Chapter 5

Summary
Cytoplasmic-genetic male sterility in plants is a phenomenon that is believed to occur as a result of incompatible nuclear-mitochondrial interactions. It has been used for hybrid seed production in several plants including rice. Rice breeders in China, Japan and in recent years in India have come out with a number of hybrids making use of the different CMS cytoplastms and their compatibility/incompatibility with indica/japonica nucleus. Hybrid rice in India is based on the use of a single CMS cytoplasm designated as wild abortive (WA). Various studies on the genetics of fertility restoration of this cytoplasm through different crosses have been carried out and have helped in utilization of different varieties for hybrid seed production. However, in spite of these useful studies it has been relatively less characterized at the molecular level. The extent of information on the organization and expression of mitochondrial genes in this cytoplasm is rather scanty and the CMS trait exhibited by this cytoplasm has not yet been associated with any specific gene.

The present study was initiated with the primary objective of characterizing the WA cytoplasm with regards to the location and expression of the important mitochondrial genes particularly those encoding for the subunits of membrane-bound complexes. Further, location of these genes in the mitochondrial genomes of various maintainer and restorers were also studied.

The studies carried out could be grouped as under.

5.1 Studies on organization of various mitochondrial genes (RFLP Studies)

Mitochondrial DNA from the sterile and fertile lines of the V20, IR58025 and IR62829 varieties and their restorers was subjected to restriction digestion using BamHI, HindIII, EcoRI and Smal and Southern blots of these lines were probed successively with 16
mitochondrial gene probes of the mitochondrial electron transport chain complex and the translational machinery.

Polymorphism in the location of coxl, coxIII, cob, atp6, rps14, rrn18 and orf156 between the sterile and maintainer lines was observed.

While polymorphism in coxl, atp6, rps14, rrn18 and orf156 was due to rearrangements in the vicinity of the respective genes, in coxIII and cob the polymorphism was due to presence of additional copies of the genes. In coxIII an additional copy of the gene was present in the sterile lines, while an additional copy of cob was present in the fertile lines. Polymorphism in coxl, cob, atp6, rrn18 and orf156 could distinguish the sterile lines from the maintainer lines while polymorphism in coxIII and rps14 could also distinguish between the maintainer and restorer lines.

Of the several enzymes used for study, BamHI was found to be most suitable to detect polymorphism. The studies also reveal linkage of the nad3 and rps12 genes.

5.2 Northern analyses

Northern blots of mtRNA from the several lines of rice were probed with the 16 mitochondrial gene probes used for Southern analyses. With the exception of orf156, no differences in transcription pattern of the genes were observed between the A and B lines. Thus polymorphism in coxl, coxIII, cob, atp6, rps14 and rrn18 did not result in any altered transcription pattern indicating that the rearrangements that led to polymorphism in these genes had occurred at sequences away from the coding portion.

The fact that transcription pattern in atp6 was identical indicated that CMS in WA cytoplasm is very different from CMS in
the Chinsurah-Boro II cytoplasm (which is used in China) where most studies have revealed alterations in transcript pattern of \textit{atp6} between sterile, fertile and hybrids and a strong correlation of the \textit{atp6} gene with CMS.

Differences were only observed in the expression of \textit{orf156} between the sterile and fertile lines. This additional transcript was present in sterile line of all the varieties studied but not in their maintainer and restorer lines.

\textbf{5.3 Western analyses}

Studies were also carried out to study if the additional transcript led to any abnormalities in the \textit{orf156} encoded product. The western blot analysis was performed using anti-wheat \textit{orf156} antisera. The anti-wheat \textit{orf156} antibody was found to hybridize very weakly to the rice protein. A single band of around 16-18 kDa was observed in sterile as well as fertile lines. No qualitative changes in the polypeptide pattern were observed.

\textbf{5.4 Biochemical studies}

The various complexes of electron transport system are made up of polypeptides encoded by mitochondrial as well as nuclear genome. Any incompatibility between these polypeptides could lead to dysfunction of the membrane bound protein complexes resulting in the alteration of electron transport rate. Therefore an analysis of the mitochondrial electron transport chain rates was carried out in IR62829A, IR62829B and CORHI. It was observed that with increase in temperature there was a steady increase in the electron transport rates. It was also observed that the cyanide sensitive respiration accounted for atleast 85-90\% of the respiration at all the temperatures in all the lines while the cyanide insensitive pathway accounted for less than 10\% of the respiration. A comparative study
of the mitochondrial F$_1$-ATPase from V20A and V20B lines was also carried out. No major difference was observed with regard to Km values between the two lines.

5.5 Sequencing of *atp6*

Sequencing of the *atp6* from WA cytoplasm was also undertaken since the *atp6* has been strongly implicated in CMS in the Chinsurah-Boro II cytoplasm of rice. No differences were observed in the sequence of *atp6* between the sterile and maintainer lines. The *atp6* had an open reading frame of 1014 nucleotides, encoding for a polypeptide of 338 amino acids. The sequence was one amino acid longer than that in Chinsurah-Boro II. Besides this, two other amino acid changes between the Chinsurah *atp6* and WA *atp6* sequences were observed. In general, the rice *atp6* had a strong homology with *atp6* sequence of monocots. Sequence homology with dicots was also high but it was less as compared to monocot *atp6* sequences.

5.6 RAPD studies

RAPD profiles were generated using mitochondrial DNA isolated from two CMS lines, two restorer lines and four maintainer lines of rice. The RAPD profiles enabled the distinction not only amongst the different lines of rice including the lines IR58025A and IR62829A that contained the same wild abortive (WA) cytoplasm. These two lines were not distinguishable from each other by mtDNA RFLP analyses with as many as 16 mtDNA probes.

The present study was confined to mtDNA of etiolated seedlings. However, the complete CMS trait expression takes place at the flowering stage or during anther formation. Therefore transcription and expression studies in other tissues specially in the flowers and anthers in the sterile and hybrid lines would be required to assign or
rule out a role for any mitochondrial gene, especially *orf156* in CMS in the WA cytoplasm of rice.