CHAPTER I

INTRODUCTION

Today, malaria is still a major health problem in the world. At the end of 2004, 107 countries and territories had areas at risk of malaria transmission. Some 3.2 billion people lived in areas at risk of malaria transmission. Malarial disease is caused by a group of protozoa parasites belonging to the genus of *Plasmodium*. Only four species are known to infect human: *P. falciparum, P. vivax, P. malariae* and *P. ovale*. The infection of parasite is introduced through the bite of female *Anopheles* mosquitoes (Geldre *et al.*, 1997). An estimated 350–500 million clinical malaria episodes occur annually; most of these are caused by infection with *P. falciparum* and *P. vivax*. Falciparum malaria causes more than 1 million deaths each year. It also contributes indirectly to many additional deaths, mainly in young children, through synergy with other infections and illnesses (WHO, 2005). Malaria in Thailand is forest-related and most prevalent along the international borders, especially on the Thai–Myanmar border. In the central plain areas, transmission has been eliminated for more than 2 decades. Malaria transmission in forested areas is intense because of highly efficient vectors, enhanced vector longevity and extensive population movement into and out of these same areas. At national level, malaria cases and deaths have fallen gradually since 1999, but the disease remains an important public health problem along the international borders. Young adult males who work in or near forests are a special group at risk in these areas (WHO, 2005).

The problems of drug resistance are greatest in Southeast Asia, where there is evidence for resistance or reduced sensitivity against all antimalarial drugs, including

mefloqine, halofantrine and even quinin. Interestingly, treatment of severe malaria now relies on the use of the latest natural antimalarials, namely artimisinin and its derivatives.

Artemisinin or qinghaosu was first isolated from *Artemisia annua* L. plant (Klayman 1985). The plant has been used in traditional Chinese medicine as a remedy for chills and fevers for more than 2000 years. However, the content of artemisinin in the leaves of this plant is present in relatively low level and is insufficient for using the compound in the treatment of the resistant strain causing the malarial disease. Therefore, there have been several attempts in many laboratories to increase the production of the artemisinin (Geldre *et al.*, 1997). Some high artemisinin production plants have been selected from the field (Liersch *et al.*, 1986), greenhouse (Ferreira *et al.*, 1994), and hydroponic conditions (Jaziri *et al.*, 1993).

A. annua is a wild growing species with relatively low artemisinin content, ranging from 0.01 to 0.8% of the plant dry weight, depending on the geographical origin, seasonal and somatic variations (Dhingra *et al.*, 2000, Wallaart *et al.*, 1999, Ferreira *et al.*, 1995). Presently, the only commercial source of artemisinin is by extraction from field-grown leaves and flowering tops of the plant. Many attempts to obtain artemisinin in a cheaper way have been made. Total synthesis of the compound has been reported (Schimid & Hofheing, 1983, Avert et al. 1992) but many chemical steps are required and the yields are low. *In vitro* cultures of *A. annua*, such as cell culture, callus (Paniego & Giulietti, 1994), shoot (Chun-Zhao et al. 2003, Ferreira & Janick, 1996) and hairy root cultures (Putalun et al. 2007, Weathers et al. 2004, Smith et al. 1997, Jaziri et al. 1995), have also been established for studying their potentials of producing artemisinin but *in vitro* culture for artemisinin production has yet to prove commercially feasible. Therefore, the whole plant of *A. annua* is still

the most economic source of artemisinin and development of high-producing plants of *A. annua* seems to be the main direction to obtain large quantities of relatively cheap artemisinin.

In recent years, ionizing radiation has been recognized as a powerful technique for plant improvement, especially in crop plants (Al-Safadi *et al.*, 2000, Chakravarty and Sen, 2001, Dong *et al.*, 2004). This technique creates genetic variability in plants which can be screened for desirable characteristics. So far, very little is known about the effect of gamma irradiation on the potential of artemisinin production in *A. annua*, which has been proposed to involve several steps in its biosynthtic pathway (Figure 11) (Bertea *et al.*, 2005, Covello, *et al.*, 2007). Among these steps, the first enzyme, namely amorpha-4,11-diene synthase (ADS) catalizing the conversion of farnesyl diphosphate (Figure 11, (**2**)) into amorpha-4,11-diene (Figure 11, (**3**)) is thought to be a possible first step of the pathway (Bouwmeester *et al.*, 1999). Gamma irradiation is a random effect on the plant genome, including various genes involved in artemisinin biosynthetic pathway. Therefore, it would be interesting to see whether the irradiation will lead to a better yield of artemisinin in *A. annua*. In order to increase a possibility in selecting high-artemisinin producing plants of *A. annua*, this study has created irradiated plantlets of *A. annua* by gamma irradiation and used a simple TLC-desitometric method for quantitative analysis of artemisinin in *A. annua* plants (Koobkokkruad *et al.*, 2007).

In this study we aimed to improve the yield of the antimalarial artemisinin production in *A. annua* by using combined techniques of gamma irradiation, tissue culture and molecular cloning. The stability of artemisinin production in irradiated *A. annua* plantlets in acclimation conditions was studied. This involves the transfer of *in vitro* irradiated plantlets to *ex vitro* plant conditions in green house and open field

followed by determining their artemisinin content during transfer process. In addition, genetic engineering of *ads* gene, our target gene, for improving the artemisinin production in transgenic *A. annua* was also another aim of this study. For this aspect, the *ads* gene was cloned and expressed in *A. annua* under expression vector pBI121/*ads*, followed by evaluated the correlation between the artemisinin content and the activity of amorpha-4,11-diene synthase (ADS).