6.0 CONCLUSION

In this study, putative *RPK1* and putative *CLV1* from *Oryza rufipogon* and *Oryza sativa* ssp. *indica* cv. MR219 were selected as candidate yield-related genes. Putative RPK1 was categorised as a LRR-like type transmembrane receptor kinase group member in the plant RLK/*Pelle* family, which contains an extracellular domain connected to an intracellular cytoplasmic protein kinase via a single pass transmembrane helix. Putative RPK1 was categorised into the LRR-III subfamily in the plant RLK/*Pelle* family based on the presence of a LRR tandem repeat. Putative RPK1 was categorised into the RD minus kinase class in the plant RLK/*Pelle* family based on the kinase domain protein. The kinase of putative RPK1 belongs to a putative kinase-defective atypical receptor kinase, which might be involved in transducing signals by phosphorylation independent mechanism. *Oryza rufipogon* and *Oryza sativa* ssp. *indica* cv. MR219 genome contain one to three copies of putative *RPK1*. BC_2F_7 line 23 is homozygous for the putative *RPK1* allele from *Oryza rufipogon*; whereas, BC_2F_7 line 7 is homozygous for the putative *RPK1* allele from *Oryza sativa* ssp. *indica* cv. MR219. Gene expression of putative *RPK1* was up-regulated at the booting stage and may be involved in gametophyte development during transition from vegetative to flowering phase. This transition period is a critical step to influence rice production.

Putative CLV1 was categorised into the LRR-like type transmembrane receptor kinase group in the plant RLK/*Pelle* family. It contains an extracellular domain

connected to an intracellular cytoplasmic protein kinase via a single pass transmembrane helix. Putative CLV1 was grouped into the LRR-IX subfamily in the plant RLK/*Pelle* family based on the presence of a LRR tandem repeat. Putative CLV1 was categorised into the RD kinase class in the plant RLK/*Pelle* family based on the kinase domain protein. The RD kinase class in the plant RLK/*Pelle* family members usually are involved in development. Southern hybridization analysis suggests that there are three to five copies of putative *CLV1* in *Oryza rufipogon* and *Oryza sativa* ssp. *indica* cv. MR219 genomes, and may belong to a multiple gene family. BC_2F_7 line 23 is homozygous for the putative *CLV1* allele from *Oryza rufipogon*; whereas, BC_2F_7 line 7 is homozygous for the putative *CLV1* allele from *Oryza sativa* ssp. *indica* cv. MR219. Gene expression of putative *CLV1* was highly up-regulated during booting and heading stages. Therefore, it is suggested that putative *CLV1* may be involved in floral meristem development, an important determinant of grain number per spikelet in rice.

The characterization of the putative *RPK1* and putative *CLV1* from *Oryza rufipogon* and *Oryza sativa* ssp. *indica* cv. MR219 should facilitate better understanding of genetic components of rice yield. This was an essential initial step toward improving agricultural traits future Malaysian crop breeding programmes.

6.1 Future Work

The biological role of putative *RPK1* and putative *CLV1* genes should be further analysed through overexpression, knockdown expression and cell localization studies in elite Malaysian *indica* rice (*Oryza sativa* L.) cv. MR219 and *Oryza rufipogon* (IRGC105491). This may provide a better correlation between putative *RPK1* and putative *CLV1* gene expressions and functions with grain yield. The extracellular domain and intracellular cytoplasmic protein kinase activity also could be analysed by overexpression of a truncated protein or a chimeric protein in order to understand the effect of amino acid substitutions of functional domains to the proteins.

Biochemical approaches can also be applied to elucidate the roles of putative *RPK1* and putative *CLV1* in the plant signal transduction. Ligands and effectors of putative *RPK1* protein and putative *CLV1* protein should be identified respectively and then analysed for example through a yeast two-hybrid system to identify interacting partners. In addition, in vitro kinase assays and in vivo analysis can be used to analyse kinase domain phosphorylation of the putative RPK1 and putative CLV1 proteins. In the future, putative *RPK1* and putative *CLV1* genes can potentially be used as a molecular breeding tool towards the development of new rice variety with high yield production.