CHAPTER 2

LITERATURE REVIEWS

2.1 Classification and origin of rose cultivars

The *Rosaceae* is a large and diverse family of approximately 100 genera and about 3000 species. Of these species 90 are economically important such as apple, peach, strawberry, plum and rose (Leus, 2005). The genus *Rosa* comprises more than hundred botanical (wild) species, of which only about ten contributed to the development of cultivated roses: *R. chinensis*, *R. foetida*, *R. gallica*, *R. gigantean*, *R. moschata*, *R. multiflora*, *R. phoenicea, R. rugosa, R. wichuraiana* and *R. rubra*(Crespel and Mouchotte, 2003). Most of the roses grown today are not true species but are derivatives of interspecific hybridization, leading to a wide diversity among cultivated roses. In this genus polyploidy occurs frequently in wild as well as cultivated roses. The majority of the wild species are diploid, whereas most cultivated roses are tetraploid or triploid. A diploid plant contains seven pairs of chromosomes (Crane and Byrne 2003).

Rose has been admired for its beauty and fragrance since its first cultivation 5000 years ago by ancient civilizations of China, Western Asia and Northern Africa (Gudin, 2000). After selection and breeding for thousand years, especially after the first hybrid-tea roses were bred and recognized around 1850, roses have become one of the most economically important ornamental crops. They are cultivated today in gardens and alongside roads for decoration, in open fields for rose oil and hip production, and in greenhouses for production of cut and pot flowers**.** Rose cultivars

are commercially used as cut flowers, garden roses and miniature pot plants. Furthermore they are also one of the major flowers used for the perfume industry and they are important for their medicinal and culinary qualities. Roses are the most ancient produced ornamentals and continue to be highly appreciated (Gudin, 2000). Only 8 to 15 species contributed to the original germplasm of the modern rose cultivars in rose domestication. Numerous crossings and hybridizations were performed between rose founder species of European origin on the one hand and of Chinese origin on the other. This domestication has allowed the introgression into modern roses of important horticultural characters such as winter-hardiness, resistance to pest, floral complexity and flower doubling which were brought by European roses, while recurrent flowering or perpetuity as well as colour brightness came from their Chinese counterparts. Important characteristics were introduced in the rose cultivar gene pool from the progenitors of the modern rose cultivars, example recurrent flowering from *R. chinensis*, cold resistance from *R. wichuraiana*, or yellow colour from *R. foetida*. Of the important ancestors mentioned, only *R. gallica* and *R. foetida* are tetraploid, whereas the rest is diploid (Wylie, 1954). In contrast, most of the modern rose cultivars are tetraploid (Vries and Dubois, 1996). Molecular work confirmed the narrow genetic background in modern rose cultivars (Matsumoto *et al.*, 1998) and the influence of some ancestors in the germplasm of the rose cultivars (Leus *et al.*, 2004; Martin *et al.*, 2001). Various taxonomical and cytological studies have concluded that only 11 species were used to create the modern rose: *Rosa canina* L., *R. chinensis* Jacq., *R. foetida* Herrm., *R. gallica* L., *R. gigantean* Colett ex Crép., *R. moschata* Herrm., *R. multiflora* Thunb. ex Murr, *R. phoenicea* Boiss., *R. rugosa* Thunb., *R. wichuraiana* Crép., and *R. rubra* Blackw. A few more species have also

been used for breeding in the past but not in use for many decades, such as *R. banksiae* Ait., *R. laevigata* Michx, *R. bracteata* Wendl. *R. roxburghii* Tratt., *R. indica*, *R. odorata* Andr., *R. clinophylla* Wendl. and *R. persica* (Michx.) Bornm, except for the recent reuse of *R. banksiae* Ait. and *R. laevigata* Michx. Traditionally, cultivars are classified as Hybrid Tea (single flower), Floribunda (cluster-flowered) and miniature roses. Most cut rose cultivars are hybrid tea, whereas garden roses are of the there types. But, the distinction between these original groups of horticultural classes including polyanthas, hybrid teas, floribundas and miniatures have faded by intensive breeding. In fact breeding will tend to narrow the original gene pool. It is suggested that the rose selection practiced over the past 100 years, based on a narrow genetic background, may have led to severe genetic erosion (Vries and Dubois, 1996).Others oppose this hypothesis and refer to the high heterozygosity in the tetraploid cultivars and the vegetative propagation (Gudin, 2001; Noack, 2003)

2.2 Rose breeding

As far breeding objectives during the twentieth century, apart from ornamental characters, disease resistance became one of the important breeding goals in garden roses whereas ornamental characters, productivity and long vase-life are more important in cut roses.

In Thailand, Samphraya (1975) studied the various characteristics of *Rosa hybrida* 'Baccara' x 'Norita' progenies. The cross between 'Baccara' x 'Norita' produced 21.8% fruit set of which each hip contained 8.9 seeds. The results from seed test showed 3.2% germination with 9.1% viable seedling. The height of the hybrid plants was approximately 123.6 centimeters with the flower stem of 41.9 centimeters.

The opening buds at full bloom were globular, approximately 7 centimeters in diameter, consisting of 36.4 petals of 'currant red' and 'cardinal red', respectively. The time required from bud stage to full bloom was approximately 3.6 days. The results indicated that all hybrids were classified below acceptable qualities.

