ABSTRACT: 116

MULTIPLY PRIMED ROLLING CIRCLE AMPLIFICATION (MPRCA) OF YELLOW MOSAIC DISEASE FROM INFECTED SOYBEAN IN CENTRAL DETECTED THE PRESENCE OF *MUNGBEAN YELLOW MOSAIC INDIAN VIRUS-[SB]*

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Genome characterization of the *yellow mosaic disease* wreaking damage on soybean in Indore-a focal point of soybean cultivation and processing in India- employing Multiply Primed Rolling Circle Amplification (MPRCA) resulted in amplification of complete genomic components of the virus. RCA, when coupled with restriction enzyme digestion investigation, revealed two components each of ~2.7 Kb unit length of genomic components of the virus. The study further revealed the absence of β- satellite DNA genomic component of the virus. The RCA derived partial genomic region on further restriction enzyme digestion investigation revealed that *Mungbean yellow mosaic Indian virus* (MYMIV) is the species of *begomovirus* causing damage on soybean in the central Indian region, Indore. The RCA together with restriction enzyme digestion has wider implications for obtaining RNAi mediated viral gene silencing via complete genome sequence characterization to identify conserved genomic regions along with the discovery of ORFs encoding for Viral Derived Suppressor Proteins (VSPs). The potent siRNA inducing regions identified, employing web based siRNA design algorithms, revealed plausible small RNA effectors to obtain RNAi based, broad spectrum resistance against *begomovirus* infection and trait stable transgenics in soybean. In addition, the RCA products followed by partial digestion and cloning in binary vector offers a prospect to develop MYMIV based virus induced knock-out vectors with a potential implication for functional genomics study on soybean.

Multiply Primed Rolling Circle Amplification (MPRCA) of *Begomovirus* genome from infected soybean in central Indian region divulges it as *Mungbean yellow mosaic Indian virus-[sb]* and its implications for RNAi mediated resistance

Ramesh S.V., Ramesh B., Nikita A., G.K.Gupta, Husain S.M.

Directorate of Soybean Research

(Formerly: National Research Centre for Soybean)

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद

DSR Staff wishes you a very-very Happy New Year 2013!

Hindi Version

DSR Profile

Soybean Variety

Staff Profile

Soy Food Uses

Welcome to www.dsrindore.org

The institution is located at Indore in Madhya Pradesh.

News and Updates

- DSR is selected for Rajarishi Tandon Award (First) for 2011-12.
- Dr. H.S. Gupta, Director IARI, New Delhi visited DSR on 02nd January 2013.
- DSR celebrated foundation day on 11th December 2012.
- IMC meeting held on 16th November 2012.
- Hindi workshop organized on 23rd November 2012.

Mandate

- Collection, evaluation and characterization of genetic resources of soybean.
- Basic, strategic and applied research to increase the productivity and oil content.
- Research on quality of oil and oilseed cake.
- Socio-economic research for assessing the sustainability of the technologies.
- Transfer of technology.
- Coordination of multilocation trials to develop varieties and technologies of national and regional importance through All India Coordinated Research.

News and Events

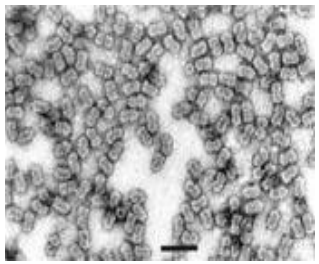
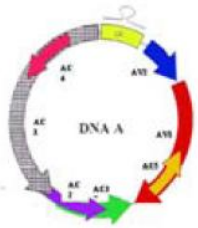
Growth Stages

