Chapter One
Introduction

1.1 Introduction

In recent years, there has been a resurgence of interest in antibody engineering due to the increasing demand for various applications, including diagnostics, therapeutics and prevention of diseases (therapeutic antibodies). Antibody molecules have specific binding activity which recognize and bind to small or large molecules such as haptens, nucleic acids, polysaccharides and proteins.

Additionally, the discovery of hybridoma technology by Kohler and Milstein in 1975 and subsequently, the advent of combinatorial display libraries which initiated the production of monoclonal antibodies (mAbs) have also paved the way for other numerous developments (Kohler and Milstein, 1975)(Daly *et al*., 2001).

Recombinant antibody display technology has been developed as an alternative approach for the generation of antibodies and antibody fragments in various expression systems such as human, rabbit, mouse, sheep, bacteria, plants and more recently (Daly *et al*., 2001). In this approach, the properties of antibody molecules can be altered to create functional-novel improved molecules under cost-effective production systems (Verma *et al*., 1998). The nature of immunoglobulins is very amenable to protein engineering approaches through the development of antibody fragments which include the antigen-binding (Fab) and single chain variable fragments (scFv) by either proteolytic cleavage of individual domains of antibodies or via cloning and expression of the PCR-amplified immunoglobulin genes in various expression systems (Padoa and Crowther, 2006).

Recently, there has been increased interest in utilizing different organisms as biorectors for production of heterologous molecules required for pharmaceutical,

diagnostic or industrial processes (Stoger *et al*, 2002). In this study, the technologies were assessed as applications for the production of diagnostic reagents. The scFv antibodies developed in the two expression systems (bacteria and plant) were against *Cucumber Mosaic Virus* (CMV). This plant virus is a virulent pathogen affecting a very broad range of host (Roossinck, 2001). Outbreaks of CMV infections could bring severe yield losses in many economically important crops. To date, most crops are protected from the infections through phytosanitary measures, by early detection and by using virus free starting material. In the field, immunoassays are advantageous because it is amenable to high throughput processes which require minute amount of samples. Also, this method is easily done, and relatively economical, sensitive and highly specific.

Immunoglobulins are widely used in research, medicine and industry, yet the extraction of proteins from their natural sources can sometimes be difficult and expensive (Ma *et al*, 2003). Recombinant antibodies such a scFvs present an alternative source of reagent especially for use as detection reagents. For commercial production, the antibody fragments need to be produced cheaply without a great deal of time or expertise.

There has been no 'universal' expression system which can guarantee high yields of recombinant protein, as each system possess its own problems in terms of expression (Verma *et al*., 1998). The major target for this research involves the comparison of two expression systems. The scFv antibodies were previously developed in *E. coli* (Tetsushi, 2004) and tobacco plants (Ng, 2004) respectively.

1.2 Objectives

The overall aim of this study was to compare the utility of scFv derived either from the bacteria *Eschericia coli* or from transgenic tobacco plants as a source of specific antibody for developing an Enzyme-Linked Immunosorbent (ELISA) for detection of *Cucumber Mosaic Virus*.

The specific objectives were :

i. To extract and purify scFv from both expression systems

ii. Standardization of CMV antigen for an ELISA test system

iii. To compare the binding affinity of the plant-derived scFv and the bacteria-derived scFv in the ELISA test system

iv. To determine the sensitivity of the antibody-antigen interaction