CHAPTER 5

SUMMARY

Seabuckthorn is a dioecious shrub which belongs to family Elaeagnaceae. The name seabuckthorn is derived from its characteristic of growing near the sea, and it possess many spines that are reminiscent of some buckthorn species of the genus Rhamnus\(^1\). The shrub has a huge potential for researchers in the field of biotechnology, neutraceutical, pharmaceutical, cosmetic and environmental sciences \(^2\). The female plants bears berries that are rich in vitamins, organic acids, fatty acids, flavonoids and antioxidants, thus provides various health related benefits \(^3\).

Early sex determination of dioecious plants has commercial applications. Financial resources and valuable time can be saved if undesired male/ female plants can be discarded at an early stage of research trials and commercial plantation. Commercial seabuckthorn plantation require only 10% males for adequate pollination \(^4\). However, the earliest detection of male and female seabuckthorn plants is possible when flowering occurs, which is too late. This presents a serious inconvenience to plant breeders who have to retain large numbers of superfluous males for several years and leads to wastage of funds, labour, field space and valuable time in case of commercial plantations. The problem is more complicated in seabuckthorn which multiply vegetatively in the field through suckers leading to excessive male or females depending upon their initial proportion. We have noticed this problem in the Kelong area of Lahul and Spiti, India, where seabuckthorn plantation was done by the Forest department, few years back. Most of the plants turned out to be male and the entire area is presently dominated by unproductive males. Molecular marker based studies like RAPD, SSR, ISSR, SCAR etc. were conducted for past several years for gender identification in *Hippophae rhamnoides*\(^5\)-\(^8\). To utilise the full potential of the seabuckthorn flora present in India, gender differentiating markers are required for all the three species existing in India. Thus in our current study the sex specific markers developed for *H. rhamnoides* were tested on the collected populations of male and female plants of *H. salicifolia* and *H. tibetana*. Female specific *HrX1* SCAR marker was able to distinguish female plants from male plants across the three species of seabuckthorn i.e. *H. rhamnoides, H. salicifolia* and *H. tibetana*\(^9\).
Moreover it is the first sex specific SCAR marker in seabuckthorn which had shown homology with known plant genes like Acyl CoA synthatase (plant lipid biosynthetic genes).

Differences between male and female plants are mainly detected in reproductive organs, which occur through differential growth, repression or abortion of sex organs in unisexual flowers [10, 11]. The genes play role in development of the flower like meristem identity genes, organ identity genes and flowering time genes could act as probable candidates for sex determination of dioecious plants. Apart from floral regulatory genes, sex determination is also dependent upon the regulatory networks which alter sex expression based on environmental cues such as photoperiod and temperature.

The genetic control of sex determination is well understood in several model plant systems like *Silene latifolia* [12-14], *Cucumissativus* [15-17], *Salix* [18, 19], etc. Molecular and genetic studies have shown that the underlying mechanisms controlling flower development are largely conserved in distantly related dicotyledonous plant species [20]. Thus, genomic resources generated from these model plants could be used to identify the potential GISD in seabuckthorn. Different spatial and temporal development stages of flower have been used to decipher the mRNA transcripts involved in sex determination in dioecious plants like *S. latifolia, Rumexacetosa, Actinidiachinensis*, etc. [21-23]. Thus for identification of potential candidates for sex determination in seabuckthorn differential expression of known flowering genes as well as transcription factors was analysed using quantitative Real Time PCR (qRT-PCR) in three temporal Floral Development Stages (FDS) of both male and female seabuckthorn flowers.

Floral Development Stage I of seabuckthorn recorded higher female specific expression for *HrAP1, HrCRY2, HrNEF1* and *HrAG*. Whereas *HrAP2, HrLFY, HrFRI* and *HrGI* recorded higher expression levels in male flowers. At II**nd** floral development stage the expression level of most of the genes studied except *HrCRY2* and *HrLFY* was observed higher in male flowers as compared to female flowers. In Floral Development Stage III of seabuckthorn, higher female specific expression of *HrAP1, HrCRY2, HrEF1* and *HrFIL* was observed, while *HrCRY1, HrCO* and *HrPHYB* had male specific expression. *HrCO* showed consistent higher expression in all male floral
development stages. On the other hand \textit{HrCRY2} recorded elevated expression levels in all the female floral development stages.

