CHAPTER 5
DISCUSSIONS

5.1 Morphological characteristics, yield evaluation and seed multiplication

The morphological characteristics of plant material, plant growth, traits and yield were determined prior to breeding program. These data were very useful to plant breeder to plan for breeding program. Sardana *et al.* (2007) used qualitative and quantitative traits, *i.e.* earliness, dwarf plants, afila type, long pods, pods per plant, number of seeds per pod, and seed yield per plant, primary branches and 100-seed weight to screen genetic variability of peas germplasm. In this study, in order to select desirable character of each line/cultivar, plant characteristics in term of plant growth, days to first flowering, flowering node, pod setting, pod characteristics, vine height, the number of node and branches, pod length and width, number of seeds per pod, number of pod and pod weight per plant were characterized.

Among 7 pea cultivars employed in this study, they could be divided into several groups according to their characteristics and habits. According to number of days from seed sown to first flowering of 7 lines/cultivar was determined. There was significantly different in flowering among studied lines/cultivar. In this study, line No.5 could be classified into earliness while line P309 was lateness. This result corresponded to Makasheva's pea classification (1986), these 7 pea lines/cultivar could be classified as early flowering *i.e.* line No.5, mid flowering *i.e* lines P175, P185, No.3, No.4 and cultivar Fang No.7, and late flowering *i.e.* line P309. However, the difference of first flowering time in 7 pea lines/cultivar would be mainly depended

on the variety, condition of cultivation and environment such as photoperiod, temperature and humidity during the vegetative period (Roche *et al*, 1999).

According to the plant height, vines height was significantly different among lines/cultivar, varied from the shortest at 60.13 cm in line P117 to the tallest at 213.10 cm in line P185. Plant height was a complex trait and influenced by the varieties, growth conditions and cultivation (Gritton, 1986). The number of node of line P185 was the tallest line and it also had the greatest number of node and branches. Meanwhile, lines No.4 and No.5, medium-tall line, showed the least number of nodes and branches. According to growth condition, the difference of the node number of each cultivar could occur (Knott, 1987). While number of branches varied among genotypes (Jeufforoy *et al.,* 1997) and were strongly influenced by environmental conditions such as soil physical conditions or soil water status (Dawkins *et al.*, 1984).

Pea flower color of all lines in this study was white, except for cultivar Fang No.7 which was purple. Most garden and processing peas have white flower, except for some edible pod cultivars (Gritton, 1986) The presence or absence of color is dependent upon a basic anthocyanin gene at the *A* locus. Dominance results in color, while homozygous recessive plant has white flower (Muehlbauer *et al.,* 1997). In addition, the lowest flowering node of pea in this experiment was also studied. The result showed that the lowest flowering node was found on line No.5, the earliness line, at the13th node and the highest flowering node on the 25th was line P309, the lateness line. Generally, flowering time and flowering node of pea were usually positively correlated, but this correlation could be negatived by genetic or environmental factors which resulted in floral bud abortion or influence plant growth (Aruminglyas and Murfet, 1994). The late cultivars of peas behaved as quantitative

long day plants. They flowered between nodes 20 and 35 under an 8 hour photoperiod (Reid, 1978).

In this current study, pod characteristics were quite variable. Type of pods in 7 lines/cultivar could be grouped into two types base on structure of the pod wall, shelling pea for edible seed and flat pod for edible pod. Generally, the inner side of the pod walls of shelling forms had hard parchment layer while snow pea did not have (Makasheva, 1986). Pod of lines P117 and 185, resistant lines, except line P309, could be classified into edible seed due to their parchment and more fiber, while lines No.3, No.4, No.5 and cultivar Fang No.7, susceptible lines/cultivar, could be classified to snow pea type for edible pod. For line P309, pod type was almost different from other resistant lines, the pod was closely to flat pod type but they still have a little of parchment. The length of fresh pod was also different among lines/cultivar. Pod length of cultivar Fang No.7, lines No.3 and No.4 was larger than the resistant lines, P117, P185 and P309. The comparison of pea pod size in this trial to Makasheva (1986) standard indicated that the pod size of lines No.3, No.4 and cultivar Fang No.7 were classified into very large size. While, pod size of the resistant lines, P117, P185 and P309, and susceptible line, No.5, were classified into large size.

