International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(4): 411-415
© 2019 IJCS
Received: 13-05-2019
Accepted: 15-06-2019

Harini V
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Varanavasiappan S
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Arul L
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Kokiladevi E
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Sudhakar D
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Sooriyanathasundaram K
Department of Horticulture, HC&RI, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Kumar KK
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Harini V
Rice Transformation Laboratory, Department of Plant Biotechnology, CPMB&B, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Molecular characterization of eukaryotic translation initiation factor *eIF4E* gene in PRSV resistant highland papaya

Harini V, Varanavasiappan S, Arul L, Kokiladevi E, Sudhakar D, Sooriyanathasundaram K and Kumar KK

Abstract
The eukaryotic translation initiation factor *eIF4E* gene was isolated from PRSV resistant highland wild papaya, *Vasconcellea cauliflora*. The *eIF4E* gene comprises of four exons separated by three intronic regions. Gene specific primers were designed in the conserved region of *eIF4E* gene, to isolate it from *V. cauliflora*. The isolated *eIF4E* cDNA of *V. cauliflora*encoded a 237 amino acid polypeptide, which was aligned with *eIF4E*protein from three PRSV resistant *Vasconcellea* species (*V. cundinamarcensis, V. goudotiana, V. monica*), two PRSV susceptible *Vasconcellea* species (*V. parviflora, V. querciflora)*and papaya. Multiple sequence alignment between PRSV susceptible and resistant species revealed two common amino acid substitutions at L227H and S230G. The *eIF4E*interacts with viral genome-linked protein (VPg) at 5' cap of viral mRNA, which is necessary for viral protein translation. These amino acid substitutions in *eIF4E* protein of PRSV resistant *Vasconcellea* spp. might be responsible for host resistance to potyvirus.

Keywords: Poty virus, *Vasconcellea cauliflora*, *eIF4E* gene, PRSV resistance

Introduction
Carica papaya Linn, commonly known as papaya, belonging to the family Caricaceae is an edible fruit in most of the tropical and sub-tropical countries. Papaya is a dicotyledonous, diploid species originated from Central America and Mexico (Kaur *et al*., 2019) [7]. A limiting factor for worldwide cultivation of papaya is the disease spread by the virus Papaya Ring spot Virus (PRSV), a destructive poty viral disease that alters the tree vigour, fruit quality and fruit set. It spreads through various species of aphids in a non-persistent manner (Han *et al*., 2014) [6]. *Vasconcellea*, a separate genus rehabilated from Caricaceae contains 21 species of 35 Caricaceae species and is commonly known as "mountain papaya" or "highland papaya". Several species of *Vasconcellea* exhibit extreme resistance to PRSV namely *V. cauliflora, V. cundinamarcensis, V. goudotiana*and, *V. stipulate* (Dillon *et al*., 2006) [2].
PRSV resistance is not identified in papaya so far. Intergeneric hybridisation of six *Vasconcellea species* (*V. cauliflora, V. cundinamarcensis, V. quercifolia, V. parviflora, V. stipulate,* and *V. goudotiana)* and *C. papaya* have been reported which results in genetic incompatibility among distantly related species, also described as sexual incompatible resulting in post zygotic barriers like abortion of embryo, infertility and poor hybrid vigour in hybrids. This incompatibility in hybridisation serves as barrier in developing PRSV resistant papaya lines (Sharma and Tripathi, 2016) [13].
There are two types of resistance genes namely, dominant resistance gene and recessive resistance genes conferring the qualitative traits based on inheritance (Maule *et al*., 2007) [8]. The dominant R gene confers resistance to fungi and bacteria (Hypersensitivity), whereas the recessive R gene confers resistance to most of the plant viruses, which requires the host factors to complete their viral infection cycle. The 14 recessive resistance gene encoding eukaryotic translation initiation factor *eIF4E* and their isoforms have been characterized in different crops like lettuce, melon, pepper, barley, pea and rice (Wang and Krishnaswamy, 2012) [16]. The *eIF4E* serves as host factors and functions as cellular translation and also controls the viral resistance/susceptibility. Being a nuclear protein, *eIF4E* regulates the cap-binding and helps in exporting the mRNA subsets into the cytoplasm and recruits the eIF4F complex, for initiating the eukaryotic mRNA translation and circularization (Pestova and Hellen, 2001) [9].

