Chapter 6

CONCLUSION

In the present we have investigated the phylogenetic relationship and functional constraints among plant \textit{CCDs} and \textit{NCEDs} sub-class genes that known to produce apocarotenoids which has various biological functions. The phylogenetic analysis displays that existence of well-conserved subfamilies. Comparative genomic analysis showed that the gene structures of the \textit{CCDs} and \textit{NCEDs} were highly conserved across some different lineage species. Through functional divergence analysis, a substantial divergence was found between \textit{CCD} and \textit{NCED} subfamilies. Finally the conclusion drawn from the phylogenetic analysis indicates \textit{CCD7} and \textit{CCD8} genes were emerge from \textit{CCD1} gene by the duplication of \textit{CCD4} gene. The codon usage bias was studied to identify whether the mutational pressure or natural selection exist in \textit{CCD} and \textit{NCED} gene family. The RSCU values indicate a high preference for CUC codon in both \textit{CCD} and \textit{NCED} gene family. The mean ENc value of \textit{NCED} genes was found to be 48.76 that suggesting a relatively higher codon bias than \textit{CCD} genes. In addition, the ENc-GC$_3S$ plot and multivariate analysis suggests that both the gene families are under mutational pressure. Moreover, the expression level of \textit{CCD} gene family was identified through codon adaptation index and relative codon bias. The codon based cluster tree result shows neatly clustered subclass of \textit{CCD} genes except BoCCD1 gene of \textit{B. orellana}. Correlation analysis of CAI values with RCB indicates an overall low level expression of \textit{CCD} across different species. In addition the \textit{CCD} genes three dimensional structure was predicted and identify their structural stability through homology modeling and dynamics simulation. The dynamic results revealed that \textit{AtCCD4} gene shows higher stability than other \textit{CCD} genes. Taken together, our study mainly focused on the evolution of \textit{CCD} and \textit{NCED} genes and their functional divergences that strongly evidence the duplication and diversification has occurred in \textit{CCD} genes but not in \textit{NCED} gene family. The codon bias analysis point out that \textit{CCD} and \textit{NCED} genes are under mutational pressure. The Codon Adaptation Index and Relative Codon Bias analysis identifies the critical factors responsible for this variation, which could aid in prediction of gene expression and function for newly reported \textit{CCD} genes. The dynamics results about \textit{CCDs} revealed their
structural conformation that can provide a better understanding of \textit{CCD} structure and identify the functional variants in \textit{CCD} genes that indicate some future applications of \textit{CCD} proteins. Finally the Plant Carotenoid Cleavage Dioxygenase Database was created which could serve as a resource for scientific community. Overall Our in-depth investigation of phylogenetic evolution, codon usage bias, gene expression and dynamic behaviour in \textit{CCO} genes have shed lights on broader sense, and brought out the most common and discriminative features at the molecular level with respect to the function in different plant species. This knowledge may helpful to increase the production of important therapeutically and industrially apocarotenoids.