

Original Research Article

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Tobacco Rattle Virus (TRV) based Virus-Induced Gene Silencing (VIGS) in Tomato (*Solanum lycopersicum*)

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ABSTRACT

VIGS is a powerful tool for assaying the effects of gene silencing in plants. Efficient silencing requires the fragments that are more than 90% homologous to the target gene. Here, we designed a set of universal primers to a region of the PDS gene that is highly conserved among species, and therefore allows to isolate a fragment of the homologous PDS gene from the species of interest i.e., tomato. We typically use silencing of PDS which is used as a marker for gene silencing in tomatoes involved in carotene biosynthesis which results in a characteristic photobleaching phenotype in the leaves. The purpose of this study was to investigate the possibility of tomato PDS gene silencing, using VIGS technique by TRV based VIGS vector containing tomato PDS. The pTRV2: PDS vector carried inserts derived from tomato PDS gene was transformed into DH5 α *E. coli* strain and then *Agrobacterium tumefaciens* GV3101 strain cells were transformed by the vectors containing PDS genes. The construct was validated for physical integrity as well as for biological functionality i.e., PDS by agroinfiltration method in the experimental host plant. The construct shows gene silencing results in photobleaching phenotype occurred in tomato plant leaves. However, plant infiltration with pTRV1 and pTRV2 (without PDS gene as control) mixed culture did not show any photobleaching. This construct found structurally as well as functionally active under plant system and can be further modified into transgene construct to be used to study the function of other genes through VIGS in tomato.

Keywords

Virus induced gene silencing,
Functional genomics,
Silencing, TRV
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Introduction

Virus-induced gene silencing (VIGS) is a fast and efficient functional genomics tool (Becker and Lange, 2009; Baulcombe, 1999) and a method for rapid silencing of plant genes in

order to discover their function (Becker and Lange, 2009; Purkayastha and Dasgupta, 2009). Virus-induced gene silencing (VIGS) offers an attractive and quick alternative for knocking out expression of a gene without the need to genetically transform the plant. VIGS

is based on Post-Transcriptional Gene Silencing (PTGS), plants exploit PTGS as their innate antiviral defense line to encounter viral multiplication, employing defense mechanism very similar to the pathways of RNA interference which refers to interference in gene expression, mediated by small RNA in a sequence specific manner (Becker and Lange, 2009; Liu and Page, 2008).

At present many plant viruses are also being used to investigate plant genomic functions (Aguero *et al.*, 2012; Hefferon, 2014; Lim *et al.*, 2015; Wang *et al.*, 2014). Impressive advances have also been made in the development of strategies for constructing viral vectors (Gleba *et al.*, 2014), as well as viral vector delivery systems (Liu *et al.*, 2002; Ryu *et al.*, 2004; Seo *et al.*, 2009; Tuttle *et al.*, 2012; Yan *et al.*, 2012).

VIGS vectors have been used to study gene function in plants, such as the potato virus X (PVX) (Ruiz *et al.*, 1998), tobacco rattle virus (TRV) is a bipartite, positive-strand RNA virus with the TRV1 and TRV2 genomes. TRV can infect meristem tissues whereas many other VIGS

vectors cannot (Ratcliff *et al.*, 2001), and bean pod mottle virus (BPMV) (Zhang and Ghabrial, 2006). The VIGS system has been established in many types of plants, including *N. benthamiana* (Ratcliff *et al.*, 2001), soybean (Zhang and Ghabrial, 2006), and potato (Brigneti *et al.*, 2004). Many VIGS vectors have been described to study gene function in plants (Kumagai *et al.*, 1995; Liu *et al.*, 2002; Ratcliff *et al.*, 2001; Ruiz *et al.*, 1998). Most of these vectors are designed to work in *Nicotiana benthamiana*. Tomato is an economically important crop and like *N. benthamiana*, it belongs to the Solanaceous family. *Nicotiana benthamiana* was the first plant species for which VIGS was reported. The PDS gene of *N. benthamiana* was silenced

by using a vector composed of hybrid sequences of both TMV and Tomato mosaic virus (ToMV) which resulted in extensive photo bleaching (Kumagai *et al.*, 1995). Vectors using VIGS were applied for *Agrobacterium tumefaciens* mediated plant transformation, contained host genes inserted in the viral infection clone. *Agrobacterium* based inoculation was used for excision of TDNA containing the viral genome (Becker and Lange, 2009; Waterhouse and Helliwell, 2003) which was transcribed by the host's RNA polymerase.

