CHAPTER I

Introduction

The aromatic prenytransferases (PTases) are a group of enzymes involved in the catalysis of prenylation reaction by transferring an isoprenoid unit to an aromatic molecule. In plants, aromatic PTases are involved in the biosynthesis of prenylated flavonoids and lipoquinones, including ubiquinones, menaquinones, and plastoquinones. Tocopherol, well known as vitamin E, is a branch of lipoquinone biosynthesis. It has been found in photosynthetic organisms such as plants and algae. Vitamin E is a highly potent antioxidant that protects and stabilize plant membrane against photo-oxidative damage or lipid peroxidation (Falk and Munne-Bosch, 2010). Moreover, it is important to plant by controlling lipid oxidation during the stages of seed germination, early seedling, and stress condition (biotic and abiotic stress) (Abbasi et al. 2007; Sattler et al. 2006). It has been shown that tocopherol disturbs lipid peroxidation chain reaction by donation proton from hydroxyl group of tocochromanol ring to polyunsaturated fatty acid (PUFA) peroxy radical (Asensi-Fabado and Munné-Bosch, 2010).

Vitamin E has been divided into two groups, tocopherols and tocotrienols which differ from each other in the degree of saturation of their hydrophobic phytyl side chain. Each group has four derivatives, including α-, β-, γ- and δ- forms by which they differ in methyl group number and position on the hydrophilic chromanol head group (DellaPenna and Mène-Saffrané, 2011). Tocopherol biosynthesis starts with the condensation of the aromatic head group precursor, homogentisate (HGA), and the phytyl tail precursor, phytyl diphosphate (PDP). HGA is derived from *p*-hydroxyphenylpyruvate (HPP) by the action of 4-hydroxyphenylpyruvate dioxygenase (HPPPD) whereas PDP is derived from the reduction of geranylgeranyl diphosphate

(GGDP) from the isoprenoid pathway (Vranová, et al., 2013). The condensation reaction of HGA and PDP is catalyzed by homogentisate phytyltransferase (HPT or VTE2), the key enzyme in the first step of tocopherol biosynthesis, to produce 2-methyl-6-phythylbenzoquinol (MPBQ). MPBQ is then methylated to yield 2,3-dimethyl-5-phythylbenzoquinol (DMPBQ) by the enzyme MPBQ methyltransferase (VET3). Subsequently, the second ring of γ-tocopherol is formed by tocopherol cyclase (TC or VTE1), and finally, α-tocopherol is formed by γ-tocopherol methyltransferase (γTMT or VTE4) (DellaPenna and Pogson, 2006).

So far, overexpression of HPT has been reported in *Arabidropsis* (Collakova and DellaPenna, 2003a), *Synechocystis* sp. PCC 6803 (Savidge, et al., 2002), apple (Seo, et al., 2011), and lettuce (Ren, et al., 2011), which usually cause the increase of α-tocopherol accumulation. HPT has therefore been considered as the enzyme catalizing the rate-limiting step of the pathway.

In this study, *Artocarpus lakoocha* Rox and *Clitoria ternatea* L. known respectively as Mahaad and Un-Chann in Thai name were used as potential sources for isolating aromatic PTase genes and enzymes. Both plants are abundant of the secondary metabolites such as flavonoids, stilbenes, and their derivative compounds which have high potential of biological activities, such as antimicrobial, anticancer, antioxidant and anti-tyrosinase activities (Likhitwitayawuid, et al., 2005; Mukherjee, et al., 2008; Sritularak, et al., 2010; Swain, et al., 2012a; Swain, et al., 2012b). The main objective of this study is to discover novel aromatic PTase genes from these plants. Methods of molecular biology were used to obtain full-length aromatic PTase genes, followed by expressing the genes in a suitable system. The expressed enzymes were then determined for their activities.