The 3' end of viral RNA is poly-adenylated, whereas 5' end is not capped but is covalently linked to VPg protein (a virus encoding protein). In poty viral RNA translation, *eIF4E* (host factors in viral infection) interacts with VPg protein at 5' end replacing the 5' cap structure and overcomes the resistance associated with recessive gene (Ruffel *et al*., 2005) [12]. In pvr2 gene from Capsicum annum, the *eIF4E* proteins from poty virus resistance cultivar ($pvr2^1$ and $pvr2^2$ allele) differed from susceptible cultivar ($pvr2^+$ allele) only at 2 amino acids, showing that changes in sequences are sufficient to achieve poty viral resistance (Ruffel *et al*., 2002) [11]**.** This study isolates and characterizes the *eIF4E* transcripts of PRSV resistance highland papaya *V. cauliflora*, to determine their nucleotide and amino acid sequence and how substitution in amino acids relates to virus disease resistance.

Materials and methods

Total RNA Extraction and first strand cDNA synthesis

Leaf sample of *V. cauliflora* (wild papaya) was obtained from Orchard, Horticultural College and Research Institute, TNAU, Coimbatore. Total RNA was extracted from leaves using TRIZOL reagent as described by Simms *et al*. (1993).The RNA quality was examined using 1% agarose gel electrophoresis (80V/cm for 45-60min) by visualizing the intactness of RNA bands (Figure 1). Using Nano-drop spectrophotometer the RNA quantity was examined based on concentration of pure ssRNA having 2.0 at 260nm absorbance and the products were stored in -80˚C until use. The first strand cDNA was synthesised with isolated total RNA as template using Revert Aid First Strand cDNA synthesis kit (Thermo Fisher Scientific, India). Following the manufacturer's procedure the reactions were carried out in eppendorf master cycler.

Fig 1: Total RNA isolated was electrophoresed on 1% agarose gel against 1kb ladder: M- 1Kb ladder. Lane 01- Isolated RNA sample from leaves of *Vasconcellea cauliflora*.

PCR primer designed for *eIF4E* gene amplification

In order to amplify the eukaryotic translation initiation factor (*eIF4E*) of *V. cauliflora*, the nucleotide sequences of *eIF4E* mRNA of five wild type papaya namely *V. quercifolia* (KP340469.1), *V. parviflora* (KP340467.1), *V. monoica* (KP340466.1), *V. cundinamarcensis* (KP340468.1), *V. goudotiana* (KP340465.1) were retrieved from National Centre for Biotechnology Information (NCBI) database. The retrieved sequences were multiple aligned to find the conserved region for primer designing. Four pairs of primers were designed covering the *eIF4E* gene using primer-3 program (Table 1). The positions of primers designed in highlighted in accession number KP340468.1 (Figure 2)**.**

Table 1: Primers used for Reverse Transcription PCR

Primer	Primer Sequence (5' to 3')	Product Size
P01	Vas F1: AAGGAACACCCAATCCATCG Vas R1: GCGCCTCTCTCATGCTTCTT	679 bp
P02	Vas F2: AAGGAACACCCAATCCATCG Vas R2: CTCCGACAGCCAACTTGCTA	336 bp
P03	Vas F3: CGTCGCGGAAGACAAACC Vas R3: GGCAAATGCTGAACAACCAG	699 bp
P04	Vas F4: ATGGGAGGACCCTGTTTGTG Vas R4: TTGGCAAATGCTGAACAACC	356 bp

RT-PCR amplification of *eIF4E* gene

The cDNA was amplified with four primer pairs by using 0.3µl (1U) of Taq DNA polymerase (Bangalore Genei Pvt, Ltd., Bangalore, India), 2.5 µl of 100 mMdNTPs, 1.5 µl of 10 µM forward and reverse primers and 2 µl (100ng) template cDNA to the final volume of 30µl. The PCR reactions were performed following the temperature and duration conditions as described in Table 2. The PCR products were electrophoresed on 1% agarose gel**.** The amplified PCR products were purified using Nucleo spin gel and PCR clean up Kit (Macherey-Nagel), following the manufacturer's procedure. The purified PCR products were sequenced by Agri Genome Labs Pvt Ltd. Kochi, India.