The RNA-dependent RNA polymerase generates dsRNA, using ssRNA viral transcripts (Becker and Lange, 2009). This dsRNA is recognized by Dicer-like proteins and cleaved into small interfering RNAs (siRNAs) which are approximately 21– 24 nucleotides (nt) in length (Becker and Lange, 2009; Bernstein *et al.*, 2001; Bass, 2000). The siRNAs are selected by RISC (RNA-induced Silencing Complex) and melted into ssRNAs which are used to target mRNAs and generate new siRNA (Becker and Lange, 2009; Agrawal *et al.*, 2003). In this case the VIGS viral RNAs and the mRNA of the target gene are cleaved.

The single stranded siRNAs are generated (Becker and Lange, 2009; Lipardi *et al.*, 2001) and spread as silencing signals throughout the plant resulting in systemic gene silencing distant from the site of infection and lead to leaf photobleaching (Becker and Lange, 2009).

In the present study, the gene for phytoene desaturase (PDS) was isolated and cloned from *Solanum lycopersicum* plant and used as VIGS reporter genes. Here, we were also used to establish a TRV-mediated VIGS system and optimize *Agrobacterium*-infiltration method in *S. lycopersicum*. This study shows that TRV-mediated VIGS technology and tomato genomic sequences can be used in future

studies that focus on gene function in *S. lycopersicum*.

Materials and Methods

Plant Material

Tomato seeds were collected from Department of Horticulture, College of Agriculture, SVPUA&T Meerut. Seedlings of a tomato (*Solanum lycopersicum*) species were grown at approximately 20-25°C. Tomato plants were raised in the field laboratory of Department of Agriculture Biotechnology of SVPUA&T.

Data mining and designing of degenerate PDS gene specific primer

The publicly available gene sequence of tomato was searched for PDS gene from NCBI database (<ftp://ftp.ncbi.nih.gov/blast/db/>). The assembled sequences were searched for identification and localization of forward and reverse primers from the flanking sequences. The primers were designed using online available software.

Isolation and cloning of the PDS cDNA from tomato

Total RNA was isolated from leaves of tomato using Gene JET Plant RNA Purification Mini Kit (Thermo Scientific). The reaction mixtures for cDNA synthesis were prepared using cDNA direct TMkit (GeNei™, Bangalore).

The PDS gene was obtained by PCR amplification from a tomato leaves cDNA using PDS gene-specific primers (forward primer: 5'-CGGGAATTCGGC(AT)CT(C/T)AACTT(C/T)ATAAA(C/T)C -3'; reverse primer: 5'-CGGTCTAGATCAGTTT(T/C)CTGTCAAACC -3'). The PCR amplification was carried out using with the following cycling parameters: heating for 3

min at 94°C, total 40 cycles of denaturation at 95°C for 1 min, annealing for 30s at 59°C, synthesis for 1min at 72°C and extension at 72°C for 10min. The gene fragment of around 406 bp was obtained and cloned into the pGEM-T Easy vector (Promega), and confirmed by sequencing.

Two clones of PDS gene were sequenced in both the directions (with SP6 and T7 universal primers). The sequencing was carried out at the DNA sequencing facility, Eurofins Genomics.

The nucleotide sequences were subjected to BLAST analysis (<http://www.ncbi.nlm.nih.gov/blast/>) to confirm the sequences thus generated are of tomato genome. Sequences generated were trimmed and assembled manually to retain only the coding regions of each gene by removing vector sequences as well as the nucleotide sequences flanking the protein coding regions.

The fragment obtained after trimming was somewhat similar to some of the PDS ortholog. The nucleotide sequence reported in this paper has been submitted to GenBank under the accession number: PDS, MN044575.

