UNDERSTANDING AND MANIPULATING POLYPLOIDY IN

GARDEN ROSES

A Thesis

by

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ABSTRACT

Rose chromosome number ranges from 14 to 56 and understanding the dynamics of rose chromosome numbers can help improve the progress made in breeding programs and enhance the understanding of certain rose populations. An analysis of the pollen diameter of 126 roses in a breeding collection suggested that 49 were diploid, 67 were tetraploid, and 10 were hexaploid. An analysis of the pollen diameter combined with pollen appearance suggested that 39 were diploid, 39 were triploid and 48 were tetraploid. Chromosome counts determined that there were 44 diploids, 28 triploids, and 54 tetraploids. Thus pollen diameter was 86.3% accurate in identifying diploids and 74% accurate in identifying tetraploids. Pollen diameter and appearance was 77.2% accurate for diploids, 71.4% accurate for triploids and 66% accurate for tetraploids. A common occurrence among the triploids was the presence of irregular and shrunken pollen grains that were likely aneuploids leading to pollen abortion. However, some triploids showed very few shrunken pollen grains and consistent pollen sizes which could suggest that these individuals have better fertility relative to other triploids. Among diploid and tetraploid plants, the frequency of 2n pollen grain production was 9% and 1.8% respectively. A series of interploidy crosses indicated that there were small differences in set, seed yield and seed germination in crosses done between diploids or between tetraploids as compared to those done between either diploid or tetraploids and the triploid 'Homerun'. The ploidy level of the seedlings of these crosses was followed and it was determined that the triploid plant produced viable n, 2n and 3n gametes. When the

triploid plant 'Homerun' was crossed with tetraploid seed parents, there was a nearly even distribution of progeny resulting from fertilization with 1n and 2n pollen grains. When 'Homerun' was crossed with diploid seed parents, there were more progeny resulting from 2n gametes than n gametes. The progeny of these diploid x triploid crosses also exhibited reduced fertility as seen in the high percentages of shrunken pollen grains in these individuals.

DEDICATION

This thesis is dedicated to three special people whose unconditional support and

encouragement have helped me become the person that I am today:

Father: Dean Ueckert

Mother: Melanie Ueckert

Brother: Sam Ueckert

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CHAPTER I

INTRODUCTION

Roses are one of the most common ornamental plants encountered around the world and have been cultivated for centuries for their uses as harvested flowers, potted plants and landscape icons. The plants are also grown for the hips which can be used for aesthetic or culinary uses (Zlesak, 2009). These characteristics along with the impressive range of plant and flower morphology have made roses one of the most popular groups of horticultural plants. Roses would not be as important as they are today if not for the incorporation of the recurrent blooming gene into once blooming genotypes. Without the addition of the ever blooming trait, rose plants would only bloom for a brief period once a year. Modern rose cultivars bloom repeatedly during the warm growing season depending on the cultivar and climatic conditions.

Roses are indigenous to the Northern Hemisphere with over 100 recognized species. Most cultivated roses come from the subgenus Rosa (Zlesak, 2009, Gudin 2000). Rose varieties were domesticated independently in Europe and in Asia for various purposes. Once these two groups, the European tetraploid and Chinese diploid, were brought together during the early 18th century, they were combined to create many of the complex rose cultivars that exist today (Gudin 2000; Zlesak 2006; 2009). These first crosses created triploid individuals such as the first widely known Hybrid Tea rose 'La France',created by Guillot in 1867. It is theorized that the early triploid individuals acted as a bridge between diploids and tetraploids through 2n gametes (Crespel et al., 2006). Among these species, the basic number of chromosomes is seven with most individuals ranging from diploid to

octoploid (Zlesak, 2006). Recently, one decaploid species was discovered in Yunnan China, the first known decaploid rose species (Jian et al., 2010). Ploidy level has a significant effect on plant performance, appearance and combining success with other individuals (Levin, 2002; Zlesak, 2009).

Breeding in roses is facilitated by using individuals that produce gametes with the same ploidy level. Altering ploidy level through gametic polyploidization can circumvent restrictions caused by dissimilar ploidy level between plants (Zlesak, 2009). The challenges of a crop with dynamic ploidy levels are made easier with the collection of the ploidy information on individuals and populations. Physically staining and counting chromosomes takes considerable time and skill to conduct (Zlesak, 2009). Several less complicated, estimation techniques have been developed to bypass chromosome counts. These include measuring pollen size and flow cytometry. Using flow cytometry with macerated leaf tissue as the nuclei source has been used to characterize sporophytic ploidy level in rose species (Jacob et al., 1996). However, DNA content among rose individuals at a common ploidy level can vary enough that it can spread into the DNA value of another ploidy level (Jacob et al., 1996; Yokoya et al., 2000). In a highly heterogeneous, multispecies complex crop such as rose, this is especially true, where wide interspecific crosses have led to frequent genome restructuring (Levin, 2002). As a result, flow cytometry is most useful when done in conjunction with another method of ploidy assessment (Zlesak, 2009).

The pollen diameter of individuals is a valuable tool to botanically classify and determine ploidy level in many groups of roses. Pollen size generally has a positive correlation with increasing ploidy level. However, the range of pollen diameters of two

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different ploidy levels can have overlapping sizes that lead to inconclusive results. Along with the natural variation in pollen size, the method in which it is harvested and prepared for viewing can affect accuracy. Despite the potential problems associated with using pollen size, it has been reported to have a good success rate for non intensive general conclusions on ploidy level (Zlesak, 2009; Jacob and Pierret, 2000). In potato, pollen diameter was 93% accurate in distinguishing between diploid and tetraploid potatoes, although it was not successful in separating tetraploid from hexaploid individuals (Bamberg and Hanneman, 1991). In Zlesak (2009), pollen diameter had a 91.1% success in identifying diploid rose individuals in a population containing diploids, triploids, and tetraploids.

Pollen size has been particularly useful in screening for individuals that produce 2n gametes (Crespel et al., 2006). Hybridization in rose with tetraploid and diploid seed parents crossed with a diploid male that had a high propensity to produce 2n grains resulted in offspring with various ploidy levels, showing that reproduction is possible with n and 2n pollen (El Mokedem et al., 2002)

2n pollen grains are usually 1.3 times the diameter of normal 1n pollen (Crespel et al., 2006). These 2n pollen grains are useful in performing crosses between diploid and tetraploid individuals. There is little information available in the literature on rose individuals that produce 3n and 4n pollen and what size increases should be expected. In potato however, 4n pollen was reported to be 1.8 times larger in diameter than 1n pollen (Bamberg and Hanneman, 1991; Zlesak, 2009).

When it comes to breeding roses, most of today's commercial rose cultivars are tetraploid and most breeding research has been conducted at the tetraploid level (Zlesak,

2006). The tetraploid roses, while they do contain many desirable horticultural qualities, lack many of the valuable traits for disease resistance and environmental adaptations that are present in the diploid species (Byrne et al., 2007). Therefore, it is important to more thoroughly examine the value of diploid germplasm as well as the methods to incorporate it into the tetraploid genome.

One example of a highly useful trait successfully transferred from diploids into tetraploids is the single recessive gene for recurrent blooming. This occurred early in the cultivation of roses where open pollination occurred in European gardens containing both groups of roses (Zlesak, 2006). Hybridization between tetraploid and diploid roses results in progeny that are triploid. These individuals exhibit ranges of reduced fertility, but due to natural variation in meiotic patterns, there are opportunities for fertile gametes to arise out of such situations (Byrne et al., 2007). When tetraploid roses are pollinated with pollen from a triploid plant, the progeny contains tetraploid and triploid individuals (Zlesak 2009; Huylenbroeck et al., 2005). This reveals that triploid plants produce 2n and 1n gametes and that both can lead to successful fertilization. In addition, some diploid plants can produce varying amounts of 2n gametes, which in the case of the early tetraploid-diploid hybridizations, would have led to some tetraploid individuals in the progeny populations (Crespel et al., 2006).

Objectives

1) Determine the ploidy level and rate of 2n gamete production of TAMU germplasm

2) Determine the accuracy in using pollen size, pollen appearance, and flow cytometry to determine ploidy level of rose germplasm.

3) Determine the ploidy level in progeny populations from parents of similar and dissimilar ploidy levels. Evaluate the usefulness of triploids in bridging the gap between the tetraploid and diploid groups of roses.

> a. Observe the differences in fertility among interploidy crosses (diploid x diploid, diploid x triploid, diploid x tetraploid, tetraploid x triploid, tetraploid x tetraploid, and tetraploid x diploid) by determining percent hip set, germination, seeds per hip as well as observing pollen size and appearance in the progeny

CHAPTER II

PLOIDY LEVELS IN THE TAMU GERMPLASM

2.1 Synopsis

One-hundred twenty-six rose cultivars and selections from the Ralph Moore, Robert E. Basye and TAMU germplasm were assayed for ploidy level by chromosome counts and it was determined to be 34.9% diploids, 22.2% triploids and 42.8% triploids. This population composition, when compared to other reports about the distributions of ploidy level in rose populations has a higher percentage of diploid plants. Observations were made on fertility based on pollen appearance such as shriveled grains and inconsistent pollen grain size. The rate of 2n gamete formation was estimated in each ploidy level and determined to be 9% in the diploids and 1.8% in tetraploids.

2.2 Introduction

Roses are some of the most common ornamental plants encountered around the world and have long been cultivated for their uses as harvested flowers, potted plants and landscape accents. The plants are also grown for the hips which can be used for aesthetic, medicinal, or culinary uses (Zlesak, 2009). These characteristics along with the expansive range of plant and flower morphology have made the rose a popular horticultural crop as well as earned it the title of being the favorite flower of the world (Cairns, 2001). The addition of the recurrent blooming gene into non recurrent blooming genotypes has also made roses much more attractive as a landscape plant. With the addition of the ever blooming trait, rose plants that would normally only bloom for a brief period once a year,

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now flower repeatedly over the course of the growing season. Hybridization in roses is sometimes facilitated by individuals that produce gametes with the same ploidy level. The challenges of a crop with dynamic ploidy levels are made easier with the collection of the ploidy information on individuals and populations. From a breeding perspective, many of today's commercial rose cultivars are thought to be tetraploid, but diploid roses still hold numerous disease resistance traits that are needed at the tetraploid level (Zlesak, 2006). The tetraploid rose contains many desirable horticultural qualities, but lack many of the valuable traits for disease resistance and environmental adaptations found in the diploid species (Byrne et al., 2007). Therefore, it is important to more thoroughly examine the value of diploid germplasm as well as methods to incorporate it into the tetraploid genome.

Objectives

1) Determine the distribution of ploidy level in the TAMU germplasm

2) Conduct observations on 2n gamete production and pollen fertility of the germplasm

2.3 Materials and Methods

2.3.1 Chromosome counts to obtain distribution of ploidy levels

Chromosome counts via root tip squashes were used to count the chromosome number in the TAMU germplasm collection. To obtain the root tips, cuttings collected from the plants to be characterized were placed under a mist bench to root. Approximately 21 days on the mist bench yielded the best quality root tips (Figure 1). The root tips were harvested directly from the cuttings on the mist bench and placed in ice water in 2 mL micro centrifuge tubes for approximately 20 hours. If the root tips were harvested a few days after

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the cuttings were removed from the mist bench, this reduced the number and quality of chromosome spreads. The harvested root tips were approximately 13-19 mm in length. The longer length was preferred as it facilitates the handling through all of the chemical treatments. After the ice water treatment the root tips were placed in Farmers fixative (3:1 v/v 95% ethanol: glacial acetic acid) (Ruzin, 1999; Zlesak, 2009) and stored in the refrigerator until characterization. The root tips were treated with 5 N hydrochloric acid for 2 hours to soften the tissue and facilitate squashing. At the end of the acid treatment, the acid was pipetted out of the tubes and replaced with distilled water. To conduct the squash, the root tips were removed one at a time and placed on a microscope slide. A longitudinal cut was made starting approximately 3 to 4 mm from the tip, continuing through the tip. The tip of the root was then spread apart and the cellular matter within pushed out onto the slide, being careful to not leave large clumps of epidermal tissue which would hinder even squashing. The remainder of the root tissue was then removed and a drop of carbol fuchsin stain (1 g basic fuchsin, 5 g phenol, 10 mL 90% EtOH, 100 mL water) (Crane and Byrne 2003) was placed onto the dispersed cells and a cover slip was added. The slide was then placed upside down onto a paper towel with another paper towel placed on top of the slide. A wooden meter stick approximately 6 mm thick was placed on top of the paper towel, centered over the slide and pressure was applied by pressing very firmly on the yard stick. The yard stick allowed the application of an adequate amount of even pressure and reduced slide breakage.

2.3.2 Observations of pollen appearance-fertility and 2n gametes

Unopened flowers were collected 1 to 2 days before anthesis, the anthers were removed and allowed to dry on paper plates for one or two days in the laboratory at approximately 24°C (Figure 2). After the anthers showed pollen dehiscence, they were placed into 2 mL plastic vials which were then placed into plastic jars filled approximately 1/3 with Drierite (W.A. Hammond Drierite Co., Ltd) and stored at -15°C until examination. At the time of examination, the anthers were first stirred with a toothpick that was previously dipped in acetocarmine stain. After the anthers were stirred, the toothpick was then placed onto a microscope slide where a drop of acetocarmine stain had already been placed, and the toothpick was twirled around in the stain to distribute the pollen grains (Zlesak, 2009). Observations were made on 126 plants from the TAMU germplasm on pollen appearance to evaluate the fertility of the plants. The observations were used to calculate the percentage of malformed pollen grains, which could possibly represent fertility issues. The pollen was viewed under 400x magnification and the diameters of 30 well formed pollen grains were recorded. Observations were also made on the occurrence of possible 2n gametes indicated by the presence of large pollen grains. The large pollen grains were compared to the relative size of n pollen, and the large grains were either classified as 2n or 4n. In roses, the diameter of 2n pollen is approximately 1.3 times longer than n pollen (Crespel et al., 2006; Zlesak 2009). There is limited information on the size of larger than 2n pollen in roses, but in potato, the diameter of 4n pollen was found to be 1.8 times larger than n pollen (Bamberg and Hanneman 1991).
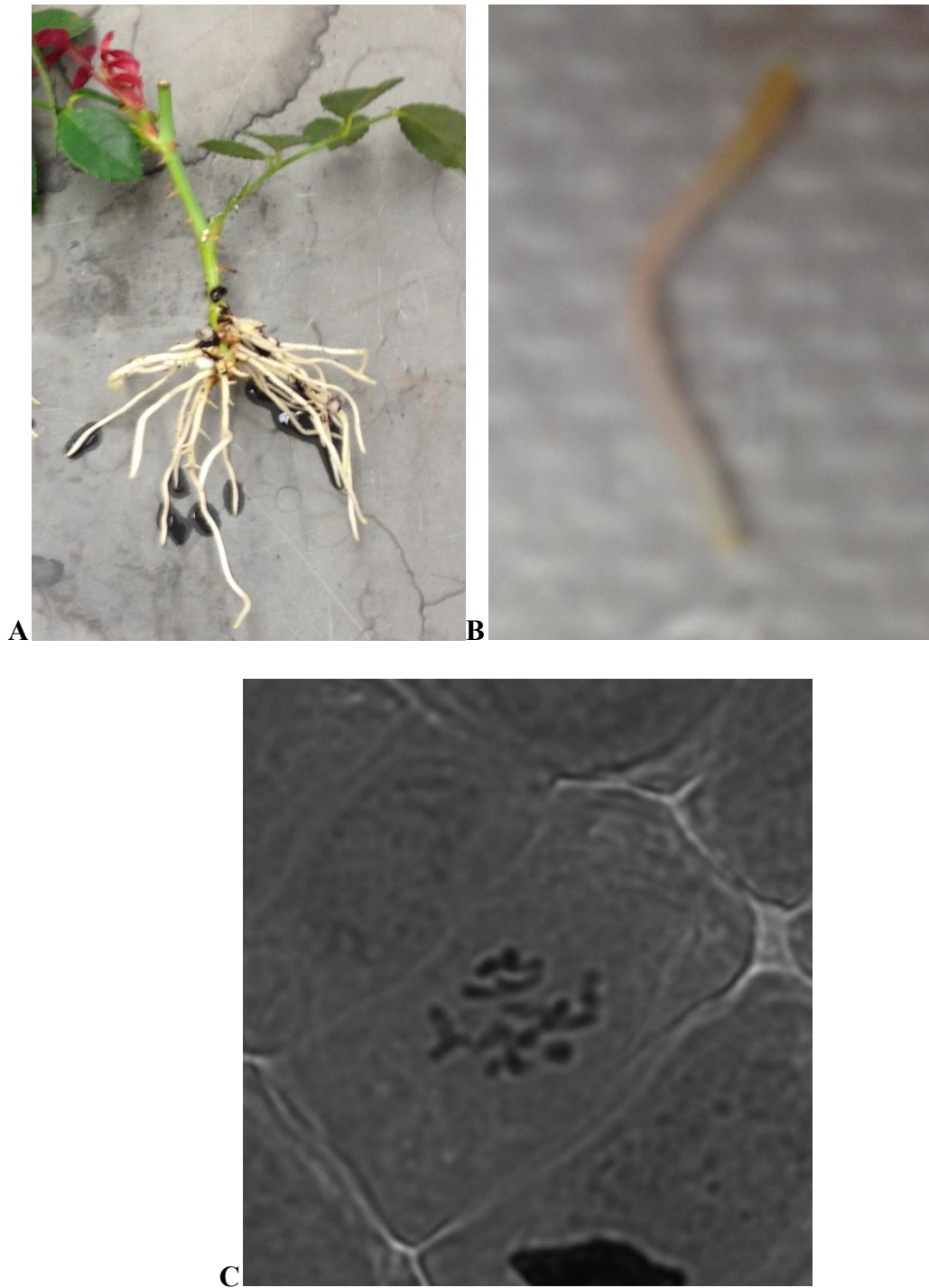
Figure 1. Process of chromosome counting in rose. (A) Rooted rose cutting after approximately 3 weeks on mist bench with roots ready for processing. (B) Approximate size of root tissue harvested to undergo chemical treatments. (C) Resulting squash of root tip cell.
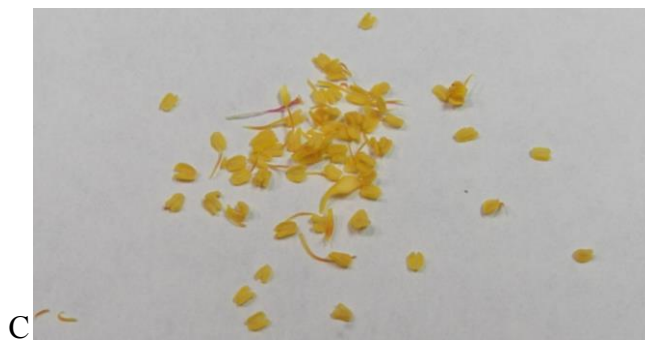
Figure 2. Process of pollen extraction in rose. (A) Flower at proper age for pollen extraction. (B) Petals removed to expose stamens. (C) Extracted anthers.

