For centuries, plant breeders have developed new varieties through breeding programs in an attempt to induce or enhance a desired trait. Although this was an effective practice, the process was strictly limited to the alleles present in the same species or closely related species populations, due to the presence of sexual barriers. Therefore, introduction of a new trait from a phylogenetically unrelated species was a limiting step to broaden the genetic variability among the cultivated species. Since the advent of recombinant DNA technology in the 1970's, the last couple of decades have shown remarkable advances in plant genetics and the rise of plant transformation. These new advances have given researchers the ability to express foreign genes, in desired plant hosts, essentially breaking "sexual" barrier among living organisms.

Plant transformation can be defined as the process where foreign genes are integrated and expressed stably in plants. The technique involves the transfer of DNA carrying genes for a desired trait following one of the several methods available and then regeneration of a whole plant from the transformed tissue. Currently, the most widely used method for transferring genes into plants is *Agrobacterium*-mediated transformation. *Agrobacterium* is a naturally occurring pathogenic bacteria in the soil that has the ability to transfer a well defined part of Ti plasmid DNA (T-DNA) into a plant's genome. Normally, *Agrobacterium* infect the wounded cells and transfer T-DNA. Expression of genes present in the T-DNA region causes a characteristic growth referred to as a crown gall tumor. Scientists have taken advantage of this naturally occurring transfer mechanism, and modified the Ti plasmid by replacing the oncogenes responsible for crown gall formation with that of desired genes into the plant (Grimsley, 1990).

Fraley and colleagues reported the successful incorporation of a kanamycin resistance gene into the tobacco genome (Fraley, *et al.*, 1983). Later year, several other researchers reported similar results (Horsch *et al.*, 1985; Paszkowski *et al.*, 1992), marking the beginning of plant transformation. Since then, successful transformations have been described for over 120 species in 35 different families

(Birch *et al*., 1997) and the number is increasing every year. Some products of plant genetic engineering are already on the market in various regions of the world. Examples include a slow-softening tomato and cotton plants resistant to herbicides and insects. With many more products in the pipeline, the genetic engineering of plants will have a profound impact on the future of agriculture all over the world.

A major challenge in transgenic approach to develop crop plants that can sustain various biotic and abiotic stresses is the lack of foreign gene expression at a desired level in a specific tissue. Various approaches have been adopted to enhance the transgene expression in plants that include the use of strong promoters such as cauliflower mosaic virus 35S (35S CaMV) (Battraw *et al*., 1990), rice actin (Park *et al*., 1996; Xu *et al*., 1996; Cao *et al*., 1992; Zang *et al.*, 1991; McElroy *et al.*, 1991), maize ubiquitin (Toki *et al*., 1992; Christensen *et al*., 1992) etc. were used. The combination of enhancer sequences with promoter such as 35S CaMV/35S enhancer (Timmermans *et al*., 1990), *Adh1*/intron, *Actin*/intron, 35S/*Adh* intron1 and *ubiquitin1* (first exon and first intron of maize ubiquitin) (Cornejo *et al*., 1993) also shown to improve the expression level when comparison with their promoter/gene directly.

The *Bacillus thuringiensis* (Bt) produce a variety of crystal proteins (Cry proteins) with insecticidal activity against a large group of insects (Schnepf *et al*., 1998). The specificity of these insecticidal Cry proteins have made *cry* genes as an attractive candidates for genetic engineering of crop plants for protection against insect predation (Brunke and Meeusen, 1991**,** Tabashnik, 1997). However, the native *cry* genes express poorly when introduced into plant nuclear genome (Vaeck *et al.*, 1987). Improvement in the expression levels of Cry proteins in plants was achieved by the modification of potential eukaryotic message destabilizing sequences (Murray *et al*., 1991), by introducing truncated forms of *cry* genes and through the modification of by eliminating the mRNA destabilizing sequences (Perlak *et al*., 1991). Considerable increase in the expression of Cry protein (ca. 0.8% of soluble protein) was obtained also through promoter optimization, protein targeting (Wong

*et al*., 1992) and codon optimization of the entire Bt gene(s) to conform the codon usage of the recipient plant (Nayak *et al.*, 1997). However, to control predation by insects and to delay the emergence of resistance using transgenic approach, these expression levels are still low and may be effective only when pest populations remain at a low density (McGaughey and Whalon, 1992)**.**