'Star of Thailand' from Chavalit Nursery was the first hybrid–tea rose seedling registered in 1977 with the American Rose Society from the cross of 'Mount Shasta x Pascali'(Nagavajara, 1999). Flower was creamy white with 40 petals.

Kanta (2003) studied varietal improvement and growing method of Rosa hybrids. Sixteen combinations of self and cross pollination of four cultivars of roses were conducted. Successful selfing and crossing was between 1.4-33.9% and 6-20%, respectively. Pollen germination depended on sucrose concentration and variety. The hips of unsuccessful pollination turned dry within 7 days; the successful ones needed 10-15 weeks for seeds to mature. It was found that the number of seeds per hip varied from 1-40. Mature seeds required $5^{o}C$ for at least 70 days to promote germination and would germinate within 7 days. The germination percentage varied from 3.4-50%. Hybrid seedlings required 11 weeks to flower and showed variation in flower colour and flower shape, demonstrating incomplete dominant gene interaction and probably multiple gene action. Three levels of X-irradiation 5, 10 and 15 Gy at 1.63 Gy/min dose rate to axillary bud of rose cultivars, Kardinal and Dallas, were also conducted. High doses reduced the first flowering stem length with early flowering dates in both cultivars. Root tip chromosome number was 2n=28. Isozyme patterns, esterase and peroxidase, did not give good results. Growing rose plants in soilless media consisting of 60% coir, 30% rice husk and 10% sand proved to be successful. Arching cultivation technique promoted stem length. However there was a varietal response to

this technique. Krasaechai *et al.* (2003) also reported the breeding for new varieties of rose.

Krasaechai (2004) studied rose pollination technique. Germination of the pollen of three varieties, Dallas, First Red and Kardinal on artificial media consisting of 15% sucrose, H3BO3 100 ppm without $Ca(NO_3)_2.4H_2O$ showed that variety 'Dallas' had the highest germination percentage (59.2%). The incubation of the anther under incandescent light bulb proved to be the best method in terms of pollen releasing time and pollen germination (35.5%). Rose flower at the earliest opening stage had the highest pollen germination. Keeping the rose flower in the vase containing rain water in the refrigerator proved to be the best method to prolong pollen germination ability.

Ketpet and Krasaechai (2005) studied the seedlings care technique. Peat moss was the best medium for germination (36.8%) and the highest transferring survival (96.7%).Seedling at cotelydon stage on growing media with the application Terraclor (20 ml. / 20 liters of water) before transplanting gave the best result. Feeding young plants with CMU-RPF nutrient solution proved to have beneficial effect.

Ketpet and Krasaechai (2006) found that the elimination of the side-shoot during seed production, using 2% $CuSO_4.5H_2O$ applied as a drop on the side buds proved to be successful. The method to remove seeds from hips, using blender, followed by 2 days fermentation in order to remove the pulp by strainers or re-blending was more effective than doing by hands. Sterilizating the seeds with 15% Clorox for 10 min followed by 10% Clorox for another 5 min gave the best result before seed sowing. Seed mixing with 20 ml/l Teraclor before sowing gave the best result.

2.3 Breeding technology

It is generally known that rose species were introduced in the Western world since antiquity and rose breeding was intensively undertaken in the eighteenth century (Gudin, 2000). However, rose cross breeding is probably much more ancient in the oriental world. There is evidence that roses were already cultivated 5000 years ago by civilizations in China, Western Asia and Northern Africa. These first acts of domestication and multiplication of species found in the wild led to the spontaneous occurrence of interspecific hybrids that have long been considered as original species. Together with the huge popularity of rose gardens, rose breeding is a feature of the nineteenth and twentieth century. The first records of aimed crosses date back to the beginning of the nineteenth century (Gudin, 2003). Roses are believed to be the first non-edible species in plant breeding. Up to now, rose breeding practices have not changed dramatically since the bursting of this activity (Gudin, 2001). World-wide there are 25 to 30 highly competitive rose breeding companies and many more amateur breeders (Gudin, 2003). Rose breeding research is carried out by highly competitive private companies, who keep their applied genetic knowledge proprietary and unpublished (Vries and Dubois, 1996).

Some companies have established associated research programmes with research groups to improve their methodology. The most applied part of the work, like research on mutation induction, fragrance, pigments or thornlessness heredity is not published (Gudin and Mouchotte, 1996). Although rose chromosomes are small and difficult to observe through cytology, since 1920, studies with historical importance were published by Hurst (Hurst 1925; Hurst, 1927). Since the 1960's, rose breeding has benefited from the general gathered knowledge, mainly concerning the sexual

reproduction of the species (Gudin, 2001). Little is really known on the genetic control of morphological or physiological characters of roses (Gudin, 2000). However, publications on topic like recurrent flowering (Semeniuk, 1971a, b; Vries and Dubois, 1978), pigmentation (Vries *et al.*, 1974; Vries and Dubois, 1978; Vries *et al.*, 1980; Marshall *et al.*, 1983), winter hardiness (Svejda, 1979) and dwarfness (Dubois and Vries, 1987) are available.

