In silico plum pox virus silencing via host retrieved miRNAs in peach plant

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Abstract

Peach (*Prunus persica*) is a deciduous, edible stone fruit tree, belongs to family Rosaceae and genus *Prunus*. This plant is prone to various pathogens and one of them is Plum Pox Virus (PPV). PPV is the lethal virus of peach plant causing pox disease of plum. Its attack is resulted in 83-100% yield loss in highly susceptible varieties of peach plant. The complete genome of PPV is 9,791 base pairs with positive sense single strand. The full-length genome of PPV encodes a

large polyprotein initially which cleaves proteolytically into 10 mature proteins; coat protein (CP), helper component proteinase (HC-Pro protein), P1 (first protein), P3, viral genome-linked protein (VPg), 6K1, 6K2, cylindrical inclusion protein (CI), cylindrical inclusion protein b (NIb) and a-Pro-proteins (C-terminal proteinase domain of Nia). The objective of this work is to identify such sites in PPV genome which can be targeted by PPV-derived miRNAs through target prediction computational tools/algorithms. A total of 214 mature miRNAs were taken from the miRNA database to check their complementarity with the PPV genome. Minimum free energy (MFE), folding energy, seed pairing, target site accessibility, pattern recognition and multiple target sites were the parameters considered for target prediction algorithms. Two out of 214 miRNAs (miR8122-3p and miR7125-5p) were predicted potential by three of four tools used for target prediction. Thus, the results encouraged generating a transgenic PPV resistant peach plant by expression of predicted miRNAs.

Keywords

Plum Pox Virus; Target Prediction; *In Silico*; Algorithms; *Prunus persica*; Pre-miRNA

Introduction

Peach (*Prunus persica*) is a temperate, deciduous, edible stone fruit producing plant. *Prunus* is an important genus in family Rosaceae which is comprised of different fruit varieties[1]. Peach is an important commercial and agronomical plant as it provides vitamins, fibers, antioxidants and minerals for a healthy diet (FAOSTAT 2010, http://faostat.fao.org/). The genus *Prunus* is infected by various viral attacks and one of the most devastating is plum pox disease, also called as pox disease of plum. This disease is transmitted in stone fruits through PPV, a member of family potyvirideae[2]. PPV is a single-stranded, positive sense, filamentous virus. Its genome size

is estimated as 9.9 kb and 750 nm long[3]. Its transmission results in premature fruit drop and decreased fruit quality which consequences in agronomic and economic loss[4]. This virus attack results in 83-100% yield loss in peach production[5,6].

Numerous strategies have been developed to control the multiplication of the virus into the plant. Certain breeding programs and genetic engineering approaches were also used to develop resistance against PPV. These strategies did not provide complete information for resistance due to the specific nature of virus strain, long juvenile seedling period[5] and unnecessary degradation of RNA-silencing pathways[7,8,9]. The computational strategy is the other technique to control this disease. Plants respond to the distinctive abiotic stress and viral infections. Viral infection or any abiotic stress is controlled both at the transcriptional and post-transcriptional level. So at post-transcriptional quality controllers' miRNAs are utilized[10]. miRNAs are small 21-22 nucleotides, endogenous and non-coding RNAs that regulate the gene expression in plants and animals through their proteolytical activities. They bind to their complementary sequences in target regions of the viral genome and repress the transcription or cleave the transcript[11]. The peach plant has 180 mature miRNAs and 214 precursors (miRNA database). To identify such miRNAs in the peach plant which have the potential to inhibit the infection through mRNA cleavage, five target prediction algorithms were used.

The plan of this investigation is to foresee the best peach-derived potential miRNAs that can develop resistance against PPV through certain computational techniques. The predicted miRNAs can be a source to inhibit the infection by cleaving the mRNA.

MATERIALS AND METHODS

Recruitment of mature miRNAs of Prunus persica

In many living organisms miRNAs are small, endogenous, non-coding and small sequences of RNA that regulate gene expression[12]. To search potential miRNA, mature miRNAs were obtained from miRbase[13,14] accessed from http://www.mirbase.org/cgi-bin/browse.pl and 214 mature miRNAs of *Prunus persica* were retrieved.

PPV Genome recruitment

PPV complete genome was downloaded from NCBI in FASTA format (https://www.Ncbi.nlm.nih.gov/nuccore) with accession number AY028309.2. Its complete genome was contained 9,791 base pairs.