Table 2: Cyclic conditions for cDNA amplification

PCR conditions	Temperature	Time	No. of cycles
Initial denaturation	94 ˚C	3minutes	1
Denaturation	94 ˚C	30 seconds	30
Annealing	55 ˚C	1 minute	
Extension	72 ˚C	45 seconds	
Final extension	72 ˚C	5 minutes	1
Hold	4 ˚C		

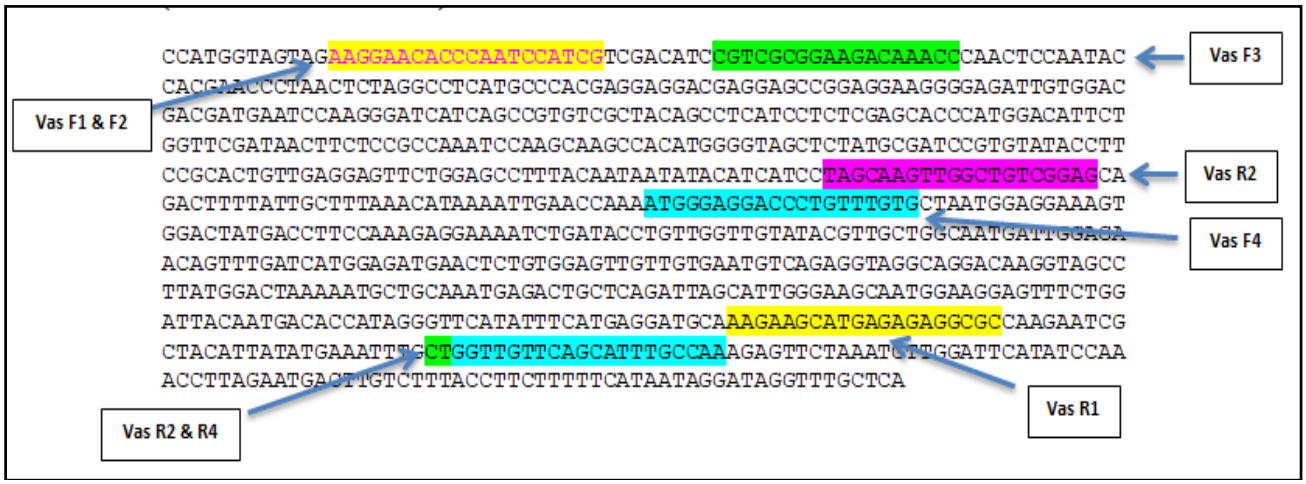

Fig 2: Indicating the designed set of primers in *eIF4E* gene mRNA of *Vasconcellea cundinamarcensis* (Accession no. KP340468.1)

Sequence analysis

Nucleotide sequences were aligned and deduced to putative amino acid sequences by using the ExPASy (Expert Protein Analysis System) translation tool. Sequence alignment was carried out by using Bio-edit software tool Clustal W (Thompson *et al*., 1994, Hall, 2001) [15, 5] with amino acid sequence obtained from National Centre for Biotechnology Information (NCBI) database of other wild type *Vasconcellea species*. MEGA X software tool was used to construct the phylogenetic tree by neighbour-joining method.

Results and Discussion

Amplification of *eIF4E* cDNA fragments using different set of primers

The amplified cDNA fragments of *V. caulifloraeIF4E* gene were subjected to 1% agarose gel electrophoresis (Figure 3). The results show that the band size observed were exactly similar to the predicted product size for all the four set of primers covering the *eIF4E* gene (i.e.,) primers 01, 02, 03 and 04 amplified at 679 bp, 336 bp, 699 bp and 356 bp respectively.

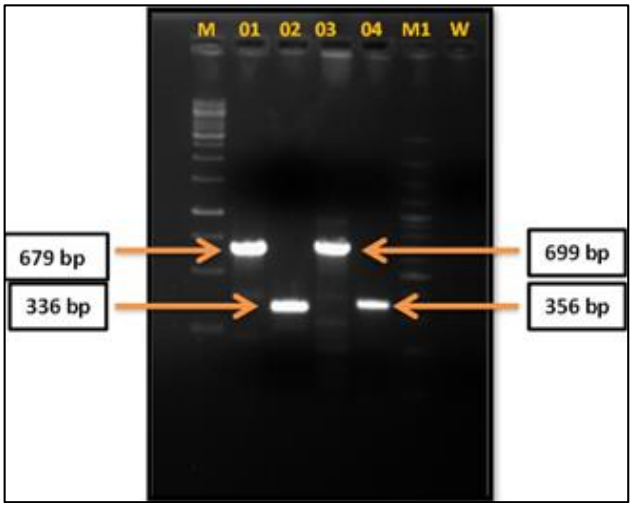

Fig 3: Amplified cDNA fragment using four set of *eIF4E* gene specific primers. M- 1Kb ladder; 01, 02, 03 and 04 indicates the cDNA amplified using four set of primers namely P01, P02, P03 and P04 respectively; M1-100bp ladder; W- Water control

