SEMINAR-III

MOLECULAR FARMING USING A GEMINIVIRAL RNAI-SUPPRESSOR PROTEIN, MYMIV-AC2 AND ITS SUPPRESSION MECHANISM IN RNAI PATHWAY

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Summary

Molecular farming is the use of plants for the large-scale production of valuable recombinant proteins. s a potential bioreactor plant has many advantage for pharmaceutically and commercially valuable proteins production. However, the effective use of plants as bioreactors depends mainly on high level accumulation of proteins and expression stability during the life of transgenic plants and in subsequent generations. Unfortunately, in many cases, the introduced transgenes are either not expressed or the expression level is too low to detect in even most favourable environment. One of the foremost barriers for expression of transgene(s) is Post-Transcriptional Gene Silencing (PTGS), a form of RNAi. Gene silencing has been considered as major obstacles for effective transgene expression in plant transformation technology as it plays dominant roles in the establishment of transgene expression variation. Therefore, PTGS has become a major impediment for production of commercial crops with predictable and stable transgene expression. Similarly, RNAi also prevents viral genes to express following infection in plants. Viral genomes, however, encode proteins called RNA silencing suppressor (s) that suppress RNAi phenomena. The use of such viral suppressors has proven to be an efficient strategy to inhibit the negative influence of RNA silencing on transgene expression in transient systems. In our study we observed that the silencing suppressor protein AC2 of Mungbean Yellow Mosaic India Virus (MYMIV) can reverse the silenced transgene in a stable transgenic system. In our approach to make a stable transgenic system, we have made AC2 expressing transgenic and transgenically silenced GFP (Green Florescent Protein) tobacco (Nicotiana tabacum cv. xanthi) and advanced to T2 generation. In transgenically silenced GFP lines, expression of GFP is silenced due to PTGS. In order to investigate the reversal of GFP expression we introduced AC2 into the GFP silenced lines by adopting genetic

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hybridization or transgenesis. Upon introduction of MYMIV-AC2in the silenced GFP-transgenic lines, the GFP expression was enhanced several fold in F1 and T0 lines. The GFP-siRNA levels were much reduced in F1 and T0 lines compared to those of the initial parental silenced lines. The enhanced GFP expression was also observed at the cellular level. This approach was also successful in enhancing the expression of another transgene, namely *topoisomeraseII (TOPOII).* We also observed that upon inintrogression of MYMIV-AC2 on the RNAi induced silenced PDS lines, the PDS silencing was suppressed and the PDS functions was resorted. This result based on transgenic tobacco as a model system proves the principle that suppression of RNA silencing by the viral suppressor, AC2 leads to the expression of silenced transgene. This finding suggests an attractive prospective application of viral-encoded suppressors to substantially elevate levels of the valuable foreign recombinant proteins in plants. Gemini virus encoded AC2 is one of the early identified suppressor protein whose mechanism of action is still elusive. The mechanism of AC2 suppression activity was determined using MYMIV encoded AC2 as a model. The mechanistic insight of MYMIV-AC2 in RNAi silencing pathway showed that AC2 directly interacts with AGO1 and RDR6 protein and thus, it interferes with the biogenesis of dsRNA and consequently siRNA. This dual site of MYMIV-AC2 interference with the RNA silencing component also justifies its strong RNA silencing suppression potential.