

DETERMINATION OF TOBACCO TRANSGENE COPY NUMBER BY REAL-TIME QUANTITATIVE PCR

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Tobacco can be easily regenerated *in vitro* and genetically transformed by *Agrobacterium* vector system, which make it ideal for gene transfer and molecular genetic studies. Efficient methods to characterize transgenic plants are important to understand the state of the transformant. Real-time PCR can be used to determine copy number and zygosity in transgenic plants. In transgenic plants, transgene copy number can greatly influence the expression level and genetic stability of the target gene, making estimation of transgene copy number an important area of genetically modified crop research. *Ax1l* gene, involved in auxin action, has been previously described to have a constant number per genome with a linear ΔCt in tobacco plants. Here we aimed to develop a sensitive real-time (RT)-PCR technique for estimating transgene copy number in transgenic tobacco. Tobacco *Petit havana* plants were transformed via *Agrobacterium tumefaciens* EHA101 strain and regenerated from selected callus in shooting and rooting medium with an empty vector (pMCG1005), rice *Phosphorus Starvation Tolerance 1* gene (*OsPstol1*), its maize (*ZmPstol3.06*, *ZmPstol8.02* and *ZmPstol8.05_1*) and sorghum (*Sb07g002840*, *Sb03g031690* and *Sb03g006765*) homologs. First, RT-PCR with $2^{-\Delta Ct}$ method was used to determine the relative copy number of *Bar* gene in the genome using *Ax1l* as an endogenous control of the T0 generation of nine transgenic tobacco lines. These results were compared with segregation ratios of *Bar* gene genotype in T1 plants of each line and were found to be general, concordant. All the three lines with the Mendelian segregation ratio of 3:1 carried one gene copy. One copy sample (*ZmPstol8.02* Ev.6) was used as a reference for the $2^{-\Delta\Delta Ct}$ method. In order to confirm the *Ax1l* copy number, two different fragments of tobacco *Ax1l* gene (727 and 975 pb) were cloned in pGEM-T Easy vector and used to generate a standard curve with plasmid dilutions using SYBR green technique. Our results confirm that *Ax1l* is a single copy gene and can be used as an endogenous control for copy number experiments with $2^{-\Delta\Delta Ct}$ method in transgenic tobacco.

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