CHAPTER VI

CONCLUSIONS

In this thesis, the preliminary screening experiment of nine PCR primer-pairs was performed with the extracted DNA of three representative tobacco cultivars from fresh-leaf samples. Eight primer pairs successfully amplified all representative DNA samples. However, *psb*D-*trn*T$^{(GGU)}$-R and 5′*rps*16x1-*trn*Q$^{(UUG)}$ primers also produced non-specific PCR products. Thus, the optimisation of the PCR condition to reduce such non-specific bands was done by increasing the annealing temperature. After optimisation, six primer pairs were selected for the second analysis with the fresh-leaf samples of 23 tobacco cultivars representing all cultivar groups. These six noncoding regions revealed various levels of sequence polymorphic characteristics. The sequence polymorphisms of all six regions can distinguish Virginia cultivar-group (K326 and PVH03 cultivars) from the other cultivars. Moreover, the *rpl*32-*trn*L region was able to separate four local cultivars (Petkhangsink, Ubon Ratchathani, Kan and Hangkai) with a large 66 bp insertion.

*rpl*32-*trn*L region was then be selected to analyse genetic relationship among all 51 tobacco samples collected in this study, including fresh-leaf and cured-leaf samples and RYO tobacco product. The aligned sequences of *rpl*32-*trn*L region were able to separate six imported tobacco cultivars (K326, PVH03, PV09, HB01, HBO04P and TN97) from the others. Moreover, eight samples of seven local tobacco cultivars (Hangkai, Kan (fresh and cured leaf), Petmakhuea, Ubon Ratchathani, Petkhangsink, Baitung (cured leaf), Bailai (cured leaf)) and Maesomsong roll-your-own tobacco were separated from the other cultivars with the expected 66 bp insertion. These sequence polymorphisms of *rpl*32-*trn*L region suggested that this region could be a specific marker for Virginia cultivars and the seven special local cultivars. Multiplex PCR technique was also tested successfully with *rpl*32F-*trn*L$^{(UAG)}$ and *ndh*F-*rpl*32R primer pairs to reduce the time and cost of the analyses. This multiplex PCR marker could be further developed and used as a solution to distinguish between some local and imported tobacco cultivars, such as in an investigation of an illegal mixing of Virginia cultivars into roll-your-own products, in a confirmation of Virginia genotype in the tobacco production.