The phylogenetic tree was constructed with the Neighbor-Joining algorithm using the software MEGA 7.0(<http://www.megasoftware.net/>).

The PDS cDNA fragment was excised using *EcoRI* and *XbaI* digestion from the pGEM-T: PDS plasmid, then subcloned into pTRV2 to generate a pTRV2:PDS construct.

Computer software and programs used

Homology study

Multiple sequence alignment was performed by CLUSTAL-W program in BioEdit software version 5.0.9 (Tom Hall, Raleigh, NC, USA) (Hall, 1999).

Phylogenetic analysis

Mega program version 7 (Kumar *et al.*, 2016) use for the construction of phylogenetic tree of various orthologous sequences of PDS genes.

Agro-infiltration

According to the described method by Velasquez *et al.*, 2009 for each experiment, *A. tumefaciens* harboring pTRV1, pTRV2 and pTRV2: tPDS were grown on LB plates supplemented with 50 µg/ml of kanamycin. Three ml liquid culture of LB containing mentioned antibiotics was inoculated with each of the strains and incubated in a shaker at 30°C for 18 hours. A 1: 25 dilution of the culture was inoculated into liquid induction media (IM) with kanamycin and 200 µM acetosyringone. The cells were harvested by centrifugation for 10 minutes at 3000 rpm and were resuspend with 10 mM MgCl₂, 10 mM MES pH 5.5. Acetosyringone was added to a final concentration of 400 µM to pTRV1 culture. Cultures containing the pTRV1, pTRV2, and pTRV2: tPDS were mixed in a 1:1 ratio. Using 1ml syringe, mixed cultures were infiltrated into the lower leaves of 2-week-old tomato plants.

RT-PCR Analysis

Total RNA was extracted from 100 mg fresh green and white tomato tissue samples (Fig.4.8) using GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, #K0801, #K0802) and treated with RNase-free DNase (Ambion) to remove any contaminating genomic DNA. The first strand cDNA was synthesized using 2 µg total RNA for cDNA synthesis were prepared using cDNA direct™ kit (GeNei™, Bangalore). The primers for amplifying PDS transcripts by quantitative PCR were designed outside the region targeted for gene silencing to avoid amplification from the pTRV:PDS construct. These primers were 5'- CTTTGCAAGCCAATTGTTGA-3' and 5'- TTGTAGGGGTCGACATGGTT-3'.

Results and Discussion

Design of primers to amplify PDS gene fragments

A BLAST search for PDS sequences from a wide range of taxa permitted us to design a set of degenerate primers that would amplify a PDS fragment from known species, and therefore, most probably from species where the gene's sequence is unknown. An approximately 406bp fragment of the PDS gene was amplified by the degenerate primers from tomato species. A comparison of the PDS fragment sequences from 4 different plant species reveals a similarity of higher than 80% but in most cases less than the 90% homology required for efficient VIGS. It seems likely that most other species will include the highly conserved region of the PDS gene amplified by this pair of degenerate primers (Fig.1).

PDS gene isolation, cloning and sequencing

The PDS gene was isolated from the cDNA of leaves samples of tomato. PCR was carried out using PDS gene specific primer for amplification of PDS gene. A coding region of around 406 bp was amplified and visualized in the 1% Agarose gel electrophoresis (Fig. 2). The PCR product of PDS gene was eluted and cloned in pGEMT cloning vector, and several transformed clones were confirmed by blue white screening.

Thereafter by colony PCR (Fig.3) as recombinant clones using the PDS gene specific primer following standard protocol (Sambrook and Russell, 2001). After colony PCR, the colony showing positive result was used for Plasmid isolation and Restriction digestion of the plasmid with EcoRI and XbaI sites to confirm that the plasmid contained the PDS gene (Fig. 4). After confirmation of recombinant clones, two clones were sequenced using the vector derived primer in automatic sequencer and presented in Fig.5.

