3 and (d) HuTy1P4.

expressed under various stress conditions but most strongly activated by in vitro culturing.

Values are means ± standard errors of three biological replicates. "*" indicates significant differences between the stressed and the control at each stage ($P < 0.05$). Control: untreated samples. TC: in vitro culture.

### 3.5. Copy numbers of Ty1-copia and Ty3-gypsy RTs in the pitaya genome

To determine the copy number of Ty1-*copia*, Ty3-*gypsy*, HuTy1C3 and HuTy1P4 in the pitaya genome, four heterogeneous populations of 260-, 430-, 236- and 196-bp fragments from the *RT* gene were used as probes for a dot-blot hybridization analysis. Serial dilutions of total gDNA of pitaya, as well as the *RT* gene fragments from Ty1-*copia*, Ty3-*gypsy*, HuTy1C3 and HuTy1P4 retrotransposons were used to compare signal intensities (Fig. 7). The *Hylocereus* species have been reported to be self-incompatible diploids (Lichtenzveig et al., 2000), and Cisneros and Tel-Zur (2013) found that *H. undatus* (2n = 2x = 22) have a 2C-DNA content that ranges from 3.76 pg to 4.40 pg (4.0 pg on average). Based on 1 pg = 978 Mb, we predicted the diploid genome size of *H. undatus* to be 1956 Mb (1C ≈ 2 pg ≈ 1956 Mb). According to the hybridization-signal intensity, we calculated the copy number of Ty1-*copia RTs* in pitaya to be approximately 22,396. Using the same method, the copy numbers of Ty3-*gypsy*, HuTy1C3 and HuTy1P4 were calculated to be 9,898, 1162 and 3,082, respectively. Assuming that all *RT* copies detected herein represented full-length retrotransposons and that the average sizes of Ty1-*copia* and Ty3-*gypsy* LTR retrotransposons were 7 kb and 10 kb, respectively (Fan et al., 2013), both types of LTR retrotransposons accounted for approximately 13.07% of the pitaya genome. Ty1-*copia* and Ty3-*gypsy* accounted for 8.01% and 5.06%, respectively. HuTy1C3 and HuTy1P4, being potentially functional retrotransposons, represented 0.41% and 1.01% of the genome, respectively.

### 3.6. HuTy1P4 transposition activity

Retrotransposon-based molecular markers can be used to sensitively detect genome changes resulting from retrotransposons transpositions. HuTy1P4, with a high copy number, was easily activated by hormone exposure and abiotic stress (Fig. 6); therefore, two primers were designed based on the conserved sequence of HuTy1P4, to detect the genetic stability of in vitro-cultured pitaya plantlets. A total of 10 bands were scored from the 66 samples by primer 5r, among which 9 bands were polymorphic, which included the presence and absence of DNA bands in comparison with the stock plant (Fig. 8). In total, 13 plantlets were detected as having 8 types of variants, accounting for 19.7% of the total plantlets (Data in Brief). Thus, HuTy1P4 probably underwent diverse transposition events in these plantlets. HuTy1P4 could be transcriptionally activated and transposon events occur during successive subculturing, leading to somatic variations.

M: DL2000 DNA ladder, Lane 1: stock plant, Lanes 2–24: plantlet No. subcultured for four cycles.

## 4. Discussion

LTR retrotransposons are usually silent, but some, such as *ONSEN*, *Tto1*, *Tnt1*, *Tnp2*, *Tos17*, *FaRE1* and *MERE1* (Liu et al., 2004; Rakocevic et al., 2009; He et al., 2010; Ito et al., 2013), can become transcriptionally and transpositionally active under abiotic stress conditions (He et al., 2012; Finatto et al., 2015). Recently, the expression of *DKRE1* in persimmon after being subjected to SA, methyl jasmonate and ABA was probably involved in bud mutations (Du et al., 2015).

