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Extended Abstract

Velvet Bean Severe Mosaic Begomovirus DNA: A Encoded RNA Silencing Suppressor Proteins

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Abstract:

Genus Begomovirus of Geminiviridae family has widest host range in plants. It can be monopartite or bipartite. These are further grouped into old world (monopartite/bipartite) and new world (bipartite) based on their genome. In the defence and counter defence strategies of plants and viruses, the latter evolved some proteins which can suppress the RNA silencing and these viral proteins are known as RSS (RNA silencing suppressors). Number of viral proteins are identified as RSS. Mucuna pruriens commonly known as velvet bean or magic bean also owing to its medicinal properties due to which its widely used in medicines for curing diseases like Parkinson's disease, bite of snake and in case of liver dysfunction. Velvet bean severe mosaic virus (VbSMV) infecting *Mucuna pruriens* has been identified. In the study suppressor proteins were identified using Agrobacterium co-infiltration assay using *Nicotiana benthamiana*. Three genes AV2, AV1 and AC2 showed suppressor activity, though only AV2 showed strong suppressor activity as compared to AV1 and AC2 genes.

Introduction: *Mucuna pruriens* (Velvet bean) is a tropical leguminous plant commonly known as velvet bean and is a member of legume family Fabaceae. Agriculturally Legumes are grown worldwide, mainly for their grain seed called pulse which are one of most cost-effective sources of proteins around the world. *M. pruriens* seeds are natural source of the amino acid L-DOPA (L-3,4-dihydroxy phenyl alanine) which act as a direct precursor of dopamine a neurotransmitter extensively used in the treatment of Parkinson's disease. Its extracts are used for the treatment of liver dysfunction, blood related diseases, ulcer, snake bite, urinary tract, neurological and menstruation disorders endocrine and male reproductive system related disorders, and helminthiasis like elephantiasis (Katzenschlager et al., 2004). Begomovirus infects large number of plants and these are categorized on the basis of geographical origin known as Old world and New World viruses with bipartite or monopartite genome respectively (Zaidi et al., 2017).Velvet bean severe mosaic virus (VbSMV) is a member of Begomovirus genera (Geminiviridae family) . VbSMV is one of the restraining factors in the cultivation of velvet bean as its infection in the plant leads to enhanced yellowing symptoms and which eventually is the cause of low yield of crop. RNA silencing is an adaptive defense mechanism in plants used against viruses. Suppressors are virus encoded genes used against host plant response to virus infection, and are known by a common name viral suppressors of RNA silencing (VSRs) (Anandalakshmi, 1998).Large number of suppressors has been identified in begomovirus such as AC2 protein (Bhindi yellow vein mosaic virus and Tomato leaf curl Java virus) , AC4 protein (Sri Lankan cassava mosaic virus and African cassava mosaic virus-[Cameroon]) (Gopal et al.2007,Wang et al. 2003,Vanitharani et al. 2004 and Jackel, et al. 2015,). The VSRs affects the plant methyl cycle, and their activities to suppress host defence, however, any interaction of begomovirus VSRs with DICER is not reported till date (Hanley-Bowdoin et al., 2013). Three ORFs of VbSMV infecting *Mucuna pruriens* were identified as suppressors of RNA silencing (Jangir et al., 2017).

Methodology: Agrobacterium co-infiltration assay was used for suppressor identification. Binary vector pCAMBIA1302 was used and oligonucleotides (for PCR) were synthesised for the amplification of various VbSMV genes (AV1, AV2, Rep/AC1, TrAP/AC2). ORFs were amplified using polymerase chain reaction using standardized thermal cycling conditions. PCR amplicons were cloned in cloning vector (TA Cloning, RBC) and the vectors harbouring genes were restricted using enzymes (enzyme sites were included in the primers designed for PCR). These genes were then cloned into pCAMBIA1302 vector. Construct prepared consists of Tobacco etch virus HcPro gene with CaMV 35S promoter (Anandalakshmi et al. 1998), and also non-recombinant pCAMBIA1302 vector (used as a positive and negative controls).

Plant transformations using Agroinfiltration: Karjee *et al.* (2008) method for Agroinfiltration was used.

HIV and AIDS Research Journal

Agrobacterium cultures were infiltrated into the leaves(underside) by applying pressure using 2 ml syringe. Plants of *N. benthamiana* were agroinfiltrated at 3-4 leaf stage using mixture of Agrobacterium culture in the ratio of 1:1 containing 35S-GFP and constructs having virus genes (for testing suppressor activity). As a control in this experiment pCAMBIA1302 was used.

Results and Discussion: Desired amplification of various candidate genes was obtained in PCR, AC1-1081, AC2-407, AVI- 771 and AV2- 346. These genes were further cloned in to pCAMBIA1302 vector. After seven days post infiltration(dpi) green inflorescence was seen in the leaves which were co-infiltrated with 35S-GFP and 35S-AV2 or 35S-HcPro. Leaves which were infiltrated with 35S-GFP and either 35S CP were not showing good signal, only week green inflorescence was observed. The inflorescence was not detected in the negative control (Fig) Result in the study shows that AV2, AC2 and AVI of VbSMV is acting as suppressor of local gene silencing in *N. Benthamiana* plants . Though AC2 and AV1 were week suppressor of gene silencing.

RNA silencing defense and counter- defense has a very important role to play in viral infection in plants. The results presented reveals that VbSMV AV2 AC2, AV1, proteins suppress local RNA silencing in test plants (*N. benthamiana*). In earlier studies, begomovirus AC4 protein has been reported. to act as suppressor of systemic silencing (Chellappan et al., 2005). AC2 (Indian cassava mosaic virus) is also identified as a suppressor of gene silencing (Vanitharani et al. 2004). Viruses encode proteins which act as suppressors of gene silencing using different mechanism of action. The mechanism by which Begomovirus tackle the host RNA silencing is not very well understood.

The results in the study suggest that AC4 might be acting as suppressor of silencing by binding and thus inactivating siRNAs. In coming time, the miRNA and siRNA pathways are required to be explored in order to study defense mechanisms of plants against newly emerging geminiviruses. Identification of host proteins which are interacting with a viral suppressor will be a very useful aspect to utilize viral suppressors for a better understating of the silencing pathway and ultimately virus management. Further detailed work will be required for deeper understanding of silencing mechanism by which VbSMV proteins interfere in the gene silencing pathways.