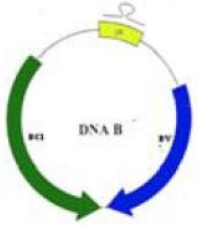
Pathogens

Background

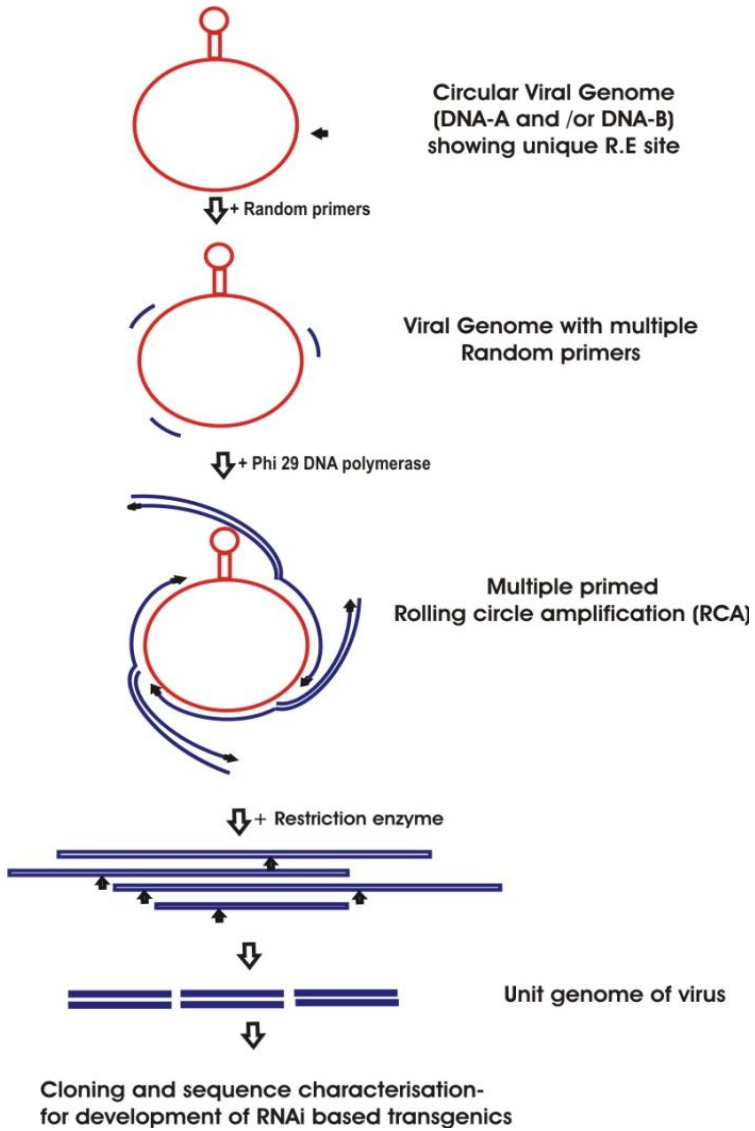
- Yellow mosaic disease (YMD) on soybean
  - Causative organism: *Yellow mosaic virus*
  - Family: Geminiviridae
  - Whitefly transmitted

- Yield loss due to YMD $ 300 million/annum
- Spread of disease to hitherto uninfected regions
- Genome diversity



Multiply Primed Rolling circle amplification (Circomics)