Sequence alignment and analysis

The nucleotide sequences obtained from sequencing the amplified cDNA of *V. caulifloraeIF4E* gene were subjected to NCBI blast search. The nucleotide sequence was deduced to amino acid sequence using Ex PASy Translation Tool (Figure 4) showing the higher degree of sequence homology with *C. papaya* and *Vasconcellea species*. The amino acid sequences were aligned using Bio-edit Clustal W program where the *eIF4E* of *V. cauliflora* shown higher degree of homology with other wild *Vasconcellea species* then papaya (Figure 5).

The changes in amino acid sequences at 227 & 230 positions from susceptible species to resistant species were observed (i.e.,) Leucine to Histidine and Serine to Glycine in 227 and 230 position respectively. This amino acid change in L227H and S230G in *V. caulifloraeIF4E* protein might define it as prominent position for host resistance to poty viral diseases by modifying the cap binding pocket. Phylogenetic analysis using software MEGA X predicts that *Homo sapiens* is the common ancestor among all other organisms in the evolution of *eIF4E* gene and *Oryza sativa* serves as ancestor for plants, whereas among the papaya species *C. papaya* serves as common ancestor. It was observed that all PRSV resistant *Vasconcellea* spp. were clustered together.

The present work was done to characterize the eukaryotic translation initiation factor 4E gene of highland papaya. Two common amino acid changes were observed at C- terminal end in both *V. cauliflora* and other PRSV resistant *Vasconcellea species*. These amino acid substitutions might be responsible for host resistance to poty virus.

Through several study, it is well established that interaction between plant *eIF4E* and poty virus VPg is necessary for translation of viral proteins for disease occurrence in plants. The earlier reports show that *eIF4E* in capsicum interacts with a virus encoded protein VPg is necessary for virus infection in Capsicum-TEV (Tobacco Etch Virus). The amino acid change in 107th position (Gly to Arg) of *eIF4E* abolished interaction with viral VPg, thereby conferring resistance to TEV. The variation in *eIF4E* encoded at pvr locus shows resistance against poty viral disease by interrupting the VPg binding and cap binding activities of eukaryotic translation initiation factor 4E gene. Over-expression of engineered *eIF4E* with impaired cap binding ability, affects the translational efficiency of mRNA in transgenic plants (Robaglia and Caranta, 2006) [10]. In addition to G107R amino acid substitution, several other amino acid substitutions were observed in recessive resistant allele pot^1, mot^1, pvr^1 and pvr^2 in tomato, lettuce and pepper respectively, but their function towards poty viral infection remains unknown (Yeam *et al*., 2007) [17].

In wheat the second form of *eIF4E* has been observed namely Eif (iso) 4E having similar activities but differ in their

physiological functions and renders resistance to poty virus in *Arabidopsis thaliana* by amino acid substitution/deletion (Dinkova *et al*., 2011) [3]. The resistance to Potato Virus Y was achieved by cloning the recessive resistance gene pot[1] and pvr[2] in tomato and pepper respectively through candidate gene approach. The pvr[2] locus in *Piper nigru*m is the first natural recessive resistance gene to be cloned. These genes also confer resistance to Tobacco Etch Virus (TEV) and Pepper Vein Mosaic Virus (PVMV) (Charron *et al*., 2008) [1]. Next to this mot[1] recessive gene cloning was achieved in lettuce against Lettuce Mosaic Virus (German-Retana *et al*., 2008) [4].

MVVEGTPNPSSTSVAEDKPNSNTTNPNSRPHGHEEDEEPEEGEIVDDDESKGSSAVSLQPHPL
EHPWTFWFDNSSAKSKQATWGSSMRSVYTFRTVEEFWSLYNNIHHPSKLAVGADFYCFKH
KIEPKWEDPVCANGGKWTLTFQRGKSDTCWLYTLLAMIGEQFDHGDELCGVVVNVRGRQE
KIALWTKNAANETAQISIGKQWKEFLDYNDTIGFIFHEDAKKHERGAKNRYII

Fig 4: Sequence of deduced amino acid of *eIF4E*gene using ExPASy Translational Tool.

