CHAPTER I

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the most important economic crops of the world as raw material for cigarette industry. This plant originated in the tropical America (Davis and Nielsen, 1999). From there, it was rapidly spread over Europe, Africa, Asia and Australia (Albert, 1996; Davis and Nielsen, 1999; Grehan, 2006). Tobacco first arrived in Thailand in the late 16th century and has been developed to be several local cultivars (ประกิต และ กรองจิต, 2547; วรวิชย์ และคณะ, 2549). Nowadays, tobacco plants are mostly grown in the northern and northeastern parts of the country. Tobacco cultivars grown in Thailand can be separated to two major groups: local cultivars and imported cultivars (further separated to three minor groups: Virginia, Turkish and Burley). Both cultivar groups have different regulations in tariff collection, i.e. the tariff for local cultivars is much less than that of the imported ones (วรวิชย์ และคณะ, 2549). However, to legally and technically separate the two groups apart is still a major problem. For example, only the tobacco cultivars which have been cultivated in the country "for a long time" could be legally called "local" cultivars, even though there is neither chemical nor physical standard method to determine their cultivation history.

Recently, several molecular markers, such as RFLP (Botstein et al., 1980), RAPD (Williams et al., 1990), AFLP (Vos et al., 1995) and microsatellite or SSR (Powell et al., 1996) have been developed and increasingly used as modern techniques to distinguish genotypes of organisms. These DNA fragment markers have been successfully used in polymorphism analysis, crop cultivar identification and phylogenetic evaluation in many plant species. Common benefits from most fragment markers include rapid analyses, highly informative results and being independent on environmental factors. However, molecular markers from DNA fragment amplification also have some limitations in the data analysing step. For instance, DNA band results may not be clear enough for the analysis and some PCR amplified fragments may not be repeatable due to a low quality of the genomic DNA (Thormann et al., 1994; Arcade et al., 2000). These problems particularly lead to an uncertainty when analysing the genetic distance between

organisms, especially in the case of cultivated crops. To avoid such problems, DNA sequencing technique would rather be used as an alternative molecular marker than DNA fragment markers.

DNA sequencing technique has been successfully used in many aspects in the recent years. For plant genomes, nucleotide sequences of chloroplasts have been proved to be a primary source of data for molecular genetic relationship studies. Many early publications usually focused on several coding-regions of chloroplast DNA (cpDNA) sequences such as *rbc*L, *mat*K, *atp*B and *ndh*F genes to elucidate genetic relationships among higher-level taxa (e.g. Chase et al., 1993; Olmstead and Sweere, 1994; Steele and Vilgalys, 1994). Not only the coding sequences of chloroplasts, but noncoding cpDNA sequences (introns and intergenic spacers) have also been used in plant molecular systematic research for more than fifteen years (since the *trn*L-*trn*F intron work of Taberlet et al. in 1991). Noncoding regions of the cpDNA have been proved to be more suitable for lower-level taxonomic studies than the coding regions (Gielly and Taberlet, 1994). Recently, Shaw et al. (2005) have evaluated the amplification and phylogenetic utility of 21 different noncoding cpDNA regions in a wide range of seed plant lineages. They have proposed that there are many more variable noncoding regions which have rarely been employed. Moreover, the same researchers have later successfully identified another 13 unexplored noncoding regions and evaluated them more thoroughly (Shaw et al., 2007). They showed that at least nine newly explored regions offered the levels of variation even higher than those of the most variable regions identified in their 2005 work. Their finding suggested that these highly variable regions of chloroplast genomes should be evaluated for their potential applicability in genetic relationship study of tobacco cultivars.

Research objective

To examine some of Shaw et al. (2007)'s primer pairs for PCR amplification of highly variable regions of tobacco chloroplast DNA and then to select the suitable primers for genetic relationship analyses of tobacco cultivars grown in Thailand.