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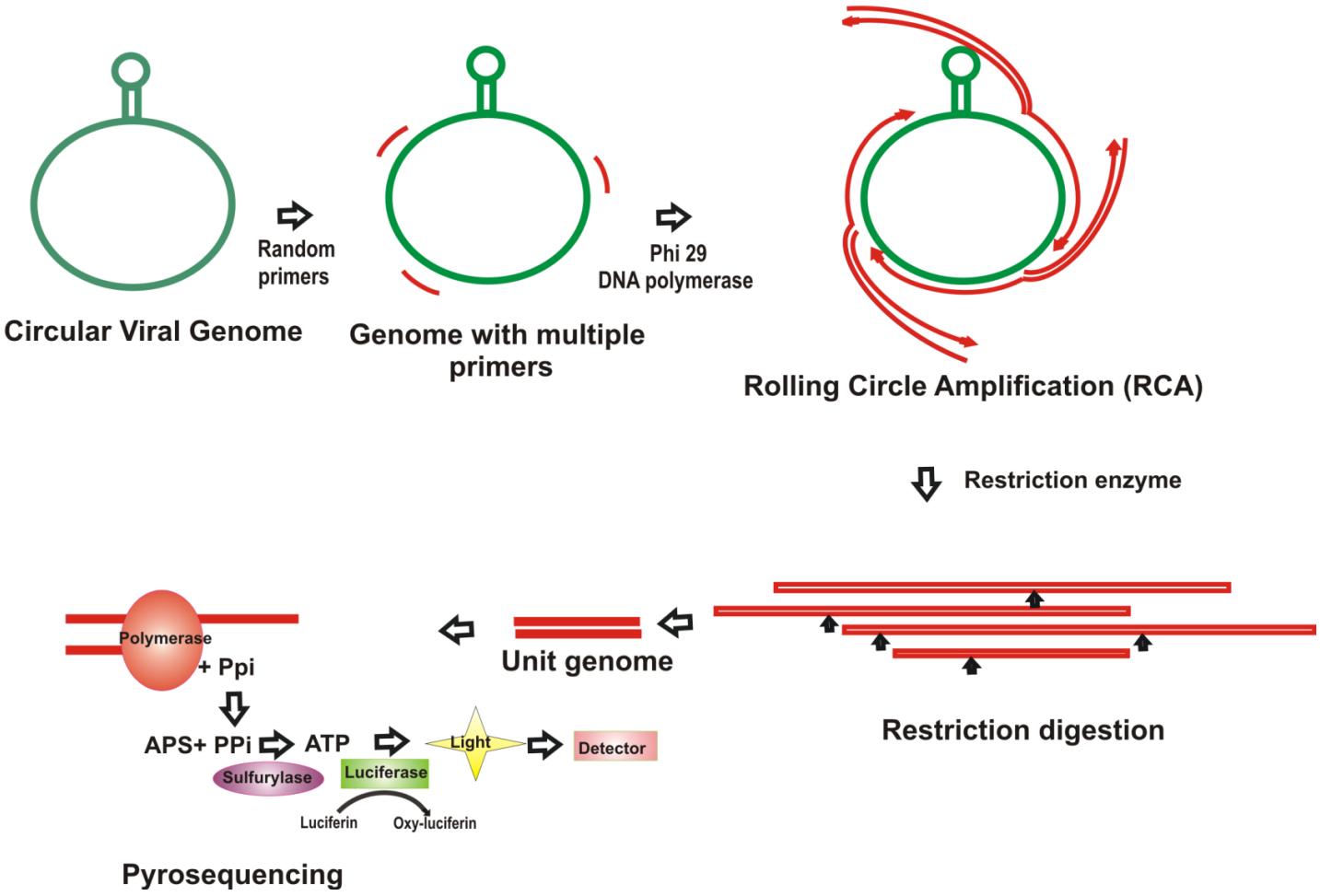
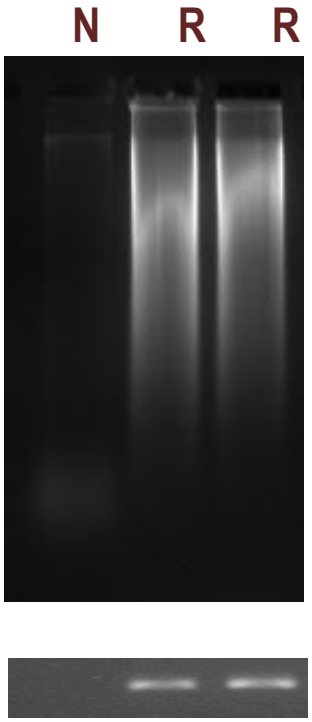


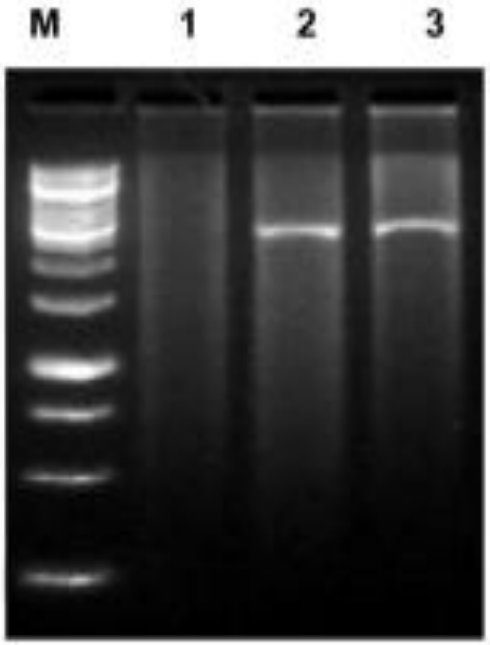
Multiply Primed Rolling circle amplification (Circomics)



