Epigenetic changes associated with physical and environmental stress in *Hevea brasiliensis*

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DNA methylation in higher eukaryotes is a major heritable epigenetic modification where the normal cytosine in the DNA is converted to 5-methylcytosine (m5C). DNA methylation of specific loci in response to stress may be associated with the alteration in gene transcription leading to morphological changes independent of nucleotide variations. Para rubber tree (Hevea brasiliensis) is the source of 99% of natural rubber produced in the world. It is commercially propagated by bud grafting where the scion of a high yielding variety is grafted on to a genetically divergent root stock. Despite this vegetative mode of propagation, great disparity in desirable characters like yield and disease tolerance is shown by individual plants of the same genotype planted together. Epigenetic changes associated with abiotic stress and stock-scion interactions may be the primary suspect responsible for this conundrum.

In the present study an attempt was made to identify the impact of cold stress and grafting on the epigenome of rubber tree. Regulatory sequences of four major genes involved in the Mevalonate pathway (rubber biosynthesis pathway) and one common defence related gene of three high yielding popular rubber clones grown at two different agroclimatic conditions were analysed for the presence of methylation changes associated with cold stress. Simultaneously grafting induced methylation changes in the genome of own rooted seedlings and their bud grafted counter parts were also analysed using MSAP markers. Several significant variations in the methylation pattern at core DNA binding motifs within the regulatory region was identified in all the five genes in addition to many consistent clone specific and location specific methylation patterns. Methylation pattern variations observed at certain pivotal cis regulatory sites indicate the impact of stress on the epigenome and support the hypothesis of site specific stress induced methylation. In the grafted plants, polymorphic DNA methylation patterns were observed despite maintaining uniform growth conditions. Methylation profiling showed that the plant specific CHG type of methylation appeared to be more sensitive to grafting than the prominent CG methylation (80%) pattern. Cloning and sequencing of the methylation sensitive bands confirmed that methylation is happening in the promoter and coding region of important genes including an LRR receptor kinase involved in signal transduction.

Based on the results It is assumed that some of the methylation patterns observed may probably be involved in the stress responsive mechanism in plants by which they adapt to extreme conditions. The study also provide clues towards the existence of divergent phenotypic characters among Hevea clones despite their high genetic similarity. Altogether these observations prove beyond doubt that there exists epigenetic variation in Hevea and environmental factors play a significant role in the induction of site specific epigenetic mutations. The study assumes importance in all crops propagated through grafting because accumulation and maintenance of epigenetic changes during subsequent cycles of vegetative propagation may eventually lead to an altered phenotype and can result in intraclonal variability.