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SILENCING THE SAM PATHWAY GENES RELATED TO CAFFEINE PRODUCTION IN TEA

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 de-caffeinated tea. Our approach is to reduce or knockout expression of caffeine biosynthetic pathway genes, *caffeine synthase (cs)* and *methyl transferase (mt)*. The genes were amplified using gene specific primers and a construct of pHELLSGATE8 was prepared by Gateway Technology. The inverted repeat sequence of *cs* and *mt* having an intronic region obtained as a NotI fragment from pHELLSGATE8 is introduced into the expression cartridge of the primary cloning vector, pART27 for use in *Agrobacterium* mediated transformation with Assam clones having known quality attributes. The transformants are being analysed for partial and complete knockdown of one or both the genes using RT-PCR and reduction in caffeine production estimated by HPLC.

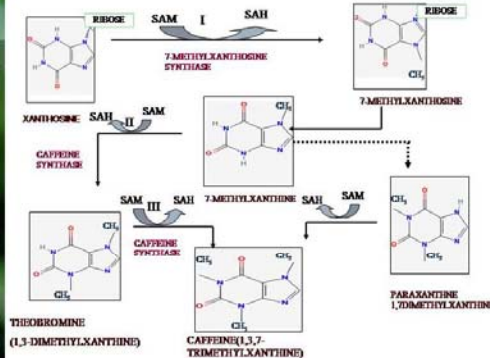
INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid present in high concentrations in TEA (*Camellia sinensis*). The caffeine biosynthetic pathway contains three S-adenosyl-L-methionine (SAM) dependent methylation steps, where N-methyltransferases plays an important role.

WHY TEA IS CONSIDERED.....

- CONSUMPTION OF CAFFEINE CAN HAVE ADVERSE EFFECT ON HEALTH AND HAS RESULTED IN AN INCREASED DEMAND FOR DE-CAFFEINATED BEVERAGES.
- KNOCKOUT OF THE TARGET GENE OF THE CAFFEINE BIOSYNTHETIC PATHWAY IN TEA PLANT THROUGH RNAi COULD CREATE FULLY FLAVOURED BEVERAGES WITHOUT CAFFEINE.

Pathways for the biosynthesis of caffeine

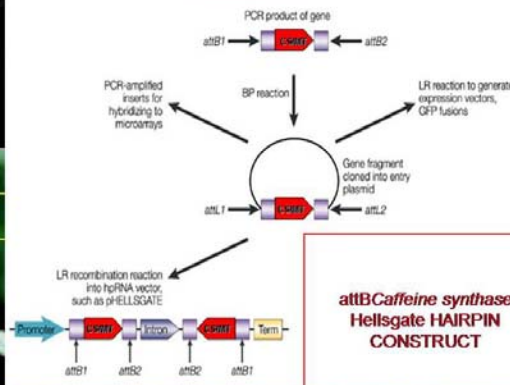


EXPERIMENTAL APPROACHES

- CLONING OF THE GENES FOR CAFFEINE SYNTHASE & METHYL TRANSFERASE OBTAINED FROM A GARDEN SERIES CLONE, S₂A₃ BY GATEWAY TECHNOLOGY.
- RE-CONSTRUCTION OF THE VECTOR pHELLSGATE8, OBTAINED AS A NotI FRAGMENT & INCORPORATION INTO THE EXPRESSION CARTRIDGE OF BINARY VECTOR pART27 FOR USE IN *AGROBACTERIUM* MEDIATED TRANSFORMATION ALREADY OPTIMIZED IN TEA BY TRA.
- THE TRANSGENIC LINES ARE BEING ANALYSED FOR PARTIAL AND COMPLETE KNOCKDOWN OF BOTH THE GENES USING RT-PCR AND BIOCHEMICAL CHARACTERIZATION WITH HPLC.



GENETIC TRANSFORMATION PROTOCOL

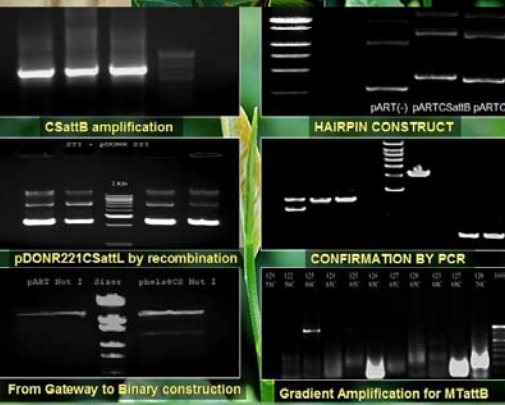
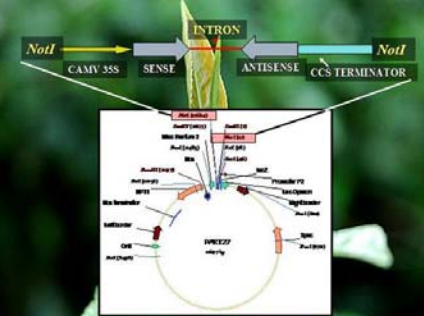


DIGESTION OF pART27 HAVING CS INSERT WITH Sac I, Sal I and EcoR V



HAIRPIN LOOP FORMATION IN RNAi CONSTRUCTS OF CAFFEINE SYNTHASE & METHYL TRANSFERASE

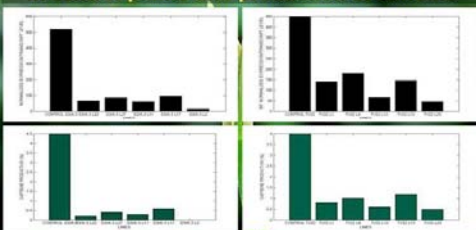
pART27 WITH HAIRPIN CONSTRUCT OF CAFFEINE SYNTHASE & METHYL TRANSFERASE



FINDINGS..

- HPLC analysis with the transformants and the control using UV absorption at the range of 150nm-550nm were performed and spectra were recorded at 276nm in the control & 150nm in the experimental for Caffeine.
- RT-PCR data showed a marked decrease in the transcript levels in the putative transformants in comparison to control.

Transcript levels in putative transformants and in control plants and production of caffeine



Representation of transcript levels of caffeine synthase (CS) and methyl transferase (MT) and production of caffeine in S2A3 and TV22

Accession	Length	GC Content	GC Content	GC Content	GC Content	GC Content	GC Content	GC Content	GC Content	GC Content
TCAAAGGGCTGCCATCTAA	9	ORF	420	47.37 %	+	+	+	+	+	+
TATGTCATCTCTGTAGA	9	ORF	503	31.58 %	+	+	+	+	+	+
GTAGGAAGTCTCTGTGA	8	ORF	456	36.84 %	+	+	+	+	+	+
GAAGCTACTTACTCAAT	8	ORF	659	36.84 %	+	+	+	+	+	+
ACGGATCAATCAATGTA	8	ORF	933	36.84 %	+	+	+	+	+	+
GCCTTCAGAGGCTATAA	8	ORF	1040	47.37 %	+	+	+	+	+	+
CACTCACATGTATGTCA	8	ORF	1108	36.84 %	+	+	+	+	+	+
ATACCGAAGATCAAGTA	8	ORF	1142	36.84 %	+	+	+	+	+	+