Multiple sequence alignment and phylogenetic analysis of isolated PDS gene with PDS genes of other species

Multiple sequence alignment of amino acid sequence of isolated PDS gene from tomato with other orthologous PDS gene of other species including *Petunia hybrida*, *Gladiolus hybrida*, *Arabidopsis thaliana*, *Oryza sativa* and *Nicotiana tabacum* along with their Accession numbers gene of other species to find out the changes in amino acid sequence performed by CLUSTAL-W program in BioEdit software version 5.0.9 (Tom Hall, Raleigh, NC, USA) (Hall, 1999) (Fig. 6).

The phylogenetic study of PDS amino acid sequences were analysed in present study using Mega program version 7 (Kumar *et al.*, 2016). The bootstrap values of the phylogenetic trees were derived from 1000 replicates run. According to amino acid-based Neighbor-Joining tree analysis the PDS gene which we isolate from tomato varieties in this study is matched with AKM12416.1 *Petunia hybrida* (Fig.7).

Construct Development

As a result of PCR amplification of PDS insert (406bp) by using specific primer (PDS-F and PDS-R). The insert thus generated was cloned successfully in pGEMT Easy cloning vector (clone was named as pGEMT:PDS). The recombinant colonies yielded the amplicon of expected size when subjected to colony PCR. The plasmid from the colony PCR positive recombinant colonies released the insert of expected size in restriction digestion analysis (Fig. 4). Sequencing of this clone yielded an insert containing 406nt (6 bp of restriction enzyme sites in both primers and 3 bp additional nucleotide on 5' end of F & R primers) than the actual size of template i.e 424bp. The gel purified product released from size from pGEMT: PDS clone was sub-cloned

successfully at EcoRI and XbaI sites through directional cloning in the MCS of pTRV2 (pYL156). This yielded several colonies after transformation in DH5 α and recovered recombinant colonies based on colony PCR (Fig. 8). This transgene insert was found to be cloned successfully into pTRV2.

Mobilization of transgene constructs into *Agrobacterium tumefaciens*

The construct was mobilized into the chemically competent cells of *Agrobacterium tumefaciens* strain GV3101 by freeze and thaw shock transformation method and obtained recombinant colonies. *Agrobacterium* colonies harboring transgene construct was confirmed by colony PCR using insert specific primers to amplify the transgene of construct and obtained the amplicon of expected size (Fig.9).

AgroInfiltration

A colony PCR confirmed clone of transgene construct (pTRV2: PDS) was used in transient expression assay in tomato plant (*Solanum lycopersicon*) through syringe (without needle) infiltration. After 15-20 days, the infiltrated leaf tissues were subjected to PDS expression assay (Fig.10).

TRV-mediated VIGS in *Solanum lycopersicum*

In order to investigate whether the TRV vectors can directly infect tomato leaves, a mixture of *Agrobacterium* cultures containing pTRV1 (pYL192) and pTRV2 (pYL156) constructs in a 1:1 ratio was infiltrated into few leaves of two-week-old tomato plant. The seedlings infected were grown at 22°C. Four weeks after agroinfiltration, more than twenty plants inoculated with TRV1 and TRV2 vectors showed no obvious differences in overall shoot and leaf morphology compared with the control (Fig.10).

Silencing of the tomato PDS gene using TRV-VIGS vector

We tested whether the TRV clones could induce gene silencing in tomato plants. In order to do so, we examined the ability of our TRV-VIGS vector to suppress the expression of the endogenous phytoene desaturase gene (PDS) in Pusa Ruby and NDT-7 variety of tomato. A mixture of *Agrobacterium* cultures containing pTRV2, carrying tomato PDS (pTRV2: tPDS), and pTRV1, was infiltrated onto the lower leaves of 2-week-old tomato plants (Fig.4.8). Tomato plants infected with pTRV-tPDS developed a photo-bleached phenotype in the upper leaves 15-20 days post Agro-infiltration and remained white for at least 1 month. The *Agrobacterium* infiltration method of infecting pTRV:tPDS resulted in 60% and 40% PDS silencing phenotype in Pusa Ruby and NDT-7 varieties of tomato respectively i.e. three out of ten plants in Pusa Ruby and two out of ten plants in NDT-7 tomato varieties (Table No.4.1). Fragments isolated from the target species using our universal primers were very effective when used in VIGS silencing of PDS. Developing leaves of pTRV2-PDS infected plants showed the photobleaching phenotype that indicated movement of the virus and its effectiveness in silencing the native PDS gene in tomato. No photo-bleached phenotype was found in the control or pTRV2 empty vector controls (Fig.10).