Target prediction tools

A number of programs and algorithms have been introduced to search target genes of miRNAs[15,16]. Four algorithms were used for miRNAs target prediction in PPV genome. These algorithms were used to analyse for their accuracy and efficiency. In the virus genome, these algorithms predicted the target sites. Most important and potential miRNAs were selected through four algorithms, which blocked translation or cleaved the mRNA to prevent synthesis of proteins. These algorithms were named as; miRanda, psRNATarget, RNA22, and RNAhybrid[17,18].

miRanda

For target prediction of plants and animals, miRanda is a frequently used software[19]. To predict potential targets, miRanda (V3.3a) run, downloaded from the web source http://cbio.mskcc.org/miRNA2003/miranda:html. This algorithm was run after setting parameters with changing energy threshold. Different energy levels (E -15, -20, -22 and 25) were used and selected the most suitable energy and assessed the one which has a suitable number of

predictions for analysis. The parameters which were set for target prediction are gap open penalty=-9.0, gap extent penalty=-4.0, score threshold=140, scaling parameter=4.0 and energy threshold=-20.

psRNA Target

This software is used for target analysis specifically for identification of target transcript of miRNAs using proven scoring schema (V1 and V2), analyzing the reverse complementary matching between miRNA and target and target site accessibility. V2 released 2017, is an improved scoring schema which was capable of discovering more miRNA target pairs without an increase in final output[20]. This algorithm is easily available at http://plantgrn.noble.org/psRNATarget/. Parameters considered were all set default then submitted and got the miRNAs which are more potential to target.

RNA22

RNA22 is a pattern-based algorithm used for target prediction. For identification of target regions of mRNA sequence that have a higher likelihood to contain miRNA binding sites. It used the pattern of miRNA that has a higher[21]. RNA22 V2.0 was easily accessed from https://cm.jefferson.edu/rna22/Interactive/. Visited this website and input up to 50 miRNAs sequences into the miRNA file and input single target sequence in a target sequence file in FASTA format. Its sensitivity and specificity values were set at 63% and 61% respectively. In seed/nucleus region seed size of 7 was selected to allowed maximum of 1 unpaired base and a minimum number of paired up bases in the heteroduplex was set 12. Maximum number of G: U wobbles allowed in seed region was set to no limit while maximum folding energy was selected to -20kcal/mol. At these parameters potential miRNA which can target the genome of virus

(target sites) were predicted with the position at which it target, folding energy and p-values.

RNAhybrid

RNAhybrid is a tool used for easy and fast miRNA target prediction. It especial predicts RNA secondary structure[22]. RNAhybrid provides with a lot of useful parameters. The first parameter is named hits per target and MFE (minimum free energy)[23]. This algorithm is easily available at the website (http://bibiserv.techfak.unibielefeld.de/rnahybrid[18]. This software was used to eliminate any kind of possible false positive attachments shown by miRanda. The E-value was set -20 kcal/mol and other parameters were set to default.

PPV gene retrieval and annotation

Retrieved gene names from https://www.ncbi.nlm.nih.gov/nuccore/9626508 and CLC genomic workbench (Version 11.0.1) was used for annotation of genomic feature of PPV.

Statistical Analysis

For statistical analysis, R studio and R language were used[18]. R studio helps to run the R language and in R studio readxl package was feed. Table 1 was uploaded into the readxl and used ggplot 2 packages. It gives the result in the form of a graph.

miRNA Genome Binding Site Conservation Analysis

MEGAX was run for alignment purpose[24]. All the PPV complete genome sequences were retrieved from NCBI nucleotide database with accession numbers as; AY028309.2, MF346290.1, MF346289.1, MF346288.1, MF346287.1, MF346286.1, and MF346285.1. ClustalW algorithm was used for alignment of the PPV genome with miRNAs. Viral genome

and putative miRNA sequences were analyzed through MEGAX to check that predicted miRNAs block other genomes too.

Result and Discussion

Plum pox disease commonly called Sharka disease is caused by the infection of PPV, can have adverse effects on the yield of peach. Various reports of peach infection with PPV have been found around the world especially in Europe resulting in a severe economic loss[25,26]. The RNA interference (RNAi) is thought to evolve as a defense mechanism against foreign organisms and it regulates gene expression[27]. miRNAs are the types of small RNAi contain 21 to 24 nucleotides and reported to enhance resistance in plants. miRNAs are reported to silence the specific gene of MCMV and in PVY. This makes the plant transgenic and resistance to the plant diseases caused by a virus[18,28].

miRNA target prediction in the genome of PPV

There four tools were used for prediction of miRNA target in the genome of PPV. Purpose of using four algorithms was to eliminate the false positive results and to maximize the accuracy of target prediction in the viral genome. miRANDA[19] was used with parameters as score threshold, scaling parameter, and energy threshold. RNA22[21] is a pattern based algorithm used for pattern recognition. psRNATarget[20] specifically used to recognize mismatch sensitive seed region. RNAhybrid is an algorithm used for the prediction of secondary structures of RNA[18]. Parameters considered in this algorithm were hits per target and energy threshold. Figure 3 demonstrates all the genome positions targeted by *Prunus persica* miRNA with different calculation utilization.

