SUMMARY
The present studies were initiated with an aim to induce haploids through anther, unpollinated ovary cultures and pollen irradiated method. The ploidy level of M₁ plants obtained after pollination with M-5 and China White pollen separately was determined using flow cytometry and cytology. The M₁ plants obtained from irradiated pollen experiments were characterized using molecular markers in order to find out the extent of similarity to parents and absence of paternal markers if any. The significant findings that emerged from the study are as follows:

- Anther culture studies led to induction of callus with a frequency of 6.8% from anthers of S-13 cultivar on MS medium with 8 mg/l 2,4-D, 0.1 mg/l NAA and 6% sucrose. Rhizogenesis was observed from the callus upon subculture on medium with 1 mg/l 2,4-D, 0.1 mg/l NAA and 3% sucrose.

- Studies on unpollinated ovary cultures of M-5 cultivar showed callus induction with a frequency of 4.5% on MS medium with 2 mg/l 2,4-D, 0.5 mg/l BAP and 3% sucrose and formation of nodular structures upon subculture on medium with 2 mg/l BAP and 3% sucrose.

- Pollen irradiation had a profound effect on pollen viability and decreased to 9.8% with the increase in the dose of gamma irradiation to 2000 Gy. The germination capacity of S-13 irradiated pollen decreased with the increase in the irradiation dose (50-1000 Gy) under in vitro and in situ conditions. The reduced pollen germination by gamma irradiation could be due to hampered protein synthesis.

- Irradiation of S-13 pollen up to 1000 Gy resulted in seed set whereas doses above 1000 Gy led to withering of the fruits.
Interspecific hybridization between M-5 and China White cultivars was successful and seed set was observed with irradiated pollen from 50 to 1000 Gy.

The germination capacity of the seeds obtained from crosses of M-5 with S-13 and China White irradiated pollen decreased with increase in irradiation dose and was lowest at 1000 Gy irradiation dose. The possible causes of germination reduction could be due to lack of completely developed or abnormal embryos within the seeds.

Morphological variations were observed in M$_1$ plants obtained after pollination with S-13 irradiated pollen. Eight variants were found to be superior with respect to leaf size and one variant viz., Plant 40 exhibited vigorous growth along with large leaf size than parents.

Interspecific hybridization of M-5 cultivar with China White irradiated pollen resulted in isolation of 18 morphological variants which displayed differences with respect to leaf size, shape and margin, plant height, stem thickness and internodal distance. While two M$_1$ interspecific hybrids exhibited larger leaf size than M-5 cultivar, eight variants exhibited slow growth with small leaves.

Flow cytometric analysis of M$_1$ plants obtained from S-13 and China White irradiated pollen showed a peak corresponding to diploid as that of parents.

Cytological studies using root tip squash method showed that the M$_1$ plants obtained after pollination with irradiated pollen were diploids (2n=28).

Four M$_1$ plants obtained from crosses of M-5 with S-13 irradiated pollen produced male catkins and mixed catkins with female and bisexual florets. The
fruits of mixed catkins of Plant 40 were larger by 2 times as compared to that of the female parent.

- Analysis of leaf protein profiles of morphological variants using SDS-PAGE showed an increase in the content of 47 kDa and 18 kDa proteins in three variants obtained from S-13 irradiated pollen and six variants obtained from China White irradiated pollen in comparison to the parents indicating differential gene expression.

- RAPD analysis of the morphological variants obtained from S-13 irradiated pollen using nineteen primers revealed polymorphism ranging from 33.3-100%. The morphological variants showed 16-29 bands specific to M-5 cultivar and 1-3 bands specific to S-13 cultivar of a total of 35 M-5 specific bands and 3 S-13 specific bands identified with different primers.

- The dendrogram derived from UPGMA cluster analysis grouped the variants into three major groups and five outliers. Three morphological variants viz., 33, 34 and 57 were placed in the sub-group of A along with M-5 cultivar depicting high similarity towards the female parent while many morphological variants were placed away from the parents indicating the existence of high level of genetic variation.

- RAPD analysis of the morphological variants obtained from China White irradiated pollen using 10 random primers showed polymorphism ranging from 56.3-100%. The $M_1$ interspecific hybrids showed the presence of 3-6 bands specific to M-5, 7-22 bands specific to China White of a total of 6 M-5 specific bands and 25 China White specific bands identified with different primers. The
dendrogram generated by the UPGMA cluster analysis grouped the morphological variants into two major groups and an outlier revealing differences with parents and among themselves in varying degrees.

- AFLP analysis of four morphological variants that produced male catkins and mixed catkins with bisexual and female florets revealed a high degree of polymorphism from 77.0% to 97.6% with eight primer combinations. The variant Plant 40 was found to be highly distinct from parents and possessed 14 bands specific to M-5 cultivar, 3 bands specific to S-13 cultivar and 135 unique bands. The dendrogram showed that the two variants viz., Plant 40 and Plant 45 got entirely separated from the tree with low similarity to parents.

In conclusion, the present study revealed a low success rate of callus induction without plant regeneration from anther and unpollinated ovary cultures in mulberry. The pollen irradiation method was chosen as an alternative for production of haploids. Although no haploid could be recovered through this approach, a large number of variants were observed which could be used in varietal improvement programmes. Thus the study showed the usefulness of pollen irradiated method for recovering promising variants in mulberry including those altered in sex expression with induction of mixed catkins consisting of bisexual and female florets. Interspecific hybridization of M-5 cultivar with China White irradiated pollen resulted in recovery of two useful variants with larger leaf size than M-5 cultivar. Molecular characterization of the morphological variants obtained from intervarietal and interspecific crosses with irradiated pollen revealed a high level of genetic variation. A detailed genetic, cytogenetic and molecular
analysis employing large number of markers will throw light on the nature of genetic variation.

**Future Scope of Work**

- The efforts aimed at isolation of haploids through *in vitro* androgenesis, gynogenesis and irradiated pollen approach have resulted in promising variants which will be very useful for basic and applied research. These promising variants can be subjected to genetic and cytogenetic analysis for understanding the basis of inheritance.

- Four M₁ plants obtained after pollination with S-13 irradiated pollen produced male catkins and mixed catkins with female and bisexual florets. These variants offer scope for understanding the molecular basis of sex expression and identification of molecular markers linked to sex if any.

- The promising variants can be multiplied and analyzed for leaf yield and quality parameters for silkworm rearing for utilization in breeding programmes of mulberry.