The measurements were obtained by analyzing digital images captured by Photometrics Cool SNAP HQ2 digital camera (Photometrics Tucson, AZ) using image software NIS-Elements AR3.0 (Nikon Instruments Inc. Melville KY) calibrated with microscopic objective lenses. The photographs were analyzed with the image analyzing software Image J developed by Wayne Rasband at the National Institutes of Health Collins TJ (July 2007). Analysis of pollen size distribution was performed using JMP software, Version 9.0, SAS Institute Inc., Cary, NC, 1989 – 2010.

2.4 Results and Discussion

2.4.1 Distribution of ploidy levels in TAMU germplasm

Chromosome counts revealed that of the 126 plants sampled from the TAMU germplasm, 44 were diploid, 28 were triploid, and 54 were tetraploid. In terms of percent composition, the population consisted of ~35% diploids, ~22 % triploids, and ~43% tetraploids. Previous reports on the distribution of ploidy in rose populations have percentages of 24.5% diploids, 29% triploids, and 46% tetraploids (in a population of 428 plants consisting of species, cultivars, germplasm releases and breeding lines) (Zlesak 2009); as well as 27.3% diploids, 41% triploids, and 31.5% tetraploids (in a population of 73 plants from the Earth-Kind® trials) (Zlesak 2010). The examination of the TAMU germplasm shows a larger percentage of diploids compared to other populations mentioned in literature. However, the collection examined in this study did contain several diploid breeding lines, which is partially responsible for the higher numbers of diploids encountered. If these materials are excluded from analysis of percent composition, the TAMU distribution is approximately 30% diploids, 25%

triploids, and 45% tetraploids. This decreases the percentage of diploids, but it is still

greater than the 2 populations mentioned from the literature.

2.4.2 Pollen fertility and 2n gamete formation

  Pollen diameters of the 126 plants from the TAMU germplasm were recorded to

search for any individuals that produced 2n gametes or that showed impaired pollen

fertility manifested through shriveled pollen grains (Tables 1, 2) (Figures 3,4). Of the 44

diploids in the population, 3 showed an increased number of shriveled pollen grains

between 20 and 30 percent of the total pollen grains observed. However, 30 percent

shriveled pollen grains does not necessarily represent a critical decrease in a plants

performance as a pollen donor. Of these 3 plants, the plant 'Fair Molly' also exhibited

2n pollen production of 20 % of the total grains observed. In addition, there were 4

other diploid plants ('Fresh Pink', 'Gold Coin', 'Mariposa Gem', and 'Pinstripe') that

exhibited 2n pollen production. Of the 28 triploids in the population, 20 individuals

exhibited significant amounts of shriveled pollen grains, ranging from 20-40 % of the

total number of pollen grains observed. The other 8 triploids ('Amber Gem', 'Iceberg',

'Jessica Rose', 'Lucy', 'Ruby Princess', 'Spotlight', 'Strawberry Swirl', 'Tangerine

Jewel') that did not exhibit significant numbers of shriveled grains (less than 10%),

suggesting that these individuals have better fertility relative to the other triploids. In

addition, while the other triploids exhibited a wide variation in pollen size within each

plant (e.g. 2n and n pollen), the triploids with few shriveled grains also appeared to

produce pollen mostly of one size, either mostly 2n or mostly n. This evidence suggests

that triploids differ in their ability to produce 1n or 2n pollen as seen in other studies of

the progenies of triploids (Huylenbroeck et al., 2005). Among the 54 tetraploid plants in the population, 14 exhibited shriveled pollen grains ranging from 15-25 percent of the total pollen grains observed. Of these plants, 4 were shown to be producing 2n (4x) gametes. Compared to the study in Zlesak (2009), the percentages of 2n pollen producing diploid plants in this study is similar (9% vs 9.8%) whereas the rate of 2n pollen production in the tetraploids of the TAMU germplasm is lower (1.8% vs. 4.8%). The rate of 2n gamete production in a population is an important factor to consider, especially in a breeding program. These 2n gametes introduce more variation in the ploidy level of progenies, which can be useful to move from one ploidy level to another, but also detrimental if the intent was to breed at one ploidy level only.

2.5 Conclusions

The ploidy composition of the TAMU rose germplasm has a higher composition of diploids compared to other reports in literature. The production of 2n gametes in tetraploids is lower than that of diploids and is lower than literature would suggest. Some triploid plants exhibited few shriveled pollen grains, as well as uniform pollen size, suggesting that these plants are potentially more fertile. Test crossing these individuals with a common set of diploid and tetraploid seed parents and comparing the results to those of other typical triploids in the same crosses could confirm this possibility. Some tetraploids exhibited high numbers of shriveled pollen grains, suggesting fertility issues. Test crosses could also be used with these individuals to evaluate their performance relative to other tetraploids with normal pollen appearance.

Table 1. Occurrence of shriveled pollen and 2n gametes in TAMU germplasm.

Cultivar	Ploidy	Pollen size (μm)	~% Shriveled / ~% 2n
Baby Austin	2x	32.1 ± 0.9	~30
Fair Molly	2x	35.7 ± 2.9	~20 / 20
Fresh Pink	2x	34.8 ± 1.5	~0 / 30
Gold Coin	2x	35.0 ± 1.0	~0 / 10
Mariposa Gem	2x	34.3 ± 3.6	~0 / 10
Patriot Song	2x	35.5 ± 3.3	~30
Pinstripe	2x	35.9 ± 2.4	~0 / 10
Angel Pink	3x	42.1 ± 2.2	~40
Apricot Twist	3x	39.4 ± 1.3	~25
Belinda's Dream	3x	38.8 ± 4.2	~25
Café Ole	3x	35.2 ± 4.7	~25
Doris Bennett	3x	39.3 ± 4.2	~20
Earthquake	3x	32.1 ± 1.2	~25
Gold Moss	3x	36.7 ± 4.1	~25
Golden Horizon	3x	41.2 ± 4.1	~35
Halo Glory	3x	45.6 ± 3.9	~25
Hi Ho	3x	45.8 ± 5.1	~20
Homerun	3x	45.2 ± 4.7	~30
Jacquie Williams	3x	39.3 ± 3.6	~40
Julie Link	3x	39.3± 2.2	~30
Lovely Lorrie	3x	37.7 ± 3.0	~25
Orange Frenzy	3x	37.3 ± 3.2	~25
Out of Yesteryear	3x	39.4 ± 2.9	~25
Quietness	3x	34.2 ± 2.1	~25
Roses are Red	3x	38.4 ± 5.1	~30
Sweet Hannah	3x	38.2 ± 4.4	~40
Twilight Skies	3x	39.1 ± 3.3	~20
Avandel	4x	44.0 ± 2.6	~25 / 25
Diamond Anniversary	4x	45.7 ± 6.8	~25 / 25
Gina's Rose	4x	34.2 ± 3.2	~20
Hoot Owl	4x	42.9 ± 2.0	~20 / 15
Kayla	4x	46.2 ± 4.9	~25
Lavender Delight	4x	45.1 ± 2.8	~15
Love and Peace	4x	48.0 ± 5.7	~20
Magseed	4x	48.5 ± 3.2	~25
O	4x	35.2 ± 1.0	~20
Orange Parfait	4x	43.3 ± 2.8	~0 / 10
Play Gold	4x	43.2 ± 3.0	~20 / 10
Renny	4x	47.3 ± 8.9	~20
Sequoia Gold	4x	39.5 ± 1.2	~20

Table 1. continued

Cultivar	Ploidy	Pollen size (μm)	~% Shriveled / ~% 2n
Splish Splash	4x	35.1 ± 5.2	~20
Yellow Jewel	4x	42.8 ± 3.3	~25

Table 2. Summary of TAMU germplasm

Cultivar	Diploid	Triploid	Tetraploid
Composition of population	34.9%	22.2%	42.8%
Plants with shriveled pollen	4.5%	71.4%	18.5%
Plants with 2n pollen	9.0%		1.8%
Plants with shriveled & 2n pollen	2.2%		7.4%

2n pollen percentages are not shown for triploid plants because of the large variation of pollen size in these plants
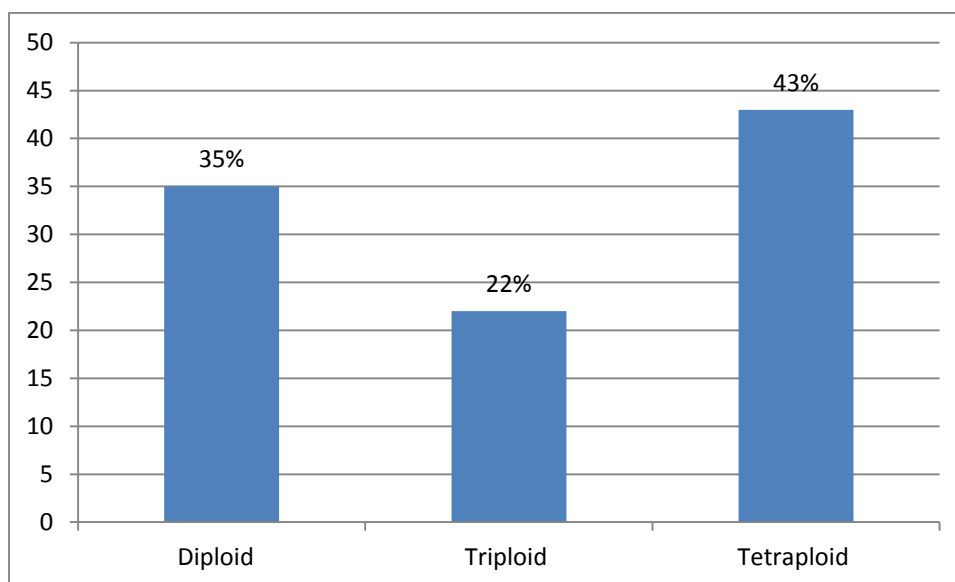
Figure 3. Distribution of ploidy level in TAMU germplasm.
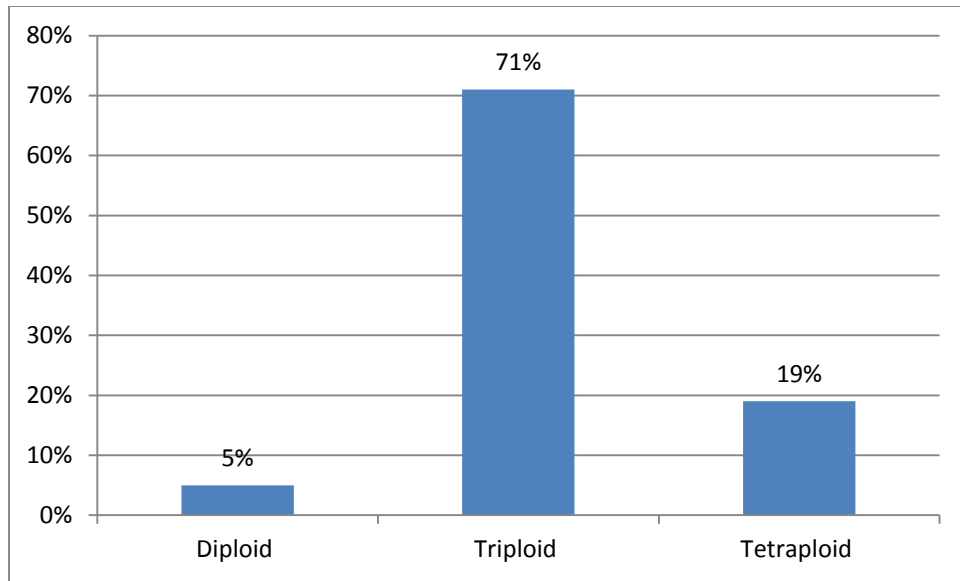
Figure 4. Percentages of plants with shriveled pollen grains in each ploidy level.

CHAPTER III

INDIRECT PLOIDY ANALYSIS VIA POLLEN MORPHOLOGY

AND FLOW CYTOMETRY

3.1 Synopsis

126 rose cultivars and selections from the Ralph Moore, Robert E. Basye and TAMU germplasm were assayed for ploidy level using pollen size, pollen appearance, flow cytometry and chromosome counts. Pollen diameter alone was 86% accurate in identifying diploids and 74% accurate in identifying tetraploids. Although the population consisted of diploid, triploid, and tetraploid individuals, the triploid individuals are not separated out by screening with pollen diameter because the range of triploid rose pollen diameter overlaps that of both diploids and tetraploids. When pollen appearance was combined with the pollen size however, 71% of the triploids were identified. A few of the diploid and tetraploid individuals were falsely grouped as triploids due to their propensity to produce irregular pollen sizes. Flow cytometry proved to be less useful in estimating ploidy than pollen morphology because of the interference with fluorescence caused by the anthocyanin compounds in roses with red/magenta coloring in the leaves. Flow cytometry was able to separate tetraploid and diploid individuals 92% of the time when plants with heavy pigmentation were excluded. Flow cytometry was only able to identify 25% of triploid individuals.

3.2 Introduction

Roses are one of the most common ornamental plants encountered around the world and have been cultivated for centuries for their uses as harvested flowers, potted plants and landscape icons. The plants are also grown for the hips which can be used for aesthetic, medicinal, or culinary uses (Zlesak, 2009). These characteristics along with the impressive range of plant and flower morphology have made the rose a popular horticultural crop as well as earned it the title of being the favorite flower of the world (Cairns, 2001). The incorporation of the recurrent blooming gene into once blooming genotypes has also made roses much more attractive as a landscape plant. With the addition of the ever blooming trait, rose plants that would normally only bloom for a brief period once a year, now flower repeatedly over the course of the growing season.

Roses are indigenous to the Northern Hemisphere from Europe to North America to Asia with over 100 recognized species. Most cultivated roses come from the subgenus Rosa (Zlesak, 2009, Gudin, 2000). Roses that are grown today are the result of numerous interspecific hybridizations of around ten different species (Gudin, 2000). Rose varieties were originally domesticated independently in Europe and Asia. It was in European gardens where the first combinations occurred that brought traits from the European tetraploid and Chinese diploid groups together to create many of the complex rose cultivars that exist today (Gudin 2000; Zlesak 2006; 2009).

Among rose species, the basic number of chromosomes is seven with most individuals ranging from diploid to octoploid (Zlesak, 2006). Recently, *Rosa praelucens* Byhouwer, originating in Yunnan, China was found to be decaploid (Jian et al., 2010). The

chromosome number of a plant is a significant factor in performance, appearance and combining success with other individuals (Levin, 2002; Zlesak, 2009).

Meiosis has two far reaching implications that apply to rose breeding. Meiotic processes govern the level of hybrid fertility in addition to establishing species distinctions. If chromosomes fail to synapse in the pachytene stage of prophase I, the spore nuclei produced at the end of meiosis will likely contain unequal numbers of each parental type of chromosome. This imbalance causes many of the spore nuclei to abort leading to varying degrees of sterility.

The pairing of chromosomes during meiosis is believed to be facilitated by similar DNA sequences at specific sites along the chromosome. The lack of pairing in meiosis suggests dissimilarities between chromosomes and a more distant relationship (Byrne and Crane, 2003). In an organism where the chromosomes are dissimilar to one another, no pairing will occur and this will result in the formation of univalents, which are single pairs of sister chromatids with no homologues to pair with. In an organism where the chromosomes are similar to each other, they pair and separate equally into spore nuclei. In a study of the metaphase I pairing of chromosomes in diploid and tetraploid roses, both exhibited largely bivalent formation, although the tetraploids also developed significant but small numbers of univalents and multivalents (Ma et al., 2000).

