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Molecular Cloning of a Novel Putative Retrotransposon From *Aloe Vera*

KEYWORDS: abiotic stress, RT-PCR, Ty3 gypsy, HVA 22, *Aloe vera*

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ABSTRACT *Aloe vera is a member of the family Liliceae and has medicinal properties. It is a sturdy plant that withstands abiotic stresses such as extreme temperature and drought conditions. It is possible that Aloe may be harboring stress inducible genes in its genome. HVA22 is a highly inducible gene for Abscisic acid and environmental stress in Barley. We followed the PCR approach and used Aloe genomic DNA as template along with primers specific against YOP1 (homolog of HVA22 in Yeast) for amplification. Amplicon obtained was 545bp; sequencing and bioinformatics analysis revealed it to be a gag_ protease region of a Ty3- gypsy retrotransposon. Aloe cDNA pool was used to obtain a transcript of the same gene. Plant retrotransposons are linked with abiotic and biotic stresses with respect to its effect on retrotransposon's activation and function. In this study, transcript of gag_protease region of a Ty3 retrotransposon was isolated from Aloe vera.*

INTRODUCTION

Excess or shortage of water, temperature, soil & salinity imparts stress upon the plant's physiological system to survive in the environment. Certain plants adapt to these changes quickly, thus growing normally, with changes as much as increase in ABA production, heat shock proteins and genes encoded by these proteins. Plants also harbor stress inducible genes. They are activated under stress, thus initiating different metabolic pathways that may support the plant's system in drastic environmental changes.

Aloe vera belongs to the family Liliaceae. It is known to possess tolerance to cold stress and a DREB1- Drought Resistant Ethylene Binding Gene 1 has been isolated from *Aloe vera* and characterized (Yang- Meng Wang, 2007). There may be genes present, which might be aiding the growth of the sturdy Aloe plant in drought like conditions.

HVA22 is one of the early ABA- responsive genes in barley, and its expression in grains appears to be correlated with dormancy status (Quingxi Shen, 2001). The yeast (*Saccharomyces cerevisiae*) HVA22 homolog, Yop1p, has been shown to interact with the GTPase-interacting protein Yip1p (Brands A, 2002). Both the genes have a conserved TB2/DP1 domain (UniProt ID: O14355). DREB transcription factor possesses a conserved AP2/EREBP DNA binding domain and functions in plant development, stress responses or in hormone responses (Yang-Meng Wang, 2007).

Retrotransposons are ubiquitous in plant kingdom (Krishna, 2011). In Liliaceae, >90% of genome is composed of LTR Retrotransposons (Feschotte C, 2002). Various biotic and abiotic stresses are shown to increase expression of various transcriptionally active LTR retrotransposons include chilling, infection etc. (Hirochika H, 2000; Grandbastein MA, 2005; Salazar M, 2007). They are of two types: the Ty3/gypsy family and the Ty1/copia family (Brown, 2005).

We aimed to target the YOP1 gene homolog from *Aloe vera* genomic DNA and cDNA. We further aimed to clone such stress inducible genes into Plant expression vectors which are then transferred to stress sensitive plants and observe if it confers any stress- resistance trait to these plants. Improvement of stress tolerance by genetic engineering overcomes the challenges faced by plant breeding methods (Sneha Lata Singla- Pareek, 2001).

Materials and Methods:

Genomic DNA studies:

Four grams of young leaf of *Aloe vera* plant were used for genomic DNA extraction. CTAB technique (Doyle J.J, 1987) with modification (Instead of liquid nitrogen, fresh leaves were directly crushed in buffer) was used to extract High molecular weight genomic DNA from *Aloe vera* leaf. It was observed by AGE & Estimation was carried out using UV spectrophotometric analysis (Thermo Electron Corp.) at 260nm & 280nm.

Primers for the PCR and RT-PCR were designed using Primer- BLAST program (Ye J, 2012) from NCBI, and the primers were synthesized by Sigma- Aldrich Pvt. Ltd.

The genomic DNA sample was purified by PCR Purelink Purification Kit (K-310001). For PCR, purified genomic DNA as template, (g_FP)5' ATATATGGATCCTTGAAAAACTGTGGAGCC 3' & (g_RP)5' ATGAACAGAAGCACCTGTAG 3' as forward and reverse primers and PCR Reaction Mix (Sigma's Red Taq PCR supermix) were used for amplification of the YOP1 homolog in ABS 2720 Thermal Cycler. AGE was performed along with low range DNA molecular ladder for reference.

mRNA studies:

One gram of stressed *Aloe vera* plant tissue was used for Total RNA extraction by TRIzol technique (Sigma's TRI Reagent) & was resuspended in sterile RNAse free Distilled water. Quality of RNA was determined by AGE using TBE Tank buffer. Concentration and Purity of RNA was analysed by UV- Spectrophotometric analysis (Thermo Electron

Corp.) at 260nm & 280nm.

cDNA pool was generated using Invitrogen's Superscript III First strand cDNA synthesis kit according to the manufacturer's manual. PCR against coding sequences was performed using with the *A. vera* cDNA pool, (c_FP) 5' TGATCCACACCGAGGGACTT 3' and (c_RP) 5' ATGAGACATCACCAGCCTCTG 3' as Forward and Reverse Primers respectively & (Sigma) Red Taq PCR Supermix. PCR product was separated on 1.5% Agarose gel, with Mid Range DNA ladder for reference.

