CHAPTER V

CONCLUSIONS

1. The cDNA encoding for Major Royal Jelly Proteins 1 and 2 were isolated from head of *A. cerana* by Reverse transcription-PCR (RT-PCR). The open reading frames (ORFs) of AcMRJP1 and AcMRJP2 were 1,302 and 1,392 bp encoding 433 and 463 amino acid residues, respectively. Deduced amino acids indicated high essential amino acid content of AcMRJP1 and AcMRJP2 (47.4% and 45%, respectively). Similarities of AcMRJP1 and AcMRJP2 and their homologues in *A. mellifera* were 93% and 92% (nucleotides) and 90% and 86% (deduced amino acids), respectively.

2. The gene encoding the AcMRJP1 and AcMRJP2 was isolated from genomic DNA of *A. cerana* by PCR strategy. The AcMRJP1 and AcMRJP2 gene sequence spans over 3,663 bp and 3,963 bp, respectively. Both AcMRJP1 and AcMRJP2 genes contain six exons interrupted by five introns and all intron-exon boundaries followed the GT/AG rule. Sequence analysis of the upstream regions revealed the putative TATA-box like element, locating approximately 31-32 bps upstream of the predicted transcription start sites of each gene. The presence of potential recognition sequences for transcription factor was observed.

3. The AcMRJP1 and AcMRJP2 cDNAs were cloned into expression vectors for expression in *E. coli* expression approach. SDS-PAGE analysis revealed protein bands of 50 and 55 kDa corresponding to the expected molecular weight of approximately 47.9 kDa and 51.7 kDa for AcMRJP1 and AcMRJP2, respectively. The expression of AcMRJP1 and AcMRJP2 in *E. coli* was maximal at 4 hours after IPTG induction. The AcMRJP1 and AcMRJP2 were expressed as insoluble forms and purified using affinity chromatography. These bands were eluted with 250 mM imidazole at 20 and 8 mg/l of induce culture. The AcMRJP1 and AcMRJP2 were confirmed by *N*–terminal sequencing and Western blot analysis.

4. *Agrobacterium*-mediated transformation can be used to transform AcMRJP1 cDNA into potato (*Solanum tuberosum* L.) and rice (*Oryza sativa* L.). The constructed recombinant plasmid for rice, AcMRJP1 cDNA was inserted under the control of the cauliflower mosaic virus 35S promoter whereas for potato the cDNA was inserted under the control of the cauliflower mosaic virus 35S, or granule bound starch synthase (GBSS) or patatin B33 promoter. Transformants were selected on antibiotic containing media and the presence of β–glucuronidase activity. The expression of AcMRJP1 in plants was detected by PCR, RT-PCR and Western blot analysis.