The morphological observation of the female sex organs i.e. ovary at FST III indicated that the ovary might have developed in stages between FST II and FST III. The expression level of the genes \textit{HrAP1}, \textit{HrCLV1}, \textit{HrFIL}, \textit{HrCRY2}, \textit{HrGI}, \textit{HrEF1} and \textit{HrETR1} increased in FST III with respect to FST II. However the expression level of the gene \textit{HrLFY} decreased at FST III with respect to FST II. In case of male floral buds the distinct male floral organs i.e. anthers were observed at MST II and which matured through MST III. Thus the development of the stamens started in between MST I and MST II while stamens matured through MST II and MST III. The expression of the genes \textit{HrAP2}, \textit{HrCLV1}, \textit{HrAG}, \textit{HrSEP3}, \textit{HrYAB5}, \textit{HrCRY1}, \textit{HrPHYB}, \textit{HrCO}, \textit{HrCOLK}, \textit{HrFRI}, \textit{HrFRILK}, \textit{HrEF1}, \textit{HrERS1}, \textit{HrETR1}, \textit{HrX1} and \textit{HrNEF1} increased in MST II with respect to MST I. But the expression of \textit{HrLFY} decreased in MST II with respect to MST I. During the maturation of male floral bud from MST II to MST III the expression of \textit{HrCO} only increased while the rest of the above mentioned genes had reduced expression levels in MST III with respect to MST II.

In Floral Development Stage I of seabuckthorn higher female specific expression of Transcription factors was observed in case of \textit{HrTF\_AP2}, \textit{HrTF\_FBZIP}, \textit{HrTF\_GRAS}, \textit{HrTF\_MYB\_DNA\_BIND} and \textit{HrTF\_PHD} while higher male specific expression was detected for \textit{HrTF\_ARF}, \textit{HrTF\_ARID}, \textit{HrTF\_AUX\_IAA} and \textit{HrTF\_SRF\_TF}. The male Floral Development Stage II recorded higher expression levels of maximum TFs under study except \textit{HrTF\_AP2}, \textit{HrTF\_FBZIP} and \textit{HrTF\_PHD}. In Floral Development Stage III of seabuckthorn higher female biased expression was showed by \textit{HrTF\_ARF} and \textit{HrTF\_ARID} while \textit{HrTF\_FBZIP}, \textit{HrTF\_GRAS}, \textit{HrTF\_MYB\_DNA\_BIND}, \textit{HrTF\_AUX\_IAA}, \textit{HrTF\_SRF\_TF} and \textit{HrTF\_SAP} recorded male biased expression. \textit{HrTF\_AUX\_IAA} and \textit{HrTF\_SRF\_TF} recorded consistent higher expression in all the male floral development stages. Out of all the TFs under study \textit{HrTF\_AP2} expressed consistently across all the female development stages.
Conclusion

- Female specific SCAR marker $HrX1$ is able to differentiate female plants from male plants in three species of seabuckthorn, namely, *H. rhamnoides*, *H. salicifolia* and *H. tibetana*. Applicability of this single marker ($HrX1$) in all the three species has circumvented the need for de-novo development of sex linked markers in *H. salicifolia* and *H. tibetana*, thus saving both the time and resources. It is the first report in seabuckthorn that sequence of sex linked marker has shown homology with known plant gene, which needs further investigation for its potential role in sex determination. Thus as per the stated hypothesis the female specific SCAR marker $HrX1$ developed for *H. rhamnoides* were able differentiate gender in other two species of seabuckthorn i.e. *H. salicifolia* and *H. tibetana*.

- $HrCO$ has shown consistent higher expression in male floral buds only. While $HrCRY2$ was expressed throughout the development of female floral buds while its expression was very low in all the development stages of male flowers. The expression level of $HrAPI$, $HrFIL$, $HrCRY2$ and $HrGI$ increased only in female flowers during the development of female floral organs while the level of expression of $HrAP2$, $HrAG$, $HrSEP3$, $HrYAB5$, $HrCRY1$, $HrPHYB$, $HrCO$, $HrCOLK$, $HrFRI$, $HrFRILK$, $HrERS1$, $HrX1$ and $HrNEF1$ increased along with the development of male floral organs in male floral buds. In case of both male and female flowers the expression of $HrLFY$ gene decreased as the sex organ development started.

- $HrTF\_AUX\_IAA$ and $HrTF\_SRF\_TF$ had consistent higher expression in male floral buds only. $HrTF\_AUX\_IAA$ had consistent higher expression levels during the first two developmental stages of male flower development of male floral organs indicating its role in initial development of male floral organs. $HrTF\_AP2$ had consistent higher expression in female floral buds only. The expression of this TF increased in second and third stage of development.

- Out of the three development stages of male and female flowers of seabuckthorn, MST II recorded the highest expression levels of maximum GISD as well as TF transcripts under study.
REFERENCES


