CHAPTER 2

LITERATURE REVIEW

2.1 Origin and production of pea

2.1.1 Origin

Pea, *Pisum sativum L.*, is a member of the cultivated legume crop belongs to the Order Fabales, Family Fabaceae (*Leguminosae*) and Tribe Vicieae (Zhang, 2004). The Fabaceae is a large and diverse family of approximately 450 genera and about 12,000 species (Myers *et al*., 2001). Peas are diploid with a chromosome number of x = 7 (Gritton, 1986). It is generally agreed that peas have been domesticated 8,000-10,000 years ago in the Fertile Crescent region of West Asia in associated with small grains and other pulses (Muehlbauer, 1997). Based on genetic diversity, four centers of origins, namely, Central Asia, the Near East, Abyssinia and the Mediterranean have been recognized (Gritton, 1980). Then, those of them spread to Russia to westward into Europe and eastward into China and India. Production then spread to the Western Hemisphere upon discovery of the new world (Muehlbauer, 1997). Peas were the first subject of the systematic plant breeding efforts of Knight and provided the tool for Mendel to elucidate the science of genetics. Genetic changes that led to domestication of pea included reduction in the pod fiber associated with thinner and more permeable testas, and increased seed size (Myers *et al.*, 2001). For most purpose, peas are divided into the grain legume types, field or dry peas, and vegetable types, the immature seeds or edible pods. Consumed peas as vegetables can be divided into the shell or garden pea (immature seeds) and edible pod peas which are comprised of two market classes, snow pea and snap pea. In addition, vegetable

types are also including pea sprouts or shoot and leaf consumption which are popular among Asian food.

Snow peas or others common name ‘sugar pea’ or ‘Chinese pea’, *P. sativum* var. *saccharatum*, is an edible pod type of peas, have been derived most likely in Europe, but it is not known exactly when or where the first snow peas were developed. Edible pod peas are not specifically named in Greek and Roman writings, although pea culture in general was discussed (Myers *et al*., 2001). In Southeast Asia, there was an evidence that snow pea was one of the earliest-known cultivated plants, with evidence of having been cultivated in a region that is now along the Thailand-Burma border, 12,000 years ago (Fondevilla *et al.*, 2008).

Snow peas have been derived from both field and garden peas, as reflected by their varietal characteristics. For example, the cultivar ‘Dwarf Gray Sugar’ was probably derived from a field pea by selection of a spontaneous mutant that had less fiber pod. While, the other snow pea cultivar ‘Mammoth Melting Sugar’, white flowered, large pods and large seeds, landrace types, was derived from garden peas groups which was occurred mutations. Contemporary snow pea cultivars have been bred from crosses between snow and garden peas. For example ‘Oregon Sugar Pod’ came from a cross of ‘Dwarf Gray Sugar’ with OSU 102 (a garden pea with typical frozen pea characteristics) (Myers *et al*., 2001).

2.1.2 Production

Snow peas may have white or purple flowers, tall or short vines, smooth or wrinkled seeds (Myers *et al*., 2001).The snow peas lack of pod parchment or fiber, unlike field or gardens peas that have fibrous pods. Within in edible pod group, the

most important trait that makes snow peas differ from snap peas is the thin walled pod, while snap pea pod wall is thick that develop tightly around the seed and become round in cross section at maturity. Pod shape can be divided into beaded forms and sword shaped forms. Immature pod color may be yellow (waxy), light green, green, dark green or in some forms with pigmented flowers (Makasheva, 1986). Seed may be pigmented when mature (Gritton, 1986). Generally, edible fresh pod types of pea are consumed when pods have enlarged, but prior to seed development, thus snow pea pods are typically large but flat at harvest stages (Myers *et al*., 2001).

Snow peas are popular in both developing and developed countries whereas garden peas and dry pea are mostly consumed in Europe and the United States. Edible pod peas are widely grown in home gardens and for fresh market consumption in small hectare that are seldom measured (Myers *et al*., 2001). In Thailand, according to Chumpirom *et al.* (1989) reported that only 2,000-3,000 rais of snow peas was cultivated. While the consumption demand of snow peas is quite high but the amount of products is less, due to lack of proper cultivars and low quality of seeds. In USA, the total production areas in 1995 were 11,246.46 ha which gave the average yield 2.62 tons per acres (Gaskell, 2010) However, about 13,000 tons of fresh edible pea pod, the majority of which are snow peas, have been still imported annually into the United States. Fifty percent of production came from Guatemala and forty-five percent imported from Mexico. China is also the big production area for exporting but it differed in cultivars with stringy type and most of them are hand-harvested and stringed (Myers *et al*., 2001).

