Effect of dual *Bt*-expression transformation vectors on transgene expression in tobacco

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ABSTRACT. This study aimed to determine the influence of vector structure on dual *Bt* gene expression and establish an efficient expression vector using *Cry1Ac* and *Cry3A* genes. Four vectors (N4, N5, N10, and S23) were developed and used for genetic transformation of tobacco to obtain insect-resistant transgenic lines. The vectors were constructed using the MAR structure, applying different promoter and enhancer sequences, and changing the transgene open-reading frame sequence. The average Cry1Ac toxalbumin expression quantity was 67 times higher in N5 than in N4 transgenic lines (8.77 and 0.13 µg/g, respectively). In contrast, the average Cry3A toxalbumin expression quantity was 1.5 times higher in N4 than in N5 lines (12.70 and 8.21 µg/g, respectively). The sequences of both *Bt* genes significantly influenced toxalbumin expression, although upstream *Bt* genes

presented lower expression levels. The average Cry1Ac toxalbumin content was 13 times higher in the transgenic lines of *AtADH* 5'-non-translated sequence N5 (8.77 μg/g) than in the *omega* N10 lines (0.67 μg/g). Furthermore, the average Cry1Ac toxalbumin content was 5 times higher in MAR N5 than in non-MAR S23 lines (8.77 and 1.63 μg/g, respectively). The average Cry3A toxalbumin content was 1.3 times higher in N5 than in S23 lines (8.21 and 6.48 μg/g, respectively). Moreover, toxalbumin expression levels differed significantly among the S23-transformed lines. The MAR structure applied on both ends of the genes increased both the level and stability of exogenous gene expression. In conclusion, N5 was the most optimal of the four tested vectors.

Key words: Tobacco; Plant expression vector; Dual *Bt* gene; Genetic transformation; Differential expression; Insect-resistance