INTRODUCTION

Artemisinin: Saving Lives, Buying Time

“A child dying from malaria every 30 seconds is completely unacceptable when we have effective and affordable ways to help children and adults avoid infection. Incredibly, one out of four child deaths in Africa is due to malaria. Yet, insecticide treated bed nets can reduce mortality by up to 25%. We need to help countries expand program to get nets and drugs for treatment to all women and children who are threatened by this silent crisis.”

Dr. Carol Bellamy, UNICEF

Plants have been an important source of medicines for thousands of years. Even today, the World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines. Secondary metabolites are those compounds that are produced exclusive of the primary (nutrition and maintenance) metabolites essential to sustain the life of an organism.

These secondary products have been shown to be very useful for both the plants as well as the animals that synthesize them (Facchini, 1999). The most abundant and structurally dissimilar group of secondary metabolites is the terpenoid family (or isoprenoids). Isoprenoids are an incredibly diverse and ubiquitous natural product group that is found in all plant species. They are the oldest class of biomolecules with evidence of their existence extending 2.5 billion years ago (Lange et al., 2000). Numbering over 30,000 identified compounds, they are involved in electron transport chains, membrane composition, hormones, photosynthetic pigments, and plant defense compounds. In industry, monoterpenes are commonly used as fragrant additives to cosmetics and food. Sesquiterpenes, such as the sesquiterpene lactones found in the...
anthemideae tribe of Asteraceae are known for their medicinal value. Being the world’s most severe parasitic infection, malaria caused more than a million deaths and 500 million cases annually. Despite tremendous efforts for the control of malaria, the global morbidity and mortality have not been significantly changed in the last 50 years (Riley, 1995). Over the last couple of decades, the rise of biotechnology has provided a cutting-edge technological platform driving innovation in the development of new therapies for life threatening diseases including malaria. These innovative approaches rely on years of research and development using technologies that are increasingly sophisticated and costly. Thus, it has been assumed that these technologies would be too expensive to apply to the creation of new medicines that are desperately needed in the developing world.

Malaria is a disease that overwhelmingly affects areas of poverty, producing 300-500 million new infections and 1–3 million deaths each year, with most of the disease burden falling on African children younger than 5 years of age (Eline et al., 2005). In the last few decades, Plasmodium falciparum; the parasite causing the most virulent form of malaria has become increasingly resistant to first-line drug therapies. However, artemisinin based combination therapies (ACTs) show nearly 100% effectiveness against these drug-resistant parasites. Unfortunately, because of their high cost, ACTs are still beyond the reach of the world’s poorest people. One of the major constraints to reduce the cost of ACTs is the low artemisinin content (0.1-1.1%) in A. annua L. plants that are still the main source of artemisinin for commercial production of ACTs.

Artemisinin, a sesquiterpene-lactone with endoperoxide bridge, isolated from Artemisia annua L. has shown strong potential as the antimalarial drug (Abdin et al., 2003; Wensdorfer, 1994; Luo et al., 1987; Li et al., 1982). The plant belongs to the family, Asteraceae
formerly Compositae and is an annual herb native to Asia. It is now distributed throughout many countries such as Europe, North America as well as Central and South America. Traditionally, *A. annua* L. is used for crafting of aromatic wreaths, flavouring of spirit, and its essential oil is distilled for perfumery and industrial use (Ferreira et al., 1995). The essential oil also possesses strong insecticidal potential (Charles et al., 1991). Due to this discovery, it is now rated as one of the top ten industrial crops of the modern world.

Artemisinin has shown to be synthesised mainly in leaves of *A. annua* L., though other green tissues of the plants also synthesize this compound. But the relative low yield of artemisinin is serious problem to the commercialization of this drug (Laughlin, 1994). The chemical synthesis of artemisinin is also possible, but it is complicated and economically unviable due to very poor yields (Ravindranathan et al., 1990). Since the total synthesis of artemisinin is difficult and expensive, a biotechnological approach has been considered as a feasible alternative for its production (Abdin et al., 2003; Dhingra et al., 2000). Studies have been conducted in different laboratories to elucidate the biochemical pathway of artemisinin and its regulation with an aim to improve artemisinin content of the plants (Avery et al., 1992; Akhila et al., 1990; Xu et al., 1986).

Mevalonate (MVA) is the primary building block for isoprenoid biosynthesis in higher plants. It serves as a common precursor for the production of a number of compounds vital to normal plant growth and development, including carotenoids, the phytol tail of chlorophylls, plastoquinone and ubiquinone, as well as the phytohormones, *viz.* abscisic acid (ABA), cytokinins and gibberellins. Mevalonate also contributes to the formation of a wide variety of plant secondary metabolites such as phytoalexins, rubber,
terpenoids, and indole alkaloids. The final step in the mevalonate production is catalyzed by the branch point enzyme, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGR) (EC 1.1.1.34), which shunts HMG-CoA into the isoprenoid pathway, leading to the synthesis of various intermediates. These intermediates are finally used for the biosynthesis of various terpenes, viz., mono, di, tri, and sesqui including artemisinin (Towler and Weathers, 2007; Akhila et al., 1987; Kudakasseril et al., 1987). It has been reported that HMGR catalyzes rate-limiting step in mevalonate pathway (MaujiRam et al., 2010; Rodriguez-Concepcion and Gruissem, 1999 Lange et al., 1998; Gondet et al., 1992; Stermer and Bostock, 1987) and increased HMGR activity has led to the higher accumulation of isoprenoids such as phytoalexins, sterols, shikonin, lycopene and artemisinin (Nafis et al., 2010, Aquil et al., 2009; Rodriguez-Concepcion and Gruissem, 1999 Lange et al., 1998; Gondet et al., 1992; Stermer and Bostock, 1987). Recently it has been shown that besides the mevalonate pathway, a non-mevalonate pathway (MEP) operating in plastids also provides carbon for the synthesis of various sesquiterpenes including artemisinin, and DXR catalyzes the rate limiting step in this pathway (Towler and Weathers, 2007). The experiments conducted in our laboratory have however, clearly demonstrated that mevalonate pathway is the main pathway providing carbon for artemisinin biosynthesis as it contributes ~80% of the carbon (MaujiRam et al., 2010).

It has also been reported very recently that amorpha-4, 11-diene synthase (ads), catalyzes the first rate limiting step in artemisinin biosynthesis (Bouwmeester et al., 1999; Mercke et al., 2000; Wallaart et al., 2001; Alam et al., 2010). It catalyzes the cyclization of farnesyl pyrophosphate (FPP) supplied by mevalonate pathway into the sesquiterpenoid skeleton, amorpha-4, 11-diene. Based on these studies, we hypothesized that by increasing the cellular mevalonate pool and diverting it to artemisinin biosynthesis in A.
annua L. through over-expression of HMG Co-A reductase (hmgr) and amorpha-4, 11-diene synthase (ads) genes respectively, the artemisinin content in transgenic Artemisia annua L plants could be increased. Keeping the above facts in view, the present study was therefore, undertaken with the following objectives:

- To clone and sequence amorpha-4, 11-diene synthase (ads) gene from A. annua L.
- To evaluate the influence of over expression of amorpha-4, 11-diene synthase (ads) and HMG-Co-A reductase (hmgr) genes in Artemisia annua L. plants on artemisinin content and yield.