CHAPTER I
INTRODUCTION

1.1 Rationale

As food demand and supply fall out of balance, the sustaining power of globalization is breaking down. The cost of food in many places has either remained static, or continued to rise. In many parts of the world, increased agricultural growth and sustainable food plant will play a key role in addressing the current world food crisis, in contributing to overall economic growth. As a major exporter in the world's agricultural food market and its agribusiness sector includes one of the world's largest multinational corporations, Thailand still remains potential for further large increases in productivity from known technologies.

Jerusalem artichoke (*Helianthus tuberosus* L.) is the member of the family Asteraceae, have originated in the North America and can be propagated by using tubers. Instead of starch form storage in the most plants, tubers of Jerusalem artichoke is rich storage sources for inulin and other fructo-oligosaccharides that may provide several health issues such as dietary health benefits for obesity, diabetes, and heart disease (Orafti, 2005). The fleshy tubers can be used as a fresh or cooked vegetable for humans, ingredient substitute for antibiotic in animal feed (Denoroy, 1996). The high-fructose syrups derived from the tubers may be used by food industries as a sweetener. Moreover, the inulin from Jerusalem artichoke tubers also has been proposed for many years as a possible substrate for the production of ethanol with possible use for biofuels (Denoroy, 1996). With its excellent potential, Jerusalem artichoke is an underutilized resource that possesses the potential to meet major health and energy challenges.

The evaluation of genetic background of germplasm is essential to plant breeding. Different breeds have been selected to fit different environmental conditions and human needs. There are several reports which have been used Sequence Related Amplified Polymorphism (SRAP) technique, for example, in *Cucurbita pepo* L. (Ferriol et al., 2003), *Bixa orellana* L. (Valdez-Ojeda et al., 2008), sugarcane (Suman et al., 2008), or wheat (Zaefizade and Goliev, 2009). Only genetic diversity

information of *H. tuberosus* derived from Randomly Amplified Polymorphic DNAs (RAPD) and Inter Simple Sequence Repeat (ISSR) are available to date, a considerable number of genetic distance studies should be carried out. So far, no studies have been reported on application of the SRAP markers in genetic diversity of *H. tuberosus*. This study was conducted to understand the genetic diversity and genetic relationships of 47 cultivars of *H. tuberosus* using SRAP markers. This result may lead to the evaluation of genetics relation between cultivars which is importance for breeding program to improved, for example, increasing gain yield and disease resistance, also valuable for germplasm management.

H. tuberosus is prone to many diseases caused by fungi, bacteria, and viruses, of which the most detrimental are those caused by fungi. Two of the most important fungal pathogens are *Sclerotinia* and *Phomopsis*. Within the genus *Sclerotinia*, *Sclerotinia sclerotiorum* is more widely distributed around the world. *S. sclerotiorum* also incites a head rot and mid-stem rot of Jerusalem arrtichoke. Since *Sclerotinia* spp. has a very wide host range and can persist in soil as dominant sclerotia for up to 10 years, the pathogens have been extremely difficult to control with either generic resistance or fungicides. The most effective control is the use of long rotations, tolerant hybrids, and sclerotium-free seed. Gray stem spot is a relatively new disease of sunflower family. It was first observed in the early 1980s in Yugoslavia and now considered the most devastating sunflower family disease in eastern Europe. Symptoms include large stem cankers and subsequent wilt. *Phomopsis helianthi* has been identified as one incitant of gray stem spot, but another *Phomopsis* species may also be involved, both in Europe and in the United States. Foliar fungicides can control gray stem spot, but the development of resistant hybrids has been much more cost-effective.

Many recognition-dependent plant disease resistance (R) genes encode proteins comprised an amino-terminal domain, a nucleotide binding site (NBS) domain, and leucine-rich repeats (LRRs) (Bent, 1996; Hammond-Kosack and Jones , 1997; Hulbert et al., 2001). NBS-LRR encoding genes are often highly duplicated and evolutionarily diverse, with hundreds of family members in plant genomes (Meyers et al., 1999; Meyers et al.,2003; Dangl and Jones, 2001; GoV et al., 2002; Richly et al., 2002; McHale et al., 2006; Jones and Dangl, 2006; Ameline-Torregrosa et al., 2007

and Kohler et al., 2008) which have been isolated from numerous species by cloning and sequencing genomic DNA fragments amplified by degenerate oligonucleotide primers complementary to conserved amino acid sequence motifs in the NBS domain, typically from the P-loop to the GLPALP motif (Kanazin et al., 1996; Leister et al., 1996; Yu et al., 1996 and Shen et al., 1998). The EST database has been used successfully in other plants to be a good source of identified NBS-LRR coding sequences. (Radwan et al., 2008, McHale et al., 2009; Rossi et al., 2003; Leng et al., 2009). Here we present the isolation of R genes from Expressed Sequence Tags (EST), *H. tuberosus* database.

1.2 Objective

1.2.1 Molecular genetic characterization of 47 *H. tuberosus* genotype, and provide the genetic relationships among them.

1.2.2 Generation of EST-SSR primers link to disease resistance gene in *H. tuberosus*.

1.3 Anticipated output

1.3.1 Information and genetic distance derived from this research available for use in *H. tuberosus* improvement programs.

1.3.2 The EST-SSR primers derived from EST sequences will be further useful for improving disease resistance in *H. tuberosus* lines.

1.4 Scope of the study

This research uses 47 *H. tuberosus* genotypes which have originated from Canada, The United States of America (USA), The Union of Soviet Socialist Republics (USSR) and France. Molecular characterization was performed using SRAP and EST-SSR marker. The research was conducted into 2 main areas.

1.4.1 The genetic diversity among 47 *H. tuberosus* genotypes were explored using Sequence Related Amplified Polymorphism (SRAP) markers. Nine primer pairs were used to generate the genetics distance. Data were analyzed with NTSYS-pc V2.0 and UPGMA WPGMA, Single Linkage and Complete Linkage clustering methods based on Jaccard's similarity, Dice's and Simple matching coefficient. The cophenetic

correlation were calculated and original matrices were compared by applying Mentel's test. The dendrograms bases on different coefficients were compared consensus fork index (CI_C). The CI_C index provides a relative estimate of the dendrogram similarities and was calculated using NTSYS pc 2.1 (Rohlf, 2000).

1.4.2 The The EST sequences from the Compositae Genome Project Database (CGPDB; http://compgenomics.ucdavis.edu/composi tae_index.php) were retrieved. Redundant sequences were removed using a local nucleotide BLAST search with ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) software. Short tandem repeats were identified using Websat software (http://wsmartins.net/websat/). Primers were designed flanking the repeat sequences. PCR amplifications were carried out and selected amplified fragment were confirmed by sequencing. Finally, to evaluate the usefulness of designed EST-SSR markers, they were used to analyze genetic diversity among 47 *H. tuberosus* genotypes.