5.2 Phenotypic evaluation of powdery mildew resistance

Two locations with different seasons were evaluated for powdery mildew resistance in order to confirm the resistant parental lines/cultivar. The natural infection from the inoculums row on pea leaves was evaluated in the field at Pang Da Royal Agricultural Station. It was found that there was significantly different in disease severity among lines/cultivar. The beginning of the incidence and severity of

powdery mildew disease occurred in the flowering stage. The greater amount of infection on the leaves in susceptible plants have appeared since after flowering until to maturing stage. According to the result of phenotypic assessment, there were only three lines, P117, P185 and P309, showed highly resistance to powdery mildew disease as lower percentage of infection on pea leaves was found. As of Nisar *et al*. (2006) stated that resistant pea cultivars to powdery mildew, Fallon, PS99102238 and PS0010128, showed highly resistance as localized infection symptom on the leaves. According to the statistical analysis of the disease occurrence percentage, tested pea lines/cultivar in this study could be divided into 3 groups as follows; resistant cultivar group (R) *i.e.* lines P117, P185 and P309, moderate cultivar group (M) *i.e.* lines No.3, No.4 and No.5 and susceptible group (S) *i.e.* cultivar Fang No.7. The difference of resistance level may occur from the resistant genetic in plant. Tiwari *et al.(*1998) stated that the resistance to the powdery mildew disease in pea (*P. sativum*) is controlled by recessive gene *er-1* and (or) *er-2* and the resistance gene *er-1* may be present in many resistant lines from around the world. However, the powdery mildew resistance which was found in this study could not be specific to what kind of *er* gene due to the resistance genetic of pea lines in this study is not matched to what has been found in other studies when using molecular markers.

However, at Inthanon Royal Agricultural Research Station, powdery mildew evaluation was conducted in greenhouse (enclosed house). It was found that rate of infection was low, but the disease severity was greater than in the open field. According to the evaluation, it indicated that all tested lines/cultivar were infected by powdery mildew disease, but high percentage of disease severity was found in four commercial lines/cultivars. However, the resistant lines, P117, P185 and P309 showed

to be susceptible to the disease. This might be hypothesized there was excessive amount of inoculums in greenhouse, due to the susceptible cultivar was planted prior to regular transplant in order to generate powdery mildew source within the greenhouse with favorable condition, high temperature and humidity caused high spore density more than normal condition. Vaid and Tygi (1997) stated that temperature rises to 25^{o}C enhanced growth of the powdery mildew. Under favorable growth condition, the pathogen rapidly grows on susceptible lines/cultivar while the resistant with late growing season may be infected when temperature rise.

In addition, Tiwari *et al.* (1998) and Fondevilla *et al.* (2006) stated that powdery mildew resistance genes in some locations may be ineffective as in the other one. These might be influenced from many factors such as pathogen races, environmental condition for pathogen and plant (Vaid and Tyagi, 1997). These could be supported by Ondrej's experiment (2005) which revealed that the resistant pea cultivars to *E. pisi* were susceptible to another powdery mildew species, *E. baeumleri*. Meanwhile the susceptible genotype to *E. pisi* (without gene *er-1*) showed resistance to *E. baeumleri*, the new race of powdery mildew. From this study, it indicated that when different race of *Oidium* was presented at different location even resistant plants were susceptible to disease. That might be due to the resistance character of lines P117, P185 and P309 was very specific to certain *Oidium* race.

5.3 DNA marker linkage to powdery mildew resistance in snow pea

5.3.1 Primer Screening

In this current study, the resistant DNA marker in 7 lines/cultivars of snow pea was identified using three primers, ScOPD-10, OPU-17 and OPO-02. None of