Arene *et al.* (1993) suggested a close link between genes controlling petal numbers, petal colours and dwarfness. Basic knowledge concerning pollen, pollination, seed maturation and germination (Gudin and Mouchotte, 1996) and the use of amphidiploids (Svejda, 1977) for a better control of hybridisation was published. Furthermore, selection procedures corresponding to new objectives, such as low temperature and disease tolerance or increased shelf life, have been used (Gudin, 2001). Selection procedures have been described for early prediction of flower productivity and in vitro tests on disease resistance. However, despite some recently acquired genetic data, rose breeding still is very dependent on breeders' experience, where aesthetical traits based on subjective selection are essential (Gudin, 2003). For cut flowers most important objectives in breeding are linked with ornamental and quality values like attractive flower colours, tough petals and double flowers, quality of stems, the size of the flowers, vase life and transport qualities and production capacity. Fragrance is linked with softer petals and a shorter vase-life and has therefore almost completely disappeared in cut roses (Chaanin, 2003).

For pot roses, inheritance of dwarfness is controlled by a single dominant gene (Dubois and Vries, 1987). Selection criteria are number of flowers per stem, flower colour, flower size, number of petals and plant habits. Other characteristics are linked

with production and shelf-life (Vries, 2003). Disease resistance is the main objective in breeding of garden roses. Despite this, aesthetical characteristics are insuperable. The demand for fragrance is often problematic since some fragrances would be linked with disease susceptibility (Gudin, 1995; Gudin, 2003).

More and more roses are used in landscaping, therefore 'carefree' types are demanded, which means pruning and crop protection measures are not necessary. Besides this the ideal rose has an all-season decorative effect like aesthetic hips during winter (Gudin, 2003).

The emphasis in breeding in the past used to be on ornamental characters like flower colour, scent and morphology, recurrent blooming and plant habit. In recent years criteria like disease resistance against the major pathogens and pests, frost tolerance in garden roses, productivity and vase life for cut roses have become increasingly important. Some studies have been conducted to reveal the inheritance of traits like flower morphology, prickles and important disease resistances (Debener, 2003) (Table 2.1).

Table 2.1 Inheritance of traits in roses

Trait	Inheritance
Prickles on petioles	Single recessive
Recurrent flowering	Single recessive
Corolla	Single dominant
Double flowers	Single dominant
Dwarf phenotype	Single dominant
Moss phenotype	Single dominant
Prickles on stems	Single dominant

Resistance to black spot	Single dominant
Resistance to powdery mildew	Single dominant
	Quantitative
Yellow flower colour	Single dominant
Pink flower colour	Single codominant
Prickle density	A major and a minor QTL
Flower colour	Quantitative
Leaf size	Quantitative
Petal number	Quantitative
Prickle size	Quantitative
Winter hardiness	Quantitative

Source: Byrne, 2009.

The advance of molecular technique makes it possible to detect specific genes or chromosome regions controlling important traits. This helps to understand the structural organization and function of the genes, and provides information for marker-assistant selection in rose breeding. A variety of molecular markers are available in roses: RFLPs (restriction fragment length polymorphisms), RAPDs (randomly amplified polymorphic DNAs), AFLPs (amplified fragment length polymorphisms), SSRs (simple sequence repeat or microsatellites) and SCARs (sequence characterized amplified regions), etc. (Rajapakse, 2003a).Genetic studies of roses are limited due to their open-pollinating mating system and difference in ploidy level. Inbred lines that could serve as parents of a classical mapping population are not easily obtained. This complication is solved through "pseudo-testcross" strategy, in which unrelated parents with a high degree of heterozygosity are crossed. The resulting mapping population is suitable for mapping of genes for the traits of interest.

Theoretically, segregation of up to four alleles for diploid roses and up to eight alleles for tetraploid roses per locus is possible in the respective populations. Molecular markers have been used in roses for genetic studies (Debener and Mattiesch, 1999; Rajapakse *et al.*, 2001; Crespel et al,. 2002a, b; Linde *et al.*, 2004), cultivar identification (Esselink *et al.*, 2003; Leus *et al.*, 2004) and genetic diversity or phylogenetic studies However, molecular marker-assisted breeding in rose still is in its infancy (Debener *et al,* 2003). Molecular genetic study of a trait of interest comprises phenotypic evaluation of the trait in a mapping population, construction of a genetic map for this population based on polymorphic molecular markers, mapping of quantitative trait loci (QTLs) for the trait, and possibly identification and cloning of the genes underlying the QTLs (Debener, 2003).

2.4 Rose genetics

Plant breeders and cytogeneticists have long valued the analysis of metaphase chromosomes for elucidating genomic relationships. Chromosome numbers in the genus *Rosa* are based on multiples of seven and range from 2n=2x=14 to 2n=8x=56. Rose chromosomes are fairly small with an average DNA content of 1.1 pg/2C for diploid roses (Yokoya *et al.* 2000). The genome size is estimated to be about four times larger than that of *Arabidopsis thaliana* (Debener and Mattiesch 1999; Rajapakse *et al.* 2001).Despite the low chromosome number and small genome size, little is known on the genetics of rose (Vries and Dubois 1996; Gudin 2000). This is largely due to characteristics like a high degree of heterozygosity, varying ploidy levels between species, difficulties in sexual reproduction, low seed set and poor seed germination. However, current advances in molecular genetic mapping have enhanced

the understanding of rose genetics and the genes controlling important traits, including resistance to fungal diseases (Debener 2003; Rajapakse *et al.* 2001; Crespel *et al.* 2002b; Von Malek *et al.* 2000; Kaufmann *et al.* 2003).

Roberts *et al.* (1990) reported the *In vitro* procedures for induction of tetraploidy in a diploid species, *Rosa wichuraiana* through treating with spindle inhibitors or tritiated thymidine.