Snow pea is a cool season crop and widely grown in the cooler temperate zones and on the highlands of tropical regions of the world (Gritton, 1986). The seeds

may be planted as soon as the soil temperature reaches 10 °C (50 °F), with the plants growing best at temperatures of 13 to 18 °C (55 to 64 °F). They do not thrive in the summer heat of warmer temperate and lowland tropical climates but do grow well in cooler high altitude tropical areas. In Thailand, the important snow pea production areas are mostly located in the northern part of Thailand especially in Chiang Mai and on the highland in Petchaboon Provinces. They are suitable to grow snow peas all year round, due to the cool climate whereas lowland production areas in Lam Pang, or some provinces in the northeast and central part of Thailand can produce only in winter season (Chumpirom *et al.*, 1989). Pongphal (2003) revealed that yield of fresh pod and seed production of snow pea in winter season during November 2003 to January 2004 in two different locations which had different attitude between lowland plot at Maejo University, and on the highland at Mae Sa Mai Royal Project Development Centre, was not different. However, on lowland, snow pea cultivation seem to be difficult referring to the experiment of Kongsombat *et al*. (1996). Five commercial garden pea cultivars were grown in winter and rainy seasons at Lam Pang Research Institute of Agricultural Technology. The result showed the trial in winter season was successful while the trial in rainy season was failure, due to environment was not suitable for the production. Wejvitan *et al*. (1996) found that the greater amount of yield of four snow pea seed cultivars production in Sakolnakon Research Institute of Agricultural Technology was obtained in September. In Pakistan, pea is cultivated under a wide range of agro-ecological zones. It is cultivated during winter on plains of Pakistan and during summer on highlands (Habib and Zamin, 2003; Nisar and Ghafoor, 2009). In addition, in USA, the important snow pea production areas are along Mediterranean coast, especially in California, due to the climate is cool all year

round (Gaskell, 2010) whereas in Kentucky, in England, snow peas can be planted only in early spring to ensure good yields (Kentucky Cooperative Extension Service, 2009).

2.1.3 Powdery mildew disease is a barrier in pea production

There are many factors affecting productivity of snow peas such as variety, climatic, soil fertility and good management. Besides, vegetative and reproductive growth of snow peas were affected by the pest. The productivity of snow pea decreased when those of them were attacked by diseases and insects. Several diseases are reported to be found in the snow pea production such as ascochyta blight (*Acochyta pisi*), mycospeaerella blight (*Mycospeaerella pinodes*), fusarium wilt (*Fusarium oxysporum* f. sp. *pisi*), virus, bacteria diseases and, especially, powdery mildew (*Erysiphe* spp.) (Kraft and Kaiser, 1993).

Powdery mildew is an economic disease of worldwide snow pea production areas (Schatz *et al.*, 2003). It causes from the one major fungus, *Erysiphy* spp., air-borne disease, which can infect in all snow peas production areas and they are easily distributed by wind into the new crops. The pathogen can affect all green parts of plant so the diseases are very harmful to the quality and yield of snow pea (Beckingham, 2001).

The pathogen of powdery mildew disease has many species but the most important species which is often reported in cool weather production areas was *E. pisi*, the obligate parasite as a perfect stage (Kraft and Kaiser, 1993), whereas in subtropical snow pea production area, especially, in the northern part of Thailand, was attacked by the *Oiduim* spp., as an imperfect stage (Wanasiri, 2007). Due to the

pathogen is seed borne fungus, it usually survives as conidia over summers on infected plant debris and on alternative hosts and produce spores, which are blown by wind into new crops. Powdery mildew causes a white “powdery” spot on the lower leaves and stems and the disease continuously spread up to the upper leaves. White mycelium (fungal threads) is built and those of them grow only on the surface of the plant. They never invade the tissues themselves. The fungi feed by sending haustoria or root-like structures, into the epidermal cells of the plant (Fondevilla *et al.*, 2006).

Disease symptoms may be systemic or expressed only on leaves or pods (Kraft and Kaiser, 1993). Severely infected plants could not mature normally (Schatz *et al.*, 2003), they always become stunt and distort (Kraft and Kaiser, 1993), the leaves turn to be yellow, wilt and fall off (Aked and Hall, 2006). According to Fondevilla (2007) the disease reduces the total biomass yield, number of pods per plant, number of seeds per pod, plant height and number of nodes. Moreover, severe pod infection can cause a gray brown discoloration of the seeds. These seeds have an objectionable flavor that lowers the quality of the grain (Aked and Hall, 2006). Kraft and Kaiser (1993) stated that periods of high relative humidity stimulate infection of the pods. Infected pods are deformed and covered with yellow to brownish area, with superficial blistering (Figure 2.1). In addition, in highly infected plants, they are killed before flowering (Kraft and Kaiser, 1993).