### 4.1. Transcriptional activation of LTR retrotransposons

In the present case, the expression levels of Ty1-*copia* and Ty3-*gypsy* retrotransposons in in vitro-subcultured pitaya plants grown in pots were frequently detected, while they were rarely expressed under non-stressful conditions. However, their expression could be activated by osmosis, cold, NaCl, SA and ABA exposure (Fig. 1), and the expression levels of different *RT*s varied depending upon the type of stress (Figs. 1 and 6). Overall, in vitro culturing was the strongest inducer, followed by cold, heat and drought. SA, ABA and 2,4-D exposure led to comparatively low RT expression levels. Ty1-*copia* retrotransposons of strawberry plants, but not the Ty3-*gypsy* retrotransposons, can be activated by SA, ABA and 2,4-D (Ma et al., 2008). SA, GA and 2,4-D induce the transcriptional activation of both Ty1-and Ty3-type retrotransposons in Masson's pine, with 2,4-D being the strongest inducer (Fan et al., 2014). Heat, SA and ABA also activated the expression of Ty1-and Ty3-type retrotransposons in Scots pine, and varying expression patterns were observed for different retrotransposons after different treatments (Voronova et al., 2014). Thus, LTR retrotransposons can become transcriptionally active after exposure to SA, ABA and 2,4-D, and the expression patterns vary with the plant species.

Although the transcriptional activity levels and the copy numbers of LTR retrotransposon could be underestimated owing to the use of degenerate primers, LTR retrotransposons are an important component of the pitaya genome, accounting for at least 13.07% (Ty1-*copia*: 8.01%; Ty3-*gypsy* 5.06%) of its total size. HuTy1C3 and HuTy1P4 constituted a considerable proportion of the gDNA (0.41% and 1.01%, respectively) and exist in active states (Fig. 6). Thus, these groups of retrotransposon copies were probably formed by recent transposition events. Active retrotransposons with high copy numbers have also been documented in other plants (Tahara et al., 2004).

### 4.2. The diversity of retrotransposons activated by abiotic stresses

The *RTs* of Ty1-*copia* and Ty3-*gypsy* retrotransposons in the pitaya genome are very heterogeneous (Fan et al., 2012; Peng et al., 2017). Here, the greater heterogeneity levels of two types of retrotransposons were also investigated in the cDNA of stress-treated pitaya shoots. Heterogeneity may be ascribed to the error-prone nature of the reverse transcription mechanism owing to the lack of proofreading repair activities of RNA polymerase and RT (Gabriel et al., 1996); therefore, base substitutions or indels could result in RNA or newly synthesized DNA copies, giving rise to divergent sequences. The *RTs* of the LTR retrotransposons in the present study were rich in AT (≥55%), which could

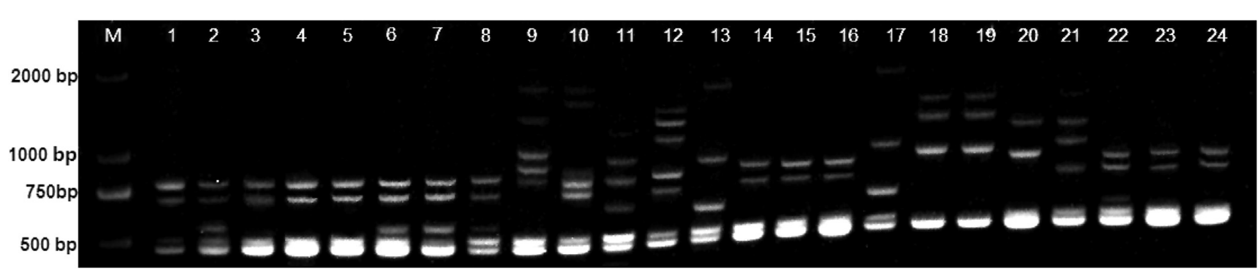


**Fig. 8.** The profile of pitaya plantlets in vitro-subcultured for four cycles as assessed by PCR (primer 5r).

increase base-pairing flexibility, resulting in the high frequency of heterogeneity observed. Furthermore, the genome mutation rate should also be considered an important factor associated with this heterogeneity.

