CHAPTER 5

DISCUSSIONS

5.1 Germplasm collection

The hybridization program was established within the breeder's progenitor collection. For this study, 27 rose varieties used for hybridization in breeding program except 'Eliza' which was used as standard check for pink colour offspring. The parentage data showed that 10 of these varieties were obtained from encyclopedia of rose science no. 3, but the remaining 18 had no known parentage record. Due to the fact that cut – rose parangage was complex and in large parts unknown and breeding history of most current varieties,was a commercial secret it was almost impossible to estimate the genetic relationships of rose varieties and the genetic diversity within rose groups on the basis of parentage data. Comparisons between dendrogram from all and optimized primers were done for evaluating the best of dendrogram for grouped progeny. Dendrogram obtained from 8 primers was the best, because it corresponded with the parentage data.It could be concluded that the selected primers affected the calculation of bootstrap value of dendrogram in classifying genetic relationships. Breeders should select the optimized primers to use in RAPD-PCR reaction and proved the results with parentage data prior to use. Utilization of optimum primer had some advantages i.e. saving cost, and giving high polymorphism and high precision results.The optimization of primer screening for RAPD analysis has been used for the analysis of diversity and identification of duplicates within the large germplasm collection (Mohapatra and Rout, 2006). In other plants in *Rosaceae*, RAPD marker could find good agreement between genetic similarity values based on RAPD markers and pedigree information (Degani *et al.*, 2001; Esselink *et al.*, 2003). In this study, the optimized primers dendrogram was classified into 3 genetic groups with high bootstrap value support, indicating that the 28 rose cultivars had high similar genetic relationship within group, and had high diversity between groups. Genetic variability is higher in cultivated roses than some of the wild species (Debener *et al.*, 1996a). The large diversity can be explained by the fact that more than 10 wild rose species have been used as crossing partners during the past centuries of rose breeding. Therefore, stringent selection on traits has obviously limited the variability to only a few characters. This is further supported by the fact that the relative genetic distance based on RAPD markers does not correlate with the classical rose in classification system.

5.2 Pairing of parents

5.2.1 Pre hybridization stage

The abundance of pollen and good pollen germination were necessary for plants to be used as male parents. Therefore, pollen germination was firstly investigated. The results found that 14 varieties could be used as male parents. The many crosses with a few top parents gave better results of good offspring than a few crosses with many parents (Nemko, 2004). This study showed that the evaluation of pollen parent by using agar medium supplemented with 15% sucrose and 100 ppm boric acid. The fresh pollen germinated ranging from 3.3 to 62.1%.

The problem of poor germination of the pollen could be summarized as follows:

a.) Genetics Rose breeders should know the hybridization property of their own germplasm.In general, pollen of *Rosa hybrida* L., had a low germination capacity, not exceeding 18% (Voyiatzi, 1995), while this study showed that some cultivars had high (i.e. Vivaldi, Diplomat, Azure Sea) or low (i.e. Jade and Raphaella) percentage of pollen germination , depending on cultivars, the average being 22%. Pearson and Harney (1984) also reported a significant difference between genotype, collected from six *Rosa* species, one botanical variety and three cultivars with a germination ranging from 1.7 to 69.2%. Kanta (2003) also observed that pollen germination rate depended on varieties and composition of medium. Dendrogram was established from HAT-RAPD, combined with pollen testing experiment data. It indicated that this method could not identify and group cultivars according to data from the experiment. This result might occur because the optimum primers were cut DNA by random positions. For further studies, DNA sequencing should be used to prove the position of DNA and to know the function of gene.

b.) Pollen quality The quality of pollen is one of the keys to the development of fruits after pollination. There is a positive correlation between the pollen fertility of the pollination parent and the growth of rose hips of the seed parent (Visser *et al.*, 1977b). The flowers to be used as male parent should be at a suitable openning stage (semi-open), and correct pollen preparation method must be used. (Krasaechai *et al.*, 2004; Ketpet and Krasaechai, 2005 and Ketpet and Krasaechai, 2006). The pollen parent varieties could be grouped according to their pollen quality into 3 categories as inferior, moderate and high (Visser *et al.*, 1977b). Furthermore, the pollen germination quality varied according to several factors. Pollen quality was influenced by season of the production (Gudin *et al.*, 1991b), bud chilling treatment of the pollen producing plants (Gudin, 1992), pollen germination which was increased by relatively high temperature (23-30^{o}C) and a good range of relative humidity (55-70%), low pH of the stigmatic exudates (Gudin and Arène, 1991) and putrescine (Gudin and Arène, 1992).

