Introduction
"This day designing God
Hath put into my hand
A wondrous thing. And God
Be praised. At his command
I have found thy secret deed
Oh million murdering Death. I know that this little thing
A million men will save
Oh death where is thy sting? Thy victory oh grave?"

Above is the famous quote from Sir Ronald Ross, which he wrote to his wife on 20 August 1897 about malaria (Ross, 1923).

Malaria is a common and serious tropical disease. It is a protozoal infection transmitted to human beings by mosquitoes. Malaria is a public health problem in some 90 countries worldwide, inhabited by 36% of the world population, i.e. over 2 billion people. From decades, people are trying to fight against malaria by different means and ways, but unfortunately none of them have been convincing enough to get rid of the disease. With the advent of parasitology, several *Plasmodium* protein molecules like, circumsporozoite protein (Doolan et al., 1996), merozoite surface protein (Holder and Freeman, 1981), apical membrane antigen-1 (Deans et al., 1982), were surfaced which have immense potentiality as vaccine candidate antigen against malaria.

The most promising candidate to date has been a merozoite surface antigen-1 (MSP-1) that was identified by monoclonal antibodies. C-terminal fragment of MSP-1 protein, i.e., MSP1₁₉ is a 19 kDa protein. The conformation of MSP₁₁₉ is also thought to be critical for protection, as this region of MSP-1 has 12 cystein residues and consists of just two epidermal growth factor-like domains, each containing three disulfide bonds (Blackman et al., 1991). The amino acid sequence of this region is largely conserved, with only limited point mutations having been identified primarily at four positions (although rare variants have
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been reported) (Jongwuitwes et al., 1993; Qari et al., 1998; Sakihama et al. 1999). The four changes are E-Q at position 1644 in the second EGF like domain and TSR-KNG at position 1691, 1700 and 1701 in the second EGF like domain (Miller et al., 1993; Kang and Long, 1995). MSP1\textsubscript{19} is the target of a series of monoclonal antibodies that have ability to inhibit the invasion of red blood cells by parasites in vitro (Blackman et al., 1990; Chappel and Holder, 1993). Furthermore, immunization with recombinant protein from this region has shown protection against lethal \textit{P. yoelii} in mice (Daly and Long, 1993) and against \textit{P. falciparum} in \textit{Aotus} monkey (Good et al., 1998).

Several expression systems have been developed to express the \textit{Plasmodium} MSP1\textsubscript{19} protein at a higher level for the purpose of using them as vaccine molecule in several models. MSP1\textsubscript{19} protein has already been produced in \textit{E. coli} (Lalitha et al., 1999), yeast (Gozalo et al., 1998), Baculo virus (Hui et al., 1994) and mammalian cell (Burghaus et al., 1999).

Transgenic plants provide experimental system in plant molecular biology and plant biotechnology. Stably transformed plants can be produced by \textit{Agrobacterium}-mediated gene transfer (Zambryski et al., 1983). Plants which traditionally served as sources of food, fuel and fiber, now being engineered as novel biomanufacturing system, with particular attention focused on the creation of plants that produce proteins of potential pharmaceuticals value (Lyons et al., 1966). Many of the basic molecular tools and methods from agricultural biotechnology are directly applicable to meet the strict endpoints of pharmaceutical production in plants. As with mammalian and bacterial systems, higher expression levels can help reduce costs, due to lower throughput volumes in purification steps (Petrides et al., 1995; Young et al., 1997).
Plant genetic engineering has provided new tools for the creation of vaccine. Small segments of DNA are isolated from the pathogen and used to genetically engineered recipients to produce the antigen "subunit" (Haq et al., 1995; Arakawa et al., 1997).

Over the past fifteen years, a new vaccination strategy has emerged as the tools of molecular biology where immunogenic proteins of the infectious organism were identified that are responsible for the protective immune response. When the genes encoding these proteins are expressed in a heterologous system, and the resultant immunogens are isolated and utilized, they provide a subunit vaccine (i.e., the immunogenic subunit of the infectious agent). Subunit vaccine offers a major advantage as they are incapable of inducing disease even in immuno compromised individuals because they are free from the infection agent (Arntzen, 1997).

Two major strategies for the production of subunit vaccine in plants have been derived (Mason and Arntzen, 1995); genetic transformation of the nuclear genome of plants using gene vectors (Mason et al., 1992) and manipulation of the genome of plant pathogenic viruses (Usha et al., 1993; Turpen et al., 1995). The fundamental difference between these two approaches is that the former gives rise to plants that have the long term capacity to produce the desired vaccine. In contrast, the viral engineering approach gives a transcript expression system in which plants produce the immunogenic protein or peptide only when the plant is injected by the engineered virus.

Utilizing the earlier mentioned process, several researchers have successfully expressed various antigens (Mason et al., 1996; Arakawa, et al., 1997) in plant system. Such studies have demonstrated the abilities of plant system to ensemble full length antibodies (Hiatt et al., 1989) or functionally active human immunological molecules (Magnuson et al., 1998).

Tobacco is a model plant for expressing foreign proteins (Crammer et al., 1996). Transformation efficiency is higher and regeneration protocol is well set
for tobacco plant. Another important feature is easy hardening process and field survival of tobacco plants in transformation study. Tobacco also produces big and profuse leaves, which could be the source for production of large amount of foreign protein.

Thus, there is ample opportunity to produce malarial antigen as subunit vaccine in plant system and to characterize the plant-derived antigen for its immunogenicity. In that direction our objective for the present study are as follows.

- To produce transgenic tobacco plants expressing MSP119, a potential vaccine candidate from *P. falciparum*.

- To determine the immune response of the plant generated protein and to check the oral immunogenicity of plant produced protein in mice by oral feeding.