them could yield band that had the same size as reported by Janila and Sharma (2004). That might be due to the difference of the origin of germplasm. Although, the SCAR marker, SCOPD-10, was developed to be used in identifying powdery mildew disease resistance in Canadian germplasm by Timmerman *et al*. (1994) and Tiwari *et al*. (1998) whereas this germplasm has been less employed in Indian pea breeding programme. Only 80% of the result was reliable when this primer was used with Indian cultivars. Meanwhile, one of the two markers developed by Tiwari *et al*. (1998) could not differentiate resistant and susceptible lines of the Indian origin (Janila and Sharma, 2004). Since the origin of snow pea lines/cultivar used in this study is not known and they might have contained different genetic of origin from those of Canadian and Indian origins. Thus, when primer, ScOPD-10, was used, it could not yield band at the size of 650 bp. as reported in Canadian cultivars (Timmerman *et al.*, 1994) and Indian cultivars (Janila and Sharma, 2004). However, band at 850 bp. was found and only the resistant lines, lines P117, P185 and P309, yielded this band. The difference in size may occur according to the difference of pea genetic background (Fondevilla *et al.*, 2008). On the other hand, other two primers, OPU-17 and OPO-02, failed to detect any MAS. That might be due to OPU-17 and OPO-02 were RAPD primers which were not specific and there were many limitations of this method, especially, high sensitivity to reaction conditions that reduced reproducibility of the results obtained in different laboratories (Ek *et al.*, 2005). However, the specific DNA band at 850 bp. could be used to confirm the powdery mildew resistance in snow pea cultivar in Thailand.

5.3.2 Sequencing of the specific SCAR marker (850 bp.)

The PCR products of the 3 powdery mildew resistant lines, P117, P185, and P309, were generated by SCAR technique using primer ScOPD-10 and the amplified fragments, size 850 bp, were sequenced. The sequencing of the 3 fragments revealed 618, 621, and 624 bases, respectively. DNA sequences were compared with DNA sequences database at National Center for Biotechnology Information (NCBI) GenBank. After BLAST searched of the sequences, the 618-, 621-, and 624- base sequences had similar banding, at 91, 90, and 91%, respectively, to the DNA sequence from clone JICPSV-598E15, complete sequence of *P. sativum*. Moreover, these sequences also similar to DNA sequence of BARE-1 gene in Barley and RIRE-1 gene in rice (Smykal *et al.*, 2009). BARE-1 gene in Barley was the resistance gene to powdery mildew which caused by *E. graminis* f. sp. *Hordei* (Wei, 1999). Thus, it could be confirmed that resistant pea lines in this study contained resistant gene as same as other powdery mildew resistant pea lines.

5.4 Hybridization for powdery mildew resistance

5.4.1 Crossing and selection

Conventional breeding can be an option for developing of resistant cultivars if resistant sources are available (Nisar *et al.*, 2006). However, resistance cultivar or wild type always carries unfavorable traits, therefore, backcross method would help to bring back all the desirable trait (Kaloo, 1988). In this breeding program, yield of snow pea pod performances as well as resistance to powdery mildew were the major goals of the improvement. The pod characteristics of twenty-three F_1 hybrids from reciprocal cross were evaluated and among of them showed various traits. Screening

for the favorable type as flat pod and no parchment found only F_1 hybrids, derived from resistant line of P309, as parental plants, was similar to snow pea or edible fresh pod. Whereas pod characteristics of F_1 hybrids derived from resistant lines of P117 and P118, as parental plants, had green round pod, more fiber and could be classified for seed consumption. Thus, in this breeding program, only F_1 hybrids derived from No.3 × P309, P309 × No.4, No.5 × P309 and Fang No.7 × P309 were used in backcrossing program. Due to F_1 hybrids of No.4 × P309 died when they were at seedling stage, F_1 hybrids of P309 × No.4 were used. According to the screening of available resistant parental lines, these help to have a short breeding time and increase the successful chance of improvement.

5.4.2 Backcrossing

Three backcross generations, BC_1, BC_2 and BC_3 in this current study, line P309 which contained resistant character, was used as a female recurrent parent instead of commercial lines No.3, No.4, No.5, and cultivar Fang No.7, the susceptible lines/cultivar, due to these lines/cultivar were heavily infected from powdery mildew disease. The hybridized flowers could not develop to be pod. Nisar *et al*. (2006) revealed that thirty-three susceptible pea cultivars which were attacked by powdery mildew (*E. pisi* Syd.) failed to initiate flowering but these genotypes flowered in the disease-free environments. However, the using of male donor parental lines/cultivar, P309, as female parental lines instead of lines No.3, No.4, No.5 and cultivar Fang No.7 could harvest F_1 seed. After selfing, some of F_2 plants showed resistant character. In addition, their pod characters were similar to edible pea pod.

