6. SUMMARY

The apocynaceous plant species *Catharanthus roseus* (2n=16; 738 Mbp) is proving useful in the genetic and genomic analyses of plant secondary metabolism. In this system many of the structural genes for the enzymes that catalyze the terpenoid indole alkaloid (TIA) biosynthetic pathway and gene regulatory network that controls the expression of the pathway have been defined and characterized. Many quantitative trait loci (QTLs) for the TIA content in the organs and overall yield have been identified and placed on the DNA marker based genetic linkage map. Certain visually apparent properties of the several salt tolerant and morphological mutants isolated in *C. roseus* have been reported in *C. roseus*. One of these called *leafless inflorescence* (*lli*) has been developed into a hyperflowering ornamental variety. The morphological mutant called *irregular leaf lamina* (*ill*) has been patented for its hyperaccumulation of terpenoid indole alkaloids in organs. In the present study *leafless inflorescence* (*lli*), *irregular leaf lamina* (*ill*) and *evergreen dwarf* (*egd*) mutants named after their most conspicuous phenotypes were characterized for their phenotypic pleiotropy, expression of a large number of genes and cytosine methylation in repeat sequences. The results demonstrated that the genetic mutations had altered the regulation of cytosine methylation process in mutants which was responsible for hypomethylation, and up- and down-regulation of many genes and consequently the pleiotropies. The results of the present study are summarized below;

6A) In *Catharanthus roseus*, three morphological-cum-salt tolerant chemically induced mutants of Mendelian inheritance and their wild type parent cv Nirmal were characterized for overall cytosine methylation at DNA repeats, expression of a large number of protein- and miRNA- coding genes and 50 quantitative traits. The mutants, named after their principal morphological feature(s), were *leafless inflorescence* (*lli*), *evergreen dwarf* (*egd*) and *irregular leaf lamina* (*ill*). The Southern blot analysis of Msp1 digested DNAs of mutants probed with centromeric and 5S and 18S rDNA probes indicated that, in comparison to wild type, the mutants were extensively demethylated at cytosine sites. Among the 126 genes investigated for transcriptional expression, 85 and 41 genes were respectively similarly up-regulated and down-regulated in mutants. All of the five genes known to be stress responsive had increased expression in mutants. Several miRNA genes showed either increased or decreased expression in mutants. The *C. roseus* counterparts of *CMT3, DRM2* and *RDR2* were down-regulated in mutants. Among the cell, organ and plant size, photosynthesis and
metabolism related traits studied, 28 traits were similarly affected in mutants as compared to wild type. Each of the mutants also expressed some traits distinctively. The *egd* mutant possessed superior photosynthesis and water retention abilities. The *lli* was characterized by largest biomass in roots, stems, leaves and seeds. The *ill* mutant was richest in the pharmaceutical alkaloids catharanthine, vindoline, vincristine and vinblastine. The nature of mutations, origins of mutant phenotypes and evolutionary importance of mutants have been described.

6B) The 5S and 18S rDNA sequences of *Catharanthus roseus* cv ‘Nirmal’ (wild type) and its leafless inflorescence (*lli*), *evergreen dwarf* (*egd*) and *irregular leaf lamina* (*ill*) single mutants and *lli egd, lli ill* and *egd ill* double mutants were characterized. The *lli, egd* and *ill* mutants of Mendelian inheritance bore the names after their most conspicuous morphological feature(s). They had been chemically induced and isolated for their salt tolerance. The double mutants were isolated as morphological segregants from crosses between single mutants. The morphological feature of the two parents accompanied salt tolerance in the double mutants. All the six mutants were hypomethylated at repeat sequences, up- and down- regulated for many genes and carried pleiotropic alterations for several traits. Here the 5S and 18S rDNAs of *C. roseus* were found to be low in cytosine content (8.5%). Cytosines were preponderantly in CG context (53%) and almost all of them were methylated (97%). The cytosines in CHH and CHG (where H = A, T or C) contexts were largely demethylated (92%) in mutants. The demethylation was attributable to reduced expression of *RDR2* and *DRM2* led RNA dependant DNA methylation and *CMT3* led maintenance methylation pathways. Mutants had gained some cytosines by substitution of C at T sites. These perhaps arose on account of errors in DNA replication. It was concluded that the regulation of cytosine methylation mechanisms was disturbed in the mutants.

6C) Contrasting point of views exist about the biology of lifespan in seed producing perennial plant species. One of the view is that the plants of perennial plant species are heritably immortal and die of accidents. The other view is that lifespan of perennial plant species is determinate and plants die from progressive senescence. The present experiment in *Catharanthus roseus* was based on the premise that the demonstration of heritable variation in survival time of its different genotypes will provide evidence for the determinate nature of lifespan in this perennial plant species and intra-genotype differences observed between the apparently normal and senescent plants will reveal the symptoms associated with mortality.
The survival frequencies of the plant populations of four genotypes derived by combining the wild type and mutant alleles of the genes *LEAFLESS INFLORESCENCE (LLI)* and *EVERGREEN DWARF (EGD)*, in a common background and different genetic backgrounds, were compared at 5 and 6 years from germination. Phenotyping showed that the surviving plants were either green leaved (normal) or bore pale leaves (senescent). The normal and senescent plants were investigated genotype-wise for the expression of plastidic and nuclear genes related to photosynthesis and certain nuclear genes involved in mitochondrial expression. The *LLI EGD, lli EGD, LLI egd* and *lli egd* genotypes were observed to differ in survival. The *lli* mutation decreased lifespan and *egd* mutation increased it and the interaction between *lli* and *egd* was additive. The leaves of senescent plants were deficient in chlorophylls, photosynthesis and expression of nuclear genes associated with plastidic and mitochondrial functions. The lifespan in the perennial plant species *C. roseus* is increased by the *LLI* gene and decreased by *EGD* gene. It is a determinate genetic trait. Degradation of nuclear-organelle interactions, manifested in the form of pale leaves, is a symptom of incipient mortality in senescent plants.

Altogether the study demonstrated that the mutations that differentially led to demethylation of cytosine marks in the *lli, egd* and *ill* mutants were responsible for a wide pleiotropic phenotype arising from the altered gene expression patterns.