Phylogenetic analyses demonstrated that a number of *RT*s from pitaya cDNA were highly divergent, while some showed remarkable similarity to pitaya gDNA (Fig. 4). The Ty1-*copia* RTs HuTy1P4, HuTy1P5 and HuTy1P8, derived from cDNA, were very similar to HuRT5, HuRT3 and HuRT23 from gDNA, respectively, (97%–98% nucleotide and 100% amino acid sequence identities; Fig. 4a), and may be the corresponding transcripts. Ty3-*gypsy* RT sequences diverged from the cDNA *RT*s but there were similarities between cDNA *RT*s and gDNA *RT*s (Fig. 4b). HuTy1C3 showed a high homology with *MERE1* from *M. Truncatula* (Fig. 5a). HuTy3C7 and CAD45567.1 and 6 of the Ty3-*gypsy* RTs from other species were located within the same family (Fig. 5b). Therefore, it might be hypothesized that horizontal transmission had occurred during the evolution of these retrotransposons. However, most of the transcriptionally active Ty1-*copia* RTs showed a high divergence level when compared with the active retrotransposons *Tntl, Tto1, Tosl 7, FaRE1, ONSEN and GBRE-1* (Fig. 5a). This further indicates that the diversity of transcriptionally active LTR retrotransposons could be ascribed to the types of retrotransposons and their responses to abiotic stresses.

### 4.3. Retrotransposon activation is involved in plant genetic variation

Retrotransposons are an important source of genetic diversity, potentially causing changes that lead to genetic variations within plant species (Zedek et al., 2010; Ito et al., 2011; Rebollo et al., 2012). The available evidence indicates that retrotransposons are a leading cause of plant mutations. Zou et al. (2011) verified that several retrotransposon insertions in genes in quantitative trait loci were putatively related to phenotype, suggesting that the de novo genetic variation was associated with retrotransposon activation in a recombinant inbred population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Yamada et al. (2014) reported that AtRE1s and AtRE2s, *copia*-type retrotransposons, differed in copy numbers and insertion loci among 12 natural variants of *Arabidopsis thaliana*. Furthermore, retrotransposons might directly influence the activation of genes by transposition inside or close to coding regions through their disruption or the addition of regulatory sequences (Yao et al., 2001; Kobayashi et al., 2004; Butelli et al., 2012; Han et al., 2017), leading to changes in plant characteristics.

In this work, HuTy1C3 with transcriptional activity, was highly similar to HuRT10 and MERE1 (Figs. 4a and 5a). HuRT10 was obtained from the pitaya mutant strain, while MERE1 is a low-copy-number *copia*-type retroelement originating from in vitro-cultured *Medicago* mutant plants (Rakocevic et al., 2009). HuTy1C3 was also strongly induced by tissue culture conditions (Fig. 6), as well as $GA_3$, SA, cold and heat exposure. Inter-retrotransposon amplified polymorphism (IRAP) markers, which were generated from primers based on the conserved regions of pitaya Ty1-*copia* RT sequences, showed considerable polymorphisms in the in vitro plantlets of this species (Nie and Wen, 2017), and 13 of 66 plantlets in vitro-subcultured for four cycles were detected as aberrant in IRAP bands in comparison with the stock untreated plant (Fig. 8). Thus, Ty1-*copia* retrotransposons are potentially involved in the occurrence of somaclonal variation in pitaya.

Retrotransposition during environmental stress is generally regarded as advantageous for the host organism because it rapidly increases the genotypic variation, which may cause rapid mutational processes in plants (Wessler, 1996; Murray, 2005). The frequent somatic mutations of pitaya under abiotic stresses could be ascribed to the activation of retrotransposons.

## Contributions

NQ and WXP conceived and designed research. NQ conducted experiments. WXP, QG and PL contributed new reagents or analytical tools. NQ analyzed data and wrote the manuscript. WXP revised and approved the article. All authors read and approved the manuscript.

## Acknowledgements

This project is supported by grants from the National Natural Science Foundation of China (31560549, 31760566).

## References

Amyotte, S.G., Tan, X., Pennerman, K., Jimenez-Gasco, M.D.M., Klosterman, S.J., Ma, L.J., 2012. Transposable elements in phytopathogenic verticillium spp.: insights into genome evolution and inter- and intra-specific diversification. BMC Genomics 13, 1–20.

Biswas, M.K., Xu, Q., Deng, X.X., 2010. Utility of RAPD, ISSR, IRAP and REMAP markers for the genetic analysis of *Citrus* spp. Sci. Hortic. 124, 254–261.