Moreover, due to heavy disease severity occurred, phenotypic evaluation for resistant plants in all F_2 generation in greenhouse condition could not differentiate the resistant and susceptible plants by using visual scoring the percentage of the powdery mildew disease on the leaves. Almost all parts of plant, especially leaves were infected. This problem might be due to excessive amount of inoculums from the susceptible cultivar which was planted prior to regular transplant in order to generate powdery mildew source within the greenhouse with favorable condition, high temperature and humidity cause high spore density more than usual condition (Vaid and Tyagi, 1997). However, the resistant and susceptible F_2 plants were differentiate by using the basal stem character.

5.4.3 DNA marker linkage to powdery mildew resistance in snow pea

When random sample of four backcross generation, F_2, BC_1F_2, BC_2F_2 and BC_3F_2 progenies derived from No.3 × P309, P309 × No.4, No.5 × P309 and Fang No.7 × P309, were tested for resistant marker using SCOPD-10 primer. It was found that about 70-100% of phenotypic resistant plant yielded marker band at 850 bp. These phenomena might have been occurred from fault phenotypic screening, due to excessive amount of inoculums in greenhouse or might have been due to deletion, mutation, random segregation or chromosome crossing over during meiosis (Tiwari *et al.*, 1998). In addition, the absence of the markers from parents to hybrids may have originated due to crossing over between positions of *er* gene and linkage gene (McClean *et al*., 2000). The results of the current study showed that screened primer, SCOPD-10_{650}, the SCAR marker, could be used to detect the powdery mildew resistance marker in backcross breeding populations, showing polymorphic DNA

band at 850 bp. Janila and Sharma (2004) identified powdery mildew resistance markers linked to *er* gene, a single recessive gene. This primer was employed in screening powdery mildew resistance marker of pea cultivar "DMR11". However, marker position of the resistance one differed from DMR11 was found at 650 bps whereas in this study in line P309 was found at 850 bp.

The results of this study proved that this marker can be used in MAS in the future to transfer the quality traits loci for powdery mildew resistance in snow pea in Thailand. The MAS for one major for powdery mildew resistance combined with phenotypic selection was highly effective in early backcross generations. The use of molecular markers in backcross breeding will be an effective strategy for improving genetic resistance for powdery mildew in snow pea. Moreover, due to MAS could be used to detect the resistant genotype since seedling stage, this help to reduce the cost production in the field and breeding time.

5.5 Morphological characteristics, yield quality and powdery mildew resistance on hybrid test

5.5.1 Morphological characteristics and yield quality

According to the various on morphological traits, first flowering and blooming, first pod setting, first flowering node, flowers number per inflorescence, first node to pod setting, plant height, pod number per inflorescence and internodes length were used to determine the genetic variation. Those characteristics and yield performances were observed in BC_3F_3 progenies comparing to their parents. The results showed that the morphological characteristics varied among lines/cultivar. However, almost morphological traits of BC_3F_3 progenies were similar to their recurrent parents, lines No.3, No.4, No.5 and cultivar No.7. In this study, the

flowering time, first flowering node and first node to pod setting of line No.5 and line of BC_3F_3 progenies derived from No.5 × P309 were similar and both of them were classified as earliness lines while lines No.4 and their hybrids, line of BC_3F_3 progenies derived from P309 × No.4, were identified as lateness lines. Sardana *et al.* (2007) stated that most pea characters such as flowering time were influenced by the variety and condition of cultivation. In addition, Roche *et al.* (1999) revealed that environment such as photoperiod, temperature and humidity during the vegetative period also influenced to the flowering time of pea plants.

Plant height varied among lines/cultivar. Plant height of BC_3F_3 progenies were also similar to their recurrent parent, lines No.4, No.5 and cultivar Fang No.7, except for BC_3F_3 progenies derived from No.3 × P309 were taller than parental plant, line No.3. Plant height is a complex trait and is influenced by the varieties, growth conditions and cultivation (Gritton, 1986). According to plant height in this study, it could be classified into two groups, this also consistent to Makasheva (1986); plant with height of 81-150 cm could be classified as medium-tall *i.e* lines No.3, No.4 and line of BC_3F_3 progenies derived from P309 × No.4, while plant with height of 151-300 cm, could be classified as tall group *i.e* lines P309, No.5, cultivar Fang No.7, and three line of BC_3F_3 progenies derived from No.3 × P309, No.5 × P 309, Fang No.7 × P309.