Breeding in roses is facilitated by individuals that produce gametes with the same ploidy level. Altering ploidy level through gametic polyploidization can circumvent restrictions caused by dissimilar ploidy levels between plants (Zlesak, 2009). The challenges of a crop with dynamic ploidy levels are made easier with the collection of the

ploidy information on individuals and populations. Physically staining and counting chromosomes takes considerable time and skill to conduct (Ma et al., 1996). Several less complicated estimation techniques have been developed to bypass chromosome counts. These include measuring pollen size and using flow cytometry to estimate ploidy level.

Flow cytometry examines the fluorescence of cell nuclei to estimate sporophytic ploidy level by comparing individuals to a known standard (Jacob et al., 1996). However, DNA content among rose individuals at a given ploidy level can have wide ranges that overlap with the DNA value of another ploidy level (Jacob et al. 1996; Yokoya et al., 2000). This is especially true in a highly heterogeneous crop such as rose where interspecific crosses have led to frequent genome restructuring (Levin, 2002). In addition, tannins, phenolics, and other plant secondary metabolites can interfere with the binding of a fluorochrome to the DNA and lead to incorrect DNA measurements (Yokoya et al., 2000). As a result, flow cytometry is most useful when done in conjunction with another method of ploidy assessment (Zlesak, 2009).

Measurements of pollen diameter as well as observing the pollen appearance in individuals can be valuable when attempting to determine the ploidy level of roses (Zlesak et al. 2005). However, the range of pollen diameter at the various ploidy levels can overlap and make ploidy estimation difficult (Lewis 1957; Jacob and Pierret, 2000; Crespel et al. 2006; Zlesak, 2009). The method in which the pollen is harvested, processed and stained for viewing can also affect accuracy (Erlanson 1931; Bamberg and Hanneman, 1991; Jacob and Pierret, 2000). The methods used for collecting and preserving pollen must remain constant throughout an experiment (Stanley and Lenskens, 1974). Despite the potential

problems associated with using pollen size, it has been shown to have a good success rate for non intensive general conclusions on ploidy level (Zlesak 2009). In potato, pollen diameter was 93% accurate in distinguishing between diploid and tetraploid potatoes, although it was not successful in separating tetraploid from hexaploid individuals (Bamberg and Hanneman, 1991). Pollen size has been particularly useful in screening for individuals that produce 2n gametes (Crespel et al., 2006). Hybridization in rose with tetraploid and diploid seed parents crossed with a diploid male that had a high propensity to produce 2n grains resulted in offspring with various ploidy levels, showing that reproduction is possible with n and 2n pollen (El Mokadem et al., 2002).

2n pollen grains in roses are 1.3 times the diameter of normal 1n pollen (Crespel et al., 2006). These 2n pollen grains are useful when crosses between two different ploidy levels are performed. There is little information available in the literature about 3n and 4n pollen production in roses and what size increases should be expected. In potato however, 4n pollen diameter was reported to be 1.8 times larger in than 1n pollen (Bamberg and Hanneman, 1991; Zlesak, 2009).

Objectives

1) Determine the accuracy in using pollen size to estimate the ploidy level of rose germplasm

2) Determine the usefulness of flow cytometry in estimating the ploidy level of rose germplasm

3.3 Materials and Methods

3.3.1 Flower collection and pollen processing

Unopened flowers were collected 1 to 2 days before anthesis and the anthers were removed and allowed to dry on paper plates for one or two days in the laboratory at approximately 24ºC. After the anthers showed pollen dehiscence, they were placed into 2 mL plastic vials which were then placed into plastic jars filled approximately 1/3 with Drierite (W.A. Hammond Drierite Co., Ltd) and stored at -15ºC until examination. At the time of examination, the anthers were first stirred with a toothpick that was previously dipped in acetocarmine stain. After the anthers were stirred, the toothpick was then placed onto a microscope slide where a drop of acetocarmine stain had already been placed, and the toothpick was twirled around in the stain to distribute the pollen grains (Zlesak, 2009). 126 plants from the TAMU rose germplasm was screened for pollen size in this manner.

3.3.2 Pollen analysis

The pollen was viewed under 400x magnification and the diameters of 30 well formed pollen grains were recorded. Observations were also made on the occurrence of possible 2N gametes indicated by the presence of large pollen grains. In addition, the lack of uniformity among pollen grains including high numbers of malformed grains

suggested that some individuals could be triploid. The measurements were obtained by analyzing digital images captured by Photometrics Cool SNAP HQ2 digital camera (Photometrics Tucson, AZ) using image software NIS-Elements AR3.0 (Nikon Instruments Inc. Melville KY) calibrated with the microscopic objective lenses. The photographs were analyzed with the image analyzing software Image J developed by Wayne Rasband at the National Institutes of Health Collins TJ (July 2007). Analysis of pollen size distribution was performed using JMP software, Version 9.0, SAS Institute Inc., Cary, NC, 1989 – 2010.

3.3.3 Flow cytometry

For the flow cytometry analysis, approximately 3-5 young leaves that had just unfurled, but not yet completely expanded were collected from multiple branches per plant and placed on ice for approximately 1 hour until they were taken to the laboratory to be processed (Figure 5). Once in the laboratory, one leaflet from each leaf was removed and placed into a Petri dish containing approximately 3-5 mL of buffer solution (Figure 5). There were 3 buffer solutions used in this experiment: Galbraith buffer (Galbraith et al. 1983), Woody plant buffer (Loureiro et al. 2007), and Nuclei isolation buffer (Mike Dobres, Managing Director NovaFlora LLC). The leaflets were hand chopped with razor blades for 10-15 seconds. Once the leaflets were chopped, 1 mL of the suspension was poured out of the Petri dish through a 30 μm filter mesh into a 2 mL microcentrifuge tube and stored on ice. Once all of the samples were prepared, 50 μL of propidium iodide stock was added to each sample. Samples were stored on ice for approximately 20 minutes before they were run through the cytometer. Known diploids and tetraploids were run as standards to obtain

a relative value to compare with the results of the unknown plants. The diploids used were 'Old Blush', 'Red Fairy' and 'Sweet Chariot'; the tetraploids used were 'Orange Honey', 'Golden Gardens', and 'Rise and Shine' (Table 3). The standards were run separately from the unknowns as well as with the unknowns (chopped separately, or chopped together at the same time in the Petri dish).
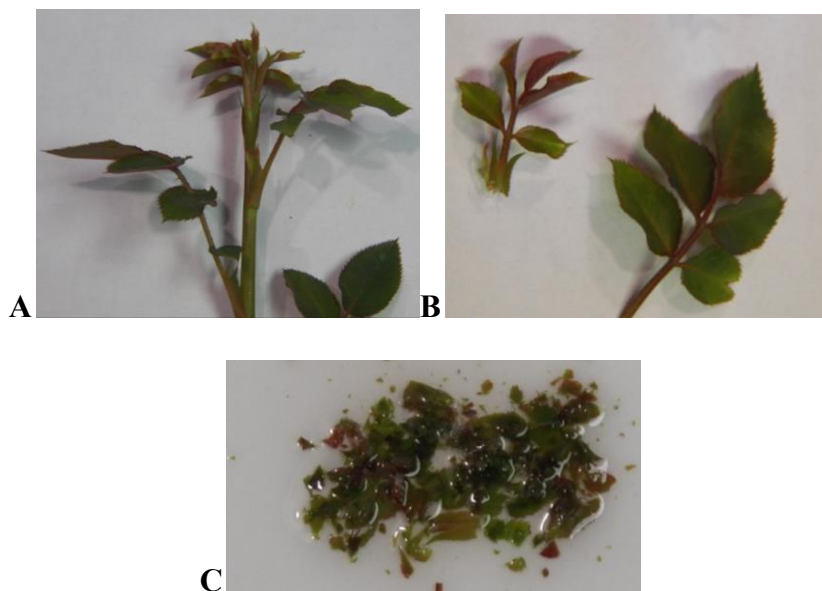
Figure 5. Tissue samples for flow cytometry (A) Shoot tip with new growth. (B) Young leaves used to supply nuclei. (C) Macerated leaf tissue containing nuclei suspension.

3.4. Results and discussion

3.4.1 Pollen size ploidy predictions of TAMU germplasm

126 roses from the TAMU rose breeding germplasm were screened using pollen size and it was predicted that 49 were diploid, 67 were tetraploid and 10 were hexaploid (Table 5, Table A-1). Pollen observation also showed that of 2n gamete production occurred in approximately 10% and 2% of the confirmed diploid and tetraploid roses respectively (2n pollen = 2x for diploid plants and 4x for tetraploid plants). Within the plant populations where 2n pollen production was observed, some individuals had only a few 2n grains observed, while others had 2n pollen in up to approximately 30% of the grains (Figure 7, Table A-1). In individuals showing 2n gamete production, between 75 and 100 pollen grains were measured to obtain 2n percentages. The individuals that displayed low rates of 2n pollen production (less than 10%) were not included in the table as most individuals in the population that did produce 2n pollen seemed to produce it in amounts of 10% or higher while individuals with amounts of 2n pollen below 10% were usually below 5% (occasional grains to ~1%) as well.

Triploids cannot be identified using pollen diameter as their sizes overlap with the diploids and tetraploids. To identify triploids, the qualitative appearance of the pollen was examined and was combined with the pollen size results. The pollen samples were observed for an increased number of malformed pollen grains as well as a large amount of variability in the range of pollen diameters (Figures 6, 8). This predicted that 39 were diploid, 39 were triploid, and 48 were tetraploid (Table 4, Table A-2). All of the previously predicted hexaploids were predicted to be triploids when looking at both pollen size and appearance.

While this helped in identifying triploids, it led to some diploid and tetraploid individuals with high numbers of shriveled pollen grains being falsely classified as triploids. The population was verified for ploidy level by conducting chromosome counts and 44 were verified as diploid, 28 as triploid, and 54 as tetraploid. The 10 plants that were predicted to be hexaploid using pollen size and triploids when using pollen size and appearance, when counted consisted of 3 triploids and 7 tetraploids.

The chromosome counts showed that pollen size was 86% and 72% accurate for identifying diploids and tetraploids respectively (Table 3, Figure 9). Combining qualitative pollen data with pollen diameter was 71% successful in identifying triploids (Table 3, Figure 10). Thus predictability using pollen size was variable. Previous work indicated that the identification of diploids using pollen size was reliable (Zlesak, 2009) whereas the present work did not. There were however, fewer rose genotypes examined in this study, (118 TAMU vs. 428 Zlesak, 2009).
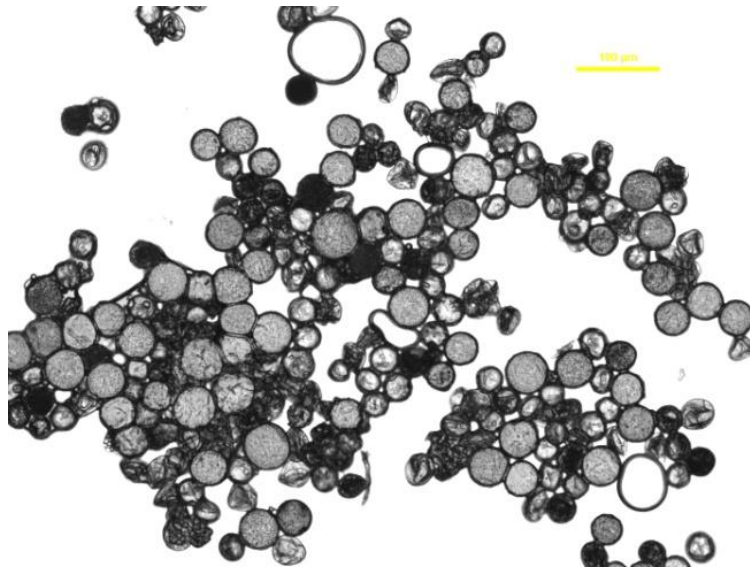
Figure 6. Pollen appearance of 'Julie Link', a triploid plant.
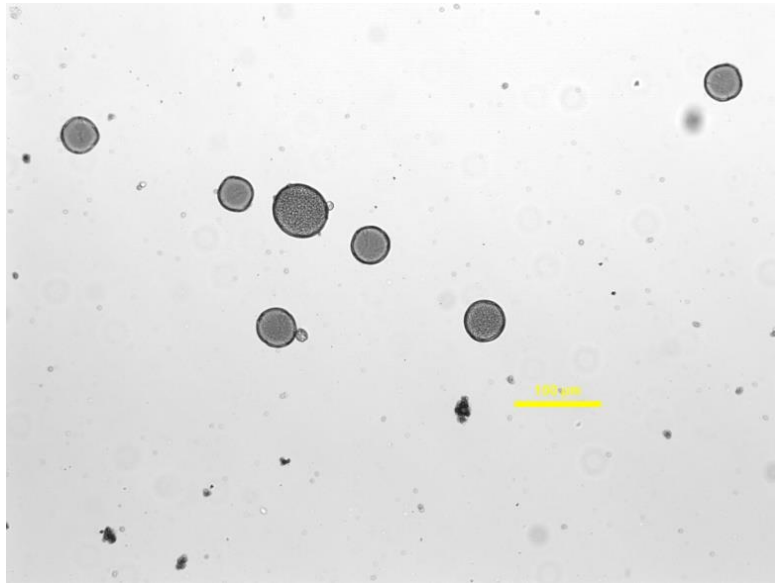
Figure 7. n and 2n pollen of 'Fresh Pink', a diploid.

Table 3. Actual vs. predicted sporophytic ploidy using only pollen size.

Actual ploidy	Predicted ploidy			
	2x	4x	6x	Total
2x	38	6	0	44
3x	4	21	3	28
4x	7	40	7	54
Total	49	67	10	126
% accuracy	86.3% 38/44	74.0% 40/54	0%	

Pollen size alone grouped the plants into predicted ploidy levels of 2x, 4x and 6x.

Pollen size correctly identified 38/44 true diploids, but also misidentified 11 non diploids as diploids.

Pollen size correctly identified 40/54 tetraploids but also misidentified 27 non tetraploids as tetraploids

Table 4. Actual vs. predicted sporophytic ploidy using pollen size and pollen appearance.

Actual ploidy	Predicted ploidy				Total
	2x	3x	4x	6x	
2x	34	5	5	0	44
3x	1	20	7	0	28
4x	4	14	36	0	54
Total	39	39	48	0	126
% accuracy	77.2 % 34/44	71.4% 20/28	66.6% 36/54	0%	