N: Negative control

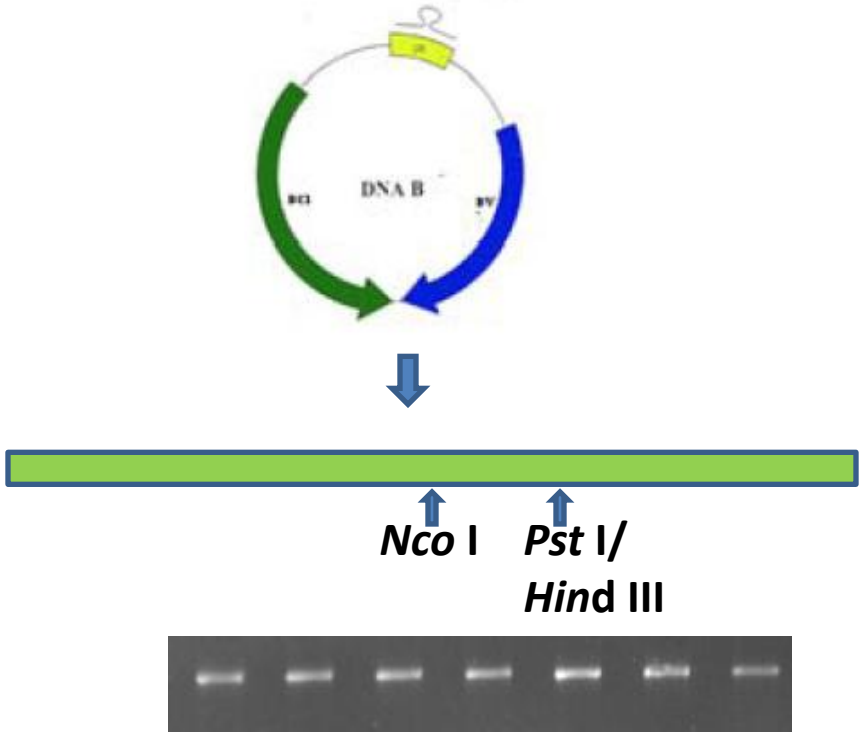
R: Partial AV-1 region amplicon

Restriction enzyme digestion of MPRCA Products



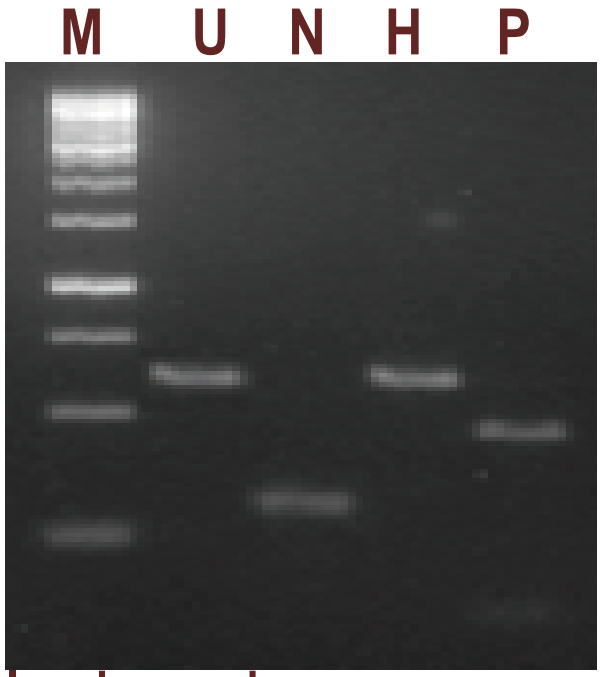
M: Molecular size marker
1- Negative control (- R.E.)
2- Restriction of RCA product with *Pst*-I
3-Restriction of RCA product with *Hin*d III