c.) <u>Pollen quantity</u> The results of this study indicated that the success of pollination might be related to sufficient density and quantity of pollen on stigma of female flower. To increase the member of successful crossings and fruit settings, it is possible to repeat the pollination of the same flower (Vries and Dubois, 1983).

d.) <u>Pollen viability</u> Pollen of rose is functional when it is freshly collected and keeps certain viability during the following days of pollen releasing.When the pollen is stored at cold temperature for several weeks, its efficiency decreases rapidly. Viability and fertility can be estimated by pollen germination test and staining pollen method (Kim *et al.*, 2007), but it dose not always indicate its true fertilizing ability. The measurement of pollen germination criterion is better related to fertilizing capacity than the traditionally used tetrazolium test (Pearson and Harney, 1984) or acetonamine (Jacob and Ferrero, 2003). Viability level is highly variable depending on the cultivars (Jacob and Ferrero, 2003).Result of crossings made between varieties and selection of hybrid tea rose varied as to fruit and seed set due to variation in pollen viability rather than to incompatibility. Thus, in an ongoing breeding programme the germination ability of pollen grains of the parent cultivars should first be checked. Not all breeders make a germination test before crossing because it is expensive and time-consuming (Spethmann and Feuerhahn, 2003).

5.2.2 Hybridization stage

Among the species of *Rosa*, there are various modes of pollination, autogamy, geitonomy, xenogamy and free-pollination, including a meiotic habit in the Genus *Rosa*. Roses are well known for their difficult sexual reproduction from pollination to seed set (Ueda and Akimoto, 2001). Ueda and Akimoto (2001) reported cross- and self-compatibility under field conditions in 48 taxa belonging to all sections of subgenus *Rosa* and subgenus *Platyrhodon* in the genus *Rosa*. Four species exhibited fruit set by self-pollination out of the 28 diploid species tested. Nine out 12 tetraploid species, all of pentaploid species, and four out of five hexaploid species also exhibited fruit set through self-pollination. Thus, it seemed that the self-incompatibility in genus Rosa broke down with polyploidization.Tetraploid species or artificially induced tetraploid had gametophytic self-incompatibility and usually displayed a self-compatible habit.

The problem of fruit setting could be summarized as follows:

a) Genetics Dendrogram showed that when analyzing the DNA from 8 primers with fruit setting and fruit drop capacity data, cultivars could be classified genetically into three groups. Group 1 had 16 cultivars and group 2 with 11 cultivars and group 3 as outgroup. Dendrogram indicated that succes was obtained in the crossing between groups and within group, especially group I and I x II. It indicated that cross-compatibility depended on male and female parents more than genetic relationship, as seen in the case of crossing combination with female parent ‘Dallas’ which was a hybridization failure. However, higher fruit set did not generally result in more seeds per pollination and seemed to be related to the fertility degree of male parentage. Both parents used had a major effect on pollination success.In hybridzing cultivars with less pollen releasing and germination, breeders should use several flowers to increase fertilization efficiency. If the quanlity of pollen was too small, hormones within to stimulate the ovaries would be limited, making it insufficient to develop into fruit. In Same case, pollen germination was not noticeably lower in incompatible pollination than in compatible ones and was not related to subsequent pollen tube growth. i.e. Kardinal had 30.9% of pollen germination and high pollen releasing, but gave high percentage of fruit drop when used as male parent. Recently developed *Rosa* cultivars had a narrow genetic base and the use of distantly related cultivars as parents would possibly give a high seed set and a greater number of progeny. Embryo rescue was also reported by many researchers who grew embryos that would otherwise had been aborted in some crosses, e.g. interspecific or intergeneric, the endosperm failed to develop and caused the abortion of the embryo and 2n gamete of male and female (Gudin, 1994; Marchant *et al.*, 1994).

