SUMMARY AND CONCLUSIONS

_Hamelia patens_ Jacq (fire bush), is a fast growing, semi woody and evergreen perennial shrub belonging to the family Rubiaceae. A native of central and southern Florida, it is chiefly grown for the showy bunch of beautiful flowers. In Tropical America, local people make use of the extract of its leaf and stem for curing diseases such as skin rashes, sores, insect sting and various fungal diseases. This plant is particularly cultivated in home gardens because of its ornamental value.

Sexual reproduction is essential for maintaining the stability and permitting reasonable degree of genetic variability within the species. In _H. patens_ sexual reproduction is beset with many problems which restrict its propagation by vegetative methods. To comprehend thoroughly the reason or reasons for the reproductive barrier, it is highly essential to have a thorough and deep understanding of reproductive biology of the plant. In order to study the failure of seed-set, a detailed analysis on the reproductive biology of _H. patens_ was done and summarised:

The study of flowering phenology, morphology and ultrastructure of plant reproductive parts are inevitable for the analyses of reproductive character and also to find whether developmental and structural abnormalities play any role in the lack of seed-set in _H. patens_. Morphological and developmental anatomy of reproductive parts showed normal growth and differentiation.

Meiotic chromosome study showed 11 bivalents at metaphase. Later stages were found to be normal. Chromosome number of _H. patens_ is reported for the first time. To estimate the correlation between the number of pollen nuclei and incompatibility mechanism operating in _H. patens_, pollen cytology has been done. Presence of
binucleate pollen grains indicated that gametophytic system of incompatibility is operating in this species.

Viable pollen grains are essential for successful sexual reproduction; hence pollen viability studies such as FCR test and in vitro pollen germinations were done. In FCR test, 90% viable pollen grains were observed. In vitro pollen germination in Brewbaker and Kwack's medium supplemented with 25% sucrose has been found to be appropriate and found optimum germination of 72.5%. This indicated that in *H. patens* male gametophyte is functional.

To analyze the pollen tube growth and behaviour after open and self pollinations, in vivo pollen germination was done using aniline blue method. In open and self pollinated stigma large numbers of pollen grains were found to adhere and germinate on the surface of the stigma. However pollen tube growth inhibition took place on the surface itself followed by abnormal development of pollen tubes such as bulging, curling and irregular callose deposition. This indicated that strong incompatible interaction may take place on the surface of the stigma.

In order to overcome incompatibility, various methods such as heat treatment, cold treatment, stump pollination and bud pollinations were tried. None of these methods have been found to overcome incompatibility in *H. patens*.

To find out the S-allelic variations within the species, RAPD analysis was done in 14 accessions of *H. patens* collected from various parts of Kerala. Based on the result, phenogram was constructed and the topology of the phenogram showed expected grouping with relation to the morphological and compatibility difference found among the accessions. Acc. No.3 has allelic difference with respect to all other accessions of *H. patens*. 
Infraspecific pollinations were done between Acc. No.1 and 3. Pollen tube growth and behaviour, and developmental anatomy of the ovary after pollination were observed. Significant differences were noticed between the self- and cross-pollinated pistils. After cross pollinations normal pollen tube growth and development was noticed. Anatomical features of the developing ovary gave clear indications of seed development. Seeds collected after 45 days of cross pollination showed 95% viability. Infraspecific pollinations have been found to be successful in overcoming self-incompatibility in *H. patens*.

To analyse the pollination induced biochemical changes in unpollinated, self- and cross-pollinated pistils, histochemical localization of primary metabolites such as proteins, carbohydrates and lipids and activity of enzymes such as ATPase, APase, SDH, peroxidase and esterase were done. Significant variations were found between unpollinated and pollinated pistils and also between self and cross-pollinated pistils. This indicated that pollinations could induce significant variation in the metabolic status of the stigma.

A comparative study of stylar protein profile has been done in unpollinated, self- and cross-pollinated pistils by SDS-PAGE. Five bands with molecular weights 23, 30, 36, 70 and 99 kDa have been found to be common to all the samples. Between self- and cross-pollinated pistils, quantitative difference was noticed but in unpollinated pistil the specific band at 36 kDa region was absent. Same protein band in cross-pollinated pistil has less concentration than in self-pollinated pistils. In the protein profile of stigma leachate, protein band at 36 kDa was also absent in unpollinated stigma leachate and present in pollinated stigma. This indicated that protein band at 36 kDa plays a significant role in the pollen-pistil interaction and self-incompatibility in *H. patens*. 
To assess the RNase activity of the stylar protein, various analyses such as diffusion plate assay, spectrophotometric analysis and *in vitro* assay were done. In diffusion plate assay, RNase activity on the stigma surface of unpollinated, self- and cross-pollinated pistils were analysed. Self-pollinated stigma (incompatible pollination) showed high RNase activity. Rate of RNase activity was studied using spectrophotometric analysis and confirmed that the activity of RNase was high in self-pollinated pistils.

*In vitro* bioassay has been done to assess whether RNase activity is essential for incompatible interaction in *H. patens* and the results confirmed that RNase activity of protein is necessary for incompatibility in this plant. The analyses concluded that RNase based incompatibility system is operating in *H. patens*. For incompatible interaction an optimum level of RNase activity is essential.

From the study it is concluded that strong RNase based gametophytic incompatibility system is operating in *H. patens* and due to this sexual reproduction is beset and seed-set was absent in this plant.