Ma *et al.* (1996) suggested technique to produce consistently high-quality slides of somatic chromosomes of roses (*Rosa sp.*) from shoot tips. The best results were obtained after pretreatment in a mixture of 0.1% colchicines and 0.001 M 8-hydroxyquinoline for 4 h, and fixation in 2 acetone: 1 acetic acid (v/v) with 2% (w/v) polyvinylpyrrolidone. The darkest-stained chromosomes were obtained with carbolfuchsin staining of air-dried cell suspensions that had been spread in 3 ethanol: 1 acetic acid (v/v).

Yokota *et al.* (2000) studied the DNA amounts in roses and suggested that in chromosome counts, steps were as follows: the tips of actively growing roots or shoots were pretreated in a saturated solution of a-bromonaphthalene for 24 h at $4^{o}C$, fixed overnight in ethanol-glacial acetic acid(3 : 1, v : v), hydrolysed in 5 M HCl at $25^{o}C$ for 30 min, stained in Feulgen reagent for 3 h, then stored in 450 g/l acetic acid for 1-24 h at $4^{o}C$. The terminal 1 mm of the tips were excised, macerated and squashed between slide and coverslip in 45% aceto-orcein. Cover slips were removed from frozen preparations, and the slides dipped in absolute ethanol and flamed. Unstained slides were observed by phase contrast microscopy. Supplementary staining was obtained with 0.05 g/l toluidine blue in citrate buffer (pH 4.0). Slides were then air-dried and mounted under a cover slip in histomount.

There are very few reports on molecular characterization of rose plants (Rajapakse *et al.*1992, Ben-Meir and Vainstein 1994, Debener *et al*. 1996a, b, Millian *et al*. 1996). DNA analysis techniques have been shown to identify rose cultivars (Vainstein & Ben-Meir 1994; Ballard *et al.*1995; Cubero *et al*. 1996). Randomly amplified polymorphic DNA technique has been widely used in many plant species for cultivar analysis, population studies and genetic linkage mapping (Debener *et al*. 1996). Optimization of the RAPD method depends on selection of primers. Although the RAPD method uses arbitrary primer sequences, many of these primers must be screened in order to select primers that provide useful amplification product.

In recent years, the random amplification of genomic DNA, mostly with short arbitrary primers (randomly amplified polymorphic DNA, RAPD) has received particular attention in the field of applied genetics. The main advantage of RAPD markers over other molecular markers, in particular to markers involving DNA-DNA hybridization techniques, is the low technical input, the short time requirements and low requirements of DNA purity and small quantity of plant DNA needed for the analysis. This allows the generation of large numbers of markers in short periods of time. The major disadvantage of RAPDs is their lower reliability compared with restriction fragment length polymorphisms (RFLPs), and microsatellites or minisatellites. However, in those cases where the laboratory equipment does not allow the use of RFLPs or amplified fragment length polymorphisms (AFLPs) , RAPDs are the markers of choice.

Vainstein *et al*. (1993) observed that the genetic similarities were small within the cultivated rose groups (hybrid tea, floribunda, polyantha and miniature) by using 28 DNA fragments from micro satellite fingerprints. 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, identification of varieties/species, phylogenetic relationship and conservation and management of genetic resources (Debener, 2003).

Debener *et al*. (1996a, b) conducted RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. The genetic variability based on randomly amplified polymorphic DNA markers was analysed among 10 cultivated rose varieties and 9 wild species from three different series of the genus *Rosa*. Using 13 different RAPD primers, 104 polymorphic DNA fragments with a high potential to differentiate rose genotypes could be produced. A dendrogram displaying the relative genetic similarities among the genotypes showed the existence of large genetic diversity among the cultivated roses as compared to the wild species. Furthermore, the main clusters found here were in agreement with known pedigrees and the classical taxonomy. However, the relationships between cultivated roses as inferred by RAPD markers did not correlate with the classical rose classification system. From the present data it was concluded that cultivated roses displayed a high level of genetic variability despite the fact that single morphological and physiological characters might be less polymorphic within rose groups. This contrasted with the widely accepted opinion of a lack of genetic variability in roses, and was also in accordance with the reported history of rose breeding which made it highly probable that rose genomes comprised mosaics of different species genomes. As a consequence, it might be possible to utilize the high genetic variability of all genetic traits not under actual selection by breeders for future breeding program.

Debener and Mattiesch (1998) investigated the effective pairwise combination of long primers for RAPD analyses in roses. Twenty-four primers of different lengths (each eight of 10, 15 and 20 bp) were tested each in RAPD reactions with DNA of *Rosa multiflora* and *Rosa canina.* The reactions with single primers of a particular length were compared with all 28 pairwise combinations within each length class in both single primer reactions and in primer combination reactions. All primer classes produced fragment patterns of comparable complexity. However, the number of new fragment patterns and the number of fragments containing repetitive DNA was dependent on the primer length. Combinations of long 15- and 20-mer primers produced more new fragments and a lower amount of repetitive DNA than shorter 10-mer primers. Implications for the use of long primer combinations in projects which required large marker numbers were discussed in comparison with other marker systems.

