Chapter 5
SUMMARY OF FINDINGS

Banana is known as the “queen of tropical fruits” as it is major staple food and export products in many countries. The banana plant is a huge monocotyledonous perennial herb and has a distinct morphological character of high leaf area and shallow root system. These two traits makes it highly susceptible to water stress.

Drought stress is one of the most insidious hazards of the nature and it is a complex phenomenon. Drought tolerance mechanism is governed by multiple traits. Initially in the current study, we have screened the banana parental line and its segregating F1 population for drought tolerance traits like total cuticular wax content, adaxial stomatal density, LWRC and stomatal conductance. We observed close to normal distribution of data for the above traits in F1 generation indicating that the traits are controlled by multiple genes. The genetic analysis of the above traits showed a high heritability with moderate to high GAM indicating its selection would be effective for future breeding program.

Compared to roots and stomata, the water retention by cuticular wax is low but increase in roots and decrease in stomatal density will finally affect the carbon assimilation. The plant cuticular wax is a hydrophobic barrier on the epidermis of the plant body. It forms a seal over the outer walls of the epidermal pavement, guard, and trichome cells, thus protect plants against uncontrolled, continuous non stomatal water loss. Thus the cuticular wax was examined in detail in the present study. Initially, we obtained a positive correlation with total cuticular wax content and LWRC indicating its role in maintaining the hydration of the banana plants. By GC-MS analysis, 71 different wax components from 13 Musa genotypes was identified and classified based on its functional group as hydrocarbons, alcohols, esters, aldehydes/ketones and others. Further, correlation analysis was carried out between different class of wax components and LWRC and observed that esters ($r = 0.597; P<0.05$) and alcohols ($r = 0.730; P<0.01$) play a major role in retaining the moisture in the banana leaf. Also, an observation was made that $>C_{28}$ compound played an important role in maintaining the hydration of banana leaf.
(r = 0.819; P<0.01). This was further confirmed by gene expression analysis of KCS11 and FATB.

An observation was made that >C<sub>28</sub> compound accumulation was higher in high LWRC musa genotypes. In order to understand the compositional variation during different leaf growth stages, GC-MS analysis in 5 different leaf growth stages of Musa balbisiana and Musa acuminata was carried out. C<sub>28</sub> compound accumulated in all the leaf stages in ‘Calcutta-4’ compared to Bee Hee Kela and vice versa for >C<sub>28</sub> wax components was observed. This was further confirmed by gene expression analysis of CUT1. Also, phylogenetic analysis was carried out for the gene CUT1 to understand the structural difference.

To further understand the regulatory mechanism involved in wax biosynthesis, computational prediction of miRNAs was done. The transcriptome of Musa balbisiana and Musa acuminata was utilized to mine the conserved miRNAs using bioinformatic tools. A total of 96 and 62 conserved-miRNA families in Musa balbisiana and Musa acuminata respectively were identified. Later, by target prediction of the above miRNA families we observed that 74 and 48 miRNA families had targets in cases of Musa balbisiana and Musa acuminata, respectively. By the pathway annotation of the above targets, 2 miRNAs were shortlisted which were involved in wax biosynthesis - MbmiR531 whose target is KCS11 and MbmiR529 whose target is KCS10/FDH. Validation of the computational prediction results was carried by qRT PCR which further revealed a negative relationship between the target gene and its miRNA, thus indicating their role in wax biosynthesis regulation. Thus, identification of drought tolerance traits, the role of cuticular wax in maintaining hydration in banana followed by identification of genes involved in wax biosynthesis and its regulation by miRNA has been done.

The next step was identification of genomic regions involved in drought tolerance in banana. The initial step in order to develop linkage maps, identification of QTLs, exhaustive comparative mapping across species, cultivar identification, genetic diversity studies, and parent selection for breeding programs is the development of molecular markers. Here, in the present study, SSR as well as SNP were identified using both
transcriptomics approach as well as WGS approach. In case of transcriptome, a total of 9857 sequences from ‘Bee hee kela’ and a total of 4424 sequences from the ‘Calcutta-4’ had SSRs and validation was done for a few selected SSRs. The mean PIC content was found slightly higher in case of ‘Bee hee kela’ SSRs compared that of ‘Calcutta-4’. In case of WGS, a total of 137850 and 145374 SSRs were identified from ‘Bee hee kela’ and Bhimaithia respectively.

By variant analysis, we identified 370100 and 103987 SNPs from ‘Bee hee kela’ and ‘Calcutta-4’ transcriptome data. In case of WGS, a total of 229444 SNPs was identified between ‘Bee hee kela’ and Bhimaithia libraries. The large number of gene-based as well as genomic based SSR and SNP markers developed will help in the development of a genetic linkage map, QTL analysis and molecular marker breeding strategies.

The final aim was to identify the genomics regions involved in the drought tolerance traits in banana. To arrive at the QTLs, a SSR based linkage map was developed using the F1 segregating population. A first generation linkage map for Musa balbisiana ‘Bee hee kela’ was constructed which is the first report for banana ‘B’ genome. The linkage map covered a total genetic distance of 1202.6 cM and 1206.8 cM in Bee hee kela and ‘Calcutta-4’ respectively. Later, by QTL analysis, a total of 6 QTLs related to drought tolerance traits – total cuticular wax content (year 2013 and 2015), LWRC, adaxial stomatal number and stomatal conductance was identified. The present study is the first report on QTLs not only on drought tolerance traits QTLs but also there are no reports on any traits in banana. Further, saturation of the linkage map will authenticate the identified QTLs and also it might lead to identification of new QTLs as the analyzed traits are all polygenic in nature.

The obtained data can be further utilized and below are the few future strategies which can be employed to improve our understanding:

- The role of cuticular wax and its components in maintaining the hydration of the banana leaves were studied in detail. A good lead was obtained with respect to
>C_{28} compounds of cuticular wax. Further this strategy can be applied for other crops and can be used as a biochemical marker for assessing high LWRC genotypes.

- The genes identified in the present study – *KCS11, FATB, FDH, CUTI*, will form valuable resource for further understanding of a complex trait like drought and also it can be utilized for marker assisted selection (MAS) in banana.

- The two miRNAs identified in the present study that is MbmiR529 and MbmiR531 whose targets are involved in the wax biosynthesis has to be further validated in a heterologous expression system or by transgenic studies to validate its function.

- A large number of genic as well genomic markers were developed in the current study. The genic SSRs developed using the pooled transcriptome data will form a valuable resource for people working on identifying QTLs related to biotic as well as abiotic stress tolerance as the data represents a global transcriptome in both A as well as B genome as the tissues used are both above and below the ground tissues.

Also, the markers developed using the whole genome sequencing data can be utilized to further saturate the available ‘A’ genome map.

- A first generation SSR based linkage map for B genome was constructed which is the first report for *Musa balbisiana*. The developed map can be further saturated using high throughput technologies such as genotype by sequencing (GBS) or Restriction-site associated DNA sequencing (RADSeq). This is important and must have high priority as a linkage map is required for correction of the draft assembly of the *Musa balbisiana*.

- A total of 6 QTLs were identified in the current study, further saturation of the linkage map can lead to identification of more QTLs as the traits are polygenic. Further, the identified QTLs have to be validated by fine mapping and gene expression studies.