Butelli, E., Licciardello, C., Zhang, Y., Liu, J., Mackay, S., Bailey, P., Reforgiato-Recupero, G., Martin, C., 2012. Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 24, 1242–1255.

Cao, Y.F., Jiang, Y.R., Ding, M.Q., He, S., Zhang, H., Lin, L.F., Rong, J.K., 2015. Molecular characterization of a transcriptionally active Ty1/copia-like retrotransposon in *Gossypium*. Plant Cell Rep. 34, 1037–1047.

Carrier, G., Cunff, L.L., Dereeper, A., Legrand, D., Sabot, F., Bouchez, O., Audeguin, L., Boursiquot, J.M., 2012. Transposable elements are amajor cause of somatic polymorphism in *Vitis vinifera* L. PloS One 7, 1250–1267.

Cisneros, A., Tel-Zur, N., 2013. Genomic analysis in three *Hylocereus* species and their progeny: evidence for introgressive hybridization and gene low. Euphytica 194, 109–124.

Collins, T.J., 2007. ImageJ for microscopy. Biotechniques 43, 25–30.

Du, J.C., Tian, Z.X., Hans, C.S., Laten, H.M., Cannon, S.B., Jackson, S.A., Shoemaker, R.C., Ma, J.X., 2010. Evolutionary conservation, diversity and specificity of LTR-retrotransposons in flowering plants: insights from genome-wide analysis and multi-specific comparison. Plant J. 63, 584–598.

Du, X., Wang, Y., Guan, C., Zeng, M., Mo, R., Xie, F., Luo, Z., 2015. DKRE1—the first full-length Ty1-*copia*-like retrotransposon in persimmon: isolation, characteristic and potential involvement in occurrence of bud mutations. Sci. Hortics. 184, 149–159.

Fan, F.H., Qiao, G., Zheng, S.C., Wen, X.P., 2012. Cloning and analysis of reverse transcriptase of Ty1-*copia* retrotransposons in *Hylocereus undatus*. Acta Hortic. Sin. 39, 265–272 (in Chinese with English Abstract).

Fan, F.H., Wen, X.P., Ding, G.J., Cui, B.W., 2013. Isolation, identification, and characterization of genomic LTR retrotransposon sequences from masson pine (*Pinus massoniana*). Tree Genet. Genomes 9, 1237–1246.

Fan, F.H., Cui, B.W., Zhang, T., Ding, G.J., Wen, X.P., 2014. LTR-retrotransposon activation, IRAP marker development and its potential in genetic diversity assessment of masson pine (*Pinus massoniana*). Tree Genet. Genomes 10, 213–222.

Felice, B.D., Wilson, R.R., Argenziano, C., Kafantaris, I., Conicella, C., 2009. A transcriptionally active copia-like retroelement in *Citrus limon*. Cell. Mol. Biol. Lett. 14, 289–304.

Finatto, T., Oliveira, A.C., Chaparro, C., Maia1, L.C., Farias, D.R., Woyann, L.G., Mistura, C.C., Soares-Bresolin, A.P., Llauro, C., Panaud, O., Picault, N., 2015. Abiotic stress and genome dynamics: specific genes and transposable elements response to iron excess in rice. Rice 8, 13. https://doi.org/10.1186/s12284-015-0045-6.

Gabriel, A., Willems, M., Mules, E.H., Boeke, J.D., 1996. Replication infidelity during a single cycle of Ty1 retrotransposition. Proc. Natl. Acad. Sci. U.S.A. 93, 7767–7771.

Gao, X., Zhou, J., Li, J., Zou, X., Zhao, J., Li, Q., et al., 2015. Efficient generation of marker-free transgenic rice plants using an improved transposon-mediated transgene reintegration strategy. Plant Physiol. 167 (1), 11.

Gaubert, H., Sanchez, D.H., Drost, H.G., Paszkowski, J., 2017. Developmental restriction of retrotransposition activated in *arabidopsis* by environmental stress. Genetics 207 (2), 813–821.

Han, M., Sun, Q., Zhou, J., Qiu, H., Guo, J., Lu, L., et al., 2017. Insertion of a solo LTR retrotransposon associates with spur mutations in 'Red Delicious' apple (*Malus × domestica*). Plant Cell Rep. 36, 1375–1385.