The full-length genome of PPV encoded into a large polyprotein. This polyprotein cleaved into ten proteins such as; coat protein, HC-Pro protein, P1, P3, VPg, 6K1, 6K2, CI, NIb and Nia-Pro-

proteins. miRNAs of *Prunus persica* was targeted these proteins at the different sites and blocked synthesis at the translational level.

P1 Protein (First protein)

It is very first protein among other proteins. P1 involved in cell to cell transportation and viral infection. These were non-proteolytic functions[29]. Suitable miRNAs for targeting P1 gene were predicted to be miR395a-3p, miR6257, miR6270, miR530, miR6274a, miR6263, miR395b-3p, miR858, miR169d, miR169e-5p, miR8131-3p and miR171e.

HC-Pro Protein

HC-Pro is a multifunctional protein of plum pox virus called helper component protease. It was involved in aphid transmission, genome amplification, and long-distance movement and it is also has been examined as a plant viral suppressors of RNA silencing. HC-Pro was also supposed to intercede the virus replication cycle at different steps[30]. The HC-Pro gene showed an interaction with 22 miRNAs (miR6273, miR167c, miR156e, miR156c, miR482a-5p, 6282, miR530, miR8126-5p, miR482d-5p, miR6297b, miR403, miR6267b, miR164d, miR6288a, miR6291c, miR6275, miR6297a, miR393b, miR8133-3p, miR156d, miR6262 and miR6277).

CI Protein (Cylindrical Inclusion Protein)

CI protein was known to involve in virus replication and cell to cell movement[31,32]. CI proteins formed pinwheel-shaped cytoplasmic inclusion bodies those were the unique features of potyvirus infection[33]. The greatest number of potential targets of *Prunus persica* miRNAs was for the C1 gene that predicted 57 targeting miRNAs (miR399h, miR160a, miR171g, iR8130-3p, miR399g, miR6273, miR2111a, miR399i, miR171o, miR2111d, miR399e, miR156g, miR390, miR393a, miR398b, miR8123-3p, miR6267c-5p, miR398a-3p, miR8129-5p, miR160b,

miR8124-5p, miR482a-5p, miR8126-3p, miR6261, miR6282, miR8130-5p, miR3627-3p, miR399n, miR2111c, miR397, miR156i, miR477-3p, miR7122a 5p, miR6284, miR3991, miR168, miR399d, miR156h, miR156a, miR399c, miR8128-3p, miR399i, miR171d-3p, miR6285, miR858, miR477a-3p, miR171a, miR6286, miR8127-3p, miR172c, miR399m, miR482c-5p, miR2111b, miR398a-5p, miR399k, miR156b and miR482b-5p.

6K1 and 6K2 Protein

6K1 and 6K2 were the two smallest proteins of PPV and both have equal molecular weight[34]. Role of 6K1 was obscured but small deletion in the sequence of 6K1 abolished viral replication[35]. 6K2 was an integral membrane protein and induced the endoplasmic reticulum (ER) originated replication vesicles that target the chloroplast for robust viral replication[36]. These two proteins were targeted by least number of miRNAs. For 6K1 gene of PPV, 10 targeting miRNAs were predicted (miR160a, miR172b, miR6292, miR160b, miR171h, miR8123-5p, miR172a-3p, miR828-3p, 172c and 172d). The 6K2 gene targeted by 6 miRNAs (miR8130-3p, miR319b, miR482a-5p, miR5225-3p, miR482f, and miR6277).

Protein

Little was known about P3 protein. It was a membrane protein and localized in ER membrane. P3 protein gene has been found to prevent replication[37]. It was targeted by more miRNAs after C1 and coat protein. Forty-one miRNAs were predicted to target P3 protein (miR169f, miR171g, miR169i, miR1691, miR171c, miR395m, miR6288c-5p, miR6293, miR156e, miR482a-5p, miR167b, miR8124-3p, miR8130-5p, miR171h, miR6288b-5p, miR169h, miR169k, miR169g, miR171b, miR395a-5p, miR7125-5p, miR535a, miR482a-3p, miR6296, miR171f, miR8122-3p,

miR6271, miR319a, miR6271, miR169i, miR6278, miR167d, miR167a,miR169d,miR169e-5p,miR171a,miR156d,miR6290,miR395b-5p.miR171d-5p, miR3950, and miR8125).

Coat Protein

Capsid protein encapsulated single stranded RNA was encoded by CP. It involved in cell-to-cell and long-distance movement[38], genome amplification[39] and aphid transmission[40]. Suitable miRNAs for targeting coat protein gene were predicted to be miR399h, miR171g, miR8130-3p, miR399g, miR399i, miR171c, miR399e, miR169c, miR172b, miR482d-3p, miR482a-5p, miR396a-3p, miR395f, miR8124-3p, miR395d, miR3627-3p, miR399n, miR164a, miR169b, miR8133-5p, miR3951, miR3991, miR399d, miR159, miR399c, miR399i, miR6260, miR172a-3p, miR395g, miR482e, miR395b-3p, miR395n, miR399a, miR171f, miR6275, miR395i, miR164b, miR395i, miR172c, miR395e, miR395k, miR399m, miR395c, miR399k, miR172d, and mi395h.