Debener *et al.* (2000) reported that sports from two cut rose varieties, as well as a garden rose variety, were analyzed with molecular markers. Between 695 and 752 randomly amplified polymorphic DNA and AFLP fragments were used to infer genetic differences between the sports, the original variety and seedlings of these varieties. Whereas no polymorphisms between the sports of the cut rose varieties and the original variety were observed, five polymorphisms could be detected between the garden rose variety and its sports. In contrast, a large number of polymorphisms occurred between all varieties and their seedlings. Therefore molecular markers could be used to verify the origin of vegetatively propagated rose plants of doubtful origin, thus enabling breeders in the future to claim plant breeder's rights on sports of varieties already registered.

Lewis (2004) reported that RAPD-PCR analysis was used to answer questions regarding the identity of numerous varieties of roses. It was previously reported that the DNA profile of 'Bremo Double Musk' did not match any of the other musk (*Rosa moschata* Herrmann) varieties. However, upon further analysis, it was determined that 'Bremo' is indeed a true musk. A parentage analysis of 'Xanadu', a recently registered modern rose, indicated that it probably resulted from a self-pollination of 'Carefree Beauty'. Numerous samples of 'Found Noisettes' were analyzed, showing multiple genetic differences among the varieties, but similarities to their assumed ancestors, 'Blush Noisette' and 'Champneys' Pink Cluster'. Utilizing 'Katie Bell's Devonianthus', it was determined that roses grown today as 'Tradd Street Yellow' and 'Devoniensis' were very likely the real, original, 'Devoniensis'. Finally, on the question of the identity of 'Spray Cécile Brunner', 'Bloomfield Abundance' was investigated, indicating that the plant currently grown under both names was truly a sport of 'Cécile Brunner', and should be classified as 'Spray Cécile Brunner'. Therefore, RAPD-PCR can be a useful tool in determining the heritage of historic and modern roses. RAPD-PCR is a powerful technique that can help deduce the genetic relatedness of many rose cultivars.

For flower production, there have been research topics on bloom habits where it has been elucidated that blooming is conditioned by a recessive allele whereas the non-recurrent blooming is conditioned by a dominant allele at one loci (Crespel *et al.*, 2002a, b; Debener, 1999; Debener, 2003; Vries and Dubois, 1978; Vries and Dubois, 1984; Rajapakse *et al.*, 2001; Semeniuk, 1971a, 1971b; Zykov and Klimenko, 1999).

For the flower colours, the pigments in rose flowers are anthocyanidins, flavonols, and carotenoids (Vries *et al.*, 1974). Pink flower colour is caused by the

accumulation of anthocyanidins in the petal cells and controlled by a major loci with white being homozygous recessive, medium pink heterozygous, and dark pink homozygous dominant for the pink allele (Debener, 1999; Debener, 2003; Lammerts, 1945b; Zykov and Klimenko, 1999). Flower colour in ornamental peaches is conditioned by multiple genes for red, pink, and white flower colours. The flower colour genes are red (rr) being recessive to pink (R_), light pink (pp) being recessive to dark pink (P_), and white (ww) being recessive to creamy white (W_) (Lammerts, 1945a).

In terms of flower forms and size, the double flower trait is controlled by a dominant allele and the single flower state is controlled by a recessive allele (Crespel *et al.*, 2002a, b; Debener, 1999; Debener, 2003; Lammerts, 1945b; Swim, 1948). Environmental interactions and additional minor genes are reported to influence the mean number of petals per flower (Debener, 1999; Debener, 2003; Lammerts, 1945b; Morey, 1959; Rajapakse *et al.*, 2001). In ornamental peaches, flower form is different from roses with the single flower form completely dominant (D_1_) to the double flower form. The number of petals in the double flower form is conditioned by Dm_1 and Dm_2 alleles (Lammerts, 1945a). Morey (1959) reported that the terminal and spring rose flowers tend to have more petals than lateral and summer flowers, that rose floral parts are arranged in whorls of five, and that most species roses have only one whorl of five petals. Morey (1959) suggests that double flowers in species roses are actually single flowers that have extra petals called petaloids. Petaloids are formed from stamen initials that fail to develop properly and make petals (Debener, 1999; Morey, 1959).

The prickleless rose has become an important trait in commercial rose production. Botanically, prickles are found at the axils of leaves and prickles are modified clusters of epidermal hairs. The number of prickles found on rose stems usually decrease from the bottom to the top (Andre, 2003). The presence or absence of stem prickles is controlled at one loci with the presence of prickles a dominant allele and the absence of prickles a recessive allele (Debener, 1999; Debener, 2003; Rajapakse *et al.*, 2001). Research with blackberries, a close relative of roses, shows that the absence of prickles on stems is also controlled by a single recessive gene. The inheritance of prickle density in roses which is quantitative (Lammerts, 1945b; Swim, 1948), has recently been reported to be controlled by two independent QTL loci (Crespel *et al.*, 2002a, b).

Breeding for disease resistance has been going on all over the world. Powdery mildew *(Podosphaera pannosa* Wallr.: Fr.) is the major disease of greenhouse roses and is also seen in field grown roses, while black spot is the most harmful fungal disease of field grown roses (Debener, 2003; Horst, 1983; Kaufmann *et al.*, 2003; Linde and Debener, 2003). Powdery mildew and black spot can be found in all countries where roses are grown (Alvarez, 2003; Horst, 1983; Linde and Shishkoff, 2003). Resistance to the rose pathogens powdery mildew and black spot (*Diplocarpon rosae* Wolf.) are reported to be controlled by a 'gene for gene' interaction (Yokoya *et al.,* 2000a; Debener, 2003; Kaufmann *et al.*, 2003). Races of both rose powdery mildew and rose black spot have been described (Linde and Debener, 2003; Malek and Debener, 1998).