Pollen size combined with qualitative inconsistency in pollen size as well as malformed pollen grouped the plants into ploidy levels of 2x, 3x and 4x
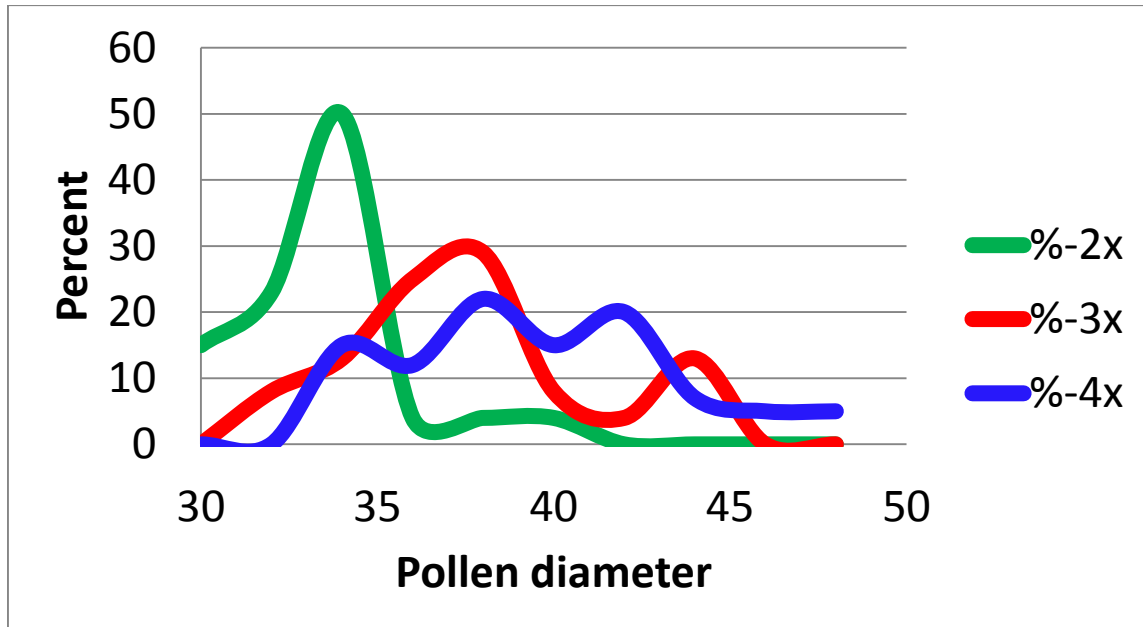
Figure 8. Overlapping ranges of pollen diameter.
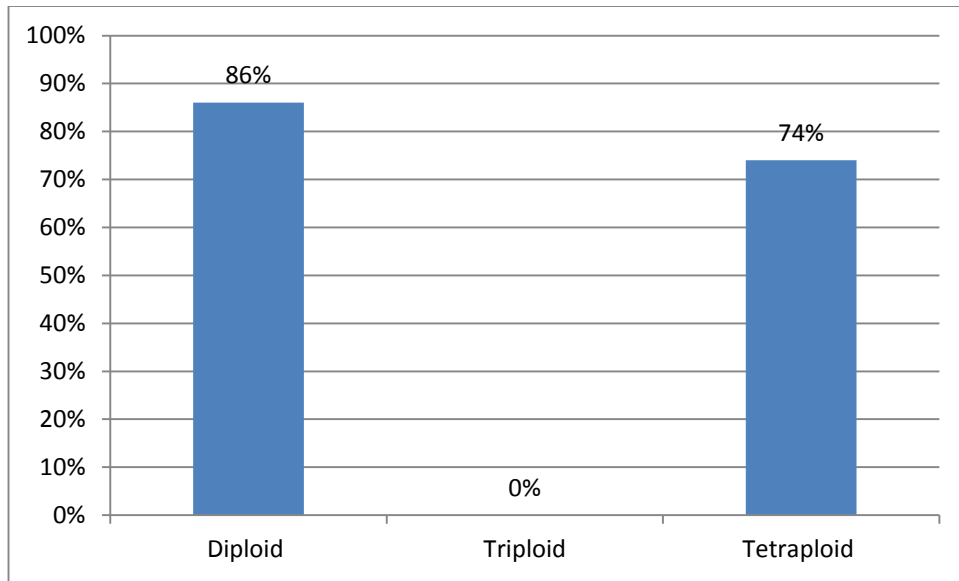
Figure 9. Percent accuracy of pollen size to predict diploid, triploid, and tetraploid plants.
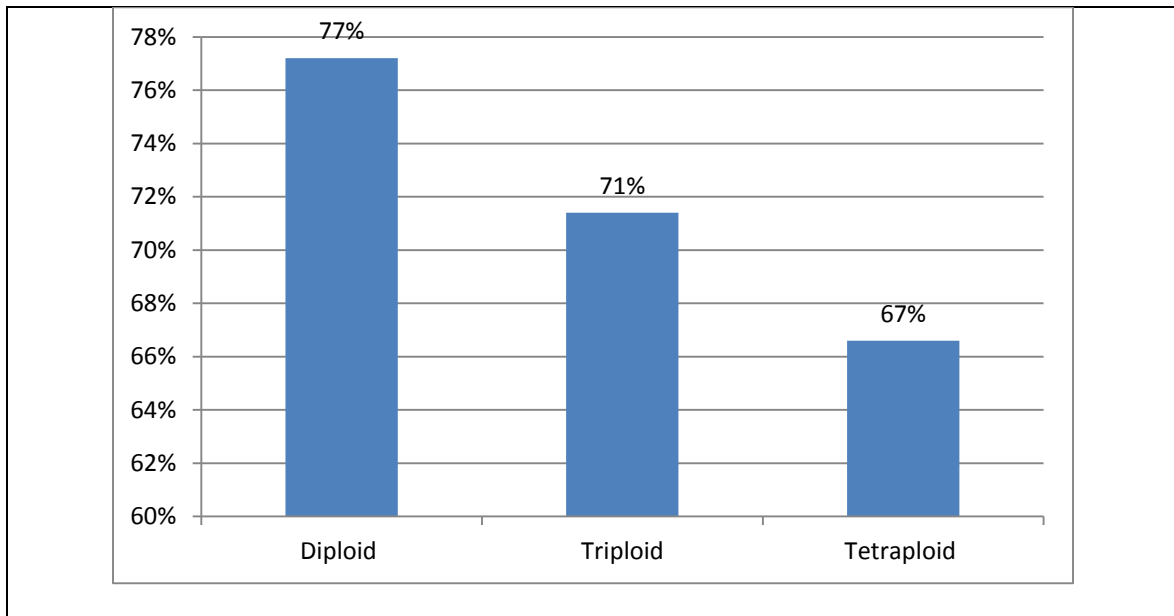
Figure 10. Percent accuracy of pollen size coupled with pollen appearance to identify triploid plants.

3.4.2 Pollen size analysis of triploid crosses to estimate ploidy level

The analysis of pollen size on the progeny of the triploid crosses predicted a ploidy distribution very different than that of the confirmed ploidy. Because of the high number of expected triploids in the progeny of theses crosses, pollen appearance was combined with the measurements of pollen diameter to accommodate the triploids. In the tetraploid by triploid cross 'Golden Gardens' x 'Homerun', pollen analysis correctly predicted 17 of 23 triploids and 15 of 26 tetraploids (Table 5). It failed to identify the one pentaploid individual. There was a large variation in pollen size in the progeny of this cross, even between plants of the same ploidy level. This caused many of the tetraploid individuals to appear to be triploid. In addition, some individuals failed to produce enough flowers and/or pollen to allow for their measurement. Because of this, only 50 of the 66 progeny in the population could be sampled for pollen characteristics. Triploid plants were predicted moderately accurately (74%), but this is still not accurate enough to allow pollen size to be considered a viable alternative to direct ploidy assessment via chromosome counts.

In the diploid by triploid cross 'WOBxOB26#212' x 'Homerun', pollen analysis was slightly more accurate at identifying the triploid individuals (86%) (Table 6). However, 2 of the 3 confirmed diploids were predicted to be triploid because of their highly malformed pollen grains. In addition, the 1 tetraploid individual was predicted to be triploid because it also had many malformed grains.

Table 5. Pollen size analysis of 'Golden Gardens' x 'Homerun'.

Actual ploidy	-----------------Predicted ploidy------------------				
	2x	3x	4x	5x	Total
2X	0	0	0	0	0
3X	0	17	6	0	23
4X	0	15	11	0	26
5X	0	1	0	0	1
Total	0	33	17	0	50
% accuracy		74%	57%		

Table 6. Pollen size analysis of 'OBxWOB26#212' x 'Homerun'.

Actual ploidy	------------------Predicted ploidy----------------------				
	2x	3x	4x	5x	Total
2X	1	2	0	0	3
3X	0	13	2	0	15
4X	0	1	0	0	1
Total	1	17	2	0	19
% accuracy	33%	86%	0%		

3.4.3 Flow cytometry ploidy predictions compared to chromosome counts

Flow cytometry analysis was conducted on 58 roses from the population that was screened with pollen size. Only 58 were used because of problems that occurred with some plant samples repeatedly displaying uninterpretable fluorescence histograms. Flow cytometry analysis consists of a histogram of each sample run (fluorescence intensity/item) containing peaks that each represents an intensity of fluorescence at a given population of particles (in this case, cell nuclei). The intensity of these peaks is demonstrated by their position on the histogram which is proportional to the amount of nuclear DNA. The positions of the peaks of sample plants were compared to those of the known standards to produce a ratio between the two intensities and therefore the two DNA amounts. All flow cytometry analysis was conducted using an Accuri C6 Flow Cytometer (BD Biosciences San Jose California).

Flow cytometry was first attempted using Galbraith buffer (Galbraith et al., 1983), but the DNA peaks in the histograms using this buffer were completely obscured by phenolic compounds interfering with the fluorochrome binding to the DNA. Nuclei isolation buffer (Mike Dobres, Managing Director NovaFlora LLC) was tested next which resulted in a slight improvement in the reduction of phenolic interference, but not consistently enough to be useful in ploidy identification. Woody plant buffer (Loureiro et al., 2007) was finally used after the limited success of the first two buffers, and was very successful in producing well formed DNA fluorescence intensity peaks that were not obscured by any background auto fluorescence, or interference from phenolic compounds. Despite these improvements however, there were other problems encountered as the experiment continued. The known

standards were run multiple times to establish an accurate value to use for creating an expected value for the unknowns. As the samples were run multiple times however, there was a significant change in the value of some of the samples from one run to another. This occurred even when samples were taken from the same branch of the same plant. Multiple leaflets from the same leaf could be chopped in separate Petri dishes and run separately and the results would still consist of widely varying values for each sample. For example, 'Red Fairy', a known diploid had values ranging from a minimum of 60,000 to a maximum of 100,000. These values go below and above the range of diploids. In addition, 'Homerun' one of the confirmed triploids, had values ranging from 116,000 to 165,000 from sample to sample. Other triploids also exhibited values that were often well into the range for tetraploid plants. It is believed that these shifting values are the result of anthocyanin pigments interfering with the fluorescence of the fluorochrome. 'Red Fairy' and 'Homerun' are plants that both have heavy magenta coloration in the new growth that would indicate an increased level of pigments, which is possibly why more problems were encountered with these individuals. Known standards that showed limited fluorochrome interference such as 'Old Blush' were used to calculate an expected range of values for each ploidy level as follows: Haploid (35,000-45,000), Diploid (70,000-90,000), Triploid (105,000-135,000), Tetraploid (140,000-180,000) (Tables 7 and 8). These ranges were created from the minimum and maximum values in a range of observed values on a group of known diploids, the minimum being 70,000 and the maximum being 90,000. The ranges for the higher ploidy levels are extrapolations from this range. These expected values were used to

estimate the ploidy level of the 54 plants (Table A-3). Flow cytometry correctly predicted

16 out of 18 diploids, 4 out of 16 triploids, and 12 out of 20 tetraploids (Table 9).

Table 7. Known standards used in flow cytometry.

Cultivar	Known ploidy	Flow cytometry value
Old Blush	Diploid	80,000
Sweet Chariot	Diploid	75,000
Golden Gardens	Tetraploid	145,000
Orange Honey	Tetraploid	160,000
Rise n Shine	Tetraploid	154,000

Table 8. Predicted ranges from known standards.

Ploidy level	Predicted flow cytometry value
Haploid	35,000-45,000
Diploid	70,000-90,000
Triploid	105,000-135,000
Tetraploid	140,000-180,000

Table 9. Flow cytometry predictions compared to known ploidy level.

Known Ploidy	Predicted 2X	Predicted 3X	Predicted 4X	Unknown	Total
2X	16	0	0	2	18
3X	1	4	10	1	16
4X	2	2	12	4	20
Total	18	6	22	7	54

Among the diploids, 2 were not identifiable because their values exceeded the expected range of diploids, although only by a small amount. Of the 16 triploids, only 4 were identifiable with 10 predicted to be tetraploids, 1 predicted to be diploid, and one unidentifiable. Of the 20 tetraploids, 12 were predicted to be tetraploid, 2 diploid and 2 triploid and 4 were unidentifiable (Table 9). These results suggest that flow cytometry is most useful in separating tetraploids from diploids. Triploid plants frequently overlap with diploids and tetraploids, greatly diminishing the success rate when they are present. In addition to the low accuracy of predicting triploids, the previously mentioned problems with shifting fluorescence peaks make flow cytometry an inferior method for ploidy identification when compared to chromosome counts and pollen size analysis.

3.4.4 Ploidy analysis of rose crosses via flow cytometry

Flow cytometry was evaluated for success in predicting the ploidy in the progeny of interploidy and intraploidy crosses. Flow cytometry was most accurate at characterizing progeny of crosses that did not have a triploid parent. The highest accuracy was in the tetraploid by diploid cross where flow cytometry was 100% accurate at separating the triploid seedlings from the tetraploid seedlings (Table 10). Flow cytometry also did very well in screening the tetraploid by tetraploid population with a 93% success rate (Table 11). In this cross, every plant that was predicted to be 4x was confirmed to be 4x. Flow cytometry encountered problems in crosses with a triploid parent. In these crosses, the triploids were usually predicted fairly well, but at the expense of falsely including many of the tetraploid individuals (Table 12, 13, 14). In the diploid by diploid crosses, flow cytometry did not detect the 1 triploid individual which was detectable only by chromosome

counts (Table 15). Flow cytometry was also hindered by shifting histograms from one sample run to another caused by anthocyanin compounds interfering with the binding of the fluorochrome to the DNA. Flow cytometry yielded better results in this study when used in crosses where the progeny contained few or no triploids. However, there is the exception of the cross between the tetraploid 'Golden Gardens' and the diploid 'Red Fairy'. In this cross, all but 1 of the progeny was triploid and flow cytometry correctly predicted them to be triploid. It also correctly predicted the 1 tetraploid. Perhaps flow cytometry had more problems in the crosses with the triploid parent because this introduces more variation into the progeny. Increased meiotic variations in the triploid pollen donor 'Homerun' could allow for the resulting triploids in the progeny to be inheriting entirely different portions of the triploid genome. It is possible that the same 2 sets of chromosomes are not being transferred into each 2N pollen grain, which increases the genomic variation from plant to plant. There was also the problem encountered with the presence of the anthocyanins in the progeny from the 'Homerun' parent.

Table 10. Flow cytometry of 'Golden Gardens' x 'Red Fairy'.

Actual ploidy	----------------Predicted ploidy-------------------				
	2x	3x	4x	5x	Total
2X	0	0	0	0	0
3X	0	16	0	0	16
4X	0	0	1	0	1
5X	0	0	0	0	0
Total	0	16	1	0	17
% accuracy		100%	100%		

Table 11. Flow cytometry of 'Golden Gardens' x 'FF'.

Actual ploidy	-----------------Predicted ploidy-------------------				
	2x	3x	4x	5x	Total
2X	0	0	0	0	0
3X	0	3	0	0	3
4X	0	2	76	4	82
5X	0	0	0	0	0
Total	0	5	76	4	85
% accuracy		100%	93%		

Table 12. Flow cytometry of 'Golden Gardens' x 'Homerun'.

Actual ploidy	-----------------Predicted ploidy-------------------				
	2x	3x	4x	5x	Total
2X	0	0	0	0	0
3X	0	2	26	0	28
4X	0	0	25	5	30
5X	0	0	0	1	1
Total	0	2	51	6	59
% accuracy		7%	83.3%	100%	

There were only 59 plants screened by flow cytometry as 7 plants did not produce usable histograms due to interference with the fluorochrome

Table 13. Flow cytometry of 'Old Blush' x 'Homerun'.

Actual ploidy	-----------------Predicted ploidy-------------------				
	2x	3x	4x	5x	Total
2X	0	3	0	0	3
3X	0	2	0	0	2
4X	0	0	0	0	0
5X	0	0	0	0	0
Total	0	5	0	0	5
% accuracy	0%	100%			

Table14. Flow cytometry of 'WOBxOB26#212' x 'Homerun'.

Actual ploidy	-----------------Predicted ploidy-------------------				
	2x	3x	4x	5x	Total
2X	0	2	1	0	3
3X	0	15	0	0	15
4X	0	0	1	0	1
5X	0	0	0	0	0
Total	0	17	2	0	19
% accuracy	0%	100%	100%		

Table 15. Flow cytometry of 'J06-20-14-3' x 'Vineyard Song'.

Actual ploidy	-----------------Predicted ploidy-------------------				
	2x	3x	4x	5x	Total
2X	28	1	0	0	29
3X	0	0	1	0	1
4X	0	0	0	0	0
5X	0	0	0	0	0
Total	28	1	1	0	30
% accuracy	96.5%	0%	0%		

3.5 Conclusions

Using pollen diameter alone to identify the ploidy level of roses in a population was moderately successful; however this method is unable to identify triploid roses as their pollen size overlaps the ranges of diploids and tetraploids. If a population does not contain triploid individuals, or only a few, then pollen size could be a viable method to get a relatively rapid estimate of the general ploidy distribution of a population. This would not be practical in most rose breeding programs as triploids make up a significant portion of the population. The success of using pollen diameter to predict ploidy level in the TAMU rose

germplasm was lower than previous studies, and it is likely that success changes from one population to another depending on genetic composition. Using pollen diameter and pollen appearance together allows triploids to be identified, but only to a modest degree. This method also results in some tetraploids and diploids being inaccurately described as triploids if they have a higher than average propensity to produce shriveled pollen as well as 2N gametes. Flow cytometry has been useful for ploidy identification in some rose populations (Zlesak, 2009), but there are occasions where DNA content can vary extensively between plants at a given ploidy level. Most modern roses have various wide interspecific crosses in their parentage, which likely leads to genomic size differences, making ploidy estimation via flow cytometry difficult (Levin 2002). In addition, triploid plants frequently have DNA contents that overlap that of tetraploids and diploids (Yokoya *et al.,* 2000). Both of these issues were encountered extensively in the TAMU germplasm, in addition to the problems with the shifting of fluorescence peaks from one sample run to another. These issues make flow cytometry less viable than pollen size and morphology at indirect ploidy estimation in the TAMU rose germplasm. Even though pollen size was more successful than flow cytometry in ploidy assessment, chromosome counts are still needed to confirm the ploidy of the plant.

When it comes to tracking the ploidy level in interploidy crosses, flow cytometry and pollen analysis both failed to perform efficiently enough to be considered as viable replacements for direct chromosome counts, especially in crosses with triploids. However, flow cytometry did provide acceptable results when used on crosses between plants of the same ploidy level, therefore it could be a useful tool to rapidly obtain a general estimate of

any outlying ploidy levels in a population of expected even numbered ploidy. When used in the crosses with a triploid parent, the triploid and tetraploid offspring frequently overlapped leading to unacceptable inaccuracies. Pollen analysis of the triploid interploidy crosses also proved to be inaccurate. The use of a triploid parent seems to introduce an increased amount of variation in the population, leading to a wide range of pollen sizes and morphology encountered.