NIb protein (Nuclear Inclusion Protein b)

The functional role of NIb protein was in RNA-dependent RNA polymerase and nuclear translocation activities41[47]. By using bioinformatics tools, 37 miRNAs were found which targeting Nib gene: miR8122-5p, miR3627-5p, miR394b, miR396a, miR319b, miR390, miR1827, miR7125-3p, miR6276, miR6269, miR3627-3p, miR6257, miR477b-3p, miR1511-3p, miR47b-5p, miR396b, miR6260, miR6266a, miR482e, miR395b-3p, miR6294, miR6280, miR6285, miR477a-5p, miR169d, miR477-5p, miR394a, miR8127-3p, miR6266c, miR6266b, miR6264, miR162, miR8129-3p, miR6274b-3p,miR6289,miR535b,miR399b, and miR6267a.

NIa-Pro Protein (C-Terminal Proteinase Domain of NIa)

NIa-Pro protein was required for proteolytic maturation of many viral proteins[42]. Bioinformatics tools were used to predict the miRNAs which target NIa pro gene. There was the least number of

miRNAs which targeted this gene. There were 18 miRNAs predicted to target Nia-Pro gene: miR6287, miR3944b, miR169c, miR828-5p, miR8127-5p, miR169b, miR395a-5p, miR169a, miR6274b-5p, miR6272, miR477b-5p, miR169k, miR8132, miR477a-5p, miR394a, miR1511-5p, miR3950.

VPg Protein

It plays a very important role in viral infection. It is a viral genome link protein involved in replication and translation of viral genome[36]. VPg may also serve as a primer for viral RNA replication[43]. Through target prediction algorithms, found only one (miR482b-3p) miRNA targeting VPg gene.

Prunus persica screened miRNAs

Two miRNAs were found to have more potential to target PPV at multiple loci. These two potential miRNAs were miR8122-3p and miR7125-5p and predicted by at least three algorithms. These screened miRNAs were most suitable *Prunus persica* miRNAs to boost defense system in the peach plant against plum pox virus.

miRNA-genome binding site conservation analysis

For the binding of miRNA to the genome of viral strains, MEGAX[24] software was used. Screened miRNAs targeted at various positions in different strains of the virus showed details of the level of conservation in binding sites.

Conflict of Interest

The authors state that the research was steered in the absence of any commercial or financial relationships that could be taken as a potential conflict of interest.

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References

1. Verde, I., Abbott, A.G., Scalabrin, S., Jung, S., Shu, S., Marroni, F., Zuccolo, A., The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nature genetics*, 2013, 45(5), 487.

2. Dunez, J. and Sutic, D., Plum pox virus. *European handbook of plant disease*, 1988, 44-46

3. Pasquini, G., Barba, M., The question of seed transmissibility of Plum pox virus. *EPPO Bulletin*, 2006, 36(2), 287-292.

4. Cambra, M., Capote, N., Myrta, A., Llácer, G., Plum pox virus and the estimated costs associated with sharka disease. *EPPO Bulletin*, 2006, 36(2), 202-204.

5. Kegler, H. and Hartmann, W., Present status of controlling conventional strains of plum pox virus. *Plant virus disease control*, 1998, 616-628.

6. Nemchinov, L., Crescenzi, A., Hadidi, A., Piazzolla, P. and Verderevskaya, T., Present status of the new cherry subgroup of Plum pox virus (PPV-C). *Plant virus disease control*, 1998, 629-638.

7. Jacquet, C., Delecolle, B., Raccah, B., Lecoq, H., Dunez, J. and Ravelonandro, M., Use of modified plum pox virus coat protein genes developed to limit heteroencapsidation-associated risks in transgenic plants. *Journal of General Virology*, 1998, 79(6), 1509-1517.

8. Tavert-Roudet, G., Ravelonandro, M., Bachelier, J. C. and Dunez, J., Transgenic *Nicotiana benthamiana* plants containing the P1 gene of plum pox virus are resistant to virus challenge. *European journal of plant pathology*, 1998, 104(1), 103-107.

9. Barajas, D., Tenllado, F., González-Jara, P., Martínez-García, B., Atencio, F.A. and Díaz-Ruíz, J. R., Resistance to Plum pox virus (PPV) in *Nicotiana benthamiana* plants transformed with the PPV HC-Pro silencing suppressor gene. *Journal of Plant Pathology*, 2004, 86(3), 239-248.

10. Eldem, V., Akcay, U.C., Ozhuner, E., Bakır, Y., Uranbey, S. and Unver, T., Genome-wide identification of miRNAs responsive to drought in peach (*Prunus persica*) by high-throughput deep sequencing. *PloS One*, 2012, 7(12), e50298.