2.5 Selection evaluation

      2.5.1 Heritability The parameter measuring the strength of the inheritance is called heritability, which is the ratio of genetic variance (V_g) to phenotypic variance (V_p). Narrow sense heritability (h^2_n) is the ratio of the additive genetic variance to the phenotypic variance (Va/Vp). Broad sense heritability (h^2_b) is the ratio of total genetic variance to the phenotypic variance (V_g/V_p). These heritabilities have to be interpreted carefully to be correct. Each heritability estimate is specific to the population, the trait, and the environment on which the estimate is based(Falconer and Mackay, 1996; Kearsey and Pooni, 1996).

      Marshall *et al.* (1983) suggested that in breeding for anthocyanin colours and winter hardiness in Rosa, more than 1200 progeny from 47 families were analyzed for anthocyanin pigment. Cyanin, peonin and pelargonin were found in 99%, 52% and 31% respectively, of the seedlings. Each pigment was highly heritable from seed or pollen parents or both. All showed quantitative inheritance, particularly cyanin and peonin. A system was proposed to explain most of the synthetic pathways and controls for anthocyanin production in roses. Heritability estimates derived from regression between parents and progeny showed that cyanin, and pelargonin and total anthocyanin were each highly heritable. There seemed little difference between the effects of seed or pollen parent; both were high for each pigment. Heriable interactions from regressions of one pigment in the parent on another pigment in the progeny were all much lower than when the pigment was the same. Again results from the seed or pollen parents did not differ greatly from each other.

      Yan *et al.* (2005b) reported that broad sense heritability based on means of the 10 traits was high and ranged from 68 to 92% measured on 88 entries of the diploid

population evaluated separately in Denmark and the Netherlands. Yan et al. (2006) also reported the broad sense heritability to describe genetic differences in response to the isolates; two contrasting subsets of genotypes were composed based on the disease scores selected at 11 day post inoculation. To understand the inheritance of mildew resistance, a tetraploid population with a size of 181 seedlings was obtained by crossing two tetraploid genotypes each having partial resistance. The estimates of broad sense heritabilities of the disease score were high for both isolates, being 57% for isolate 2 and 62% for isolate F_1.The large diversity of responses of the genotypes, the significant genetic variation and the relatively high heritability of resistance found in the present population might facilitate the selection of highly resistant genotypes.

Cherri-Martin et al. (2007) studied the fragrance heritability in hybrid tea rose. Variations in scent quality were mostly linked to the quantity of monoterpenes that was present. Offspring emitting a pleasant fragrance was found to be rare.

2.5.2 Multi-stage and Multiple-trait Selection The effectiveness of rose breeding program depends on the breeder's ability to select superior individuals for many traits of interest. One method of idenifying superior individuals for multiple traits is the use of selection criterion, available to help the breeder in the selection process (Strefeler and Wehner, 1986).

Improvement of efficiency and quality of cut-rose production can be achieved by improving several characteristics of the rose plants. The ideal cut-rose should produce not only a high yield of flowers, but also a good percentage of high quality flowers. The rose plants should be fast growing with good disease resistance. Flowers should have long vase-life, good transportability and favorable market response. Selecting for multiple traits at each stage is called multi-stage selections.

Multi-stage selection which is refered to as selection criteria, combines several desired aspects of independent culling level in several stages (Tang and Li, 2006). Methods of multiple trait selection consist of tandem, independent culling levels and selection index. Independent culling levels is a frequently used method of improvement and used in combination with multi-traits and multi-stage selection. The developments for independent culling level selection may possibly make multiple traits available at each stage (Muir and Xu, 1991; Xu and Muir, 1992). Selection on multiple traits and multi-stage relationship means that in selecting for one trait, genetic value (mean, strandard deviation, etc.) change in the other trait and other stage may be resulted (Xie and Xu, 1997).