CHAPTER IV

PLOIDY TRANSMISSION IN AND SUCCESS OF INTRAPLOIDY

AND INTERPLOIDY CROSSES

4.1 Synopsis

A series of interploidy and intraploidy crosses were conducted that consisted of: (female x male) diploid x diploid, diploid x tetraploid, diploid x triploid, tetraploid x tetraploid, tetraploid x triploid, and tetraploid x diploid. These were done to evaluate the transmission of ploidy in various ploidy crosses, but especially the transmission of ploidy from a triploid male. These interploidy crosses indicated that the highest hip set, seeds per hip, seed germination, and seedlings per pollination were in crosses done between diploid – tetraploid, diploid-tetraploid, tetraploid-triploid, and tetraploid-diploid roses respectively. The ploidy level of the seedlings of these crosses was followed to determine the frequency of haploid and diploid pollen from the triploid parent 'Homerun' that resulted in a viable seedling. In crosses with a tetraploid female parent and the triploid pollen parent, 55% of the progeny were triploid, 41% of the progeny were tetraploid, and 3% were pentaploid. In crosses with a diploid female and a triploid male, 20% of the progeny were diploid, 75% were triploid and 4% were tetraploid.

4.2 Introduction

Roses are one of the most popular ornamental plants grown around the world and have been grown for centuries for their multiple horticultural and culinary uses (Zlesak, 2009). In addition, roses would not be as successful as they are today if recurrent blooming cultivars were not widely available. With the addition of the ever blooming trait, rose plants that would normally only bloom for a brief period once a year, now flower repeatedly over the course of the warm growing season. Roses originated in the Northern Hemisphere. There are currently over 100 recognized species with most of the cultivated roses coming from the subgenus *Rosa* (Zlesak, 2009, Gudin, 2000).

Roses were independently domesticated in Europe and Asia. When the domesticated Chinese roses were taken to Europe, they intercrossed and transferred the ever blooming trait to the European species which ultimately led to the modern roses encountered today. These first interploidy hybrids are the ancestors of many of the highly heterogeneous rose cultivars encountered today (Gudin, 2000; Zlesak 2006; 2009). Among these species, the basic number of chromosomes is seven with individuals ranging in ploidy level from diploid to decaploid (Zlesak, 2006; Jian et al. 2010). Ploidy level is an important factor that must be taken into consideration in any plant breeding program as it has an influence on plant performance, appearance and combining success with other individuals (Levin, 2002; Zlesak, 2009).

When it comes to breeding roses, most of today's commercial rose cultivars are tetraploid (Zlesak, 2006). Tetraploid roses carry many useful horticultural traits, but can be hindered by their lack of critical traits such as disease resistance and environmental

adaptations (Byrne et al., 2007). Because of this, it is important to adequately examine the value of diploid germplasm, which contains valuable disease resistance and environmental tolerance genes, and the methods to incorporate useful diploid traits into the tetraploid genome.

One example of a highly valuable trait successfully transferred from diploids into tetraploids is the single recessive gene for recurrent blooming. This occurred early in the cultivation of roses where open pollination occurred in European gardens containing groups of roses with diploid and tetraploid genomes (Zlesak, 2006). Hybridization between tetraploid and diploid roses generally results in progeny that are triploid. These individuals have reduced fertility, but due to natural variation in meiotic patterns, there are opportunities for viable gametes to be produced by triploid individuals (Byrne et al., 2007).

This variation in meiosis also leads to the formation of gametes with various even and odd numbered ploidy levels. In one study, when tetraploid roses were pollinated with pollen from a triploid plant, the progeny contained nearly equal numbers of tetraploid and triploid individuals (Table 17) (Zlesak 2009).   In a different study however, 98% of the progeny was found to be tetraploid when tetraploid females were pollinated with triploid pollen (Table 16) (Huylenbroeck et al. 2005). In this study there were also 5x and 6x seedlings produced, which suggests that the tetraploid parent was possibly producing 2n gametes. This indicates the different triploids can produce a range of viable 1n and 2n gametes. Zlesak 2009 suggested that these differences could be the result of different degrees of pollen competition between n and 2n pollen, or variation in meiotic patterns from one triploid individual to another.

Another important factor to be considered when attempting interploidy crosses is the success of the cross related to hip set and seed germination. In one study, triploids performed just as well as diploids with respect to hip set and seed germination when each was used as a pollen parent in a cross with a tetraploid (Huylenbroeck et al. 2005). However, triploids do perform poorly when used as female parents, leading to a low hip set rate of 14% (Huylenbroeck et al. 2005).

The objectives of this experiment were to:

1) Compare hip set, seeds per hip, seed germination, and seedlings per pollination in interploidy and intraploidy crosses.

2) Examine the transmission of ploidy level in interploidy and intraploidy crosses.

3) Examine morphological traits among the different ploidy levels in the progeny of crosses with the triploid 'Homerun', as the pollen donor.

4) Conduct pollen analysis to estimate fertility and the propensity of triploid progeny to produce consistent pollen grains

Table 16. Interploidy cross summary from Huylenbroeck et al., 2005.

Seed parent	Pollen parent	% hip formation	# seeds/ hip	# tested seedlings	3x	4x	5x	6x
'Kasteel van Ooidonk' 4x	'Jacky's Favorite' 3x	43.2	4.7	125	1	123		1
'Jacky's Favorite' 3x	'Kasteel van Ooidonk' 4x	23.4	1.1	15	3	11	1	

Huylenbroeck, J.V., L Leus. E.V. Bockstaele. 2005. Interploidy crosses in roses: use of triploids. Acta Hort. 690: 109-112.   http://www.actahort.org/books/690/690_15.htm

Table 17. Summary of interploidy crosses from Zlesak et al. 2009.

Seed parent 4x	Pollen parent 3x	Offspring		Total
		4x seedlings	3x seedlings	
BUCbi	KORbin	0	2	2
	1G84	3	5	8
	2G102	5	9	14
	1990-6	0	1	1
1A10	1990-6	1	3	4
4A29	1G84	2	0	2
1B30	1B43	2	0	2
	2G102	1	0	1
	1990-6	6	1	7
1990-1	2G102	0	2	2
Total		20	23	43

Zlesak, D. C. 2009. Pollen diameter and guard cell length as predictors of ploidy in diverse rose cultivars, species and breeding lines. J. of Floriculture and Ornamental Biotechnology 3:53-70.

4.3 Materials and Methods

4.3.1 Plant Material

Pollinations to create the interploidy and intraploidy populations used in this experiment were made in the spring of 2010 and the resulting hips collected in October and November of 2010. The populations used in this study contained various interploidy and intraploidy combinations (Table 18). The seeds were extracted from the hips by placing the hips into a blender for 5 to 10 seconds with enough water to cover the hips. After the blending, the excess water was drained away and the suspension was spread out to dry. After drying was complete, the seeds were separated either by hand or mechanically and were counted. In mid-December, the seeds were planted in 45 x 76 cm trays containing Metro-Mix® (Sun Gro Horticulture, Bellevue, WA) with approximately 150 seeds per tray, watered and allowed to drain and then wrapped in plastic and placed in cold storage at 4°C for approximately ten weeks. In early March, the seed trays were moved to the greenhouse (approximately 30°C during the day and 18°C during the night) for germination and grown for about 4 months. At his point,
 the seedlings were moved into individual 1 gallon pots in an outside location where they remained for the winter and spring months. The seedlings were transplanted into field plots in the following summer (2012).

4.3.2 Hip and seed measurements

To obtain a better understanding of how interploidy crosses affect the progress of a breeding program from a fertility standpoint, several parameters regarding hips and seeds

were examined. These included hip set, seeds per hip, and germination rate. These

parameters were compared between interploidy and intraploidy crosses.

Table 18. Summary of interploidy crosses.

Seed parent	Ploidy	Pollen parent	Ploidy	Population size
Vineyard song	2X	J06-20-14-3	2X	12
Sweet Chariot	2X	M4-4	2X	41
Old Blush	2X	Homerun	3X	5
WOB26xOB	2X	Homerun	3X	21
J06-28-8-1	2X	Homerun	3X	6
J06-30-3-6	2X	Homerun	3X	4
J06-28-8-1	2X	O	4X	48
Jacquie Williams	3X	Basye's Blueberry	4X	10
Golden Gardens	4X	Red Fairy	2X	19
Golden Gardens	4X	Homerun	3X	69
Golden Gardens	4X	FF	4X	84
Orange Honey	4X	FF	4X	21

4.3.3 Chromosome counts to conduct ploidy analysis

Chromosome counts were used to verify the ploidy level that the pollen predicted and were not previously recorded in literature. Root tip squashes were used to obtain the cells for the chromosome spreads. To obtain the root tips, cuttings were collected from plants to be characterized and were placed under a mist bench to root. Approximately 21 days on the mist bench yielded the best quality root tips. The root tips were harvested directly from the cuttings on the mist bench and placed in ice water in 2 mL micro centrifuge tubes for approximately 20 hours. If the root tips were harvested a few days after the cuttings were removed from the mist bench, this seemed to significantly reduce the number and quality of chromosome spreads. The harvested root tips were approximately 13-19 mm in length. The longer length preferred as it facilitates the handling through all of the chemical treatments. After the ice water treatment the root tips were placed in Farmers fixative (3:1 v/v 95% ethanol: glacial acetic acid) (Ruzin, 1999; Zlesak, 2009) in the refrigerator until characterization. The root tips were treated with 5 N hydrochloric acid for 2 hours to soften the tissue and facilitate squashing. At the end of the acid treatment, the acid was pipetted out of the tubes and replaced with distilled water. To conduct the squash, the root tips were removed one at a time and placed on a microscope slide. A longitudinal cut was made starting approximately 3 to 4 mm from the tip, continuing through the tip. The tip of the root was then spread apart and the cellular matter within pushed out onto the slide, being careful to not leave large clumps of epidermal tissue which would hinder even squashing. The remainder of the root tissue was then removed and a drop of carbol fuchsin stain (1 g basic fuchsin, 5 g phenol, 10 mL 90% EtOH, 100 mL water) (Crane and Byrne

2003) was placed onto the dispersed cells and a cover slip was added. The slide was then placed upside down onto a paper towel with another paper towel placed on top of the slide. A wooden meter stick approximately 6 mm thick was placed on top of the paper towel, centered over the slide and pressure was applied by pressing very firmly on the yard stick. The yard stick allowed the application of an adequate amount of even pressure and reduced slide breakage.

4.3.4 Morphological trait and pollen analysis in triploid crosses

Morphological traits were examined in the tetraploid x triploid cross and the diploid x triploid crosses. The first trait examined was the color of the new growth, which was categorized by the major color. New growth was classified as being green or red depending which color made up the majority of the appearance. The next trait was flower type classified as either single or double. Plants were classified as being single if the petal number was 8 or fewer. The final trait examined was leaf form, being either miniature or normal. The progeny in the tetraploid x triploid cross 'Golden Gardens' x 'Homerun' was screened for resistance to the fungal disease black spot (*Diplocarpon rosae*) to determine if any correlation exists between resistance and ploidy level of the progeny. Pollen was also examined in the progeny of these crosses to estimate fertility and determine if any of the triploid offspring vary in the ratio of 1n and 2n pollen grains that they produce.

4.4 Results and Discussion

4.4.1 Hip and seed measurements

When hip set was evaluated in the crosses, the highest set (80.6%) occurred in the diploid x tetraploid crosses, while the lowest (43%) occurred in the tetraploid x diploid cross (Figure 11,Table 19). Among the 2x x 4x and 4x x 2x crosses, the highest hip set was obtained when the tetraploid parent was the pollen donor. This is similar to the results of interploidy crosses in citrus where the best fruit set was obtained by using the higher ploidy plants as males (Muhammad et al. 2005). The number of seeds per hip had a large variation from one cross to another, with no apparent association with the ploidy level of either of the parents. In contrast, the tetraploid x diploid cross had the highest number of seeds per hip at 15, while the diploid x tetraploid had the lowest at 3 (Table 19). The germination rate also showed significant variation with the highest germination occurring in the tetraploid x triploid cross and the lowest germination occurring in the diploid x diploid crosses (Figure 12, Table 19). In addition, all crosses where diploids were used as the female parent had lower germination rates compared to crosses where tetraploids were used as the female parents. When diploid females are crossed with diploid, triploid and tetraploid males, the germination rate increased with the ploidy of the male parent. Overall it seems that hip set is highest with diploid seed parents while seed germination is highest with tetraploid seed parents. Furthermore, in both diploid and tetraploid groups of female parents there is a positive correlation with increasing male parent ploidy and increasing hip set. This correlation also occurred with seed germination in crosses with diploid females. The number of seedlings per pollination was mostly dependent on the ploidy of the female parent, being

higher in crosses with tetraploid female parents when compared to diploid female parents (Figure 13, Table 19).
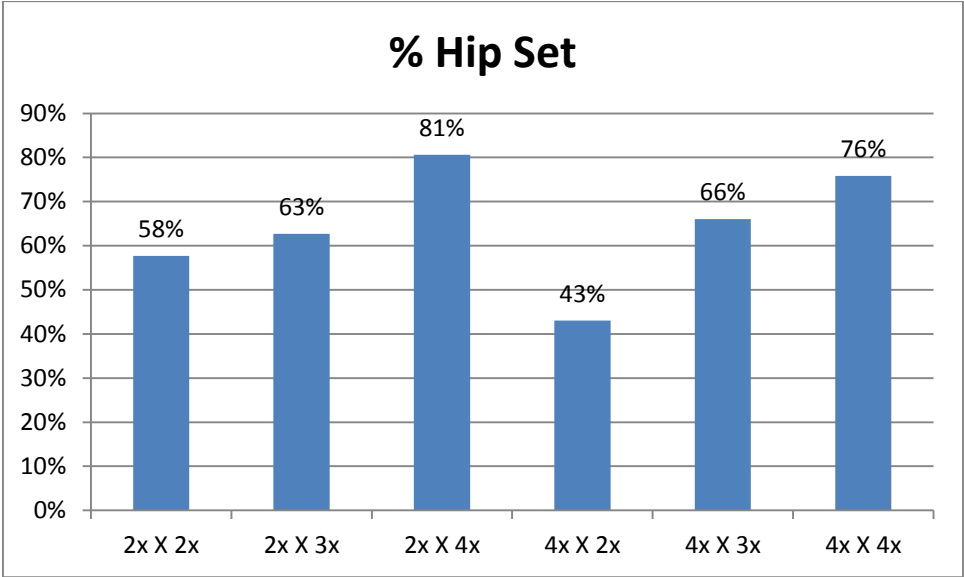
% Hip Set

Figure 11. Hip set in interploidy and intraploidy crosses.
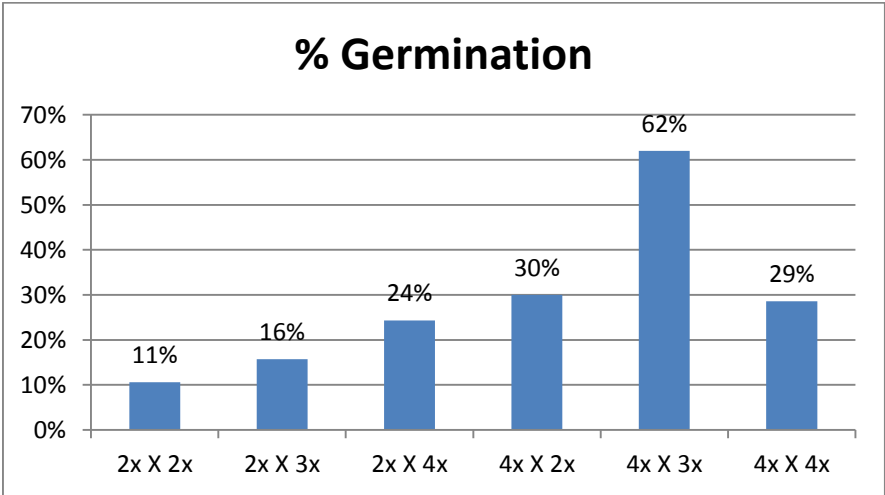
% Germination

Figure 12. Germination success in interploidy and intraploidy crosses.

Table 19. Hip set, seeds per hip and % germination in crosses of various ploidy.