The number of flower per inflorescence was similar among lines/cultivar, except cultivar Fang No.7 and BC_3F_3 progenies derived from Fang No.7 × P309. In both trial locations, line P309 gave the greatest number of flowers per inflorescence while the least number of flowers per inflorescence was found in cultivar Fang No.7 and BC_3F_3 progenies derived from Fang No.7 × P309. Gritton (1986) stated that the

number of flower per inflorescence depended on the genotype and environmental conditions. They might have varied greatly in length and bore from one to many flowers per inflorescence. The number of flowers was not constant on a plant, in early cultivars, they often could yield single flower or bear some single and some double flowers whereas late cultivars were mostly double or triple flower. However, the total number of flower per inflorescence may not completely develop to pod.

The similar result of first flowering node was found in both trial locations. The lowest first flowering node was found in line No.5 and BC_3F_3 progenies derived from No.5 × P309 at the 9^{th} to 11^{st} nodes. Whereas others lines/progenies had the first flowering node higher than the 16^{th} node. In both locations, the first flowering node of other lines /cultivar was found between the 16^{th} to 20^{th} nodes and 16^{th} to18^{th} nodes, respectively. Gritton (1986) stated that early lines/cultivar would produce the first flower from the 5^{th} to 11^{st} node while the lateness lines/cultivar would start flowering at the 13^{rd} to15^{th} nodes.

First pod setting of BC_3F_3 progenies at Ang Khang Royal Agricultural Station was similar to their recurrent parents, lines No.3, No.4, No.5 and cultivar Fang No.7. The lowest node to first pod setting in both locations was found in line No.5 and BC_3F_3 progenies derived from No.5 × P309. The result showed that the first flowering node was not the same as the first pod setting node. In addition, the result of pod number per inflorescence of all cultivars/progenies in both trial locations was similar. Generally, the pod number per inflorescence in this study was about one pod. While line P309 showed the greatest number of pod per plant.

Various data of branches were observed in two locations. The highest node to first branch was found in cultivar Fang No.7 and BC_3F_3 progenies derived from Fang

No.7 × P309. The node to first branch and the number of branch per plant of BC_3F_3 progenies was similar to their recurrent parent. However, the greatest branch number per plant at Khun Wang Royal Project Development Centre was line P309 whereas at Ang Khang Royal Agricultural Station was cultivar Fang No.7. The different result might be due to environment. Dawkins *et al.* (1984) revealed that branching is strongly influenced by environmental conditions such as soil physical conditions or soil water status.

Internode length differed among cultivars. At Khun Wang Royal Development Centre, internode length of five lines/cultivars was the same, ranged from 9.64 to 10.22 cm. Meanwhile, at Ang Khang Royal Agricultural Station, internode length between backcross progenies and their parents was different. Internode length on plant was the characteristics property of a variety but it could be changes only depending upon condition of growth (Makasheva, 1986). In pea, internode length was controlled by three alleles loci *Le, Na* and *Cry*. These genes had effected on growth in complete darkness as well as in the light (Reid, 1983).

Harvesting date characteristics was similar in both locations and it could be classified into three groups; early harvesting groups *i.e.* line No.5, cultivar Fang No.7, BC_3F_3 progenies derived from No.3 × P309, No.5 × P309 and Fang No.7 × P309, mid harvesting group *i.e.* lines P309, No.3, No.4, and the late group *i.e.* BC_3F_3 progenies derived from P309 × No.4. In addition, the most harvesting duration of BC_3F_3 progenies was similar to their recurrent parents, especially in line No.5 and cultivar Fang No.7.

In this study, pod length, pod width, number of seed per pod and pod weight were different among lines/cultivar. However, the average of those characters among

BC_3F_3 progenies and their recurrent parents were similar. In addition, number of pod per plant and total pod weight per plant were also different among cultivars in both locations. At Khun Wang Royal Project Development Centre, number of pod per plant and total pod weight per plant of BC_3F_3 progenies were greatest than their recurrent parents, lines No.3, No.4, No.5 and cultivar Fang No.7 but lower than donor parent, P309. This may occur from all recurrent parents were heavily infected by powdery mildew disease and the disease reduced the snow pea production (Tiwari *et al*., 1998) Whereas, at Ang Khang Royal Agricultural Station, number of pod per plant of some parentals, line No.4 and cultivar Fang No.7, were slightly greater than their BC_3F_3 progenies. This might be occurred from less infection of powdery mildew disease than other lines/cultivar. On the other hand, total pod weight of BC_3F_3 progenies was similar to their recurrent parents except for BC_3F_3 progenies derived from P309 × No.4 gave the lowest yields. Pod length and number of seeds per pod were the major green pod yield contributing characters in garden pea. In addition, more number of pods per plant would contribute more pod weight per plant (Naweb *et al.*, 2008).