A) B)

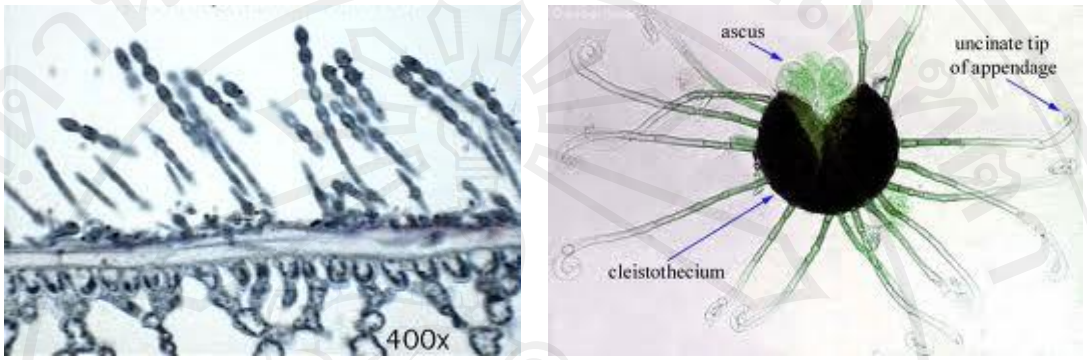
Figure 2.1 Powdery mildew diseases infected on pea

A) Leaf and stipule and

B) Pod

Weather plays an important part in the occurrence of powdery mildew (Fondevilla *et al*., 2006). Under favorable conditions, the disease may completely colonies a plant in 5-6 days. Once a few plants become infected, the disease rapidly spreads to adjacent areas. It is most severe when day temperatures are warm and night temperatures cool (Kraft and Kaiser, 1993). The high relative humidity of the air is needed for pathogen's spore germination (Fondevella *et al*., 2006). The favorable conditions for this disease are temperature between 15-25°C, humidity over 70% RH during growing season, flowering and pod filling can cause severe damage (Richardson, 2008). In addition, Schatz *et al.* (2003) reported that night time dews are sufficient for the disease development. Wet or heavy dew conditions help to spread the disease to upper leaves, flowers and pods. Moreover, crowded snow pea plantings where air circulation is poor and damp, shaded area, help to increase the diseases. On the other hand, heavy rainfall is not favorable for the disease, as it will actually wash

spores off plants. In additional, sprinkle irrigation can help to reduce the disease severity (Kraft and Kaiser, 1993).



A) B)

Figure 2.2 Characteristics of powdery mildew

A) Conidia of *Oidium* spp.

B) Cleistocarps with appendages of *Erysiphe* spp.

According to various planting times and stages of plant, the combination of these two factors can make different in disease severity. Young and succulent growth is more susceptible than older plant tissues. Moreover, late planting of snow pea in the field will increase more disease severity. In UK, Schatz *et al.* (2003) indicated that late planting field pea beyond mid-May resulted in plants more susceptible to powdery mildew and yield loss typically doesn't occur unless the infection occurs prior to or during early pod set. In western Canada, this parasite caused severe damage to late seeded crops or when hot and dry conditions occur in July (Tiwari *et al.*, 1998). In Thailand, all snow pea production areas and cultivation seasons can be infected by powdery mildew but the heavily infected time was found during November to March, and decreased in rainy season (Buakhao, 1993).

Generally, the decrease in pea production is due to severity of infection (Tiwari *et al*., 1998) and the disease can cause 25-50% yield losses and instability in yield of pea (Fondevilla, 2007) and heavily infected may make snow pea plants dies. Beckingham (2001) revealed that the diseases could affect all green parts of plant including pods. Due to the pathogen is seed borne fungus, it usually survives as conidia over summers, on infected plant debris and on alternative hosts and produce spores, which are blown by wind into new crops. In India, pea cultivation area was reduced from 1,325,000 ha in 1963 to 443,000 ha in 1983 causing by this disease (Janila and Sharma, 2004).

To control the disease, several methods are used to protect and decrease the disease severity. Chemical usage method is very popular to farmers, due to their results in rapid controlling, whereas this method extends to high cost of production, and not safe to the farmers, the consumers and environment. However, many controlled methods were referred such as cultivation methods. Iannotti (2010) stated that controlling the amount of nitrogen fertilization usage, avoiding overhead watering to reduce the relative humidity, and destroyed the infected plants, could reduce the disease occurrence. Schatz *et al.* (2003) indicated that the disease controlling by using the combination at early planting and tolerant varieties in UK production areas could aid in reducing risk with this disease. However, planting of resistant cultivars is one of the most efficient and effective methods in controlling disease. Resistant phenotype reduces the growth, reproduction, or disease-producing this activities of the pathogen. Eventhough host resistance eliminates or minimizes losses due to diseases and reduces the cost and other controls. Resistance is compatible with other methods of disease control and it can be integrated easily in

pest management programs (Pataky and Carson, 2004). Thus, resistant cultivars seem to be the favorable method that is widely used to reduce the powdery mildew diseases in the current snow pea productions, due to the natural controlling method with safe and cheap.