b) Cross-and self-incompatibility Many inter-varietal or inter-specific crosses are difficult or impossible because of cross incompatibility and either viability or sterility of microgametophytes. Sexual incompatibility which was due to the activity of the pollen, although functional, to grow down and was arrested in the style of stigma, and failure in of the process of fertilization and development, embryo-sac, embryo and endosperm. The cross and self-incompatibility in cut rose crossing was also found in this study. The data on appendix I showed self-incompatible habit as fruit drop, low number of seeds or seedlings in some crosses. The results of this study showed that of the 12 self-crossings done, 42% of crossing was lost. The hybridization was made between 14 fixed male parents and 27 female parents. In this study, with 268 cross combinations, 7844 flowers were pollinated. 196 successful crossings were obtained, 3299 hips harvested. 42% fruit setting was recorded. Self-incompatibility of rosaceous species was involved in monofactorial gametophytic system by S-alleles. The known product of the rosaceous S-gene was an extracellular ribonuclease produced in the style (the S-RNase). The S-RNase degrades the RNA of a pollen tube in matching combinations of S-alleles (Sassa *et al.*, 1992, 1994, 1996). Although the genome of *Rosa* had not been clarified, the concept of septet corresponding to the genome was proposed by Hurst (Hurst 1925; 1927). According to his septet theory, it was presumed that most tetraploid might have many S-alleles originated from 10 wild ancestors.

c) <u>Incomplete fertilization process</u> Germination of pollen may be abortive or unable to prenetrate into the ovary for fertilization and produce no seed. Without seeds, rose hips could not develop due to lack of food and adequate hormonal stimulation. It indicated that not only cross-ability of genus *Rosa* had effect on hybridization, but some other factors also did so i.e. chromosomal aberration, genetic unbalance between micro- and megaspores, embryo abortion before fertilization in several stages i.e. pollen tube entering, egg-break down, embryo degeneration after pollination, competition between ovules for nutritional supply and seed abortion due to poor nutrition (Vries and Dubois, 1983).

d) <u>Low seed/hip</u> Rose breeders frequently complained about the low seed production in crosses between rose cultivars or, a still lower production when species roses were involved. Although, the environment seemed optimal, the number of seeds obtained per pollinated hybrid tea flower was only between 5 to15 (Vries and Dubois, 1983). For this study, pollination index (PI) was measured for seed production and classified into 3 categories as low (<5 seeds/pollinated flower), moderate (6-12 seeds/

pollinated flower) and high number of seeds (>12 seeds/pollinated flower) by one single pollination. It indicated that PI could measure the efficiency of pollination. Vries and Dubois (1983) compared the effect of repeated pollination on fruit and seed set in crosses between the hybrid tea 'Sonia' with 'Ilona' for 0 to 7 times at 24 h intervals. The numbers of seeds per pollinated flower (PI) were increased fourfold the number of achenes per pollinated flower, compared with 1 time pollination and 5 times successive pollinations (4.1 vs. 15.6). It could be concluded that the PI related not only to repeated pollination, but also to cross-ability. These factors were likely to play a role in relatively few seeds per fruit. The results from the breeding pairs in this study elucidated that some varieties were suitable to be male plants while others were suitable to be female plants.

e) <u>Condition</u> In *Rosa* the temperature has effect on fertilization, fruit ripening and seed setting. The poor seed set in temperature below 18 ^{o}C is likely to be due to slow pollen tube growth, poor fertilization and embryo abortion, rather than under-developed or defective ovules.

To improve both fruit and seed set, Vries and Dubois (1983) studied the effect of repeated pollination of fruit and seed set in 'Sonia' x 'Ilona' crosses and found that by pollinating more than once, the number of seeds per fruit was more than doubled and the seed set was still 30% only. The effect of GA_{4+7} on fruit and seed set of 'Sonia' unpollinated and pollinated with 'Ilona' was studied. Dubois and Vries (1986) found that GA improved fruit and seed set, and induced heavier, earlier ripening of fruits. In the unpollinated category all achenes were parthenocarpic. In the pollinated category, 10 and 50 ppm GA after 14 days more than doubled the number of achenes per pollinated flower. Ogilvie *et al.* (1991) also reported the effect of gibberellic acid on fruit and seed set in crosses of garden and winter hardy *Rosa* accessions. Although in general, theses crosses gave fewer seeds per fruit, but fruit set was higher in most crosses when GA_3 was applied to the stigma at the rate of 250 ppm ten days after pollination.