Expression analysis of PDS gene

To confirm gene silencing at the molecular level, we performed quantitative RT-PCR analyses to quantify the transcript levels of the silenced endogenous genes. Gene-specific primers for tPDS were used to amplify the respective genes. After amplification, PDS gene transcripts were clearly evident in non-

silenced leaves and vector control, but barely detected in photo-bleaching leaves. The result suggested that the photo-bleaching phenotype was caused by the down-regulation of the PDS gene (Fig. 11). These findings reveal the potential for functional characterization of genes expressed in leaves that are involved in diverse biological functions (physiological, biochemical, and plant-microbe interactions).

Virus-induced gene silencing is an excellent functional genomics tool for gene function analyses (Liu *et al.*, 2002; Robertson, 2004; Lange *et al.*, 2013). In previous VIGS studies of a wide range of different ornamentals (Chen *et al.*, 2003), only *N. benthamiana* and *P. hybrida* (both from the *Solanaceae*) showed the PDS phenotype. PDS silencing in *N. benthamiana* inhibits carotenoid biosynthesis, causing the plants to exhibit a photo-bleached phenotype (Kumagi *et al.*, 1995; Burch-Smith *et al.*, 2004; Howes, 2005; Goodin *et al.*, 2008). PDS gene which encodes the important enzymes in the carotenoid biosynthesis pathway was targeted. PDS silencing is an indicator for gene silencing and shows a clear mutant phenotype (Lu *et al.*, 2003). We used TRV [bipartite RNA virus; TRV-RNA1 (TRV1) and TRV-RNA2 (TRV2)] as a VIGS vector. This virus has already been used for VIGS in many plants, including dicots and monocots (Ratcliff *et al.*, 2001; Burch-Smith *et al.*, 2004; Brigneti *et al.*, 2004; Becker and Lange, 2010) and recently in potato (Brigneti *et al.*, 2004). Ye *et al.*, (2009) reported that TRV-based vector could trigger virus-induced gene silencing in *Jatropha curcas* L., which is a small tropical, woody tree belonging to the Euphorbiaceae family. Recently, an *Agrobacterium*-mediated VIGS assay was successfully developed in several cotton cultivars with various genetic backgrounds (Gao *et al.*, 2011).

Table.1 Phenotypic efficiency showed in green tissues plant and white tissues plant (PDS silenced)

| S.No. | Variety | Treatment | Green tissues plant (%) | White tissues plant (PDS Silenced %) |
|-------|-----------|--------------------------------|-------------------------|--------------------------------------|
| 1 | Pusa Ruby | Control | 100% | Nil* |
| | | Agroinoculation With pTRV-tPDS | 40% | 60% |
| 2 | NDT-7 | Control | 100% | Nil* |
| | | Agroinoculation With pTRV-tPDS | 60% | 40% |

*Nil indicates the no photo-bleached phenotypic expression.

Fig.1 Multiple sequence alignment and sequence comparison of the PDS fragments from the four different plants for designing of degenerate primers



Fig.2 PDS gene fragments were amplified by the universal primers from tomato varieties. The size of the PDS fragment is approximately 406 bp. Lane 1: amplification of PDS gene from Pusa Ruby variety. Lane; M: 100bp DNA Ladder, GeneRuler (Fermentas); Lane 2: amplification of PDS from NDT-7 variety

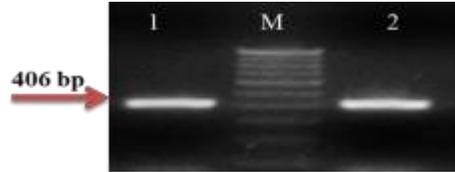


Fig.3 Colony PCR for PDS amplification from pGEMT vector transformed in DH5 α cells. Lane M: 100bp DNA Ladder, GeneRuler (Fermentas); Lane 1-4: four colonies grown on antibiotic media.