2.2 Genetic basis for powdery disease resistance

Keller *et al*. (2000) stated that there are two types of genetic mechanisms for disease resistance, monogenic resistance based on single gene whereas quantitative resistance depends on two or more genes (non-race specific). In most case single resistant genes confer complete resistance but are only active against certain races of the pathogen (race-specific) while quantitative resistance shows on obvious genetic interaction with the pathogen and slows down the disease development by increasing latency period and other parameters related to the epidemic.

Resistance to powdery mildew was firstly described by Harland (1948) as the single recessive *er-1* gene. Another recessive gene, designated *er-2* was found by Heringa *et al.* (1969) and both of two recessive gene control of powdery mildew resistance, *er-1* and *er-2,* are independent. Combining both resistance genes in a cultivar could increase the durability of resistance (Tiwari *et al*. 1998). In addition, Fondevilla (2007) discovered a new gene, *Er-3,* which resist to powdery mildew in *P. fulvum* and this gene displayed a dominant nature and phenotypic expression. Sharma and Yadav (2003) revealed that the new resistant genes which found in *P. fulvum*, was *Adi Umb Astr Le R.* The resistant gene of *P. fulvum* is more resistant than *P. sativum* to several pea diseases and insects. Timmerman *et al*. (1997) stated that disease resistance can either be monogenic, encodes by a single gene, or quantitative,

encoded by a number of genes. There are several researches about these recessive genes. Genetic analyses have shown that gene *er-1* is present in many resistant lines (Tiwari *et al.*, 1998). Vaid and Tyagi (1997) revealed that the pea cultivars HPPC-63, HPPC-95, DPP-26, DPP-54, Mexique-4, SVP-950, Wisconsin-7104 and P-6588, and JI2302 of Fondevilla *et al*. (2007), had a single recessive gene and showed resistance to *E. pisi*. In addition, none of the qualitative trait was linked to powdery mildew disease (Nisar and Ghafoor, 2009).

Sharma and Yadav (2003) confirmed the nature of resistance and defined allelism from 2 crossing, Pusa 10 × Dharwad and P 1542 × Dharwad. Dharwad is powdery mildew resistant accession while the others are susceptible accessions. The materials derived from the 2 crosses were advanced as single progenies (single seed descent), and all possible gene combinations have been evolved. The results suggested that only a single locus controlling powdery mildew resistance exists in the genus *Pisum.* This locus recognized as *er-1*, and mapped on chromosome 6.

Fondevilla *et al.* (2006) and Fondevilla *et al.* (2007) stated that the resistance mode governed by *er-1* was based on a pre-penetration barrier, the penetration to epidermal cell was prevented and very few haustoria or colonies were formed while the *er-2* and *er-3* was expressed as a post-penetration hypersensitive response. Gene *er-1* could bring about full resistance, while gene *er-2* found in the pea resistance line JI2480 showed complete resistance only when the temperature was at 25˚C or only in mature leaves, whereas the expressed of *er-3* was independent of the temperature. Both genes are inherited independently from each other (Nisar *et al*., 2006; Ondrej *et al*., 2003; Tiwari *et al.* 1998 and Heringa *et al.* 1969).

The resistance encompasses a wide variety of host-pathogen interaction. Resistant reaction varies in both degree and kind (Pataky and Carson, 2004). Nisar *et al*. (2006) investigated the resistance to powdery mildew of 177 genotypes of *P. sativum* collecting from 23 countries. The result showed the disease severity was different among cultivars. Only three cultivars of pea, Fallon, PS99102238 and PS0010128, showed highly resistant genotype as localized infection symptom and eleven cultivars, Shawnee, Lifter, Franklin, PS610152, PS810240, PS710048, PS610324, PS810192, CGN3273, CGN3272 and PS9910181, showed symptoms after inoculation but the infection was not severe and recovery was rapid.

McDonald and Linde (2002) reported that generally, single gene-controlled resistances are ephemeral due to the rapid evolution of pathogen virulence. Particularly, pathogens such as the powdery mildew fungi show a high risk of resistance erosion due to the coexistence of sexual and asexual stages and their high dispersal capability, while the races of *E. pisi* with specific virulence have not been described. There are many reports presented that the particular host resistance to the specific race pathogen change to be susceptible to others race of powdery mildew disease. According to Ondrej *et al*. (2005) revealed that sixteen cultivars of pea resistance to *E. pisi* had been attacked by another powdery mildew species, *E. baeumleri*. Only one cultivar, Tudor (Cebeco 4119), was found to be completely resistant to *E. baeumleri*. In addition, a few out of nineteen pea genotypes with *er-1*, Fallon, AC Melfort and Joel, demonstrated at a high level of resistance in field condition while the other cultivars, Consort R, SGL 2024, SGL 1977 and Franklin, were very susceptible to *E. baeumleri*. The susceptible controlled check genotypes without gene *er-1*, Komet, Adept and Gotik, were not attacked by *E. baeumleri*.

