

Post Transcriptional Gene Silencing for Down Regulating Caffeine Biosynthesis in *Coffea canephora* P. ex Fr.

**V. KUMAR, K.V. SATYANARAYANA, S. SARALA ITTY, P. GIRIDHAR,
A. CHANDRASHEKAR, G.A. RAVISHANKAR**

Plant Cell Biotechnology Department, Central Food Technological Research Institute,
Mysore – 570 020, Karnataka State, India

SUMMARY

RNAi, co-suppression and antisense technologies are powerful tools of post transcriptional gene silencing in sequence specific manner. *Agrobacterium* mediated genetic transformation was achieved in *Coffea canephora* for silencing of N-methyl transferase involved in caffeine biosynthesis. In the present study, we report the transformation and direct regeneration of transformants through secondary embryos in the presence of selection pressure and hormonal regimes. Three different constructs were made for silencing caffeine biosynthesis in *Coffea canephora*. pSAT 201 is a co-suppression construct, consists of a fragment of methyl transferase in sense orientation with respect to the promoter. pSAT 202 is an antisense construct, consists of a fragment of methyl transferase in antisense orientation with respect to the promoter. pSAT 222 is an invert repeat construct, which is expected to induce target gene silencing through RNAi. These constructs were mobilised to *A. tumefaciens* and sonication assisted *Agrobacterium* mediated transformation method was adopted for plant transformation. Transformation efficiency of 0.5-3.0% was obtained depending on the regeneration medium. The putative transgenic secondary embryos were selected under 20mg/L hygromycin for at least 8 months. The transgenic nature was confirmed by PCR and southern blots. The purine alkaloids were extracted and estimated by HPLC and characterized by LCMS. Transformation using pSAT 222 construct was found to be most efficient and the caffeine content in one transformant was 3.8% of the control value. Transformants with antisense construct resulted in caffeine levels 10% of the control value, transformants with co-suppression construct resulted in 0.78% caffeine, when compared to control with 0.8% caffeine. These data clearly demonstrated reduction in caffeine levels. However the extent of silencing varied in independent transformants.