11. Pillai, R.S., Bhattacharyya, S. N. and Filipowicz, W.. Repression of protein synthesis by miRNAs: how many mechanisms? *Trends Cell Biol*., 2007, 17, 118-126.

12. Ritchie, W., Flamant, S., Rasko, J. E., miRNA: a microRNA expression profiler and classification resource designed to identify functional correlations between microRNAs and their targets. *Bioinformatics*, 2009, 26(2), 223-227.

13. Pani, A., Mahapatra, R. K., Behera, N. and Naik, P. K., Computational identification of sweet wormwood (*Artemisia annua*) microRNA and their mRNA targets. Genomics, *proteomics & bioinformatics*, 2011, 9(6), 200-210.

14. Griffiths-Jones, S., Saini, H.K., van Dongen, S. and Enright, A. J., miRBase: tools for microRNA genomics. *Nucleic acids research*, 2007, 36, D154-D158.

15. Chaudhuri, K. and Chatterjee, R., MicroRNA detection and target prediction: integration of computational and experimental approaches. *DNA and Cell Biology*, 2007, 26(5), 321-337.

16. Li, L., Xu, J., Yang, D., Tan, X. and Wang, H., Computational approaches for microRNA studies: a review. *Mammalian Genome*, 2010, 21(1-2), 1-12.

17. Witkos, M. T., Koscianska, E. and Krzyzosiak, J. W., Practical aspects of microRNA target prediction. *Current molecular medicine*, 2011, 11(2), 93-109.

18. Iqbal, M. S., Jabbar, B., Sharif, M. N., Ali, Q., Husnain, T. and Nasir, I. A., In silico MCMV silencing concludes potential host-derived miRNAs in maize. *Frontiers in plant science*, 2017, 8, 372.

19. Liu, H. W., Luo, L.X., Liang, C. Q., Jiang, N., Liu, P. F. and Li, J. Q., High-throughput sequencing identifies novel and conserved cucumber (*Cucumis sativus* L.) microRNAs in response to cucumber green mottle mosaic virus infection. *PloS One*, 2015, 10(6), e0129002.

20. Dai, X. and Zhao, P. X., psRNATarget: a plant small RNA target analysis server. *Nucleic acids research*, 2011, 39(2), 155-159.

21. Miranda, K. C., Huynh, T., Tay, Y., Ang, Y. S., Tam, W. L., Thomson, A. M. and Rigoutsos, I., A pattern-based method for the identification of microRNA binding sites and their corresponding heteroduplexes. *Cell*, 2006, 126(6), 1203-1217.

22. Rehmsmeier, M., Steffen, P., Hochsmann, M. and Giegerich, R., Fast and effective prediction of microRNA/target duplexes. *RNA*, 2004, 10, 1507-1517.

23. Lewis, B. P., Burge, C. B. and Bartel, D. P., Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, 2005, 120(1), 15-20.

24. Kumar, S. and Stecher, G., Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 2016, 33(7), 1870-1874.

25. Díaz-Vivancos, P., Rubio, M. and Mesonero, V., The apoplastic antioxidant system in Prunus: response to long-term plum pox virus infection. *Journal of Experimental Botany*, 2006, 57(14), 3813-3824.

26. Hernández, J. A., Rubio, M., Olmos, E., Ros-Barcelo, A. and Martínez-Gómez, P., Oxidative stress induced by long-term plum pox virus infection in peach (*Prunus persica*). *Physiologia Plantarum*, 2004, 122(4), 486-495.

27. Jolliffe, K., RNAi collection. *Sigma Aldrich*, 2 2002

28. Iqbal, M. S., Hafeez, M. N., Wattoo, J. I., Ali, A., Sharif, M. N., Rashid, B. and Nasir, I. A., Prediction of host-derived miRNAs with the potential to target PVY in potato plants. *Frontiers in genetics*, 2016, 7, 159.

29. Pan, Q., Molecular Characterization of the Potyviral First Protein (P1 Protein). *Electronic Thesis and Dissertation Repository*, 2016, 4115.

30. Kasschau, K. D., Cronin, S. and Carrington, J. C., Genome amplification and long-distance movement functions associated with the central domain of tobacco etch potyvirus helper component–proteinase. *Virology*, 1997, 228(2), 251-262.

31. Jenner, C. E., Sanchez, F., Nettleship, S. B., Foster, G. D., Ponz, F. and Walsh, J. A., The cylindrical inclusion gene of Turnip mosaic virus encodes a pathogenic determinant to the *Brassica* resistance gene TuRB01. *Molecular Plant-Microbe Interactions*, 2000, 13(10), 1102-1108.

