Rapid screening of hpRNAi constructs for imparting plant viral disease resistance by employing Agro-infiltration

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Introduction

• Plant virus cause huge loss in many important crops like tomato and chilli.

• Integrated disease management is inefficient to control the virus diseases.

• RNA interference- impart durable multiple virus resistance.

• A broad spectrum of hpRNAi gene construct were developed- Tospovirus (GBNV & CaCv), Cucumovirus (CMV), Potyvirus (ChiVMV).

• An indicator crop (cowpea) selected to check the efficacy of hpRNAi construct for Tospovirus and optimize different parameters for transient expression by Agro-infiltration.
Materials and methods

1. Selection of virus genes and off-target minimized regions to develop dsRNA construct:
   - Individual virus fragments were amplified, cloned and sequenced.

2. Development of broad spectrum Multiple virus hpRNAi construct:
   - All fragments were joined by overlap PCR.
   - Sense, Intron and Antisense fragments were cloned in pBluescript(KS\(^+\)) vector and confirmed the fragments by sequencing.
   - Finally, confirmed fragment were cloned in pBlI121 Binary vector using restriction sites and called as MVR-pBI vector. The confirmed clone were mobilized into *EHA105 Agrobacterium* strain by electroporation

3. Optimization of Agro-infiltration in cowpea plant for Tospovirus:
   - Virus infection in cowpea plants were optimized for tospovirus by different temperature conditions and different buffers and different dilutions.
   - *Agro-infiltration* were optimized by different bacterial concentrations and different methods

4. Analysis of the silencing efficacy of RNAi construct by virus infection:
   - Virus symptoms were recorded in all treated and control plants
   - Viral load were detected by DAS-ELISA and qRT PCR
   - Different bacterial concentrations of MVR and pBI 121 expression levels were recorded by qRT and GUS assay respectively.
Results

GUS assay for pBI121 vector control infiltrated plants

MVR expression by qRT-PCR

Optimization of vacuum agro-infiltration in cowpea cotyledons with different bacterial concentrations (OD₆₀₀) using pBI121 35S-GUS Binary vector. A-Buffer control, B-OD 0.1, C-OD 0.25, D-OD 0.5, E-OD 0.75, F-OD 1.0
Optimization of different bacterial concentrations (OD\textsubscript{600}) of MVR construct by Agroinfiltration to evaluate the silencing of tospovirus: a- Un-infected pBI vector control OD 0.5; b- infected pBI vector control OD 0.5; c- MVR-OD 0.1 infected; d- MVR-OD 0.25 infected; e- MVR-OD 0.5 infected; f- MVR-OD 0.75 infected; g- MVR-OD 1.0 infected

**Relative accumulation of GBNV**

**Estimation of Viral load by DAS-ELISA**
**Conclusion**

- Due to lack of resistant source, current management involves roguing and insect vector control as resistant gene pools for breeding are unavailable in most crops.
- RNAi is a potential tool imparting durable multiple virus resistance.
- Transient expression in cowpea through *Agro-infiltration* method is fast, time consuming to study the efficacy of viral gene constructs.
- This approach is simple, rapid and efficient to screen the efficiency of the RNAi constructs.

**Future work:** The MVR vector used in this study, is further carried over for stable transformation in both tomato and chilli plants to impart Multiple virus resistance.