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TRANSIENT EXPRESSION OF HDA19 RECOMBINANT PROTEIN IN NICOTIANA BENTAMIANA

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Expression of recombinant proteins in plants has been the center of significant attention in the last two decades. Plants are among promising and appropriate platform systems for the production of recombinant biopharmaceutical proteins due to several features such as safety, no need for fermentation, inexpensive investment, and fast and easy scale-up. Transient gene expression has been developed to provide a more rapid means of assessing plant tissues as a protein production platform because generating stably transformed transgenic plants is a laborintensive and time-consuming process. This study reports the expression of HDA19 gene in Nicotiana bentamiana by means of transient transformation. Upon biotic stresses such as wounding and pathogen infection, HDA19 regulates gene expression in jasmonic acid and ethylene signaling pathway. It has also been shown to regulate light mediated processes. After the extraction of RNA and generation of cDNA, Gateway adapters and His6-tag encoding nucleotides at the 3'or 5'end were attached by PCR in order to enable the production of HDA19 with a C-terminal or N-terminal His 6 tag. Then, the constructs were shifted into the plant expression vector pB2GW7 by homologous recombination using the Gateway technology. The plasmids were transformed into E.coli DH5a; and after purification, the correct nucleotide sequences were confirmed by restriction analyses and sequencing. The recombinant construct was transferred into Agrobacterium tumefaciens strain GV3101 and was used for Agrobacterium mediated transformation of plants. The expression of the protein in transgenic lines was confirmed by immune-dot blot assay and SDS-PAGE.

Keywords: HDA19, Recombinant protein, Nicotiana bentamiana, pB2GW7