Keller *et al*. (2000) stated that some plant lines were resistant to a particular disease where others were susceptible. In addition, resistant crops could become susceptible, even after showing good resistance in the field for several years. This problems cause from the genetic characterization of disease resistance in plant-pathogen interaction. Thus, for the developing stable resistance in a variety, sources of resistance with a broad genetic base would be imperative (Kalloo, 1988).

2.3 Breeding for powdery mildew disease resistance in pea

Breeding for disease resistance has assumed greater importance in a number of vegetable crops than improvement for yield or quality. Consequently, there has been remarkable achievement in the development of cultivars resistance to diseases and extensive use of such resistant cultivars impedes epidemics of pathogens and maintains a biological balance in the environment (Kalloo, 1988). The introducing resistance genes through plant breeding, plant species with carry genes for disease resistance are the prerequisite. Sources of resistance must be identified by the well-established technique of the screening of germplasm and further assessment of the genetic material (Timmerman *et al.*, 1997).

Current breeding programs for many crops, concentrate on more durable forms of resistance rather than a resistance based on major genes. Cultivars of crop plants possessing quantitative resistance to plant disease show continuous variation ranging from very low to high levels of resistance and the rate of epidemic progress is usually reduced for these cultivars. Quantitative resistance is preferable to qualitative resistance as it has proved, in many cases, to be long lasting. However, identify

quantitative resistance, and using it in plant breeding programs, is more difficult than for qualitative resistance (Vilijanen *et al*., 1997).

Due to peas is self-pollinated crop, the most common breeding strategies employed in pea improvement programs are backcross, pedigree, single-seed descent and bulk, and various modifications of those methods (Gritton, 1986). The backcross method is a form of recurrent hybridization by which superior characteristics may be added to an otherwise desirable variety. The goal of most backcrossing programs is to improve a particular strain (recurrent parent) for specific characteristics, usually a single gene, obtained from a donor parent (Baenziger, 2005). The advantage of backcrossing method is less influence of environment. For example, Mutlu *et al*. (2004) improved the resistance of pinto bean, *Phaseolus vulgaris* L., to common bacterial blight cultivar. The cultivar 'XAN 159', the highly resistant to CBB, was used as resistant donor parent, introgressed into the recurrent parent 'Chase' using classical backcross breeding.

Beside backcrossing, other breeding methods such as single seed descent was reported in improving pea to powdery mildew resistant cultivar. For example, Bing *et al.* (2006) developed field pea 'Reward' cultivars which were derived from the cross 4-0359, resistant parent × MP1491, susceptible parent. Powdery mildew resistant plants were selected from F_2 population and advanced to the F_4 by single seed descent in the greenhouse on the basis of plant type resistance to powdery mildew and lodging resistance. The selected F_9 from the line was tested in 13 locations in Canada. Reward was derived from a bulk harvested F_9 single line. This cultivar has high seed yield with medium seed size and round seed shape, and excellent powdery mildew resistance.

Although the introgression of resistant gene to recurrent cultivar was successful but some other characteristic traits, flowering day, and yield performance, pod character and number of pod per plant of new line had been considered. De Ron *et al.* (2005) evaluated 33 edible-pod pea (*P. sativum* L.) lines selected from single plant within 11 northwestern Spain landraces snow pea and three elite cultivars for their horticultural value in three field trials. Field performance was estimated according to six traits related to earliness and duration, while horticultural value was determined by five pod traits. Moreover, Sardana *et al.* (2007) observed the genetic variability in 210 accessions of pea germplasm assembled from diverse eco-geographic regions of the world. Quantitative traits such as earliness, dwarf plants, afila type, long pods, high pods/plant, high number of seeds/pod, and high seed yield/plant were used for selecting the high germplasm. However, none of the qualitative trait was linked to powdery mildew disease. (Nisar and Ghafoor, 2009)

In addition, evaluating stability of performance and range of adaptation has become increasingly important for breeding programs. Genotype (G) × environment (E) interaction is more important in breeding for other traits. First, pathogens may vary in their aggressiveness under different environments. Furthermore, physiological races may be different across environments. Second, the growth, development and physiological status of genotypes may be change across environments (Singh and Chaudhary, 1977).

The different levels of aggressiveness among isolates from different locations and the recent identification of pathogen suggest that G × E interaction could be important. Therefore, it is needless to mention the importance of breeding for disease resistant cultivars with high and stable seed yield across the intended environments.