Hawkins, J.S., Hu, G., Rapp, R.A., Grafenberg, J.L., Wendel, J.F., 2008. Phylogenetic determination of the pace of transposable element proliferation in plants: copia and LINE-like elements in *Gossypium*. Genome 51, 11–18.

He, P., Ma, Y., Zhao, G., Dai, H., Li, H., Chang, L., Zhang, Z.H., 2010. FaRE1: a transcriptionally active Ty1-*copia* retrotransposon in strawberry. J. Plant Res. 123, 707–714.

He, P., Ma, Y., Dai, H.Y., Li, L.G., Liu, Y.X., Li, H., Zhao, G.L., Zhang, Z.H., 2012. Characterization of the hormone and stress-induced expression of FaRE1 retrotransposon promoter in strawberry. J. Plant Biol. 55, 1–7.

Ito, H., Gaubert, H., Bucher, E., Mirouze, M., Vaillant, I., Paszkowski, J., 2011. An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. Nature 472, 115–119.

Ito, H., Yoshida, T., Tsukahara, S., Kawabe, A., 2013. Evolution of the ONSEN retrotransposon family activated upon heat stress in *Brassicaceae*. Gene 518, 256–261.

Jiang, S.Y., Ramachandran, S., 2013. Genome-wide survey and comparative analysis of LTR retrotransposons and their captured genes in rice and sorghum. PloS One 7, e71118.
Kapitonov, V.V., Jurka, J., 2008. A universal classification of eukaryotic transposable elements implemented in Repbase. Nat. Rev. Genet. 9, 411–414.
Kashkush, K., Feldman, M., Levy, A.A., 2003. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat. Genet. 33, 102–106.
Kim, S., Park, J.Y., Yang, T.J., 2015. Characterization of three active transposable elements recently inserted in three independent DFRA alleles and one high-copy DNA transposon isolated from the pink allele of the ANS gene in onion (*Allium cepa* L.). Mol. Genet. Genom. 290, 1027–1037.
Kobayashi, S., Goto-Yamamoto, N., Hirochika, H., 2004. Retrotransposon-induced mutations in grape skin color. Science 304, 982.
Li, J., Loua, Q.F., Biao, J., Wang, D., Chen, J.F., 2014. LTR retrotransposons cause expression changes of adjacent genes in early generations of the newly formed allotetraploid *Cucumis hytivus*. Sci. Hortics. 174, 171–177.
Lichtenzveig, J., Abbo, S., Nerd, A., Tel-Zur, N., Mizhrahi, Y., 2000. Cytology and mating systems in the climbing cacti *Hylocereus* and *Selenicereus*. Am. J. Bot. 87, 1058–1106.
Lim, T.K., 2012. Edible Medicinal and Non-medicinal Plants, vol. 1. Springer Science +Business Media B.V, pp. 643–655 Fruits.
Liu, Z., Han, P.F., Tan, M., Shan, X.H., Dong, Y.Z., Wang, X.Z., Fedak, G., Hao, S., Liu, B., 2004. Activation of a rice endogenous retrotransposon Tos17 in tissue culture is accompanied by cytosine demethylation and causes heritable alteration in methylation pattern of flanking genomic regions. Theor. Appl. Genet. 109, 200–209.
Ma, Y., Sun, H.Y., Zhao, G.L., Dai, H.Y., Gao, X.Y., Li, H., Zhang, Z.H., 2008. Isolation and characterization of genomic retrotransposon sequences from octoploid strawberry (*Fragaria × ananassa* Duch.). Plant Cell Rep. 27, 499–507.
MacRae, A.F., 1998. A pentamer-repeat-containing DNA sequence in Texas bluebonnet (*Lupinus texensis* Hook.). Genome 41, 553–559.
Murray, B.G., 2005. When does intraspecific C-value variation become taxonomically significant? Ann. Bot. 95, 119–125.
Nie, Q., Wen, X.P., 2017. Somaclonal variation of pitaya (*Hylocereus undatus) in vitro* plants and identification by molecular markers. J. Fruit Sci. https://doi.org/10.13925/j.cnki.gsxb.20170157. (in Chinese with English Abstract).