RCA + RFLP Studies on Partial DNA-B region



Nco* I is conserved in legume infecting *Begomoviruses
***Pst* I- *Mungbean yellow mosaic Indian virus* (MYMIV)**
***Hind* III-*Mungbean yellow mosaic virus* (MYMV)**

RCA + RFLP Studies on Partial DNA-B region



M:1Kb molecular size marker

U: Uncut DNA B genomic fragment

N: DNA B genomic fragment treated with *Nco* I

H: DNA B genomic fragment treated with *Hind* III

P: DNA B genomic fragment treated with *Pst* I

RCA Derived Complete Genome

Development of virus induced knock-out vectors

Small non-coding RNAs mediated antiviral resistance

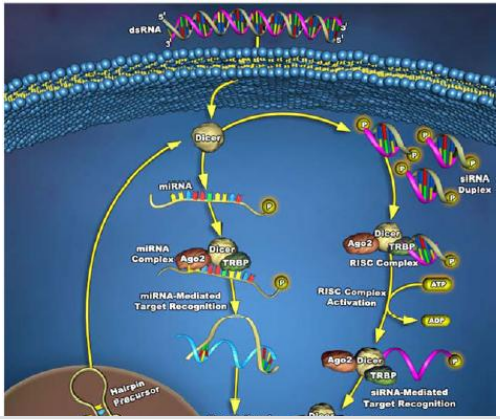
Host derived and artificial miRNAs

siRNAs mediated gene silencing

Small non coding RNAs based virus resistance

- **RNA interference (RNAi)-hope for nucleotide sequence specific down-regulation of viral genes**

- **Successful deployment of RNAi in control of SS DNA viruses**
  ***African cassava mosaic virus*,**
  ***Tomato leaf curl New Delhi virus* etc.,**



siRNAs mediated gene silencing

Concomitant silencing of AC1 and AC2 ORFs

- Replication initiator protein (rep)

and

- Transcriptional activator protein (TrAP) respectively

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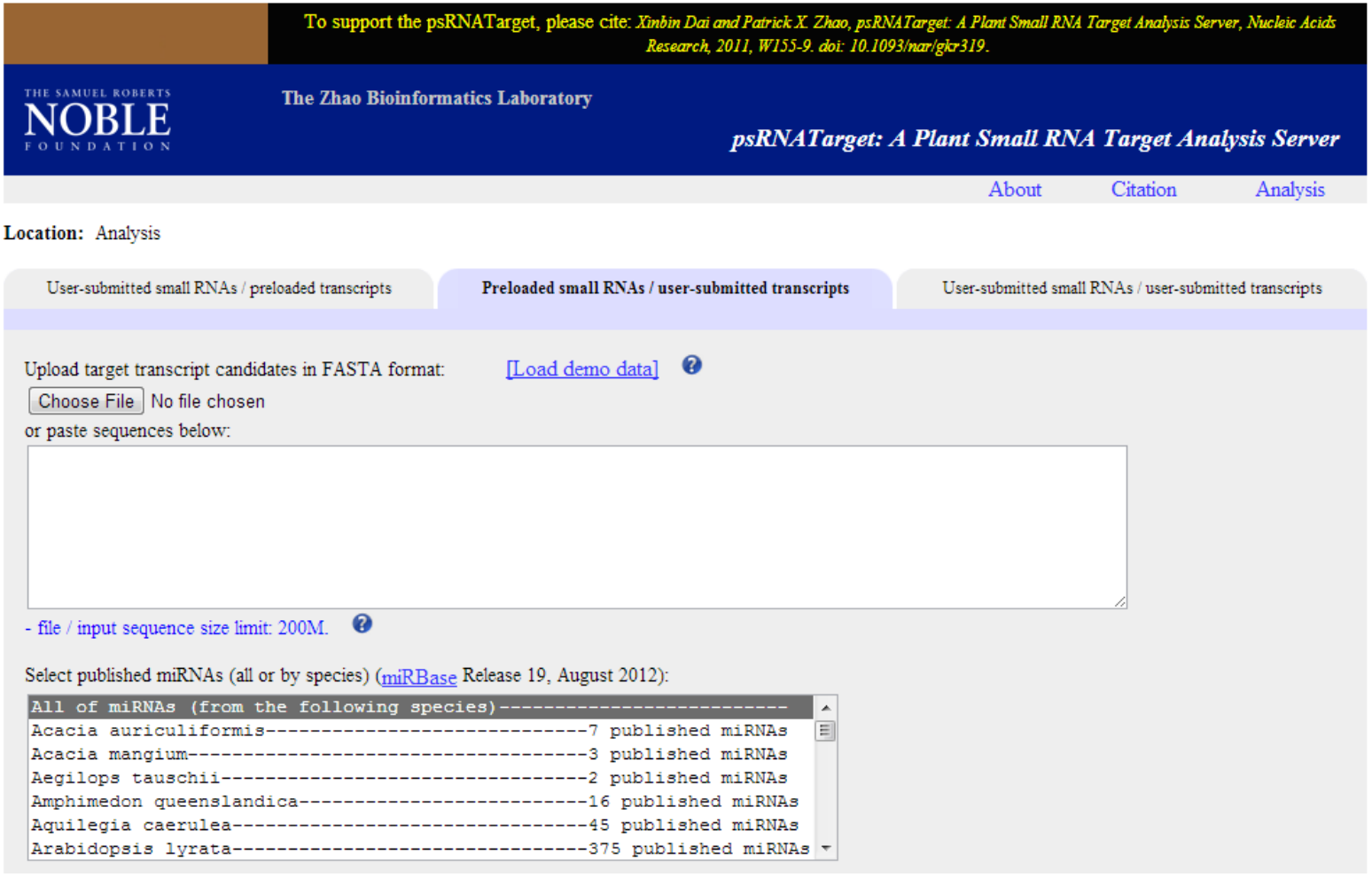
Prediction of miRNA cross silencing activity with viral genome