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5.2.3 Post hybridization stage

For post hybridization stage, with 118 crosses combinations, 39,707 seeds were sowed, 7,132 seedlings obtained. Germination rate was 18% and 15.6% with healthy seedlings. 6,202 seedlings were selected for the next evaluation stage. Ma and Junyu (1995) showed some wild Chinese rose species which had been crossed or back-crosses with Chinese traditional old rose or modern rose cvs. With 227 cross combinations, 6,001 flowers were pollinated and 2,149 distant hybrid seeds gave 313 hybrid seedlings. The efficiency of hybridization of these crosses was comparatively low, both in fruit setting percentage and seed germination. It indicated that cross-ability had effect on fruit setting and number of seed.

Vries and Dubois (1987) suggested the use of pollination efficiency (PE) to measure the effect of temperature on fruit set, seed set and seed germination in ‘Sonia’ x ‘Hadley’ crosses. In general, seed propagation was used in breeding new varieties and in raising rootstocks of certain species. The germination of viable rose seeds was function of the genetic constitution and of the climatic condition under which the seeds matured (Rowley, 1956; Von Abrams and Hand, 1956). Growth and germination of seeds were controlled through a balance of endogenous growth promoters and growth inhibitors (Ueda *et al.*, 2001) in pericarp (Svejda, 1972; Svejda and Poapst, 1972). Generally, there was dormancy in the seed of roses, and it has been shown that cold stratification was necessary to break down this dormancy and to promote germination. The seed dormancy was related to the germination inhibitor which had been thought to be abscisic acid (ABA) and the pericarp thickness. Yambe and Takeno (1992) found that seed germination could be improved by treatments with 1% driselase, a macerating enzyme, for 36 hours.

In the current rose breeding for world market, crossing strategies had to be changed every year. The parents usually were from the existing varieties available in the market. Careful choice of parents was therefor a very important step to reduce risks. Large-scale crosses were carried out to be sure of good results in the progenies (Chaanin, 2003). Seedling populations needed to be large due to extreme heterozygosity, quantitative inheritance of economic traits, and the very large number of traits which must be considered. 99% of the offspring in each seedling population were discarded during some stage of selection (Vries and Dubois, 1996).

5.4 Selection

This study was intended to introduce a suitable rose breeding pattern for Thailand's condition. Chaanin (2003) concluded that the conventional breeding of cut roses proceeded in 3 principal stages: (1) creating new genetic variability by crossing (2) selection and test of elite plants and (3) propagation of the selected plants and introduction into the market. These principal stages did not differ essentially from those of most breeding companies. In the different stages of test, certain characteristics were taken as the main selection criteria for keeping or eliminating unsuitable seedlings. The breeding pattern of this study was to be discussed

Production of seeds and seedlings: Previously Thai's researchers (Samphraya, 1975, Kanta, 2003; Krasaechai *et al.*, 2003) reported that many crossings obtained low number of seeds and seedlings. After 2004-2005, studies on rose pollination and seedling techniques helped raise the number of seeds and seedlings (Krasaechai *et al.*, 2004; Ketpet and Krasaechai, 2005; Ketpet and Krasaechai, 2006).

Seedling stage: In this study, the seeds had germinated and the first true leaves appeared. This was the best time to recognize the resistance to some of the important diseases as initial infection such as powdery mildew normally occurred on young leaves. The susceptible seedlings could be discarded. Selection for healthy seedlings alone could reduce the size of the progeny by 13%, 6,202 plants of the total 7,132 plants. The more seedlings that were eliminated at seedling stage the better, since it greatly reduced the number to be grown in the nursery.

The concept of selection criteria of rose growth stage (mean±sd) was developed from the plant and animal breeding model from selection methods of independent culling level (Yamada, 1977; Xu and Muir, 1992), multi-traits (Strefeler and Wehner, 1986; Tang and Li, 2006), multi-stage (Muir and Xu, 1991; Xie and Xu, 1996a,b; Xu, 1997, Jemain et al., 2007), correlated characters (Lande and Arnold, 1983).