Seed parent x Pollen parent	# seeds	# pollinations	% hip set	# seeds/hip set	% germination	seedlings per pollination
Diploid x diploid	598	108	57.7	9.8	10.6	0.66
Diploid x triploid	308	150	62.7	7.2	15.7	0.46
Diploid x Tetraploid	218	62	80.6	4.4	24.3	0.77
Tetraploid x diploid	135	21	43.0	15	30.0	1.57
Tetraploid x triploid	241	119	66.0	3	62.0	1.25
Tetraploid x tetraploid	1227	271	75.8	5.6	28.6	1.03
Mean	454.5	121.8	64.3	7.5	28.5	0.95
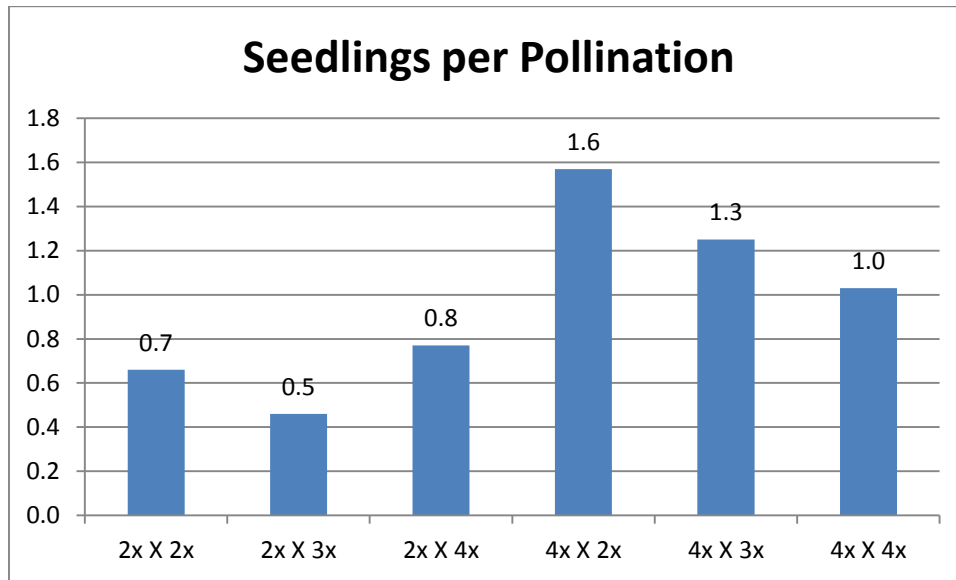
Figure 13. Seedlings resulting from every pollination in various interploidy and
intraploidy crosses.

4.4.2 Discussion of ploidy transmission

Ploidy transmission is an important item to consider in any crop containing

polyploidy. Ploidy transmission has significant implications in rose development because

of the use of triploids in breeding programs. Triploids can produce fertile offspring and can

be important in the transfer of genes from the diploid genome to the tetraploid genome or

genes from the tetraploid genome to the diploid genome. In the tetraploid by triploid cross,

there was a nearly even distribution of triploid and tetraploid offspring. This shows that in

this case, the triploid pollen parent 'Homerun', produces viable n and 2n gametes (Tables

20-21). There was one plant in this progeny population that was pentaploid, meaning that

the entire genome of the triploid parent was transferred to that seedling via a 3n pollen

grain. These results differ significantly from Huylenbroeck 2005 in that most of the progeny in the tetraploid x triploid cross in that study (98%) were tetraploid. Zlesak 2009 however, also reported nearly equal numbers of tetraploids and triploids in the progeny of tetraploid x triploid crosses. In the pollen analysis of chapter II, some triploids seemed to have uniform pollen with little variation in size. This could suggest that triploids have different meiotic patterns and that some can produce significantly different ratios of n and 2n gametes which could also explain the differing results of this experiment compared to Huylenbroeck 2005.

In the crosses between the diploids 'Old Blush', 'J06-28-8-1', 'J06-30-3-6' and triploid 'Homerun', the distribution between diploids and triploids was also nearly equal, although these were all small populations (Table 21). The progeny in these crosses, along with the tetraploid x triploid cross both show a similar distribution between N and 2N pollen grains produced by the triploid pollen parent. In the population of seedlings from the diploid x triploid cross 'WOB x OB212' x 'Homerun', the distribution of seedlings was skewed toward triploid plants, with 15 out of 19 plants being confirmed as triploid (Table 20). The difference in the ratios of ploidy levels between the different progenies could be the result of the small and inconsistent population sizes. It could also be that the competiveness of 2n pollen is affected somehow by the female parent. In the tetraploid x tetraploid and diploid x diploid crosses, the progeny population consisted almost entirely of individuals with the same ploidy level as the parents. There were however, 3 individuals in the tetraploid population ('Golden Gardens' x 'FF') that were confirmed as triploids, meaning that one of the parents was producing 1n gametes, or that there was outcrossing

with a diploid individual. Morphological markers are not likely to be of assistance in this situation due to the female parent having the phenotype of prickles, doubled-yellow flowers, and miniature growth type, which are all dominant alleles (De Vries and Dubois, 1984) which would mask the effect of any outcrossing. The pollen of 'Golden Gardens' does show a slight inconsistency, with a small amount of the pollen grains (~5%) being significantly larger (3n) and smaller (1n) than expected sizes for tetraploids. There were no observed pentaploids in the population. In the diploid x diploid cross J06-20-14-3 x 'Vineyard Song', there was one individual that was confirmed to be triploid, which is not unexpected as the female parent 'J06-20-14-3' did show a few (less than 5%) large pollen grains. Morphological markers are not likely to be helpful in this case either. The female parent 'J06-20-14-3' has prickles and single flowers (dominant, recessive respectively) while the pollen parent has prickles, doubled flowers and miniature growth type (all dominant). If any selfing occurred, the progeny would have the single flowers of the female parent 'J06-20-14-3. However, unless the pollen parent 'Vineyard Song' is homozygous for doubled flowers, it could still produce offspring with single flowers.

4.4.3 Morphological analysis and pollen observations

A few morphological traits were tracked in the progenies of the triploid crosses to determine if the inherited ploidy level had any correlation with certain morphological characteristics. In the tetraploid x triploid cross 'Golden Gardens' x 'Homerun', the population was approximately evenly distributed between triploids and tetraploids. Furthermore, approximately 50% of the triploids and 50% of the tetraploids displayed all 3 observed morphological traits from the triploid pollen parent (single flowers, normal leaf

size and red coloring in new growth) (Table 22). The remaining 50% of the population in each ploidy level were evenly distributed among the other morphology traits, with most progeny having at least 1 trait from the triploid parent. In the diploid x triploid crosses, the relationship between ploidy level and morphology was harder to establish due to the low numbers of individuals in these seedling populations. In the cross between WOBxOB26#212 x 'Homerun', the diploid and triploid seedlings (being equivalent to the 3x and 4x seedlings in the tetraploid cross) were distributed with a majority of the seedlings displaying at least one of the traits from the pollen parent 'Homerun' (Tables 22-26). Screening the progeny of the cross 'Golden Gardens' x 'Homerun' for resistance to black spot showed a significant difference in distribution of resistance in the 3x and 4x populations of the progeny (Table 27). The triploid population had an even distribution of resistant and susceptible individuals, while the tetraploid population contained mostly susceptible individuals (28% R, 72% S). Despite the fact that the tetraploid individuals possess an extra set of chromosomes from the triploid parent, they still fail to inherit resistance. It is possible that the genome with the chromosome containing the resistance gene is transmitted more frequently in 1n gametes than 2n gametes. Pollen size analysis of the 'Golden Gardens' x 'Homerun' cross showed that triploid progeny have a higher rate of inconsistent pollen size (70% vs 37%) as well as a slightly higher average percentage of shriveled pollen grains (30% vs 20%) when compared to the tetraploid progeny (Table 28, A-4). Analysis of the 'OBWOB26#212' x 'Homerun' cross showed that all triploid progeny, one tetraploid individual and 1 of the 3 diploid individuals exhibited inconsistent size (Table 29, A-5). A noticeable difference in this cross was the dramatic increase in the

number of shriveled grains present. The diploid offspring suffered the worst from this

condition with an average rate of 76% of the pollen grains being shriveled, with one of the

diploid individuals having ~90% of the pollen grains be deformed. The fact that these

diploid plants exhibit such poor fertility is an important thing to consider if they are to be

used in further breeding efforts.

Table 20. Summary of confirmed ploidy in progeny of interploidy and intraploidy crosses.

	4X x 4X 'Golden Gardens' x' FF'	4X x 2X 'Golden Gardens' x 'Red Fairy'	4X x 3X 'Golden Gardens' x 'Homerun'
Diploid	0	0	0
Triploid	3	16	36
Tetraploid	82	1	31
Pentaploid	0	0	1
Unknown	0	1	0
Total	85	18	66

Table21. Summary of confirmed ploidy in progeny of interploidy and intraploidy crosses.

	2X x 3X 'J06-28-8-1' x 'Homerun'	2X x 3X 'J06-30-3-6' x 'Homerun'	2X x 3X 'Old Blush x 'Homerun'	2X x 3X ' WOBxOld Blush' x 'Homerun'	2X x 2X 'J06-20-14-3' x'Vineyard Song'
Diploid	2	2	3	3	29
Triploid	3	2	2	15	1
Tetraploid	0	0	0	1	0
Pentaploid	0	0	0	0	0
Unknown	0	0	0	0	0
Total	5	4	5	19	30

Table 22. Distribution of morphological traits in progeny of 'Golden Gardens' x 'Homerun'.

	1/M/R	1/N/R	2/M/R	2/N/R	1/M/G	1/N/G	2/M/G	2/N/G
Diploid	0	0	0	0	0	0	0	0
Triploid	4	10	1	1	3	2	1	0
Tetraploid	6	9	1	0	2	1	1	0
Pentaploid	0	0	0	0	0	0	1	0
Total	10	19	2	1	5	3	3	0

1= single flowers, 2= doubled flowers, N= normal leaf size, M= miniature leaf size
R= new growth has significant red coloring, G= new growth is green, no red color
'Golden Gardens'= 2/M/G, 'Homerun'= 1/N/R
Only 43 plants characterized due to some not flowering and exhibiting poor growth

Table 23. Distribution of morphological traits in progeny of 'J06-28-8-1' x 'Homerun'.

	1/M/R	1/N/R	2/M/R	2/N/R	1/M/G	1/N/G	2/M/G	2/N/G
Diploid	0	0	0	1	0	1	0	0
Triploid	0	1	0	0	0	2	0	0
Tetraploid	0	0	0	0	0	0	0	0
Pentaploid	0	0	0	0	0	0	0	0
Total	0	1	0	1	0	3	0	0

1= single flowers, 2= doubled flowers, N= normal leaf size, M= miniature leaf size
R= new growth has significant red coloring, G= new growth is green, no red color
'Homerun'= 1/N/R, 'J06-28-8-1'=1/M/G

Table 24. Distribution of morphological traits in progeny of 'J06-30-3-6' x 'Homerun'.

	1/M/R	1/N/R	2/M/R	2/N/R	1/M/G	1/N/G	2/M/G	2/N/G
Diploid	1	0	0	0	0	1	0	0
Triploid	1	0	0	0	0	1	0	0
Tetraploid	0	0	0	0	0	0	0	0
Pentaploid	0	0	0	0	0	0	0	0
Total	2	0	0	0	0	2	0	0

1= single flowers, 2= doubled flowers, N= normal leaf size, M= miniature leaf size
R= new growth has significant red coloring, G= new growth is green, no red color
'Homerun'= 1/N/R, 'J06-30-3-6'=1/M/G

Table 25. Distribution of morphological traits in progeny of 'Old Blush' x 'Homerun'.

	1/M/R	1/N/R	2/M/R	2/N/R	1/M/G	1/N/G	2/M/G	2/N/G
Diploid	0	1	0	0	0	1	0	0
Triploid	0	1	0	0	1	0	0	0
Tetraploid	0	0	0	0	0	0	0	0
Pentaploid	0	0	0	0	0	0	0	0
Total	0	2	0	0	1	1	0	0

1= single flowers, 2= doubled flowers, N= normal leaf size, M= miniature leaf size
R= new growth has significant red coloring, G= new growth is green, no red color
'Homerun'= 1/N/R, 'Old Blush'=2/N/G
One diploid plant was not characterized due to poor growth

Table 26. Distribution of traits in progeny of 'WOBxOB26#212'x 'Homerun'.

	1/M/R	1/N/R	2/M/R	2/N/R	1/M/G	1/N/G	2/M/G	2/N/G
Diploid	1	2	0	0	0	0	0	0
Triploid	0	7	0	0	1	4	0	0
Tetraploid	0	1	0	0	0	0	0	0
Pentaploid	0	0	0	0	0	0	0	0
Total	1	10	0	0	1	4	0	0

1= single flowers, 2= doubled flowers, N= normal leaf size, M= miniature leaf size
R= new growth has significant red coloring, G= new growth is green, no red color
'Homerun'= 1/N/R, WOBxOB26#212=2/N/G

Table 27. Resistance to black spot among triploid and tetraploid progeny.

Ploidy	Resistant	Susceptible	Total	Chi square (p-value 0.05,1DF =3.8)
3x[1]	17	17	34	1.8
4x[2]	7	18	25	7.4[**]
Total	24	35	59	

[1]expected ratio of 1 resistant to 2 susceptible

[2]expected ratio of 2 resistant to 1 susceptible

[**]significantly different than expected

Table 28. Pollen analysis of 'Golden Gardens' x 'Homerun'.

Ploidy	Consistent size	Inconsistent size	Average % shriveled
3x	5	21	30
4x	15	9	24
Total	20	30	

Table 29. Pollen analysis of 'OBWOB26#212' x 'Homerun'.

Ploidy	Consistent size	Inconsistent size	Average % shriveled
2x	2	1	76
3x	0	15	60
4x	0	1	40
Total	2	17	

4.4 Conclusions

Analysis of hip set and seed germination shows that as compared to same ploidy crosses, interploidy combinations do not suffer from significantly decreased hip set, seed germination, or the number of seedlings produced per pollination. Among crosses with diploid female parents, triploid male parents produced lower numbers of seedlings per

pollination than diploid or tetraploid male parents. When crossing tetraploids and diploids to produce triploids, hip set was higher when the male parent was tetraploid, but the germination rate and seedlings per pollination were higher when the male parent was diploid.

In the progeny of a tetraploid x triploid cross, half of the triploids and half of the tetraploids appeared to inherit all 3 morphological traits from the triploid parent. The remaining 50% of each population of ploidy level were distributed among the other morphology traits, with all but 3 progeny displaying at least 1 trait from the triploid parent.

In the progeny of diploid x triploid crosses, the relationship between ploidy level and morphology was difficult to establish because of the small population size, although the majority of plants containing all 3 traits from the pollen parent were triploids (result of 2n pollen).

Examining ploidy transmission in triploid interploidy crosses suggests that on average the triploid parent plant 'Homerun' produced approximately even amounts of 1n and 2n gametes, which comprise the majority of the pollen, with a smaller population of 3n gametes. In this study, the triploid plant proved to be a potential pathway to move genetic material between the tetraploid and diploid genomes. However, there was more success in moving traits to the tetraploid level than the diploid level. In the tetraploid by triploid cross, 1 tetraploid plant with all 3 morphological traits from 'Homerun' was obtained for every 13 pollinations performed. One plant with at least 1 of the traits was obtained for every 6 pollinations performed. In the diploid by triploid crosses, the transfer of traits from 'Homerun' was less successful. For every diploid plant containing all 3 traits from

'Homerun', about 75 pollinations were conducted. For every diploid plant containing at least 1 trait, about 50 pollinations were conducted.

Pollen screening of the tetraploid x triploid cross showed that triploid progeny have lower pollen fertility as well as inconsistent pollen size when compared to their tetraploid counterparts. Progeny of the diploid x triploid crosses had lower fertility regardless of ploidy level, but diploid individuals were observed to have extremely decreased fertility with the majority of the pollen grains that they produce being shriveled. In a breeding program where the objective is to move traits from the triploid/tetraploid level to the diploid level for further breeding, these low fertility diploids could prove to be a significant obstacle. Further study is suggested to determine if altering the triploid parent and/or diploid parent could improve the fertility in progeny of these crosses.

CHAPTER V

CONCLUSION

The TAMU germplasm has a higher percentage of diploids than other populations in the literature. Diploid plants in the TAMU germplasm produce 2n gametes at a rate of ~9%, while tetraploids produce 2n pollen grains at a rate of ~1.8%. The triploid plants in the population were shown to have reduced fertility when pollen grains were examined. Triploid plants frequently had higher numbers of shriveled pollen grains as well as inconsistent pollen sizes than did even ploidy roses. However, a few triploids had pollen with few shriveled grains and of a consistent size range. This is a possible indication that these particular triploids are more fertile relative to other triploids and may produce different ratios of 1n and 2n gametes. This finding supports the fact that different studies with different triploids in literature show significantly different ploidy level transmission among triploid parents.

Of the methods tested to screen for ploidy level, chromosome counts were the most successful. Indirectly estimating ploidy level through pollen size and flow cytometry did not perform well enough to merit their use as primary ploidy predictors capable of replacing direct chromosome counts. Populations containing triploid individuals present the greatest difficulties. Triploid plants overlap in pollen size as well as flow cytometry readings which greatly reduces the accuracy of these 2 methods. It was thought that if the ploidy level screen was done within the progenies of interploidy crosses, that the common parentage of the population would reduce some of the variation in pollen size and flow cytometry readings. However, the variability in pollen size as well as flow cytometry results was still

high among individuals with the same parents. In addition to problems with distinguishing triploids from other ploidy levels in the populations, flow cytometry encountered problems with some of the pigments and other chemicals found in rose tissue. Despite multiple variations in the buffers used with flow cytometry, the anthocyanin pigments caused interference with readings that further decreased the accuracy of flow cytometry. Further study is needed on the composition of buffers used in flow cytometry and their ability to combat the secondary metabolites found in roses.