In general, the selection criteria are divided into 3 criteria i.e. below average threshold, equal to the average and above-average threshold. Suppose n traits are to be selected in m stage (m<n), the phenotypic values of n traits can be partitioned in three levels; mean-sd, mean, mean+sd (Figure 2.1).
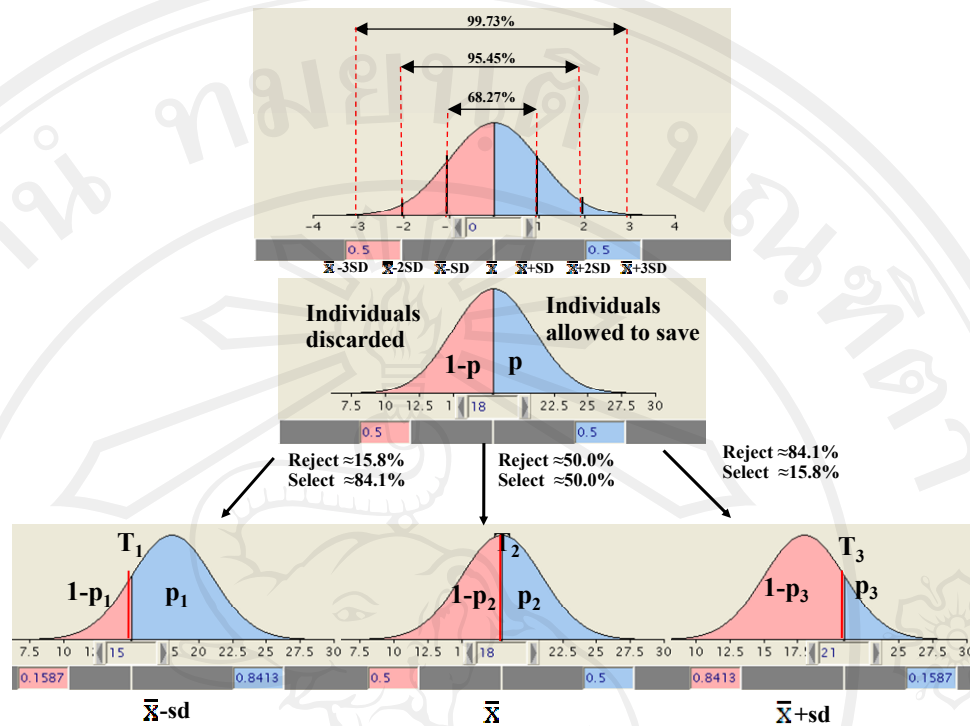
Figure 2.1 The proposed scheme for selecting "random samples" or targeted group for selection. Sd=standard diviation, $\bar{x}$=mean, model 1=mean-sd was the lower selection intensity, model 2=mean was standard to select the plants and model 3 was the common truncation points. Under truncation selection the uppermost (most or lowermost) fraction *p* of a population is selected to be saved. Althernatively, one could set a threshold level *T* for which individuals are allowed to be saved.To predict response given either *p* or *T*, it is necessary to know the mean of the selected *p* (p_1, p_2, p_3), from which selection diffrentials could be computed.

This study set the following selection criteria,

1) If the study population is too large to measure the actual value of individual plants directly. It can be estimated from the sampling (Jemain *et al.*, 2007). The relationships of each characteristic were conducted in order to select the appropriate criteria, but an appropriate manner of the same stage (Figure 2.2) or between stages has to be selected (Figure 2.3).
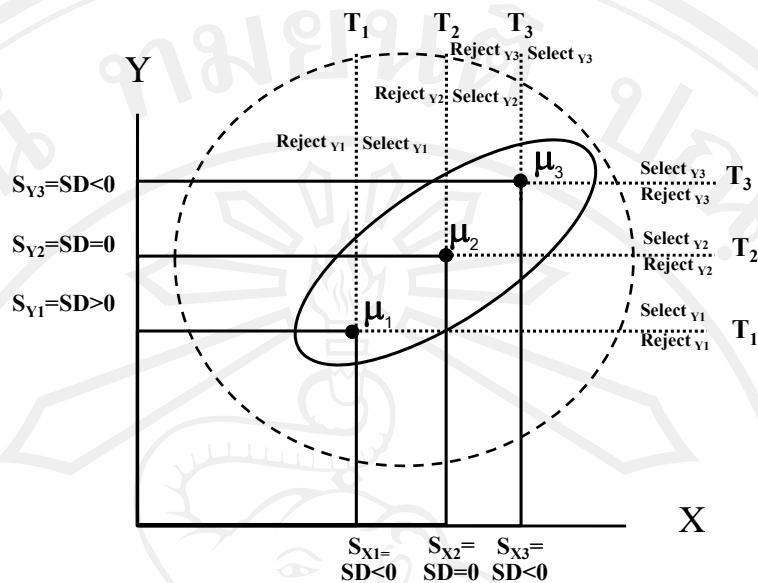
2) If the study population is small and can be measured directly, suitable selection criteria can be set from the study of the relationship of each characteristic. These relationships can set the selection criteria for superior plants with best possible characteristics.
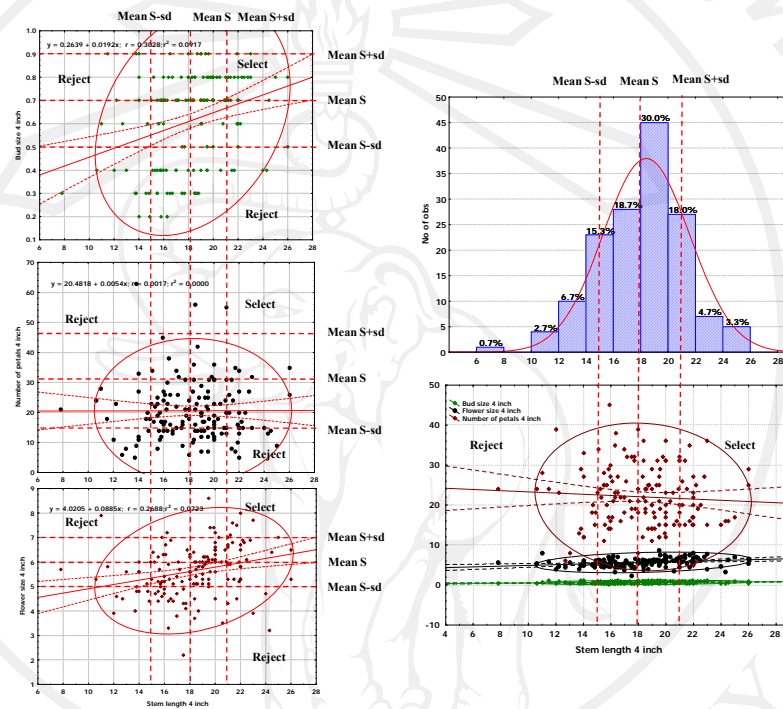
Figure 2.2 The relationship between 3 traits against stem length in small plant stage
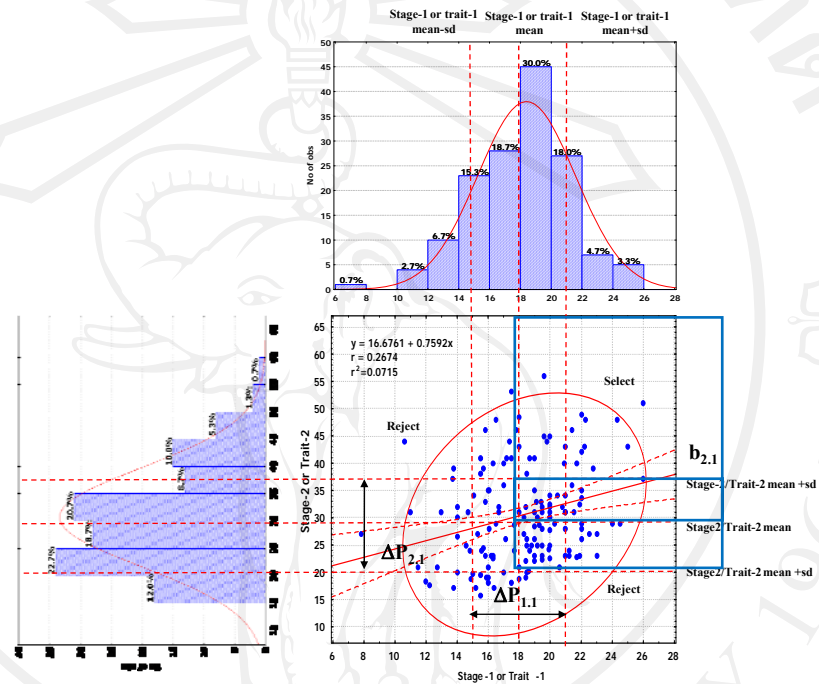
Figure 2.3 the relationship between stem length of small and medium plant size stage