5.5.2 Powdery mildew resistance

The progenies and their parents showed similar result of powdery mildew resistance. Line P309 was used as donor parent of resistance character. Backcrossing was employed and it was found that resistant character would be transferred from line P309 to all recurrent parents, lines No.3, No.4, No.5 and cultivar Fang No.7.

After selection for good characteristics of progenies, they were tested for their performance in two different locations. At Khun Wang Royal Project

Development centre, all BC_3F_3 progenies which derived from four crosses showed resistance to powdery mildew as well as their donor parent, P309. From this result, it could be summarized that *Oidium* race at Khun Wang Royal Project development Centre might be similar race which was found at Pang Da Royal Agricultural Station. Whereas all female recurrent, lines No.3, No.4, No.5 and cultivar Fang No.7, may not contain resistant genetic, therefore all of them showed highly susceptible to the disease.

At Ang Khang Royal Agricultural Station, the BC_3F_3 progenies of all crosses were susceptible to powdery mildew as well as their parent lines/cultivar. That might be due to the resistance character of line P309 was very specific to certain *Oidium* race. When different race of *Oidium* was presented at different location even resistant plants were susceptible to disease (Fondevilla *et al*., 2006 ; Tiwari *et al.,* 1998). As Buakhao's (1993) experiment revealed that the resistant pea cultivar, line P131 was resistant to powdery mildew when grown in Doi Musur, Tak Province but they were susceptible to powdery mildew when grown in Kao Ko Agriculture Research Station in Petchaboon Province. Moreover, Ang Khang Royal Agricultural Station is located at nearly 1,400 meters above sea level, within the valley. The climate is cool all year round and has high humidity. This point may make the pathogen races differ from other location. In, addition, when the specific race of resistance was found, major *Oidium* race could not attack, minor race of *Oidium* could develop and invade the snow pea plant due to no competitiveness. Ondrej *et al*. (2005) stated that the resistant pea cultivars to *E. pisi* were susceptible to different race of *Oidium, E. baeumler*, which found in the different locations. In addition, Tiwari *et al.* (1998) and Fondevilla *et al*. (2006) stated that powdery mildew

resistance genes in some locations may be ineffective to the other one. These might be influenced from many factors such as pathogen races, environment condition for pathogen and plant (Vaid and Tyagi, 1997).

5.5.3 Consumer response trial

At present, market requirements of pea can be divided into fresh market and processing market. Both markets need different varieties of pea (especially pod characteristics). For fresh market, pod quality is often more important than productivity. The parameters for edible fresh pod are more variable than those for cultivars used for processing (Myers *et al*, 2001). The appearance of the pod is very important. Many characters, pod size, shape, stingy pod, pod wall thickness, sweetness, flavor at maturity and low in pod fiber, must be considered in breeding selections.

Pod of four BC_3F_3 progenies derived from No.3 × P309, P309 × No.4, No.5 × P309 and Fang No.7 × P309 and their parents, cultivars No.3, No.4, No5, Fang No.7 and P309 were tested for their 6 characters, *i.e.* pod size, pod shape, crispness, sweetness, colors and scent. In this study, consumer's satisfaction levels to 6 parameters of yield quality were distributed among cultivars/lines. Over all, consumer satisfaction scoring of new lines was similar to their parents. Although cultivar P309 was the best among tested cultivars and progenies, their progenies derived from No.5 × P309 and P309 × No.4 were quite favorable whereas the consumer acceptance of cultivar Fang No.7 got the lowest point. It indicated that the BC_3F_3 progenies from this breeding program have been favorable to consumers as well as their parents.

Customer trial was conducted at Royal Project Shop and the result might not represent the major market need. Large group of customers such as a Chinese restaurant and other locations, might provide better information on preference of this new snow pea variety.