NAME OF THE THEME: Life Sciences & Agricultural Sciences

Development of *Tobamo virus* resistance Tobacco by using CRISPR/Cas9 Technology

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Abstract: CRISPR/Cas9 mediated genome engineering has emerged as a powerful approach for targeted alteration in the genome of plants. This technology opens a new platform for genetic and metabolic engineering in numerous plant species. Tobacco is one of the commercial crops grown in all parts of the world. The Tobacco Mosaic Virus and Tomato mosaic viruses cause heavy yield loss in tobacco. Host proteins TOM1 and TOM3 associated with tonoplast membrane are shown to be required for efficient multiplication of Tobamoviruses. Simultaneous mutations in both these genes completely inhibit the Tobamoviruses multiplication. The target was selected for TOM1, TOM3 and cloned in pHSE401 CRISPR/Cas9 vector in which Cas9 was regulated by CaMV and gRNA was regulated by the AtU6 promoter. After confirmation of target site cloning the vector mobilized into Agrobacterium tumefaciens LBA4404 strain by freeze-thaw method for plant genetic transformation. PCR confirmation of transgenics was achieved by HptII and Cas9 primers. Detection of mutation in NtTOM1 and NtTOM3 genes by nucleotide sequencing. Out of 30 regenerated transgenic lines of tobacco, ten plants showed mutation at a target site.

Keywords: TOM1, TOM3, TMV, Genome editing