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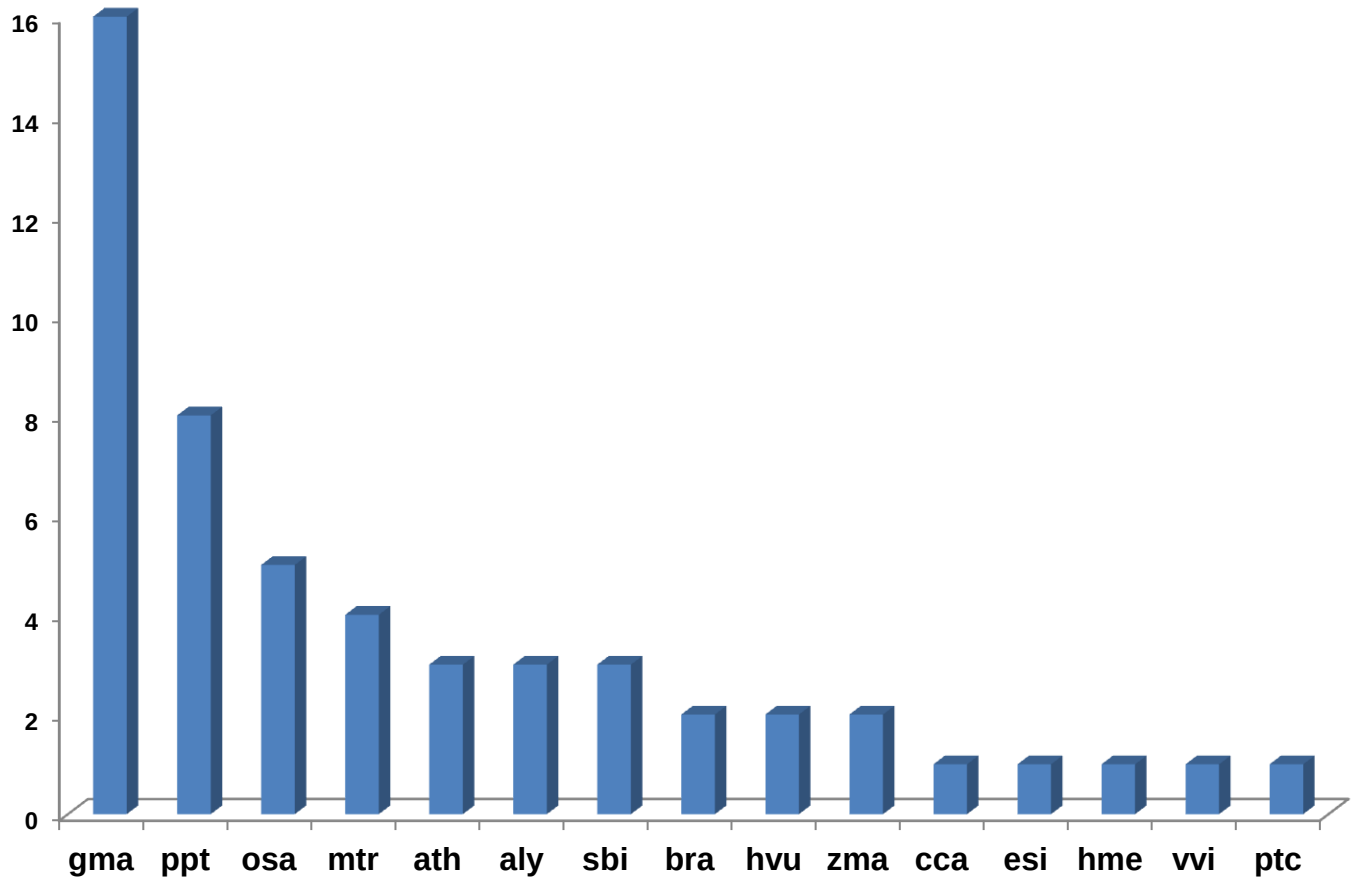
Plant miRNAome targeting MYMIV-sb