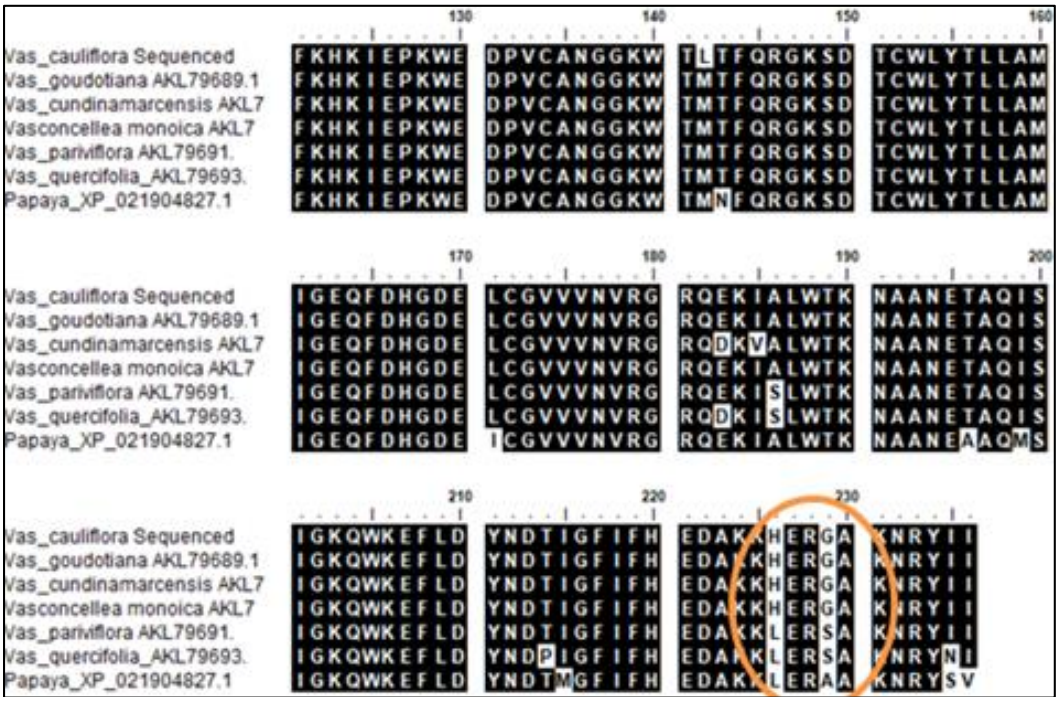

Fig 5: Clustal W alignment of *eIF4E* gene deduced to amino acid sequences of *Vasconcellea cauliflora* with selected organisms