Small plant stage: When the seedlings had emerged and produced flower, the most important part of seedling evaluation began in order to screen for characters such as growth and other undesirable features in the early stage. There were also some correlated characters which could be detected in order to save time and labor in measuring each characteristic by prediction formulas, such as the flower bud size, which was associated with the number of petals. Some useful correlations and regression were established between small and medium plant size stages for a selection of best elite plants in the population. Selection at the small plant stage could reduce the size of the progeny by 68.5% of total 6,202 plants. Visser and Vries (1976) studied the juvenile period (J.P.) of apple and pear seedlings. The relation between the vigour and J.P. were found and used to shorten pre-selection periods. Vries (1976a) demonstrated that the time from seedling emergence to flowering (juvenile period) could be useful to predict flower production. Hybrid tea seedlings of the same age and juvenile period showed non-uniform appearance of flowering after first flowering. It indicated that the flowering of several sets referred to genetical and environment interaction. Vries and Smeets (1978a, b) also reported the effects of temperature on juvenile period in various temperatures. The juvenile period and flower bud appearance were shorter at higher temperatures, but taller at increasing irradiances. Vries and Smeets (1979) studied the effects of temperature on growth and development of hybrid tea rose seedlings. it was found that when temperatures increased, the juvenile period, the number of days to first flowering, the length of leaf part of the shoot, the length of flower stalk, the fresh weight of the shoot and the root decreased,but leaf number was not affected. Vries *et al.*, (1982) also studied the interaction of temperature and light on growth and development of hybrid tea seedlings, with reference to breeding for low-energy requirement (low temperature and low light intensity) in 9 controlled environments. It seemed possible to use criteria of percentage and rapidity of flowering and stem length at anthesis for seedling selection.

Medium plant stage: The selection criteria for flower and stem quality were on characteristics such as flower shape, opening capability. Correlations and regressions from medium plant stage could be used to predict large plant stage and as discard criteria. Selection at medium plant stage could reduce the size of the progeny by 16.5%. Vries and Dubois (1977) also used the correlations as a tool for selection. The selection was primarily for short juvenile periods and subsequently for long stemmed seedlings, prior to flower characters, because in young seedlings few characters appeared to be related to flower production. In the process, 50% of plants with short juvenile periods were retained, 20% of them having above average stem length.

Large plant stage: The selection was for plants with yield and stem quality suitable for the arching technique. This was to ensure both productivity and plant performance. Selection for large plant stage could reduce the size of the progeny by 3.4%. There were many studies on arching technique in cut rose cultivation (Zieslin *et al.*, 1976; Kool and Lensen, 1997; Ohkawa and Suematsu, 1991; Brito, 1999; Hoog et al, 2001; Lieth and Kim, 2001; Kim and Lieth, 2004). At this stage seedlings (non replicate and own roots) were evaluated under arching cultivation technique with partial bending of stem, to stimulate a new strong and long stem at the base of the plant. The quality of the stems could be evaluated for plant performance and yield potentials.

First budding stage: When the superior selections were interplanted (6 plants replicates with budded clones onto rootstocks) with standard check varieties, the relationships among variables needed to be determined i.e. rose type, number of flowers/plants, number of strong canes, number of petals, peduncle/flower ratio, bud size, flower size and stem length. Selection for first budding stage could reduce the size of the progeny by 1.2%. The choice of rootstock was discussed by Zieslin (2002) a good rootstock gave strong root system, soil - born disease resistance, earlier flowering, high productivity, good adaptation to various conditions, stress resistance, etc. Shock proteins of rootstocks were the main reason of vigor. Safi (2005) also reported a comparison of productivity of three rose cultivars (First Red, Versilia and Virginia) grown on their own roots and grafted onto three rootstocks (*R. indica, R. canina and R. hybrida* Natal Briar). Natal Briar showed the highest flower yield and required the shortest time to re-bloom. The other rootstocks gave lower yield but were still superior to own-roots plants. Vries (1977) also reported the productivity of 62 clones of cut roses grafted on the rootstock 'Brögs Stachellose' with reference to breeding for winter flowering. Progenitors for winter flowering roses should be selected among clones that combined a constant total shoot production with a low percentage of blind shoots. Plant performance and flower production was affected by rose rootstock selection (Cabrera, 2002).