Crossing plants with dissimilar ploidy levels did not adversely affect the success of hip set or seed germination compared to same ploidy crosses. The number of seedlings per pollination was slightly affected when diploids were pollinated with triploids as opposed to diploids. Diploid x diploid crosses frequently have low germination rates, but diploids pollinated with triploids had higher germination rates. The triploid 'Homerun' was shown in this study to not only be fertile, but capable of combining successfully with diploids and tetraploids. This allows for genetic material to be moved from the diploid level to the tetraploid level and vice versa via triploid individuals. In the crosses conducted with a triploid pollen donor and a tetraploid seed parent, 1n and 2n gametes appear to be produced in approximately equal amounts, which facilitates movement from one ploidy level to another. However, in some of the crosses with the triploid pollen parent and diploid seed parent, the distribution of successful 1n and 2n fertilizations was skewed more toward ~15% and ~80% respectively, with ~5% 3n gametes.

Analysis of the pollen of a tetraploid x triploid cross showed that triploid progeny had lower pollen fertility as determined by % shriveled grains as well as greater

inconsistencies in pollen size when compared to their tetraploid counterparts. Diploid and triploid progeny of the diploid x triploid crosses had lower estimated fertility regardless of ploidy level, but diploid individuals were observed to have very low fertility, with the majority of the grains being shriveled. In a breeding program where the objective is to move traits from the triploid/tetraploid level to the diploid level for further breeding, these low fertility diploids could prove to be a significant obstacle. Further study is suggested to determine if altering the triploid parent and/or diploid parent could improve the fertility in progeny of these crosses.

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APPENDIX

Table A-1. Confirmed ploidy level of rose cultivars and the predicted ploidy level using pollen diameter.

Cultivar	Confirmed ploidy	Pollen Mean ± SD (µm)	% Shriveled grains	Predicted Ploidy
0-47-19	Diploid	33.3 ± 2.4		Diploid
145-95-3	Tetraploid	43.4 ± 4.7		Tetraploid
46-03-04	Tetraploid	42.5 ± 4.6		Tetraploid
Albuquerque Enchantment	Tetraploid	38.3 ± 2.4		Tetraploid
Amber Gem	Triploid	36.2 ± 2.7		Tetraploid
Angel Pink	Triploid	42.1 ± 2.2	~40	Tetraploid
Annie R. Mitchell	Tetraploid	34.3 ± 1.9		Diploid
Antique rose	Diploid	32.1 ± 1.2		Diploid
Apricot Twist	Triploid	39.4 ± 1.3	~25	Tetraploid
Avandel	Tetraploid	44.0 ± 2.6	~25	Hexaploid
2n pollen (20% of total)		49.0 ± 3.2		
Baby Austin	Diploid	32.1 ± 0.9	~30	Diploid
Baby Eclipse	Diploid	35.1 ± 4.2		Diploid
Belinda's Dream	Triploid	38.8 ± 4.2	~25	Tetraploid
Butter Mint	Tetraploid	38.1 ± 4.3		Tetraploid
Café Ole	Triploid	35.2 ± 4.7	~25	Diploid
Cal Poly	Tetraploid	39.1 ± 2.1		Tetraploid
Carol Jean	Diploid	39.9 ± 4.0		Tetraploid
Cee Dee Moss	Tetraploid	38.5 ± 3.3		Tetraploid
Centennial Miss	Diploid	35.4 ± 2.1		Diploid
Charlie Brown	Tetraploid	36.7 ± 2.6		Tetraploid
Chiquita	Diploid	35.0 ± 3.0		Diploid
Courier	Diploid	36.6 ± 2.8		Tetraploid
Crimson Shower	Diploid	33.0 ± 3.0		Diploid
Diamond Anniversary	Tetraploid	45.7 ± 6.8	~25	Hexaploid
2n pollen (~25% of total)		54.2 ± 4.3		

Don Marshall	Diploid	33.3 ± 3.2		Diploid
Doris Bennett	Triploid	39.3 ± 4.2	~20	Tetraploid
Double Treat	Tetraploid	37.8 ± 4.8		Tetraploid
Dresden Doll	Diploid	34.6 ± 1.3		Diploid
Earthquake	Triploid	32.1 ± 1.2	~25	Diploid
Edna Marie	Tetraploid	43.3 ± 4.2		Tetraploid
English Porcelain	Diploid	34.3 ± 3.1		Diploid
FF	Tetraploid	39.7 ± 3.5		Tetraploid
Fair Molly	Diploid	35.7 ± 2.9	~20	Tetraploid
2n pollen (~20% of total)		42.1 ± 3.2		
Fiesta Gold	Tetraploid	38.9 ± 3.9		Tetraploid
Finger Paint	Tetraploid	39.2 ± 3.1		Tetraploid
Fingerpaint X Shadow Dancer	Tetraploid	35.8 ± 4.0		Tetraploid
Fresh Pink	Diploid	34.8 ± 1.5		Diploid
2n pollen (~30% of total)		47.1 ± 3.4		
Fuzzy Wuzzy Red	Tetraploid	35.1 ± 2.7		Diploid
Gina's Rose	Tetraploid	34.2 ± 3.2	~20	Diploid
Gold Coin	Diploid	35.0 ± 1.0		Diploid
2n pollen (~10% of total)		39.7 ± 1.1		
Gold Moss	Triploid	36.7 ± 4.1	~25	Tetraploid
Golden Century	Diploid	34.8 ± 1.4		Diploid
Golden Gardens	Tetraploid	39.5 ± 2.6		Tetraploid
Golden Horizon	Triploid	41.2 ± 4.1	~35	Tetraploid
Hall of Flowers	Tetraploid	34.8 ± 3.4		Diploid
Halo Fire	Tetraploid	42.5 ± 5.1		Tetraploid
Halo Glory	Triploid	45.6 ± 3.9	~25	Hexaploid
Halo Today	Tetraploid	42.4 ± 4.3		Tetraploid
Hi Ho	Triploid	45.8 ± 5.1	~20	Hexaploid
Homerun	Triploid	45.2 ± 4.7	~30	Hexaploid

Hoot Owl	Tetraploid	42.9 ± 2.0	~20	Tetraploid
2n pollen (~15% of total)		46.3 ± 3.1		
Hope & Joy	Tetraploid	37.8 ± 3.2		Tetraploid
Iceberg	Triploid	32.2 ± 1.2		Diploid
Ice Tea	Tetraploid	39.2 ± 4.2		Tetraploid
J06-20-14-3	Diploid	34.4 ± 1.2		Diploid
J06-28-8-1	Diploid	32.8 ± 1.8		Diploid
J06-30-3-3	Diploid	31.3 ± 3.7		Diploid
J06-30-5-1	Diploid	35.2 ± 1.4		Diploid
J06-32-4-1	Diploid	34.3 ± 3.2		Diploid
Jacquie Williams	Triploid	39.3 ± 3.6	~40	Tetraploid
Jessica Rose	Triploid	35.7 ± 1.3		Tetraploid
Julie Link	Triploid	39.3± 2.2	~30	Tetraploid
Just for You	Tetraploid	43.3 ± 4.5		Tetraploid
Kayla	Tetraploid	46.2 ± 4.9	~25	Hexaploid
Lavender Delight	Tetraploid	45.1 ± 2.8	~15	Hexaploid
Lavender Jewel	Diploid	34.7 ± 1.2		Diploid
Little Buckaroo	Diploid	33.4 ± 3.6		Diploid
Little Chief	Diploid	31.2 ± 2.4		Diploid
Little Darling X Yellow Magic	Tetraploid	39.7 ± 5.3		Tetraploid
Little Emma	Tetraploid	40.3 ± 1.2		Tetraploid
Love and Peace	Tetraploid	48.0 ± 5.7	~20	Hexaploid
Lovely Lorrie	Triploid	37.7 ± 3.0	~25	Tetraploid
Lucy	Triploid	37.1 ± 5.7		Tetraploid
M4-4	Diploid	32.7 ± 5.7		Diploid
Magseed	Tetraploid	48.5 ± 3.2	~25	Hexaploid
Make Believe	Diploid	34.5 ± 1.6		Diploid
Mariposa Gem	Diploid	34.3 ± 3.6		Diploid
2n pollen (~10% of total)		38.6 ± 4.1		

Max Colwell	Diploid	40.9 ± 3.5		Tetraploid
Millie Walters	Tetraploid	43.1 ± 2.2		Tetraploid
Moores Striped Rugosa	Diploid	35.4 ± 2.1		Diploid
My Stars	Tetraploid	37.7 ± 1.2		Tetraploid
Nurse Donna	Tetraploid	38.7 ± 2.9		Tetraploid
O	Tetraploid	35.2 ± 1.0	~20	Diploid
Old Blush	Diploid	35.1 ± 3.4		Diploid
Ora Kelly	Tetraploid	39.3 ± 3.4		Tetraploid
Orange Frenzy	Triploid	37.3 ± 3.2	~25	Tetraploid
Orange Honey	Tetraploid	42.8 ± 3.6		Tetraploid
Orange Parfait	Tetraploid	43.3 ± 2.8		Tetraploid
2n pollen (~10% of total)		49.6 ± 3.7		
Out of Yesteryear	Triploid	39.4 ± 2.9	~25	Tetraploid
Papoose	Diploid	34.3 ± 2.2		Diploid
Patriot Song	Diploid	35.5 ± 3.3	~30	Diploid
Persian Autumn	Tetraploid	35.1 ± 2.4		Diploid
Pink Cameo	Tetraploid	40.7 ± 3.9		Tetraploid
Pink Elf	Diploid	30.8 ± 3.4		Diploid
Pinstripe	Diploid	35.9 ± 2.4		Diploid
2n pollen (10% of total)		40.1 ± 2.7		
Playgold	Tetraploid	43.2 ± 3.0	~20	Tetraploid
2n pollen (10% of total)		56.4 ± 4.4		
Quietness	Triploid	34.2 ± 2.1	~25	Diploid
Rain Forest	Tetraploid	42.8 ± 5.7		Tetraploid
Red Fairy	Diploid	31.9 ± 3.2		Diploid
Renny	Tetraploid	47.3 ± 8.9	~20	Hexaploid
Rise n Shine	Tetraploid	41.0 ± 2.2		Tetraploid
Rosa wichuriana	Diploid	35.1 ± 1.6		Diploid
Rose Gilardi	Tetraploid	40.5 ± 4.8		Tetraploid

Roseberry Blanket	Diploid	34.6 ± 1.8		Diploid
Roses are Red	Triploid	38.4 ± 5.1	~30	Tetraploid
Ruby Princess	Triploid	36.7 ± 5.1		Tetraploid
Scarlet Moss	Tetraploid	41.2 ± 3.2		Tetraploid
Sequoia Gold	Tetraploid	39.5 ± 1.2	~20	Tetraploid
Sheri Anne	Tetraploid	41.9 ± 4.3		Tetraploid
Show N Tell X Joycie	Tetraploid	40.5 ± 3.2		Tetraploid
Southern Delight	Tetraploid	36.9 ± 3.8		Tetraploid
Splish Splash	Tetraploid	35.1 ± 5.2	~20	Diploid
Spotlight	Triploid	38.1 ± 8.2		Tetraploid
Star Delight	Diploid	35.6 ± 8.2		Tetraploid
Stars n Stripes	Tetraploid	36.0 ± 4.9		Tetraploid
Strawberry Swirl	Triploid	37.9 ± 4.0		Tetraploid
Sweet Chariot	Diploid	30.1 ± 2.1		Diploid
Sweet Hannah	Triploid	38.2 ± 4.4	~40	Tetraploid
Tangerine Jewel	Triploid	41.4 ± 3.6		Tetraploid
The Fairy	Diploid	31.3 ± 1.3		Diploid
Topaz Jewel	Diploid	33.9 ± 5.4		Diploid
Trinket	Diploid	35.6 ± 2.8		Tetraploid
Twilight Skies	Triploid	39.1 ± 3.3	~20	Tetraploid
Vineyard Song	Diploid	28.9 ± 3.5		Diploid
WOB26xOB#212	Diploid	29.9 ± 3.5		Diploid
Yellow Jewel	Tetraploid	42.8 ± 3.3	~25	Tetraploid

Plants were categorized as follows: Diploid:<35.6 μm, Tetraploid:35.6-43.7μm, Hexaploid: 43.7-47μm

2n pollen grains were not included in mean diameter calculations for ploidy analysis

Table A-2. Confirmed ploidy level of rose cultivars and predicted ploidy level using pollen diameter and appearance

Cultivar	Confirmed ploidy	Pollen Mean ± SD	Pollen appearance	Predicted Ploidy
0-47-19	Diploid	33.3 ± 2.4		Diploid
145-95-3	Tetraploid	43.4 ± 4.7		Tetraploid
46-03-04	Tetraploid	42.5 ± 4.6		Tetraploid
Albuquerque Enchantment	Tetraploid	38.3 ± 2.4		Tetraploid
Amber Gem	Triploid	36.2 ± 2.7		Tetraploid
Angel Pink	Triploid	42.1 ± 2.2	Inconsistent/malformed	Triploid
Annie R. Mitchell	Tetraploid	34.3 ± 1.9		Diploid
Antique Rose	Diploid	32.1 ± 1.2		Diploid
Apricot Twist	Triploid	39.4 ± 1.3	Inconsistent/malformed	Triploid
Avandel	Tetraploid	44.0 ± 2.6	Inconsistent/malformed	Triploid
2n pollen (20% of total)		49.0 ± 3.2		
Baby Austin	Diploid	32.1 ± 0.9	Inconsistent/malformed	Diploid
Baby Eclipse	Diploid	35.1 ± 4.2		Diploid
Belinda's Dream	Triploid	38.8 ± 4.2	Inconsistent/malformed	Triploid
Butter Mint	Tetraploid	38.1 ± 4.3		Tetraploid
Café Ole	Triploid	35.2 ± 4.7	Inconsistent/malformed	Triploid
Cal Poly	Tetraploid	39.1 ± 2.1		Tetraploid
Carol Jean	Diploid	39.9 ± 4.0		Tetraploid
Cee Dee Moss	Tetraploid	38.5 ± 3.3		Tetraploid
Centennial Miss	Diploid	35.4 ± 2.1		Diploid
Charlie Brown	Tetraploid	36.7 ± 2.6		Tetraploid
Chiquita	Diploid	35.0 ± 3.0		Diploid
Courier	Diploid	36.6 ± 2.8		Tetraploid
Crimson Shower	Diploid	33.0 ± 3.0		Diploid
Diamond Anniversary	Tetraploid	45.7 ± 6.8	Inconsistent/malformed	Triploid
2n pollen (~25% of total)		54.2 ± 4.3		
Don Marshall	Diploid	33.3 ± 3.2		Diploid
Doris Bennett	Triploid	39.3 ± 4.2	Inconsistent/malformed	Triploid

Double Treat	Tetraploid	37.8 ± 4.8		Tetraploid
Dresden Doll	Diploid	34.6 ± 1.3		Diploid
Earthquake	Triploid	32.1 ± 1.2	Inconsistent/malformed	Triploid
Edna Marie	Tetraploid	43.3 ± 4.2		Tetraploid
English Porcelain	Diploid	34.3 ± 3.1		Diploid
FF	Tetraploid	39.7 ± 3.5		Tetraploid
Fair Molly	Diploid	35.7 ± 2.9	Inconsistent/malformed	Triploid
2n pollen (~20% of total)		42.1 ± 3.2		
Fiesta Gold	Tetraploid	38.9 ± 3.9		Tetraploid
Finger Paint	Tetraploid	39.2 ± 3.1		Tetraploid
Fingerpaint X Shadow	Tetraploid	35.8 ± 4.0		Tetraploid
Fresh Pink	Diploid	34.8 ± 1.5	Inconsistent/malformed	Triploid
2n pollen (~30% of total)		47.1 ± 3.4		
Fuzzy Wuzzy Red	Tetraploid	35.1 ± 2.7		Diploid
Gina's Rose	Tetraploid	34.2 ± 3.2	Inconsistent/malformed	Triploid
Gold Coin	Diploid	35.0 ± 1.0		Diploid
2n pollen (~10% of total)		39.7 ± 1.1		
Gold Moss	Triploid	36.7 ± 4.1	Inconsistent/malformed	Triploid
Golden Century	Diploid	34.8 ± 1.4		Diploid
Golden Gardens	Tetraploid	39.5 ± 2.6		Tetraploid
Golden Horizon	Triploid	41.2 ± 4.1	Inconsistent/malformed	Triploid
Hall of Flowers	Tetraploid	34.8 ± 3.4		Diploid
Halo Fire	Tetraploid	42.5 ± 5.1		Tetraploid
Halo Glory	Triploid	45.6 ± 3.9	Inconsistent/malformed	Triploid
Halo Today	Tetraploid	42.4 ± 4.3		Tetraploid
Hi Ho	Triploid	45.8 ± 5.1	Inconsistent/malformed	Triploid
Homerun	Triploid	45.2 ± 4.7	Inconsistent/malformed	Triploid
Hoot Owl	Tetraploid	42.9 ± 2.0	Inconsistent/malformed	Triploid
2n pollen (~15% of total)		46.3 ± 3.1		