Nie, Q., Gao, G.L., Fan, Q.J., Qiao, G., Wen, X.P., et al., 2015. Isolation and characterization of a catalase gene "*HuCAT3*" from pitaya (*Hylocereus undatus*) and its expression under abiotic stress. Gene 563, 63–71.
Nystedt, B., Street, N.R., Wetterbom, A., Zuccolo, A., Lin, Y.C., Scofield, D.G., Vezzi, F., Delhomme, N., et al., 2013. The Norway spruce genome sequence and conifer genome evolution. Nature 497, 579–584.
Peng, L., Wu, Y., Liu, X.C., Yang, K., Fan, F.H., Wen, X.P., 2017. Cloning and analysis of reverse transcriptase of Ty3-*gypsy* retrotransposon in *Hylocereus undatus*. J. Fruit Sci. 34, 186–195 (in Chinese with English Abstract).
Rakocevic, A., Mondy, S., Tirichine, L., Cosson, V., Brocard, L., Iantcheva, A., Cayrel, A., Devier, B., Ghada, A., Abu El-Heba, Ratet, P., 2009. MERE1, a low-copy-number *copia*-type retroelement in *Medicago truncatula* active during tissue culture. Plant Physiol. 151, 1250–1263.
Rebollo, R., Romanish, M.T., Mager, D.L., 2012. Transposable elements: an abundant and natural source of regulatory sequences for host genes. Annu. Rev. Genet. 46, 21–42.
Rico-Cabanas, L., Martínez-Izquierdo, J.A., 2007. CIRE1, a novel transcriptionally active Ty1-*copia* retrotransposon from *Citrus sinensis*. Mol. Genet. Genom. 277, 365.
Tahara, M., Aoki, T., Suzuka, S., Yamashita, H., Tanaka, M., Matsunaga, S., et al., 2004. Isolation of a transcriptionally active element of high copy number retrotransposons in sweetpotato genome. Mol. Genet. Genom. 272, 116–127.
Takeda, S., Sugimoto, K., Otsuki, H., Hirochika, H., 1999. A 13-bp *cis*-regulatory element in the LTR promoter of the tobacco retrotransposon Tto1 is involved in responsiveness to tissue culture, wounding, methyl jasmonate and fungal elicitors. Plant J. 18, 383–393.
Viñas, M., Fernández-Brenes, M., Azofeifa, A., Jiménez, V.M., 2012. *In vitro* propagation of purple pitahaya (*Hylocereus costaricensis* [FAC weber] britton & Rose) cv. Cebra. *In vitro* cell. Dev. Biol. Plant 48, 469–477.
Voronova, A., Belevich, V., Rungis, D., Jansons, A., 2014. Stress-induced transcriptional activation of retrotransposon-like sequences in the Scots pine (*Pinus sylvestris* L.) genome. Tree Genet. Genomes 10, 937–951.
Wessler, S.R., 1996. Plant retrotransposons: turned on by stress. Curr. Biol. 6, 959–961.
Yamada, M., Yamagishi, Y., Akaoka, M., Ito, H., Atsushi, K., 2014. Genomic localization of AtRE1 and AtRE2, *copia*-type retrotransposons, in natural variants of *Arabidopsis thaliana*. Mol. Genet. Genom. 289, 821–835.
Yao, J.L., Dong, Y.H., BretA, M., 2001. Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. Proc. Natl. Acad. Sci. U.S.A. 98, 1306–1311.
Zedek, F., Šmerda, J., Šmarda, P., Bureš, P., 2010. Correlated evolution of LTR retrotransposons and genome size in the genus *Eleocharis*. BMC Plant Biol. 10, 1–10.
Zhuang, Y., Zhang, Y., Sun, L., 2012. Characteristics of fibre-rich powder and antioxidant activity of pitaya (*Hylocereus undatus*) peels. Int. J. Food Sci. Technol. 47, 1279–1285.
Zou, J., Fu, D.H., Gong, H.H., Qian, W., Xia, W., Pires, J.C., Li, R.Y., Long, Y., Mason, A., Yang, T., Lim, Y., Beom, A.S., Meng, T.J., 2011. De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Plant J. 68, 212–224.