Second budding stage: The elite selections were interplanted (4 replicates with 6 plants per replicates) with standard check varieties. First period, the relationships among variables needed to be determined i.e. bud size, flower size, number of petals, stem length and bud union. Second period, screening was to eliminate seedlings with questionable characteristics i.e.flower neck, yield and quality. Those that met all the requirements were selected. For disease resistance screening, Uggla and Carlson-Nilsson (2005) studied the screening for important diseases of black spot, leaf spot, powdery mildew and rust in 649 field-grown offspring from eleven families between *Rosa* sections *Caninae* and *Cinnamomomeae*. It indicated the possibility to select for multi-resistance traits to these diseases in seedlings from several crosses. Yan et al, 2006 developed the spore-suspension inoculation method to screen powdery mildew resistance of tetraploid rose population. Field problems of bent necks, breaking stems and propagation were also investigated. Chaanin (2003) suggested that plants with nicking necks should be eliminated at seedling stage as well as those with a small number of petals. This study suggested that elimination was a means to ensure flower quality. Plants with characteristics other than cut-rose (garden rose, pot rose) were ascertained by peduncle shaking and cross-section and discarded.

For yield and quality, an experimental design was used to rank the superiority of offspring, scoring plants with quality characters and summation of overall characteristics. The selection for yield and quality had also been reported in many publications (Vries, 1976a, b; Vries and Dubois, 1977). Selection for second budding stage could reduce the size of the progeny by 0.3%. Most characteristics of first and second budded clones were different from original plants, while those of first and second budded plants also were different, especially in the number of flower/plants and flower size, first budded being better on number of flower, but second budded better on flower size. Dubois and Vries (1997) studied the comparison of *R. chinensis* minima (Voss) Sims miniature seedlings and their clonal plants, with reference to selection for pot rose cultivars from various populations. In principle, seedlings and their clones performed similarly although the level of expression of seven corresponding characters could differ significantly as seedlings had earlier flowering, shorter stem length, fewer leaves, higher productivity, higher percentage of sprouted axillary buds and fewer petals than their clonal plants. Hybrid tea rose seedlings on their own roots had flower yields similar to plants on a rootstock (Vries, 1976b). Grouping of offspring colour was done before yield trial to be able to compare them with commercial varieties as standard check.

5.5 Inheritance of parent

Published literature about rose genetics is relatively rare. Rose breeding is mainly carried out by highly competitive private companies and their applied genetic knowledge is proprietary and unpublished. Furthermore, some technical factors make the rose a difficult model system for geneticists. It has indeed high heterozygote and ploidy levels, plus well known difficulties in sexual reproduction, from pollination to seed germination. This behavior reduced the efficiency of breeding programs and genetic understanding. Such studies of Visser *et al.* (1977b) studied from the analysis of genetic variation of inheritance of pollen viability. It was concluded that only significant GCA effects and no significant SCA effects occurred. Pollen abortion and sterility appeared to occur frequently in rose species and their hybrids, being related to the number of chromosomes (Erlanson, 1930). Vries *et al.* (1980) also studied long-term experiment of combining ability from analysis of variance of four flavoniod in F_1 populations. The study analyzed an incomplete diallel cross of 18 parents, with 114 crosses, including 9 selfing and 19 reciprocals in a 5-year period from 1972 to 1976. It showed highly significant difference between GCA of the varieties.

As for cytology studies, the results of this study showed that the comparison of the chromosome number between the 12 offspring of different colours and their parents was similar with 2n=28. Kanta (2003) also recorded similar observation. It indicated that crossing with the same chromosome number parents had the same number of chromosome in all of the offspring despite difference in colours. It indicated that crossing with the same chromosome number parent had the same number of chromosome and no difference in offspring of different colours.