Stability in performance is one of the most desirable properties of a genotype to be released as a variety for wide cultivation (Singh and Chaudhary, 1977)

2.4 Marker-assisted selection in breeding for disease resistance

2.4.1 Marker-assisted selection in breeding

The process of conventional disease breeding for resistance involves making controlled crosses and selecting progeny. This process becomes progressively more difficult due to the influence of the environment, especially when weather conditions do not favor strong fungal growth and hot spots of natural powdery mildew epidemics are not always available, and time-consuming for resistance encoded by single recessive gene inheritance and for quantitatively inherited resistance. Through the process of genetic linkage mapping, molecular markers which are linked to disease resistant genes can be identified, and these can then be applied in plant breeding program to assist in resistant gene introgression (Timmerman *et al*., 1997; Janila and Sharma, 2004).

Genetic markers may provide an attractive alternative to powdery mildew resistance selection, making the breeding process more efficient and less resource demanding. Once molecular markers that are closely linked to powdery mildew resistance have been identified, marker-assisted selection (MAS) can be performed at early stages of plant development, thus avoiding selection through disease exposure (Rakshit *et al*., 2001). In addition, markers tightly linked to genes of interest are useful in breeding programs since they can enable marker-assisted selection to overcome inaccuracy in field evaluation caused by environmental factors (Tanksley et al., 1989). Thus, they can help to increase the efficiency and accuracy of selection

(Sanchez *et al.,* 2000). MAS have been widely used to speed up the processes of crop improvement. (The British Society of Plant Breeders, 2010). Certain monogenic traits, like susceptibility to diseases and insect, are difficult or expensive to evaluate during cycles of backcross. Moreover, in the case of transferring recessive genes, it is necessary to intercalate one generation of self-fertilization after crossing with the recurrent parent to reveal the recessive homozygotes, which lengthens the transfer process in comparison with that for a dominant gene (Vienne, 2003).

Although the potential benefits of using markers linked to genes of interest in breeding program by moving from phenotype-based towards genotype-based selection, have been obvious for many decades. However, realization of this potential has been limited by the lack of markers. With the advent of DNA-based genetic markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of markers to detect associations with traits of interest, thus allowing MAS finally to become a reality (Guimaraes *et al*., 2007).

Markers have been widely used in plant breeding program for many purposes. Tiwari *et al.* (1998) stated that for introgression purposes, the recessive nature of *er-1* requires a generation of selfing after every second to third backcross generation to obtain homozygous resistant BC_nF_2 parents for the next backcross cycle. MAS provides an ideal strategy for transferring *er-1* into agronomically superior pea cultivars. In addition, in case of disease resistance is a quantitative; the presence of phenotype is highly affected by environment condition. Markers can provide the accuracy of selection more than phenotypic selection.

Tiwari *et al.* (1998) increased the durability of resistance to powdery mildew in pea by using gene pyramiding, combining between the *er-1* and *er-2 gene*. The

reliable and user-friendly specific primers closely linked to *er-1* were developed for investigating pea genotypes containing both *er-1* and *er-2* instead of visual scoring selection, which was very difficult, due to *er1* alone provides a high level of resistance. In addition, Timmerman *et al*. (1997) reported that the molecular markers linked to three monogenic disease resistant genes, *P-1* resistance to pea seed –borne mosaic virus, *sbm-1,*and ,*er-1,* resistance to powdery mildew, have been applied in the field pea breeding program to develop germplasm containing multiple disease resistant phenotypes. DNA tags linked to *sbm-1* and *er-1* have been used in conjunction with limited direct testing for disease resistance.

2.4.2 Qualitative markers associated with powdery mildew resistance in pea

Genetic variability of pea has been studied using several other genetic markers such as RFLP, AFLP, micro satellite markers and diversity array technology (Ahmad *et al*., 2010). Many reports with using DNA markers which linked to powdery mildew resistance in pea breeding program were published, due to their advantages. RAPD, SCAR and microsatellite markers linked to the gene *er-1*, have already been described (Timmermand *et al*., 1997, Tiwari *et al*., 1998, Janila and Sharma, 2004, Ek *et al*., 2005, Fondevilla *et al*., 2007).

2.4.2.1 RAPD

Random amplified polymorphic DNA does not require sequence information, and involves amplifying random pieces of DNA in which PCR is primed by a single 10 base primer at low stringency, such that random sequences of DNA amplified based on homologous sequences to the primer being present in the target DNA. It is a useful initial approach to identify polymorphisms.