There is a steady increase in the number of recombinant proteins expressed in plants as plants are considered as cost-effective “natural bioreactors” for large scale production of foreign proteins for industrial application (Giddings, 2001). A number of antigens that have been expressed in plants include cholorea toxin, subunit B from Vibro cholerae, capsind protein from Norwalk virus, Surface antigen from Hepatitis B virus Drg24 antigen from Rabies virus. Hiatt *et al*. (1989) demonstrated for the first time the production of functional antibodies in plants. Since then, a considerable amount of effort has been put in developing transgenic plants for the production of antibodies, also known as “plantbodies”. Other biopharmaceuticals produced in plants include Human protein such as serum protease (Cramer, *et al.*, 1996)**,** hirudin (Parmenter, *et al*., 1995), granulocyte-macrophage**,** colony-stimulating factor (Kwon, *et al*., 2003)**,** ctxB (Jani, *et al*. 2002), erythropoietin (Matsumoto *et al*., 1995)**,** serum albumin (Farran *et al*., 2002)**,** aprotenin phytoene synthase (Lindgren e*t al*., 2003), Ama1 seed albumin (Chakraborty *et al*., 2000)**,** ferritin (Semenyuk *et al.*, 2002). However, high level expression of these heterologous proteins in plants is critical for any successful application of this important technology.

Micronutrients are very important for humans, especially where vegetable based diets are primary food source. Although supplements added to food or taken in tablet form are effective in preventing and controlling micronutrient, such treatment are difficult to implement in developing counties because of the associated costs and the small number of primary health care program. Identifying the genes needed to increase the levels of essential micronutrients in staple crops is an immediate goal that would have a significant impact on human nutrition world-

wide. All plants synthesize vitamins and nutrients, genes for their synthesis can be isolated, reconstructed and transferred from any plant system.

Addition to micronutrients, production of antigens in plants is very attractive due to low costs involved in the production. More than two billion people worldwide are infected with hepatitis B virus, a serious liver infection that can result in jaundice, cirrhosis, and liver cancer (Walmsley and Arntzen, 2000; Richter *et al*., 2000). The currently hepatitis B vaccine is produced using yeast that requires some chemical modification to become active, increasing the cost of the vaccine, which also must be stored under refrigeration. This has severely limited its utility in third world regions where the disease in rampant, it is unavailable to more than one-third of the world's population, especially in poor countries where the vaccine is in urgent need. Plant-based vaccines can be grown locally, reducing the cost and complications of transportation, while the stability of proteins in intact plants removes the need for refrigeration. Furthermore, the edible nature of the vaccines eliminates the need for syringe-based delivery, saving money and reducing the risk of infections. However, the problem was to increase vaccine protein amount in transgenic plant which is very low presently. A stronger immune response could be triggered if they were able to increase the amount of protein produce.

Recently, an alternative approach was developed for overexpression of foreign genes in plants through chloroplast genetic engineering. Plastids of higher plants are cellular organelles with circular, double-stranded genomes of 120-160 kb in size. The genome of each plastid encodes approximately 120 genes: each cell contain up to 10,000 identical copies of each plastid gene (Maliga, 1993, 2002). Thus, it is ideal system for high level of recombinant protein expression in plants. However, chloroplast transformation is achieved routinely so far only in tobacco (Maliga, 2003). Moreover, posttranslational modifications such as glycosylation of recombinant proteins may be a limiting factor when expressed in chloroplasts that resemble prokaryotes. Keeping in view the limitations posed, we have developed a

new expression system based on the transcription of the foreign gene directed by an introduced T7 RNA polymerase (T7 RNAP).

Bacteriophage T7 RNA polymerase (T7 RNAP) is known to be one of the simplest enzymes catalyzing RNA synthesis. In contrast to most RNA polymerases known, this enzyme consists of one subunit and is able to carry out transcription in the absence of additional protein factors. Owing to its molecular properties, the enzyme is widely used as a tool for synthesis of specific transcripts, as well as being a suitable model for studying the mechanisms of transcription.

Our major objectives in this study are:

1. To develop an expression system that can increase the production of recombinant proteins in plants.
2. To develop a regulated expression of recombinant proteins with chemically inducible mechanisms
3. To express recombinant molecules in a highly tissue specific manner.

In this study, the T7 RNAP based expression system has been tested for the overexpression of a reporter (*uid*A) gene using a number of plant tissue specific promoters. In addition, the T7 RNAP has been also tested for the overexpression of *uid*A in rice. Thesis also contained the results on our attempt to induce the expression of foreign genes through the application of low amounts of tetracycline[(*)]. Also attempts were made to express ferritin in rice endosperm and Hepatitis B virus surface protein (HBsAg, an oral vaccine candidate) and lycopene (pro-vitamin A) in tomato fruits using the newly developed expression system.

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[(*)] Part of the work with title: "Bacteriophage T7 RNA polymerase directed inducible and tissue specific overexpression of foreign genes in transgenic plant" has been applied for the patent in ICGEB and results were accepted for publication in Plant Biotechnology Journal.