Sequencing:
PCR products obtained with genomic DNA and g_FP& g_RP primer was purified using Purelink PCR purification Kit (K-310001). The purified sample was sent for bi- directional sequencing to Chromous Biotech Pvt Ltd. Sanger's Dideoxy technique of sequencing was performed. The RT-PCR product obtained with c_FP and c_RP primers was outsourced to GenomBiotech Pvt. Ltd for Purification and Sequencing (3130 Genetic Analyzer by Applied Biosystems)

Insilico analysis:
PCR and RT- PCR Product sequences obtained were subjected to NCBI BLAST (Sense & Antisense strands) (Altschul, 1990). A BLASTn, BLASTx (Gish, 1993) and CDD (Conserved Domain Database) search was performed (Bauer, 2011). ORF was found using NCBI's ORF finder program. The potential exons present within the fragment were analysed using FGENESH 2.0 version Softberry Program (Solovyev V, 2006). Protein prediction was performed at Predict Protein website (Yachdav, et al., 2014).

RESULTS
DNA extraction & PCR:
Concentration of Genomic DNA was found to be 0.53µg/µl and of good purity. AGE analysis of unpurified and purified DNA revealed a single band with minimum smear and no RNA contamination (Figure 1A). PCR amplified an approximately 550bp fragment that was approximately same as expected band size; which on sequencing gave 543bp sequence information (Figure 1B). BLASTx analysis and Conserved Domain Database search (Figure 2A) (Bauer, 2011) revealed a Retrotransposon_gag domain with a score in the range of 80-200 on scorecard for identity with Ty3- Gypsy type gag_protease polyprotein region of *Cucumis melo, Oryza sativa Japonica group, Vitis vinifera, Prunus persica* etc, showing 101 BLAST hits. The percentage identity differed from 36% to 33% and the query coverage in all cases was between 93% and 77%. The conserved domain analysis on CDD search showed that the domain was present within the sequence, which putatively is a part of a complete Retrotransposon (Figure 2B). NCBI's ORF finder revealed a single ORF stretching between 4 and 543 on the +1 frame. On subjecting the sequence to Softberry's FGENESH program 2.6 [20] (Solovyev V, 2006), a 423bp long stretch within the 543bp sequence was detected. This 543bp gene fragment was submitted to Genbank via Bankit and is available on NCBI, DDBJ and EMBL under the **Accession ID KF892820.**

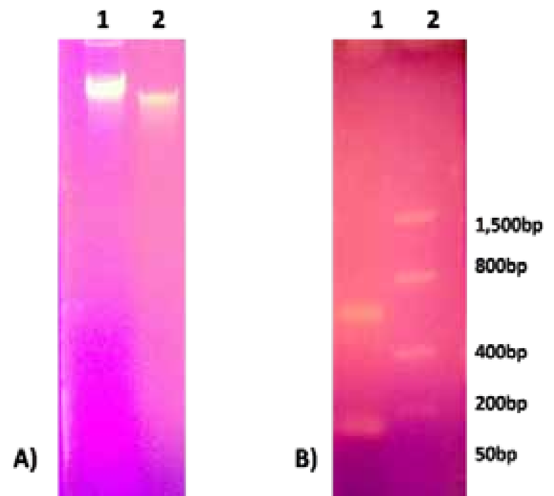

Figure 1: A. Lane 1- Genomic DNA isolated from young Aloe vera leaves; Lane 2- Spin- column purified Genomic DNA of Aloe. B. Lane 1- ~545bp PCR product obtained using g_FP & g_RP primer pair and purified genomic DNA as template; Lane 2: Low range molecular weight marker ladder (from top: 1500bp, 800bp, 400bp, 200bp, 50bp)

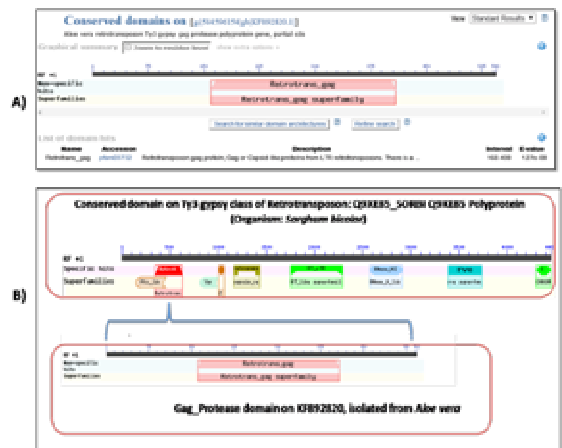

Figure 2: A) Conserved domain on the sequence amplified from Aloe vera genomic DNA detected using CDD search program (Bauer et al., 2011). B) Diagrams of conserved domain of a complete Ty3 gypsy Retrotransposon (Sorghum bicolor) aligned with Aloe vera gag_ protease sequence shows that this sequence is small part of a complete retrotransposon.