By using RAPD markers, Samec and Nasinec (1995) detected DNA polymorphism among 6 economically important pea cultivars, finally *P. sativum* and *P. sativum* subsp. *arvense* cultivars were separated into 2 different clusters, according to their result of RAPD data cluster analysis. Moreover, Ahmad *et al*. (2010) used RAPD marker to investigate the genetic diversity in pea lines. Whereas, in the study of disease resistance, Tiwari *et al.* (1998) identified RAPD markers, 416 Operon primers, for powdery mildew resistant gene *er-1* in the resistant pea cultivar 'Highlight' (carrying *er-1*), the susceptible cultivar 'Radley', and their F_3 progenies which derived from the cross between 'Highlight' and 'Radley'. Only three primers, OPO-18, OPE-16, and OPL-6, were found to be linked to *er-1*. OPO-18_{1200} was linked in coupling (*trans* to *er-1*) and no recombinant was found. OPE-16_{1600} (4 ± 2 cM) and OPL-6_{1900} (2 ± 2 cM) were linked in repulsion (*cis* to *er-1*).

In addition, Janila and Sharma (2004) identified molecular markers linked to *er* gene, a single recessive gene which linked to powdery mildew resistance caused by *E. pisi*. Only RAPD primers, OPU-17, and a SCAR primer, ScOPD-10_{650}, could amplify polymorphic bands in resistant cultivar 'DMR11'. The primer OPU-17 and ScOPD-10_{650} mapped in coupling phase at a distance of 10.3 and 4.5 cM, respectively to the resistant allele *er.* Whereas OPO-02 could amplify polymorphic bands only in the susceptible cultivar. The markers ScOPD-10_{650} and OPU-17 being coupled with the allele causing resistance would substantially increase the efficiency of MAS in pea breeding for powdery mildew. According to the result of Fondevilla *et al*. (2008)'s experiment, six RAPD markers linked to *er-3* which consist of four RAPD markers linked in coupling phase, OPW04_{637}, OPC04_{640}, OPF14_{1103}, and OPAH06_{539}, and 2 RAPD markers in repulsion phase, OPAB01_{874} and OPAG05_{1240},

were identified by using Bulk Segregant Analysis (BSA). These markers increased the efficiency in breeding program. However, the RAPD procedure has several disadvantages. One of the main limitations of the method is high sensitivity to reaction conditions that reduces reproducibility of the results obtained in different laboratories (Fondevilla *et al*. 2008). In addition, many RAPD markers tightly linked to *er* gene have been identified and converted into SCARs.

2.4.2.2 SCAR markers

Sequence Characterized Amplified Region derived from the polymorphic RAPD marker lack most of their limitations (Melotto *et al*., 1996). This led to the creation of various studies of SCAR markers in pea. For example, Koveza *et al*. (2001) identified a 750 bp fragment RAPD marker which specific to particular pea genotype (line L-111 and the Nord cultivar) and SCAR was obtained from the marker. SCAR inheritance in the first and second generations was studied and its dominant character was shown. In other revisions, Koveza and Gostimsky (2005) developed more specific markers that characterized particular regions of the pea genome from the data on nucleotide sequences of RAPD fragments. Inheritance of the developed SCAR markers was studied in F_1 and F_2, and 12 polymorphic SCAR markers were obtained. These SCAR markers were used to identify various pea lines.

However, many SCAR markers were developed for investigation the powdery mildew resistance. Timmerman *et al.* (1994) reported a SCAR primer, ScOPD-10_{650}, for the powdery mildew resistance gene of pea, *er*. Similarly, Tiwari *et al*. (1998) reported two SCAR markers, ScOPD-16_{1600} and ScOPD-$18_{1200,}$ linked to *er-1* in repulsion phase and coupling phase, respectively. These three specific primer pairs were synthesized and tested in lines of Indian origin. In addition, Fondevilla *et*

al. (2008) revealed that two out of six of RAPD markers linked to *er-3*, $OPW04_{637}$, $OPC04_{640}$, $OPF14_{1103}$, $OPAH06_{539}$, $OPAB01_{874}$ and $OPAG05_{1240}$, were converted to SCAR markers. SCAR marker $SCW4_{637}$ co-segregated with the resistant gene and the SCAR marker $SCAB1_{874}$, in repulsion phase with *er-3*, was located at 2.8 cM from the gene and, in combination with $SCW4_{637}$, was capable to distinguish homozygous resistant individuals from heterozygous with a high efficiency.

2.4.2.3 Microsatellites or SSRs

Simple sequence repeats are short repeats of 1-5 nucleotides in length that are present in genomes of all higher eukaryote (Tautz and Renz, 1984). Variation in tandem repeat number at a particular locus causes the length of the microsatellite to vary (Zhang, 2004). The regions flanking microsatellite repeats are conserved and are sources for the design of locus specific primers to amplify the internal repeated regions (Pandian *et al*., 2000). SSR has become popular for genetic mapping purposes (Holton, 2003). They have several advantages over other molecular marker systems, they are frequent, are dispersed throughout the genome of most eukaryotic organisms and generally show a high level of polymorphism. SSR genotyping relies on a simple and robust PCR methodology, requiring only small amounts of crudely extracted DNA. Furthermore, Microsatellites are attractive because they have a high level of polymorphism, are widely dispersed throughout the genome, and are usually co dominant, which allows heterozygotes to be identified (Pandian *et al*, 2000). SSR markers have been used in many crops for breeding or genetic analysis, such as in pea (Loridon *et al*, 2005), maize (Taramino and Tingey, 1996), rice (Wu and Tanksley, 1993), and wheat (Gupta and Varshhney, 2000). Some pea SSR primers have been developed for investigating the marker with linked to