On DNA fingerprint, this study used RAPD fingerprinting for hybrid identification in rose parents and offspring. The results showed that it could show the genetic relationships in parent rose, but could not identify the hybrid in rose offspring. Kanta (2003) also reported that Isozyme patterns from esterase and peroxidase did not give good results. Grossi *et al.* (1997) studied Isozyme polymorphism of *Rosa spp.* and cultivar identification by referring to the classification named groups by Wylies (1954). The Isozyme polymorphism was based on esterase, leucine aminopeptidase and superoxide dismutase systems to identify ancient rose cultivars. The comparison of electrophoretic results and taxonomical ordering obviously was not clear. Raymond *et al.* (1995) also reported the DNA fingerpring in the selection process of ancient roses by means of floral phenolic metabolism. RAPD marker was popular for its low cost and technical simplicity, but its extensive use was limited by its poor reproducibility when amplification conditions changed. Lewis *et al.* (2004) suggested that RAPD-PCR analysis was used to answer two questions concerning the identities of open-pollinated seedlings. The first of these questions centered on 'Xanadu', a newly registered seedling of 'Carefree Beauty'. The suspected pollen parent was 'Fragrant Cloud', a rose grown in close proximity to 'Carefree Beauty'. DNA analysis of all three varieties was used to investigate parentage. If 'Xanadu' resulted from a cross-pollination of 'Carefree Beauty' with 'Fragrant Cloud', then half of the bands resulting from the 'Xanadu' RAPD-PCR analysis would match with 'Carefree Beauty', and the other half would match with 'Fragrant Cloud'. Every band in 'Xanadu' was found in 'Carefree Beauty', and while some bands did match up between 'Xanadu' and 'Fragrant Cloud', there were no bands that were unique to these two varieties and not to 'Carefree Beauty'. Therefore, it appeared that 'Xanadu' was not a hybrid of ‘Fragrant Cloud’, but a result of a self-pollination of ‘Carefree Beauty’. For the further study, HATT-RAPD should used combination with molecular markers such as: RAPD, PCR-RFLP, AFLP, ISSR or DNA sequencing.

On heritability, in this study, the heritability of male and female effect from offspring of same male with 6 different females and same female with 6 different males were analyzed with 2 methods i.e. sib analysis and regression of offspring on parents. The regression of offspring on parent method displayed all heritability traits more than the sib analysis. High heritability on female effect was observed with sib analysis in number of petals, flower bud size, and peduncle length traits, and with parent-offspring regression analysis in number of petals trait. High heritability of male effect was also conducted with sib analysis in stem length and flower diameter, and with parent-offspring regression analysis in peduncle length trait. It indicated that the heritability value depended on the comparison methods i.e. sib analysis (comparing within family) and parent-offspring regression (comparing mid-parent and offspring in each family). Female effects might occur with cytoplasmic inheritance and male effect with nuclear-inherited (Debener, 2003). In general, traits of high heritability were subject to large genetic gain, under selection, per generation; those with low heritability might not be capable of significant advance through selection. Some information on *Rosaceae* family as pear (White et al, 2000), apple (Currie et al, 2000; Oraguzie *et al.*, 2001) had been published. Marshell *et al.* (1983) estimated heritability of three anthocyanin in *Rosa* i.e. cyanin, peonin and pelagonin. It indicated that colour intensity and quality of parents should be as near as possible to the desired colour of the progeny because of the quantitative factors influencing pigment intensity. Yan *et al.* (2005b) studied the heritability of 10 vigour-related traits from 88 diploid offspring and their parents (P119 and P117). Cherri-Martin *et al.* (2007) studied fragrance heritability in hybrid tea rose and reported that most of the cut flowers were non-scent. A well-balance combination of 'European' and 'Chinese' scents could be introduced to the offspring to create a new variety like the world's favorite 'Double Delight'. One of the major problems in heritability estimates was that the amount of traits of yield and quality of cut rose varied from case to case and difficult to measure with the limited number of progenies and seedlings.

Chaanin (2003) suggested that in the current developments in the world market for roses, crossing strategies had to be changed every year. Varieties with high production and good bud size in different colours were normally used. Thornless and fragrant varieties were also included in the crossing programme. The parents usually were from the existing varieties on the market. Test materials from the last years which had desirable characteristics were also taken to make backcrosses with one of the parents. Careful choice of parents was a very important step to reduce risks. Large-scale crosses were carried out to be sure of good results in the progenies. Seedling populations needed to be large due to extreme heterozygosity, quantitative inheritance of economic traits, and the very large number of traits which must be considered. 99% of the offspring in each seedling population were discarded during some stage of selection (Vries and Dubois, 1996).

