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ORIGINAL ARTICLE

Expression of short hairpin RNA (shRNA) targeting AC2 gene of *Mungbean yellow mosaic India virus* (MYMIV) reduces the viral titre in soybean

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Abstract
Mungbean yellow mosaic India virus (MYMIV) belonging to the family *Geminiviridae* and the genus *Begomovirus* is a severe pathogen of tropical legumes including soybean. The absence of genetically mapped loci conferring resistance together with the genetic diversity of begomoviruses infecting soybean warrants the utilization of RNA interference (RNAi) technology to develop virus resistance. However, viral suppressors of RNAi (VSRs) reduce the effectiveness of RNA silencing. Here, we report the effectiveness of *Agrobacterium*-mediated transient expression of shRNA, targeting a conserved region of AC2 ORF (a VSR) of MYMIV, in conferring virus resistance in soybean. Transient expression of shRNA showed progressive reduction of the viral titre estimated by the MYMIV-derived AC2 gene copy numbers from the initial inoculum by approximately 80-fold 20 days post-application. In addition, the newly emerging leaves exhibited symptom recovery. Thus, this study proves that AC2 of MYMIV is a potent target gene for obtaining RNAi-mediated virus resistance in soybean. Agro-infiltration-based delivery of shRNA was an efficient means of gene silencing and could pave way for the development of transgenic virus-resistant soybean genotype.

Keywords Begomovirus · MYMIV · RNAi · shRNAs · Soybean · Virus resistance

Introduction

RNA interference (RNAi) is a sequence-specific gene regulatory mechanism that has been exploited to engineer antiviral resistance in plants (Ding and Voinnet 2007). RNA-based gene silencing functions on the principle of nucleotide sequence complementarity. Hence, meticulous identification and expression of a target viral region, such as self-complementary RNA, could potentially downregulate cognate viral gene and provide immunity to the plants. *Geminiviridae* is a family of phytopathogenic viruses that has characteristic geminate virion particles encompassing single-stranded DNA molecules as genomic components. Numerous strategies have been adopted to develop geminivirus resistance in plants utilizing virus-derived nucleic acids. Initially, viral-derived complete, truncated or mutated coat protein (Kunik et al. 1994), movement protein, nuclear shuttle protein (Hou et al. 2000), and replication-associated protein (Antignus et al. 2004; Shivaprasad et al. 2006) were ectopically expressed in plants to develop geminivirus resistance. Later, antisense RNA-based virus resistance strategy was exploited to confer geminivirus resistance (Bendahmane and Gronenborn 1997; Haq et al. 2010). RNAi has been exploited by the plant virologists to express small interfering RNAs (siRNAs) (Vanitharani et al. 2003), intron-spliced hairpin RNAs (Ramesh et al. 2007; Praveen et al. 2010) and artificial microRNAs (miRNAs) (Vu et al. 2013) to target various geminiviruses.

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