powdery mildew resistance gene. Ek *et al.* (2005) used SSRs to find markers linked to powdery mildew resistance in resistant pea cultivar '955180', susceptible pea cultivar 'Majoret', and their F_2 plants. A total of 315 SSR markers was screened. Five markers showed linkage to powdery mildew resistant gene, two of the markers can be used in plant identification with 1.6% incorrectly result.

Katoch *et al*. (2010) investigated the segregation of F_2 progeny derived from crosses between Lincoln, susceptible cultivar and JI2480, resistant cultivar, for the resistant phenotype, inheritance, and genomic location of gene(s) controlling resistance to powdery mildew caused by *E. pisi*. The resistance was controlled by a single recessive gene, *er-2*. The linkage analysis of 111 resistant F_2 progeny plants were developed by SSR and RAPD markers from the published linkage maps, the *er-2* gene was localized on pea linkage group III (LGIII), different position of *er-1*. RAPD marker OPX-17_{1400}, exhibiting *cis* phase linkage (2.6 cM) to *er-2* was successfully converted to a SCAR marker, ScX17_{1400}. The marker would be studied to ensure speedy and precise introgression of *er-2* into susceptible cultivars by permitting selection of *er-2* heterozygotes amongst $BC_n F_1$s without progeny tests and resistance screening.

2.4.2.4 Amplified fragment length polymorphisms (AFLP)

Amplified fragment length polymorphisms AFLP is also a PCR-based technique, in which selective pre-amplification and amplification steps are carried out to amplify a subset of fragments of the genome, depending on the linkers added and primers used. Many potentially polymorphic fragments are generated by this approach. Polymorphic bands between parents can be identified and linked to useful traits. The developed AFLP marker was widely useful in fine mapping of

genomic regions containing genes of interest in cultivated plants. In mungbean, new marker loci associated with powdery mildew resistance were discovered and mapped by AFLP and RFLP analysis. One of the potential probe has been developed to assist in breeding for powdery mildew resistance in mungbean (Chaitieng *et al*., 2002). In pea, Stackelberg *et al*. (2003) used AFLP technique to identify markers closely linked to the *def* gene. While, Taran *et al*. (2003) identified genetic loci associated with lodging resistance, plant height and reaction to mycosphaerella blight. In addition, Coyne *et al.* (2000) indicated that AFLP have been used in pea to map *er-2*, a gene that confers resistance to powdery mildew. Furthermore, Tiwari *et al*. (1998) developed AFLPs marker to identify *er-2* gene which was introgressed in lines carrying *er-1* in pea breeding program by combining both resistance genes *er-1* and *er-2* in a cultivar which aims to increase the durability of resistance. The result indicated that AFLPs had a clear advantage over RAPDs in term of number of amplicons amplified per reaction. Therefore, this technique has been successfully used to identify markers for disease resistance genes, due to dominant markers.

2.4.2.5 Restriction fragment length polymorphisms (RFLP)

Restriction fragment length polymorphisms RFLP rely on the combination of a probe and restriction enzymes to identify polymorphic DNA sequences using Southern blotting. This approach requires either radioactive or non-radioactive detection methods to identify polymorphic DNA bands. This technique was employed by many researchers in order to investigate the marker which linked many diseases resistance and interested trait characters in pea. Dirlewanger *et al.* (1994) found the *er* gene at 9.8 cM distance from a restriction fragment length polymorphism (RFLP) maker, *p236*. Furthermore, other RFLP markers linked to each

resistance gene were also found such as Fusarium wilt (6 cM from *Fw*) and pea common Mosaic virus (15 cM from *mo*). Moreover, plant height, flowering earliness, and number of nodes of 172 F_2 plants of pea were analyzed in order to map the genes responsible for their variation by RFLPs.

In addition, Humphry *et al.* (2003) identified a major locus conferring resistance to powdery mildew, *E. polygoni* DC, in mungbean. A linkage map was generated by using RFLP markers. The 51 probes generated 52 mapped loci, which were used to construct a linkage map spanning 350 cM of the mungbean genome over 10 linkage groups. Using these markers, a single locus was identified that explained up to a maximum of 86% of the total variation in the resistance response to the pathogen. Although, RFLPs are reliable and yield co-dominant but this technique required more time-consuming than PCR-based methods and also expensive, requiring relatively large amount of highly purified DNA and they do not lend themselves to automation (Gupta *et al*., 1999).