ABSTRACT

Caffeine (1, 3, 7-trimethyl xanthine), known to have sensory and stimulatory effects, causes adverse effects on health which has resulted in an increased demand for decaffeinated tea. The present study is carried out to investigate the functional role of two reported genes of caffeine biosynthetic pathway, *Caffeine synthase (cs)*, *n-methyltransferase (nmt)* in *Camellia assamica* and to isolate and characterize the genes from S3.A/3 genotype, for further in-depth study at molecular and functional level through *Agrobacterium tumefaciens* mediated genetic transformation using RNAi based silencing approaches. To set the bottom line for genetic transformation in *Camellia assamica* (S3.A/3), having known quality attributes, our approach was to optimize the genetic transformation protocols for Assam clones. For which, standardized protocol for somatic embryogenesis established in our lab were followed and highly repeatable secondary somatic embryos were considered for gene transfer studies. The genes were amplified using gene specific primers and RNAi construct of pHELLSGATE8 and pWATERGATE encompassing cs and nmt genes respectively, were prepared following Gateway Technology. The gateway cassette with an inverted repeat sequence of cs and nmt along with a spacer fragment forms hairpin loop after transcription. The *Not*I fragment from pHELLSGATE8-cs was introduced into the expression cartridge of primary cloning vector, pART27. The resulting hpRNAi vectors, pART27-cs and pWATERGATE-nmt were taken for use in *Agrobacterium* mediated transformation with secondary somatic embryos of S3.A/3 clone. Leaf samples collected from putative transformants were analyzed
using RT-PCR and reduction of caffeine production estimated by HPLC. The present study have shown the various changes taking place in the tea transformants in response to silencing of cs an nmt genes in tea, which plays active role in caffeine biosynthetic pathway. We have demonstrated that cloning of both the genes form C.assamica and transforming separately with two different RNAi vectors (pART27-cs and pWATERGATE-nmt) resulted in the production of transgenic plantlets with varying purine alkaloid contents and this differences in expression is mediated at least in part by its multigene activity or activation of alternate pathway genes. The functionality of both the genes was confirmed by the altered regulation in transcript level in the transgenic plantlet compared to the untransformed control plants. Molecular analysis carried out in the study suggests that there is a complex interaction of gene regulation operating as various functional units that contributed to the silencing in the genotype examined.