Change in the mean variable:

The Selection Differential (*S*) is the mean before selection (μ_0) – mean after selection

(μ_1)

$$S = \mu_0 - \mu_1$$

Given a threshold cutoff T, the expected mean of the selected plants is given by the conditional mean, mean±sd (Figure 2.4). The plants should first be selected by using minimum level in each trait to identify plants with traits of best possible performance and, were again selected in the next stage criteria.

Figure 2.4 The differentials effects of phenotypic and genetic correlation on selection response. The dotted circle is the jointed distribution of the phenotypes for traits X and Y, the solid ellipse was the phenotype correlation and the points were the mean-sd (sd<0), mean(sd=0) and mean+sd (sd>0). Truncation selection between individuals with X_1, X_2, X_3 and Y_1, Y_2, Y_3 in same satge saved, with mean μ_1(the mean-SD; SD<0) , μ_2 (the mean; SD=0) and μ_3(the mean+SD; SD>0). Phynotypic traits in same or different stage correlation and regression were measured. Following simulation selection, the mean, sd, selection of both X and Y have changed, and all parameters could be used as the optimum of minimum level selection.

The mean±sd of one trait are selected to make phenotypic value change in other trait. The mean-sd of n traits will set the minimum level for selection, which will be used in another stage.

For simplicity, $b_{2.1}$ is the regression coefficient of stage-2 or traits-2 on stage-1 or traits-1, ΔP_{ij} is the selection differential of the first subscripted trait (i) when selection is intended for the second subscripted trait (j) as is illustrated in Figure 2.5.

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Figure 2.5 The relationship between multiple traits or multistage selection

Selection on a character can result in a within- population change in the mean of other phenotypically corelated characters not themselves under direct selection (Xie and Xu, 1996; Yamada, 1977).

Selection criteria (SC) = SC trait 1+SC trait 2 +SC trait 3+…+SC trait n

When considering multiple trait selection, the genetic correlation among traits is important, possitvely or negative, which means as you select to change one trait, the second trait moves in the same direction. Overall phenotypic mean change takes place

through selection. Selection criteria can be derived from information on rose plants' phenotypes and yield. Phenotypic information is geneally based on objective measurement of roses' characteristics.Some traits can not be measured directtly on the individual, some traits are unseen by time limited. When these traits are included in the selection objective, the selection criterion should include correlated sources of information such as other traits and information on relatives.Correlation among traits may be exploited to reduce testing plants (Lande and Arnold, 1983). It is difficult to accurately measure directly on individual plants. Therefore, the selection on quality of roses can be achieved by using the traits of random sampling plants for setting up minimum culling levels as criteria.

A problem of multi-stage criteria selection is the criteria corelated between stages. As such, selection at an earlier stage will cause the distritution of the correlated trait or criteria to change at a latter stage. Therefore, general solutions for optimum truncation points must be used to calculate proper truncation points. The efficiency of transformed culling may greatly exceed that of multi-stage selection because the latter does not incorporate information from previous stage of selection into the current stage.

A characteristic feature of this study is to provide a technique which enables one to evaluate objectively the realized selection as actually practiced by a breeder without knowing his selection criteria, provided that the data on all traits that contributed to the selection criteria are available. Few investigators have attempted to quantify such data.This data describes a useful technique designed to evaluate the result of selection in rose.