32. Jenner, C. E., Tomimura, K., Ohshima, K., Hughes, S. L. and Walsh, J. A., Mutations in Turnip mosaic virus P3 and cylindrical inclusion proteins are separately required to overcome two *Brassica napus* resistance genes. *Virology*, 2002, 300(1), 50-59.

33. Eagles, R. M., Balmori-Melián, E., Beck, D. L., Gardner, R. C. and Forster, R. L., Characterization of NTPase, RNA-binding and RNA-helicase activities of the cytoplasmic

inclusion protein of tamarillo mosaic potyvirus. *European Journal of Biochemistry*, 1994, 224(2), 677-684.

34. Kekarainen, T., Savilahti, H. and Valkonen, J. P., Functional genomics on potato virus A: virus genome-wide map of sites essential for virus propagation. *Genome research*, 2002, 12(4), 584-594.

35. Cui, H. and Wang, A., The Plum pox virus 6K1 protein is required for viral replication and targets the viral replication complex at the early infection stage. *Journal of virology*, 2016, JVI-00024.

36. Huang, T.S., Wei, T., McNeil, J., Laliberté, J. F., Hong, J., Nelson, R. S. and Wang, A., Sequential recruitment of the endoplasmic reticulum and chloroplasts for plant potyvirus replication. *Journal of virology*, 2010, 84(2), 799-809.

37. Klein, P.G., Klein, R. R., Rodriguez-Cerezo, E., Hunt, A. G. and Shaw, J. G., Mutational analysis of the tobacco vein mottling virus genome. *Virology*, 1994, 204(2), 759-769.

38. Dolja, V. V., Haldeman-Cahill, R., Montgomery, A. E., Vandenbosch, K. A. and Carrington, J. C., Capsid protein determinants involved in cell-to-cell and long distance movement of tobacco etch potyvirus. *Virology*, 1995, 206(2), 1007-1016.

39. Missiou, A., Kalantidis, K., Boutla, A., Tzortzakaki, S., Tabler, M, and Tsagris, M., Generation of transgenic potato plants highly resistant to potato virus Y (PVY) through RNA silencing. *Molecular Breeding*, 2004, 14(2), 185-197.

40. Brigneti, G., Voinne, O., Li, W. X., Ji, L. H., Ding, S. W. and Baulcombe, D. C., Retracted: Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *The EMBO journal*, 1998, 17(22), 6739-6746.

41. Li, X. H., Valdez, P., Olvera, R.E. and Carrington, J. C., Functions of the tobacco etch virus RNA polymerase (NIb): subcellular transport and protein-protein interaction with VPg/proteinase (NIa). *Journal of virology*, 1997, 71(2), 1598-1607.

42. Garcia, J. A., Riechmann, J. and Lain, S., Proteolytic activity of the plum pox potyvirusNla-like protein in Escherichia coli. *Virology*, 1989, 170(2), 362-369.

43. Puustinen, P. and Mäkinen, K. M., Uridylylation of the potyvirusVPg by viral replicase NIb correlates with the nucleotide binding capacity of VPg. *Journal of Biological Chemistry*, 2004, 279, 38103-38110.

Figures Legends

Figure 1**.** Genome annotation of plum pox virus**.** Ten genes of PPV represented along with their positions. Arrows indicated the position of ORF. Genes were translated from these positions. Long arrow represented polyprotein, cleaved into ten mature proteins.

Figure 2. Target prediction results of miRNAs against PPV genome indicated target prediction obtained from miRanda**.**

Figure 3. Target prediction results of miRNAs against PPV genome indicated target prediction obtained from RNA22.

Figure 4. Target prediction results of miRNAs against PPV genome indicated target prediction obtained from psRNATarget.

Figure 5. Target prediction results of miRNAs against PPV genome indicated target prediction obtained from TapirHybrid.

Figure 6. Secondary structures of identified potential miRNAs which formed from precursors of the mature miRNAs (pre-miRNAs)

Figure 7. Multiple sequence alignment of PPV genome showed the conservation of binding site of a particular miRNAs.