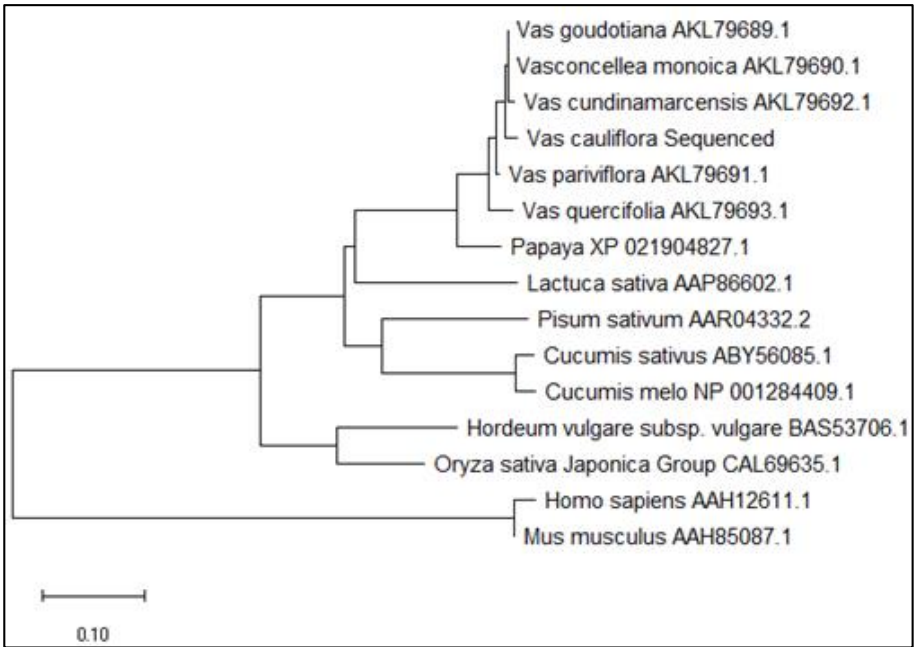

Fig 6: Phylogenetic relation of *Vasconcellea cauliflora* with other selected organisms using MEGA X software. The evolutionary history was inferred using the Neighbour-Joining method.

Acknowledgement
The authors are thankful to Department of Plant Biotechnology, CPMB & B, Tamil Nadu Agricultural University, Coimbatore, India for providing facility for this research work. We greatly acknowledge the Department of Horticulture, HC&RI, TNAU, Coimbatore, India for providing the samples for this study.

Reference

1. Charron0 C, Nicolaï M, Gallois JL, Robaglia C, Moury B, Palloix A, *et al.*. Natural variation and functional analyses provide evidence for co-evolution between plant eIF4E and poty viral VPg. The Plant Journal. 2008; 54:56-68.
2. Dillon S, Ramage C, Ashmore S, Drew R. Development of a codominant CAPS marker linked to PRSV-P resistance in highland papaya. Theoretical and Applied Genetics. 2006; 113:1159-1169.
3. Dinkova TD, Márquez-Velázquez NA, aguilar R, Lázaro-Mixteco PE, DE Jiménez ES. Tight translational control by the initiation factors eIF4E and eIF (iso) 4E is required for maize seed germination. Seed Science Research. 2011; 21:85-93.
4. German-Retana S, Walter J, LE Gall O. Lettuce mosaic virus: from pathogen diversity to host interactors. Molecular plant pathology. 2008; 9:127-136.
5. Hall T. Bio Edit version 5.0. 6. North Carolina State University, Department of Microbiology, Raleigh, North Carolina, 2001; 192.
6. Han J, Murray JE, YU Q, Moore PH, Ming R. The effects of gibberellic acid on sex expression and secondary sexual characteristics in papaya. Hort. Science. 2014; 49:378-383.
7. Kaur M, Talniya NC, Sahrawat S, Kumar A, Stashenko EE. Ethnomedicinal Uses, Phytochemistry and Pharmacology of Carica papaya Plant: A Compendious Review. Mini-Reviews in Organic Chemistry. 2019; 16:1-18.
8. Maule AJ, Caranta C, Boulton MI. Sources of natural resistance to plant viruses: status and prospects. Molecular plant pathology. 2007; 8:223-231.
9. Pestova T, Hellen C. Functions of eukaryotic factors in initiation of translation. Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor Laboratory Press, 2001; 389-396.
10. Robaglia C, Caranta C. Translation initiation factors: a weak link in plant RNA virus infection. Trends in plant science. 2006; 11:40-45.
11. Ruffel S, Dussaul MH, Palloix A, Moury B, Bendahmane A, Robaglia C, Caranta C. A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). The Plant Journal. 2002; 32:1067-1075.
12. Ruffel S, Gallois J, Lesage M, Caranta C. The recessive potyvirus resistance gene pot-1 is the tomato orthologue of the pepper pvr2-eIF4E gene. Molecular Genetics and Genomics. 2005; 274:346-353.
13. Sharma SK, Tripathi S. Resistance against papaya ringspot virus in Vasconcellea species: present and potential uses. Plant Viruses: Evolution and Management. Springer, 2016.
14. Simms D, Cizdziel PE, Chomczynski P. Trizol: A new reagent for optimal single-step isolation of RNA. Focus. 1993; 15:532-535.
15. Thompson JD, Higgins DG, Gibson TJ. Clustal W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research. 1994; 22:4673-4680.
16. Wang A, Krishnaswamy S. Eukaryotic translation initiation factor 4E-mediated recessive resistance to plant viruses and its utility in crop improvement. *Molecular plant pathology.* 2012; 13:795-803.
17. Yeam I, Cavatorta JR, Ripoll DR, Kang BC, Jahn MM. Functional dissection of naturally occurring amino acid substitutions in eIF4E that confers recessive potyvirus resistance in plants. The Plant Cell. 2007; 19:2913-2928.