Number of miRNA Hits



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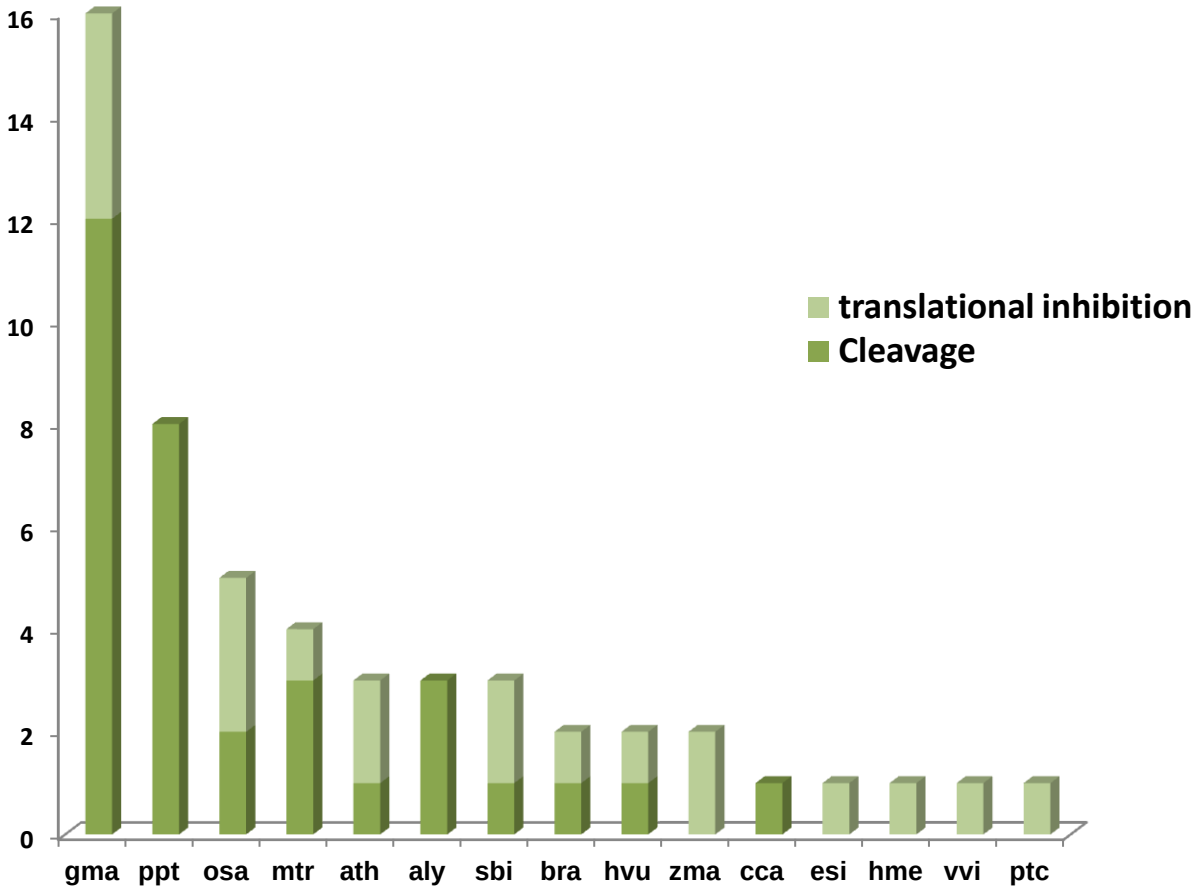
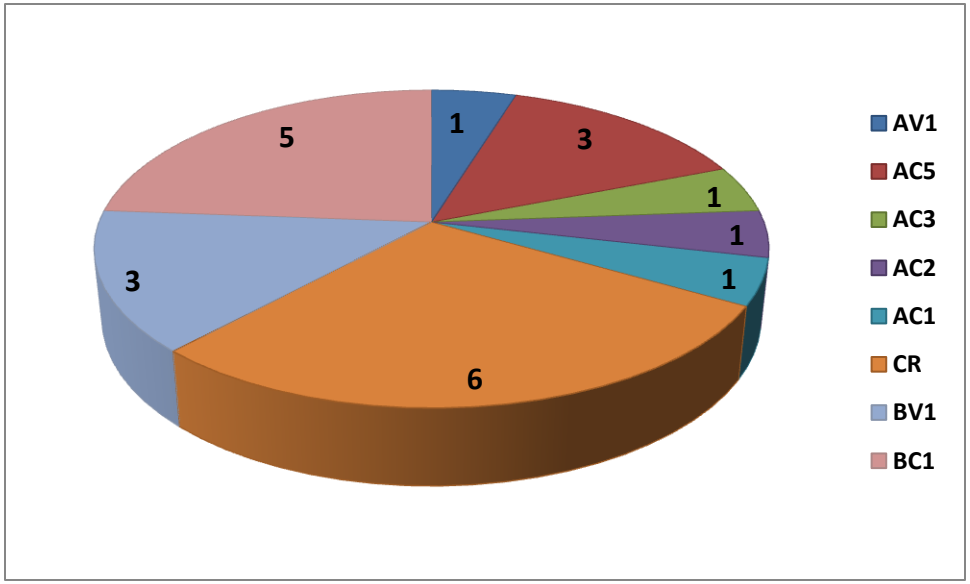
Mode of action of Plant miRNAome against MYMIV-sb



Genomic regions targeted by plant miRNAome



Glycine max miRNAs repress MYMIV movement

miRNA family	Viral genome Targets	Host transcriptome targets/functions
gmamiR482b	Common region	Root specific expression during nodulation
gmamiR1519	Non coding region	miRNA isolated from *B. japonicum*-inoculated soybean roots
gmamiR5764	BV 1 movement protein	Regulators of NB-LRR defense related genes
gmamiR1520	Non coding region	miRNA isolated from *B. japonicum*-inoculated soybean roots
gmamiR4371	AC 5	Non- annotated Protein targets in seed cotyledons
gmamiR5785	BC1 Nuclear shuttle protein	Regulators of NB-LRR defense related genes

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Conclusions

- Complete Genome of soybean-infecting *Begomovirus* in central Indian region studied
- RCA-RFLP studies identifies it as MYMIV
- Small ncRNAs based gene silencing is explored to develop antiviral resistance
- Host derived miRNAs display propensity to repress virus movement

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Acknowledgements

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