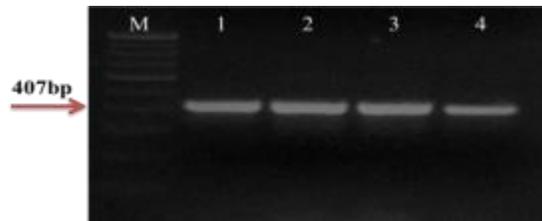
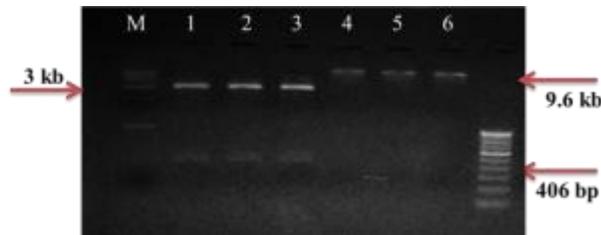


Fig.4 Restriction Digestion of PDS and TRV2 vector with EcoRI&XbaI: Lane M: 1Kb DNA Ladder, GeneRuler (Fermentas); Lane 1-3: Restriction release of PDS from pGEMT cloning vector; Lane 4-6: Restriction of TRV2



Nucleotide and amino acid sequence

Fig.5 PDS gene nucleotide and amino acid sequence after Sequencing

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      10      20      30      40      50      60      70      80      90     100
PDS NT  GGCACTCAACTTTATAAACCCCGGTGAANTTTCATGCAGTGCATTTTGATCGCATTGAACAGGTTTCTTCAGGAGAACATGGTTCAAAAATGCCCTTTT
      110     120     130     140     150     160     170     180     190     200
PDS NT  TAGATGGTAACTCCTCCTGAGAGACTTGCATGCCGATGTTGAACACATTTGAGTCAAAAGGTGGCCAACTCAGACTGAATCAGCAATAAAAAAGATTGA
      210     220     230     240     250     260     270     280     290     300
PDS NT  GCTGAATGAGGATGGAAGTGTCAAGAGTTTATACTGAGTGACGGTAGTCAAATCGAGGGAGATGCTTTTGTGTTGCCCTCCAGTGGATATTTTCAAG
      310     320     330     340     350     360     370     380     390     400
PDS NT  CTTCTATGCTGAAGACTGGAAGAGATTCCATATTTCCAAAAGTTGGAGAAGTTAGTCGGAGTACCTGTGATAAATGTACATATATGGTTTGACAGAA
PDS NT  AACTGA
    
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Amino acid sequence

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      10      20      30      40      50      60      70      80      90     100
PDS AA  MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
      110     120
PDS AA  YFCKLEKLVGVFVINVEHINFDREL
    