mRNA Studies:
Total RNA extracted from *Aloe vera* was estimated to be 0.1µg/µl and was found to be of good purity. AGE analysis revealed 2 bands which were intact and degradation was minimum (Figure 3A). The primer pairs c_FP & c_RP was designed to target 368bp nucleotide stretch within the predicted Exon sequence (423bp). RT-PCR amplified approximately 350bp fragment, as observed after analysis of the PCR products by AGE (Figure 3B). On sequencing, 318bp sequence information was obtained.

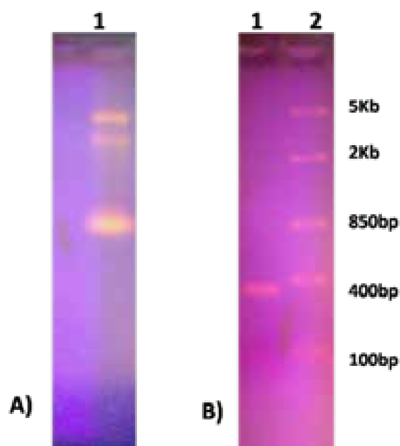

Figure 3: A. Lane 1- RNA isolated from Aloe vera plant; B. Lane 1: ~350bp long PCR product amplified using c_FP& c_RP and cDNA pool obtained from Aloe total RNA as template; Lane 2: Mid Range Molecular weight marker ladder (from top: 5Kb, 2Kb, 850bp, 400bp, 100bp)

BLASTx (Gish, 1993) results of the 318bp sequence showed highest identity (88%) with gag protease polyprotein of *Aloe vera* with a score of 196 and query coverage of 99%. Maximum hits were observed in the range of 50-80 on the scorecard and showing identity with gag protease polyprotein region of same plants as with "gag_ protease gene". The percentage identity ranged between 48% and 30%; query coverage ranged between 93% and 70%. This amplified cDNA is a transcript of the gene fragment that was investigated before in this paper, gag protease polyprotein of *Aloe vera*, thus confirming its expression.

Discussion:

The 543bp sequence obtained from genomic DNA of *Aloe vera* shows similarity with the gag domain of Ty3 gypsy class of plant retrotransposons. The transcript analysis of the coding region within the gene gave approximately 350bp RT- PCR product, which confirms the insilico information on the coding sequence of gag domain and also its active transcription. This data shows presence of a gene fragment from *Aloe vera* which is homologous to plant retrotransposon, specifically TY3/Gypsy class. The point to be specified here is the sample of *Aloe vera* is a non-stress treated plant. *Aloe vera* is a different plant with respect to its genetic and physiological make up. There are two well studied stress-response genes already known from *Aloe vera* which are**,** DREB1 (Yang- Meng Wang, 2007) and NADP-ME (Sun SB, 2003)**.** This study adds to identification of one more transcript fragment which is interestingly a retrotransposon. Retrotransposons are present in all plant genomes and can constitute very large part of some of them (Grandbastien, 1998). All known active plant retrotransposons are largely quiescent during development but activated by stresses, including wounding, pathogen attack and cell culture (Wessler, 1996). But abiotic stress also decreased normalized expression of genes including retrotransposon, as was observed in one study (Ungerer, 2013). Ty3 gypsy is known to be transcriptionally active, along with Ty1 and Ty2 in Yeast particularly. Evidence for being transcriptionally active also comes from a recent study conducted on *Helianthus annus*, where it was seen that sublineage- specific elements of Ty3/gypsy that proliferated in the sunflower hybrid taxa were transcriptionally active (Ungerer, 2013). Expression of Ty3 results in production of RNA, Gag3 and Gag3-Pol3 polyproteins (Clemens, 2011).

Further studies on various stress treatments on *Aloe vera* can reveal the expression patterns of retrotransposon and its role in stress-tolerance mechanism.

Conclusion:

A novel putative gag_protease fragment of a Ty3 gypsy retrotransposon was isolated from genomic DNA of *Aloe vera*, and its active transcription was confirmed. The function of the transcript and the gene can further be characterized by means of cloning into pGEMT and cloning into pCAMBIA, to study its sub cellular localization in plants. In a study conducted, defective capsid proteins induced resistance in plants against the attack of viruses after a brief period under salt stress (Krishna Karajol, 2011). Therefore, expressing this transcript in different model systems may reveal it's potential role in aiding the plant against stress.

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