Figures

Figure 1

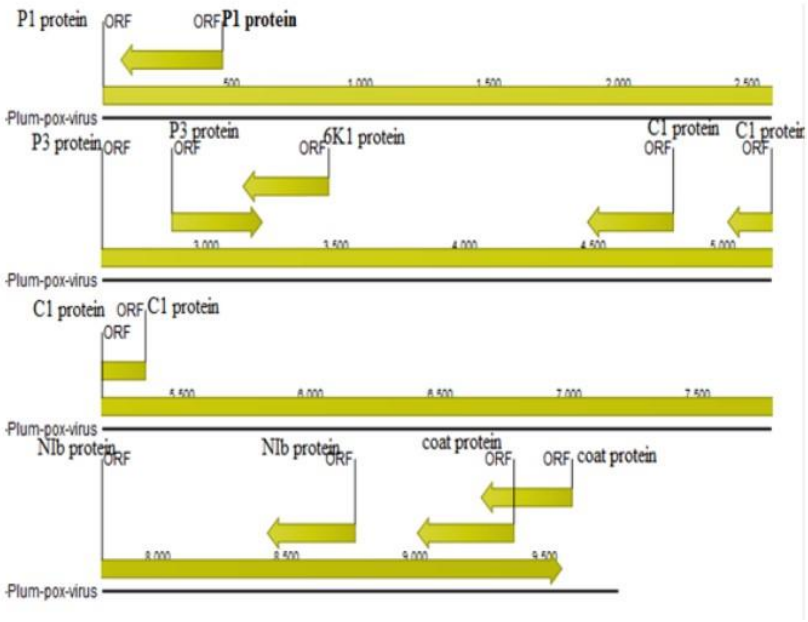
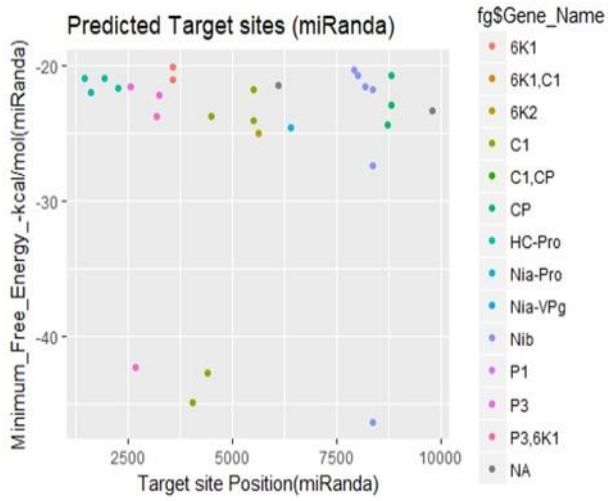
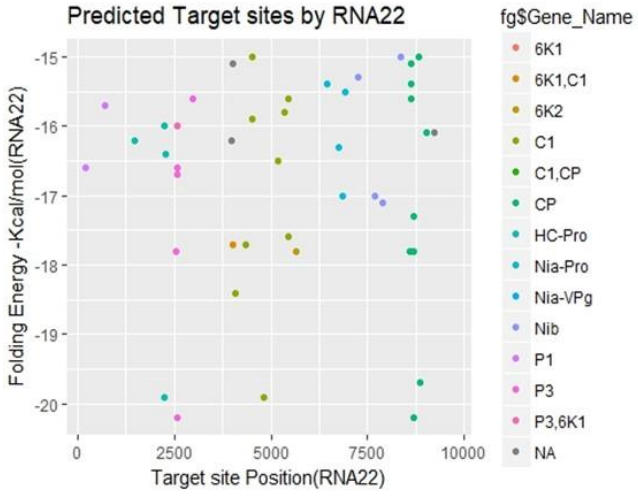