```

Fig.6 Multiple sequence alignment of amino acid sequences of PDS gene and PDS gene from tomato, Petunia, Gladiolus, Arabidopsis and other species to identify the conserved region among these species performed by Clustal W program using BioEdit software version 5.0.9.

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      10      20      30      40      50      60      70      80      90     100
PDS AA  MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
AGI17588.1 Gladiolus hybrida MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
AAD02489.1 Oryza sativa MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
AKM12416.1 Petunia hybrida MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
NP_001234095.1 Solanum lycoper MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
NM_001329033.1 Arabidopsis tha MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
CAE83576.1 Nicotiana tabacum MQCILIALNRFLQEKHGSEMAFLDGNPPERLQMPIVEHIESKGGVRLNSRIKIELNEDGSVRSFILSDGSAIRGDAFVFAAFVDIFKLLLPEDWKEIP
Clustal Consensus  ***  *****  *****  *****  *****  *****  *****  *****  *****  *****

      110     120
PDS AA  YFCKLEKLVGVFVINVEHINFDREL
AGI17588.1 Gladiolus hybrida YFCKLEKLVGVFVINVEHINFDREL
AAD02489.1 Oryza sativa YFCKLEKLVGVFVINVEHINFDREL
AKM12416.1 Petunia hybrida YFCKLEKLVGVFVINVEHINFDREL
NP_001234095.1 Solanum lycoper YFCKLEKLVGVFVINVEHINFDREL
NM_001329033.1 Arabidopsis tha YFCKLEKLVGVFVINVEHINFDREL
CAE83576.1 Nicotiana tabacum YFCKLEKLVGVFVINVEHINFDREL
Clustal Consensus  **  *  *****
    
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Fig.7 Neighbor-joining tree of PDS genes from tomato, Petunia, Arabidopsis and other species. 100 bootstrap samples were generated to assess support for the informed relationship. Local bootstrap probabilities (%) are indicated near the branching points using the Mega 7 software

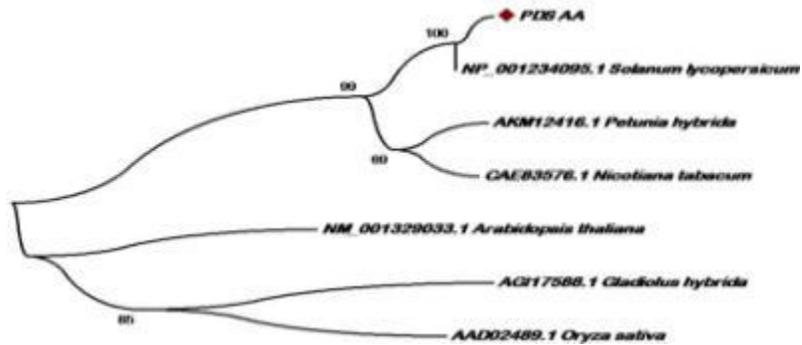


Fig.8 Colony PCR amplification of PDS from TRV2, vector transformed in DH5 α cells. Lane M: 100 bp DNA Ladder, GeneRuler (Fermentas); Lane 1-3: three colonies grown on antibiotic media; Lane 4: Negative control; Lane 5: Positive Control.

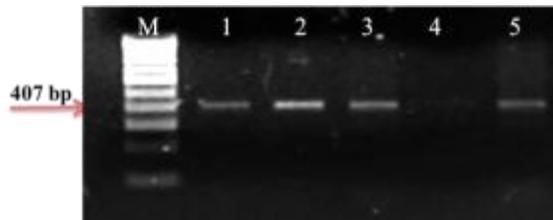


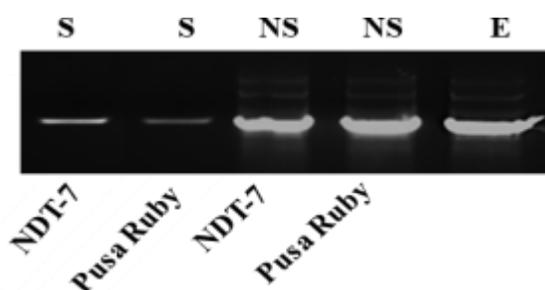
Fig.9 Colony PCR amplification of PDS from recombinant TRV2 vector transformed in GV3101 cells; Lane M: 100 bp DNA Ladder, GeneRuler (Fermentas); Lane 1-4: four colonies grown on antibiotic media; Lane 5: Positive control



Fig.10 A and B; Photobleaching phenotype resulting from VIGS silencing of TRV:tPDS gene in Pusa Ruby and NDT-7 plant variety respectively. C; control plant and D; TRV infected plant.



Fig.11 Abundance of PDS transcripts in tomato plants after VIGS silencing. S-silenced tissue, NS- non-silenced tissue, E-empty vector control



This method will be useful for functional analysis of many genes interest of tomato and will fulfill its promise of being a fast and efficient functional genomicstool.

Virus-induced gene silencing is an excellent functional genomics tool for gene function analyses. Agro infiltration is a novel and simple method to elicit VIGS with greater rapidity and convenience, whole-plant level gene silencing, adequately stable transformation and suitability for studying functions of genes involved in seed germination and early plant development stages. The ease of the agroinfiltration method suggests it would be a better choice for high throughput gene silencing. Agroinfiltration

method can be used for efficient VIGS in various Solanaceae species. We have demonstrated that agroinfiltration shuts down transcript levels to a greater degree in leaves. This may allow us to do gene functional analysis.

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