Summary and Conclusion
6. Summary and conclusions

*Garcinia gummi-gutta, G. indica, and G. morella* are dioecious trees which belong to family Clusiaceae. The tree species are found in Western Ghats of India. Both *G. gummi-gutta* and *G. indica* are endemic to this region. These trees are a source for various researchers especially in the field of pharmacology and are industrial valuable. The fruits obtained from the female trees are the major source of various phytochemicals and for the preparation of drugs especially HCA which is a weight loss agent.

Determination of sex at seedling or juvenile stage in dioecious crops has commercial applications. Selection of desirable sex can eliminate the wastage of financial resources and time. Commercially, female tree is important to the farmers and earliest detection of male and female trees is only possible when the trees reach reproductive maturity i.e. after 8-12 years. This may lead to serious problems to plant breeders in commercial plantations that have to retain the larger number of male trees for several years, leading to wastage of resources. Molecular marker techniques, like RAPD have been carried out to determine the sex of the plant in *G. indica* in the past; however reproducibility serves as a major problem. In order to retain more fruit-bearing female trees, determination of sex is required for all the three species existing in India. Thus, in the present study male-specific SCAR markers were developed in the trees studied *Garcinia* species. 150 decamer primers each was used to screen the sex-specific fragment in these species. One primer each in these species was able to differentiate between the sexes. The RAPD primer, OPBD-20 developed a 566 bp fragment in *G. gummi-gutta* male tree samples, which was absent in females. In the case of *G. indica*, the decamer primer OPN-15, was able develop a 1,501 bp fragment only in male trees samples. Similarly, in case of *G. morella*, a 634 bp fragment was obtained only in male samples. The sex-specific fragments were isolated and cloned into a vector and transformed. Based on the sequence of the unique fragment, sex-specific SCAR primers were designed. These primers were further standardized for PCR analysis. The CAM-566, IND-1501, and MOR-634 were able to differentiate male and female trees in *G. gummi-gutta, G. indica, and G. morella*, respectively. The primers amplified single, sharp, and highly specific bands of the specific male fragment in all the three species. The SCAR primers developed were further validated.
for its reproducibility and selectivity using plant samples whose sexes were determined morphologically and also in seedlings with unknown sex. The sequences of the sex-specific fragment also did not show any similarity with any known sequence in the NCBI database and was submitted to the NCBI GenBank Database. Moreover, this is the first report of sex-specific SCAR marker in the tree species of *Garcinia*.

Based on the above results the following conclusions were drawn

1. The designed sex-specific SCAR marker CAM-566 developed from the sequence of the unique male-specific fragment obtained from RAPD primer OPBD-20 was able to differentiate between male and female sexes of *G. gummi-gutta*. This primer develops a 566 bp fragment which is unique to male samples only.

2. The IND-1501 SCAR marker developed for sex determination in *G. indica* was able to differentiate between male and female tree samples. The primer was designed from the sequence of male-specific fragment obtained from the fragment of male samples by using RAPD primer OPN-15. The SCAR primer IND-1501 develops a 1501 bp fragment unique to male samples and absent in female samples.

3. In *G. morella*, the 634 bp sequence of the male sex-specific fragment obtained from the decamer primer OPL-05 was used to develop SCAR primer MOR-634. The primer was able to differentiate between male and female samples. The SCAR primer amplified a 634 bp fragment in all the male samples.

4. The SCAR primers developed is the first report for the identification of sex in these *Garcinia* species.