5.6 Yield trial

In this stage the elite selections (6 red and 6 pink offspring) were interplanted (3 replicates with 90 plants per replicates) with standard check varieties (1 red and 1 pink) in a statistical design with conduct replicated tests that would insure the reliability of the selections within the usual statistical probability limits. There were many criteria by which the flowers would be judged. Plants with flowers falling below the standard would be eliminated. Selection of individuals as candidates for the introduction as new cultivars was largely subjective. Each individual selection was scored at bloom, as well as at appropriate times for disease ratings, propagation trials, harvesting evaluation and transport ability screening. The 40 characterizations of the selections were made by subjective scoring against a standard for each character, such as the performance of certain cultivar, a scale of five intervals around a selected arbitrary value or dividing the range of variation into each class. Scores were subject to the usual statistical tests. Scalar standards could be changed as populations improved. Several characteristics of the total yield, size, grading quality, disease, pest, etc. were recorded for the period of 2 years, several times to represent seasonal variations (Pompodakis et al, 2005). The undesired problems in production such as fading, blackening, blueing, bent neck should be noted. The main quality problems, which could occur in post-harvest phases of roses were: heterogeneous maturity, mechanical damage, grey mould (*Botytis cinera*) and mildew infections, cold storage (Serrano *et al.*, 1992), bacterial growth (Wittee and van Doorn, 1988; Wouter *et al.*, 1991), problems with flower opening, wilting and over heating during transport (Haines, 1999, Armstrong, 2000; Botden, 2002;). Suitable harvesting stage should be studied since correct harvesting involved flower opening capability and vase life. Rose varieties and seasonal variation also determined the harvesting stage. The good opening variety probably was more able to import carbohydrate from leaves and stem than the bad opening variety (Marrissen and Brijin, 1995). When testing new varieties in regard to their suitability for the market, a comparison with the commercial varieties regarding their production qualities could not be overlooked. Candidates with lower production or shorter stem had a poor chance of being established on the market. Two best offspring of each colour were selected for the advance market response. In this study, the number of clonal plants was too small to take several tests at one time. There should be 1 row per code. Chaanin (2003) suggested that this stage should have more materials available for several tests to find out suitable methods i.e. rootstocks and propagation methods, because intensity of colour, size of flower, stem length as well as production characteristics depended on it. Morey (1969) suggested that promising cultivar should be carefully observed and data collected on it for at least two years before final decision was made as to whether or not it was a failure, a success, or a possible intermediary in one' s breeding program.

5.7 Market response trial

The traditional breeding practice relied on the experience and opinions of one or two persons in identifying the most promising and favorable selections from the great number of controlled crosses. This naturally raised a question of the soundness of the method. Many releases never achieved commercial success for lack of the necessary cut rose qualities. Consumer tests were somehow impossible for routine screening of breeding selections, because of the limited availability of flowers and other resources required a comprehensive evaluation. The prediction of consumer response would be useful for screening breeding selections. A new variety should be accepted by both florists and general public. Usually introductions were made after such tests, and had proven to be beneficial in terms of time and investment of rose breeders, growers and all other parties in the chain from production to marketing.

Growers believe that success is based on choosing the right variety which involves market response, experience of growers, production area, and trades competition, average length of the stem, susceptibility to diseases, transport ability, vase life, labor requirement, and breeder's distribution policy (Pertwee, 2002a; 2002b; Kordes Soehne, 2003;Law, 2003, Evans, 2005). Several trends in production worldwide also depended on region, tradition, culture and marketing response (Armstrong, 2003; Bos, 2000; Law, 2001; Priel, 2002; Law, 2007). Trotter (1969) concluded that consumer preference for rose depended on several factors, such as purpose, price, colour and form of roses available in comparison with alternative choices. Today the ideal rose depended on the grower's location, target markets and marketing strategy. A few years ago, a grower in Europe would try only two rows of a new hybrid, and if it looked good, would order more. But today's growers must move quickly, purchasing large quantities of varieties that would be a success in the market right at the start. Colours also varied by country ; peach colour roses were popular in England and South of France, but not in Germany, where they required roses in red, yellow, orange, copper and green, as well as champagene and old rose pink. In Milan, Italy, the users liked big, long red and fuchia roses, but in Russia, yellow rose was not wanted. In the United States, the public like red, white and pink. Even stem length varied by market. Italy and Hungary wanted 90-100 cm roses; France and Poland 80-90 cm. Germany went for 60-70 cm hybrid teas and sweethearts. Russia used to take 100 cm-roses, but now import only 80-cm stems. The United States like 50-60 cm roses. Botden (2002) suggested that each grower tried to achieve the higher quality possible at the points of sale, in this case being when the characteristics of the flower, which the consumer perceived, meet or exceeded his or her expectations. Hampson *et* *al.* (2000) carried out the sensory evaluation as a selection tool in apple breeding selections for dessert quality. The results showed that sensory evaluation could be used successfully for screening breeding selection, and might provide more reliable data from customers than the opinions of only few people.