Figure 2



1

2 Figure 3



3

4 Figure 4

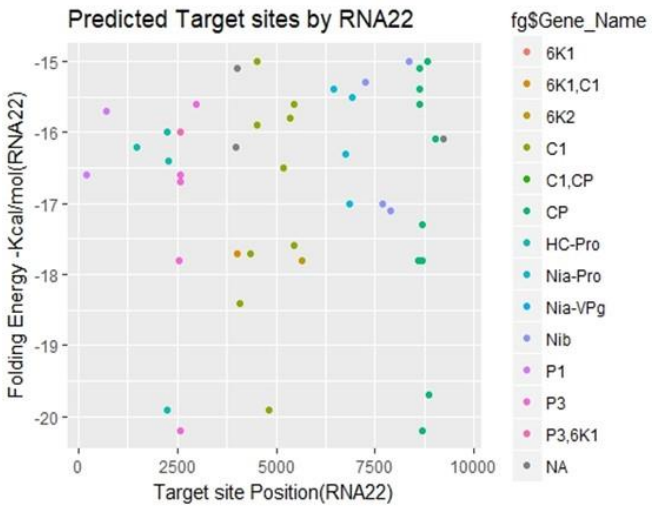


Figure 5

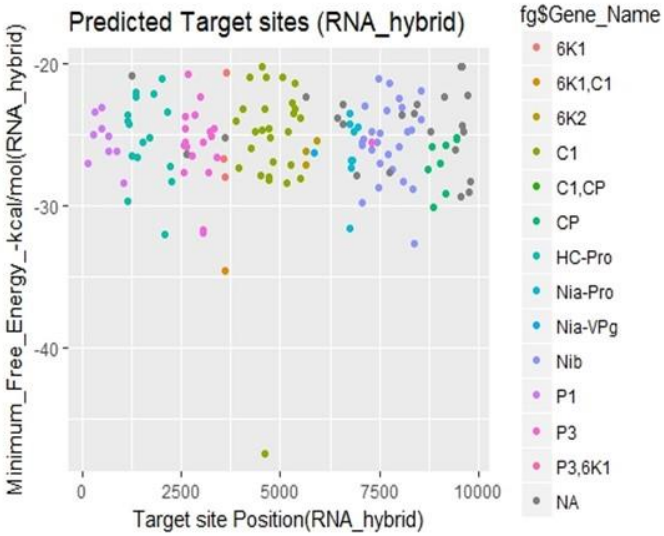
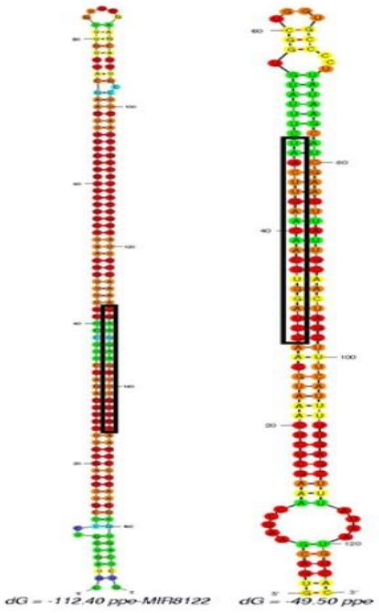


Figure 6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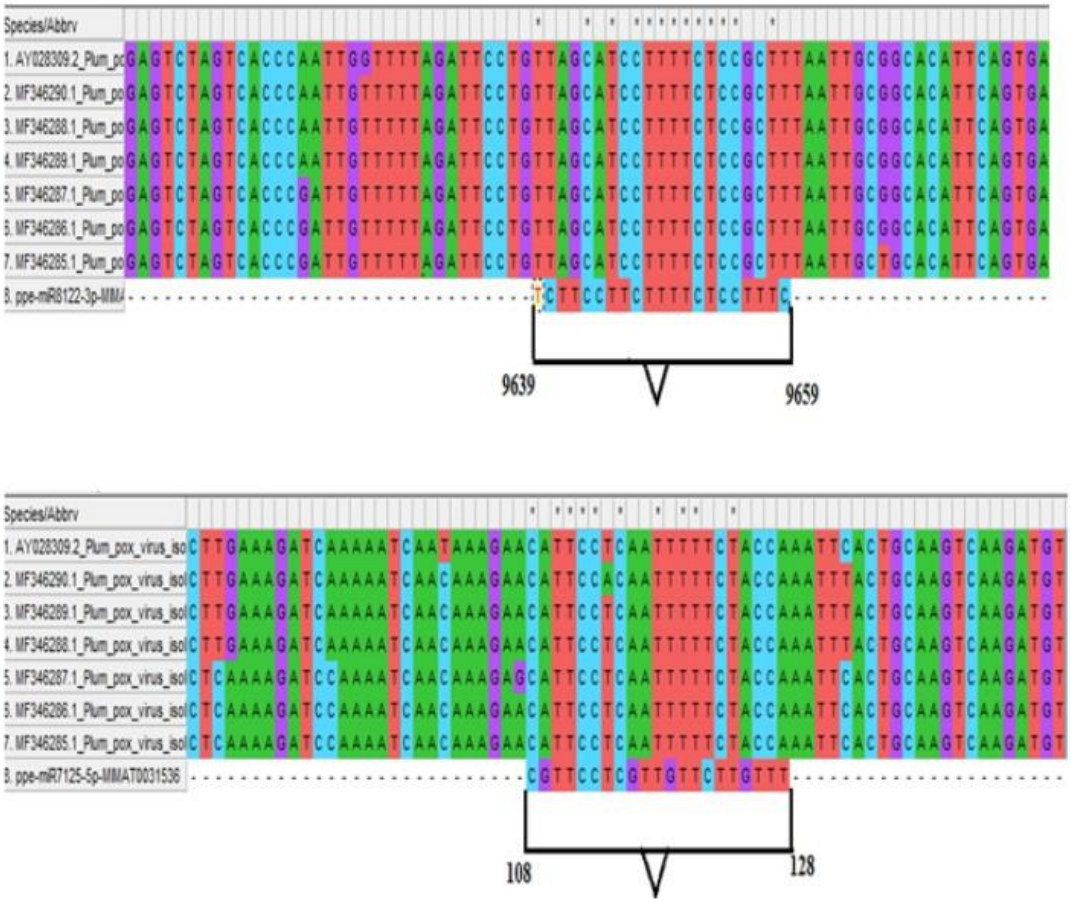
dG = -49.50 ppe

Figure 7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3. MF346288.1_Plum_po
4. MF346289.1_Plum_po
5. MF346287.1_Plum_po
6. MF346286.1_Plum_po
7. MF346285.1_Plum_po
8. ppe-miR8122-3p-MIM
9639
9659
Species/Abbrv
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3. MF346289.1_Plum_pox_virus_iso
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128