Hope & Joy	Tetraploid	37.8 ± 3.2		Tetraploid
Iceberg	Triploid	32.2 ± 1.2		Diploid
Ice Tea	Tetraploid	39.2 ± 4.2		Tetraploid
J06-20-14-3	Diploid	34,4 ± 1.2		Diploid
J06-28-8-1	Diploid	32.8 ± 1.8		Diploid
J06,30-3-3	Diploid	31.3 ± 3.7		Diploid
J06-30-5-1	Diploid	35.2 ± 1.4		Diploid
J06-32-4-1	Diploid	34.3 ± 3.2		Diploid
Jacquie Williams	Triploid	39.3 ± 3.6	Inconsistent/malformed	Triploid
Jessica Rose	Triploid	35.7 ± 1.3		Tetraploid
Julie Link	Triploid	39.3± 2.2	Inconsistent/malformed	Triploid
Just for You	Tetraploid	43.3 ± 4.5		Tetraploid
Kayla	Tetraploid	46.2 ± 4.9	Inconsistent/malformed	Triploid
Lavender Delight	Tetraploid	45.1 ± 2.8	Inconsistent/malformed	Triploid
Lavender Jewel	Diploid	34.7 ± 1.2		Diploid
Little Buckaroo	Diploid	33.4 ± 3.6		Diploid
Little Chief	Diploid	31.2 ± 2.4		Diploid
Little Darling X Yellow	Tetraploid	39.7 ± 5.3		Tetraploid
Little Emma	Tetraploid	40.3 ± 1.2		Tetraploid
Love And Peace	Tetraploid	48.0 ± 5.7	Inconsistent/malformed	Triploid
Lovely Lorrie	Triploid	37.7 ± 3.0	Inconsistent/malformed	Triploid
Lucy	Triploid	37.1 ± 5.7		Tetraploid
M4-4	Diploid	32.7 ± 5.7		Diploid
Magseed	Tetraploid	48.5 ± 3.2	Inconsistent/malformed	Triploid
Make Believe	Diploid	34.5 ± 1.6		Diploid
Mariposa Gem	Diploid	34.3 ± 3.6	Inconsistent/malformed	Triploid
2n pollen (~10% of total)		38.6 ± 4.1		
Max Colwell	Diploid	40.9 ± 3.5		Tetraploid
Millie Walters	Tetraploid	43.1 ± 2.2		Tetraploid

Moores Striped Rugosa	Diploid	35.4 ± 2.1		Diploid
My Stars	Tetraploid	37.7 ± 1.2		Tetraploid
Nurse Donna	Tetraploid	38.7 ± 2.9		Tetraploid
O	Tetraploid	35.2 ± 1.0	Inconsistent/malformed	Triploid
Old Blush	Diploid	35.1 ± 3.4		Diploid
Ora Kelly	Tetraploid	39.3 ± 3.4		Tetraploid
Orange Frenzy	Triploid	37.3 ± 3.2	Inconsistent/malformed	Triploid
Orange Honey	Tetraploid	42.8 ± 3.6		Tetraploid
Orange Parfait	Tetraploid	43.3 ± 2.8		Tetraploid
2n pollen (~10% of total)		49.6 ± 3.7		
Out of Yesteryear	Triploid	39.4 ± 2.9	Inconsistent/malformed	Triploid
Papoose	Diploid	34.3 ± 2.2		Diploid
Patriot Song	Diploid	35.5 ± 3.3	Inconsistent/malformed	Triploid
Persian Autumn	Tetraploid	35.1 ± 2.4		Diploid
Pink Cameo	Tetraploid	40.7 ± 3.9		Tetraploid
Pink Elf	Diploid	30.8 ± 3.4		Diploid
Pinstripe	Diploid	35.9 ± 2.4	Inconsistent/malformed	Tetraploid
2n pollen (10% of total)		40.1 ± 2.7		
Quietness	Triploid	34.2 ± 2.1	Inconsistent/malformed	Triploid
Playgold	Tetraploid	43.2 ± 3.0	Inconsistent/malformed	Triploid
2n pollen (10% of total)		56.4 ± 4.4		
Rain Forest	Tetraploid	42.8 ± 5.7		Tetraploid
Red Fairy	Diploid	31.9 ± 3.2		Diploid
Renny	Tetraploid	47.3 ± 8.9	Inconsistent/malformed	Triploid
Rise n Shine	Tetraploid	41.0 ± 2.2		Tetraploid
Rosa wichuriana	Diploid	35.1 ± 1.6		Diploid
Rose Gilardi	Tetraploid	40.5 ± 4.8		Tetraploid
Roseberry Blanket	Diploid	34.6 ± 1.8		Diploid
Roses are Red	Triploid	38.4 ± 5.1	Inconsistent/malformed	Triploid

Ruby Princess	Triploid	36.7 ± 5.1		Tetraploid
Scarlet Moss	Tetraploid	41.2 ± 3.2		Tetraploid
Sequoia Gold	Tetraploid	39.5 ± 1.2	Inconsistent/malformed	Triploid
Sheri Anne	Tetraploid	41.9 ± 4.3		Tetraploid
Show N Tell X Joycie	Tetraploid	40.5 ± 3.2		Tetraploid
Southern Delight	Tetraploid	36.9 ± 3.8		Tetraploid
Splish Splash	Tetraploid	35.1 ± 5.2	Inconsistent/malformed	Triploid
Spotlight	Triploid	38.1 ± 8.2		Tetraploid
Star Delight	Diploid	35.6 ± 8.2		Tetraploid
Stars n Stripes	Tetraploid	36.0 ± 4.9		Tetraploid
Strawberry Swirl	Triploid	37.9 ± 4.0		Tetraploid
Sweet Chariot	Diploid	30.1 ± 2.1		Diploid
Sweet Hannah	Triploid	38.2 ± 4.4	Inconsistent/malformed	Triploid
Tangerine Jewel	Triploid	41.4 ± 3.6		Tetraploid
The Fairy	Diploid	31.3 ± 1.3		Diploid
Topaz Jewel	Diploid	33.9 ± 5.4		Diploid
Trinket	Diploid	35.6 ± 2.8		Tetraploid
Twilight Skies	Triploid	39.1 ± 3.3	Inconsistent/malformed	Triploid
Vineyard Song	Diploid	28.9 ± 3.5		Diploid
WOB26xOB	Diploid	29.9 ± 3.5		Diploid
Yellow Jewel	Tetraploid	42.8 ± 3.3	Inconsistent/malformed	Triploid

Plants with irregular pollen size and/or multiple malformed pollen grains were categorized as triploid

Table A-3 Flow cytometry ploidy predictions compared to chromosome counts

Cultivar	Confirmed Ploidy	Flow Cytometry Value	Predicted Ploidy
0-47-19	Diploid	80,000	Diploid
145-95-3	Tetraploid	155,000	Tetraploid
46-03-04	Tetraploid	142,000	Tetraploid
Albuquerque Enchantment	Tetraploid	150,000	Tetraploid
Amber Gem	Triploid	145,000	Tetraploid
Antique Rose	Diploid	85,000	Diploid
Baby Austin	Diploid	80,000	Diploid
Baby Eclipse	Diploid	80,000	Diploid
Belinda's Dream	Triploid	143,000	Tetraploid
Café Ole	Triploid	127,000	Triploid
Cal Poly	Tetraploid	164,000	Tetraploid
Carol Jean	Diploid	75,000	Diploid
Crimson Shower	Diploid	70,000	Diploid
Doris Bennett	Triploid	148,000	Tetraploid
Earthquake	Triploid	143,000	Tetraploid
Fiesta Gold	Tetraploid	160,000	Tetraploid
Fuzzy Wuzzy Red	Tetraploid	156,000	Tetraploid
Gina's Rose	Tetraploid	96,000	Diploid
Gold Moss	Triploid	160,000	Tetraploid
Golden Century	Diploid	97,000	Unknown
Golden Horizon	Triploid	96,000	Unknown
Halo Today	Tetraploid	140,000	Tetraploid
Homerun	Triploid	145,000	Tetraploid
Hoot Owl	Tetraploid	130,000	Triploid
Hope & Joy	Tetraploid	147,000	Tetraploid
Iceberg	Triploid	91,000	Diploid
J06-30-5-1	Diploid	97,000	Unknown
J06-32-4-1	Diploid	70,000	Diploid

Table A-3 Flow cytometry ploidy predictions compared to chromosome counts

Cultivar	Confirmed Ploidy	Flow Cytometry Value	Predicted Ploidy
Jacquie Williams	Triploid	150,000	Tetraploid
Julie Link	Triploid	122,000	Triploid
Little Buckaroo	Diploid	88,000	Diploid
Little Chief	Diploid	74,000	Diploid
Lucy	Triploid	124,000	Triploid
M4-4	Diploid	78,000	Diploid
Magseed	Tetraploid	138,000	Unknown
My Stars	Tetraploid	143,000	Tetraploid
Nurse Donna	Tetraploid	85,000	Diploid
O	Tetraploid	152,000	Tetraploid
Out of Yesteryear	Triploid	185,000	Tetraploid
Patriot Song	Diploid	70,000	Diploid
Persian Autumn	Tetraploid	177,000	Tetraploid
Pink Elf	Diploid	78,000	Diploid
Playgold	Tetraploid	115,000	Triploid
Renny	Tetraploid	96,000	Unknown
Rosa wichuriana	Diploid	85,000	Diploid
Rose Gilardi	Tetraploid	138,000	Unknown
Roses are Red	Triploid	160,000	Tetraploid
Sequoia Gold	Tetraploid	136,000	Unknown
Splish Splash	Tetraploid	156,000	Tetraploid
Spotlight	Triploid	143,000	Tetraploid
Sweet Hannah	Triploid	135,000	Triploid
Tangerine Jewel	Triploid	140,000	Tetraploid
The Fairy	Diploid	75,000	Diploid
Topaz Jewel	Diploid	89,000	Diploid
Trinket	Diploid	77,000	Diploid

Table A-4 Pollen analysis 'Golden Gardens' x 'Homerun'

Progeny	Ploidy	Pollen size	% Shriveled	Consistent/inconsistent
2	3x	34.7 ± 2.6	40	Inconsistent
3	3x	40.2 ± 3.1	20	Inconsistent
4	4x	34.7 ± 2.6	40	Inconsistent
5	4x	35.0 ± 2	30	Consistent
7	3x	38.0 ± 4.5	20	Consistent
8	4x	38.4 ± 2.5	20	Inconsistent
9	3x	36.6 ± 3.5	30	Consistent
10	3x	35.8± 2.5	40	Inconsistent
12	4x	39.7 ± 3.5	30	Consistent
13	3x	32.6 ± 3.6	40	Inconsistent
20	4x	36.9 ± 5.6	10	Consistent
23	4x	37.6 ± 2.1	30	Inconsistent
25	4x	39.2 ± 2.8	45	Consistent
26	4x	36.0 ± 1.5	20	Inconsistent
27	3x	38.2 ± 4.6	30	Consistent
28	3x	37.6 ± 3.3	30	Inconsistent
29	4x	36.9 ± 3.5	20	Inconsistent
31	3x	39.0 ± 3.1	40	Inconsistent
32	4x	39.1 ± 2.1	10	Consistent
33	4x	41.4 ± 2.4	20	Consistent
34	4x	40.4 ± 2.5	10	Consistent
35	4x	39.6 ± 2.1	10	Consistent
36	3x	38.7 ± 2.0	40	Inconsistent
37	3x	39.8 ± 2.3	20	Consistent

39	3x	34.4 ± 2.5	30	Inconsistent
40	3x	37.9 ± 2.9	30	Inconsistent
42	3x	36.1 ± 3.7	20	Inconsistent
43	4x	38.1 ± 2.3	10	Consistent
44	4x	39.2 ± 3.5	20	Consistent
46	4x	37.4 ± 2.4	20	Consistent
47	4x	37.1 ± 2.8	45	Consistent
48	4x	37.0 ± 3.5	20	Inconsistent
51	3x	36.0 ± 1.8	30	Inconsistent
53	3x	35.8 ± 2.3	30	Inconsistent
56	3x	41.2 ± 3.6	20	Consistent
57	4x	39.2 ± 2.4	30	Inconsistent
58	3x	33.6 ± 1.7	30	Inconsistent
59	3x	34.9 ± 2.8	40	Inconsistent
60	4x	38.2 ± 2.9	45	Consistent
61	3x	38.3 ± 2.9	20	Inconsistent
62	4x	38.2 ± 3.1	10	Consistent
63	3x	36.7 ± 3.5	30	Inconsistent
65	3x	33.2 ± 1.8	20	Inconsistent
66	3x	38.7 ± 3.7	40	Inconsistent
67	5x	39.9 ± 4.6	50	Inconsistent
70	3x	40.5 ± 3.4	30	Inconsistent
71	3x	37.2 ± 2.2	40	Inconsistent
73	4x	39.3 ± 2.7	20	Consistent
74	4x	36.9 ± 2.3	30	Inconsistent
79	3x	39.6 ± 3.5	20	Inconsistent

Table A-5 Pollen analysis 'OBxWOB26#212' x 'Homerun'

Progeny	Ploidy	Pollen size	% Shriveled	Consistent/inconsistent
52	3x	39.7 ± 2.6	80	Inconsistent
54	3x	38.2 ± 2.1	60	Inconsistent
56	3x	35.7 ± 2.9	40	Inconsistent
58	3x	42.0 ± 2.8	80	Inconsistent
59	4x	39.0 ± 3.5	40	Inconsistent
60	3x	35.4 ± 2.8	20	Inconsistent
61	3x	34.6 ± 2.5	90	Inconsistent
64	2x	35.8± 2.9	80	Inconsistent
65	3x	35.7 ± 2.5	40	Inconsistent
68	3x	36.6 ± 3.6	70	Inconsistent
69	2x	34.9 ± 1.6	70	Consistent
71	3x	35.6 ± 2.2	90	Inconsistent
74	3x	36.0 ± 1.5	40	Inconsistent
76	3x	37.2 ± 4.6	80	Inconsistent
77	3x	38.8 ± 3.4	60	Inconsistent
78	2x	34.6 ± 2.5	80	Consistent
79	3x	36.3 ± 3.0	60	Inconsistent
80	3x	38.2 ± 2.5	50	Inconsistent
81	3x	40.3 ± 2.3	40	Inconsistent

Table A-6 Parentage of cultivars involved in this study

Cultivar	Name	Female Parent	Male Parent
0-47-19	Morwichflo	*Rosa wichuraiana*	Floradora
145-95-3		Peach Halo	Halo Rainbow
22-94-12		Fingerpaint	shadow dancer
46-03-04		Sequoia Ruby	Playboy
6-97-07		Show N Tell	Joycie
Albuquerque Enchantment	MORalbuque	Poker Chip	Cherry Magic
Amber Gem	MORamber	Joycie	Out of Yesteryear
Angel Pink	MORgel	Little Darling	Eleanor
Annie R. Mitchell		Mary Hill SPORT	
Antique rose	MORcana	Baccara	Little Chief
Apricot Twist	MORbrown	Golden Angel	Sequoia Gold
Avandel	MORvandel	Little Darling	New Penny
Baby Austin	MORbaby	Joycie	String of Pearls
Baby Eclipse	MORedi	0-47-9	Yellow Jewel
Belinda's Dream	Belinda's Dream	Jersey Beauty	Tiffany
Butter Mint	MORsnop	Pink Petticoat	Gold Badge
Café Ole	MORole	Winter Magic SPORT	
Cal Poly	MORpoly	1-72-1	Gold Badge
Carol Jean	Carol Jean	Pinocchio	Little Chief
Cee Dee Moss	MORceedee	Carolyn Dean	14st
Centennial Miss	Centennial Miss	Oakington Ruby	Oakington Ruby
Charlie Brown	MORcharlie	Anygold	Pinstripe
Chiquita	MORkita	Anytime	Happy Hour (1983)
Courier	Courier	R. gigantea	Unknown
Crimson Shower	Crimson Shower	Excelsa	Unknown
Diamond Anniversary	MORsixty	Joycie	Cherry Magic
Don Marshall	MORblack	Baccara	Little Chief
Dorris Bennet	MORben	Joycie	Red Fairy
Double Treat	MORtreat	Arizona	13St

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Dresden Doll	Dresden Doll	Fairy Moss	34-69-15
Earthquake	MORquake	Golden Angel	44st
Edna Marie	MORed	Pinocchio (1940)	Peachy White
English Porcelain	MORporc	Pink Petticoat	Happy Time
FF		Basye Selection	Unknown
Fair Molly	MORfairpol	Rosa polyantha x unknown	Fairy Moss
Fiesta Gold	Fiesta Gold	Golden Glow (1937)	Magic Wand
Finger Paint	MORfing	Orangeade	Little Artist
Fresh Pink	Fresh Pink	0-47-19	Little Buckaroo
Fuzzy Wuzzy Red	Fuzzy Wuzzy Red	Scarlet Moss	Scarlet Moss
Gina's Rose	Morgina	Playboy	Basyes Legacy
Gold coin	Gold coin	Ginas Rose	Ginas Rose
Gold Moss	Goldmoss	Rumba	44-59-4
Golden Century	Golden Century	0-47-19	1953
Golden Gardens	MORgogard	1-72-1	Gold Badge
Golden Horizon	Morhorizon	Cal Poly	Strawberry Ice
Hall of Flowers	MORmint	Avandel	Gold Badge
Halo Fire	MORhalfire	Orangeade	Halo 8
Halo Glory	MORglory	Gold Badge x (Anytime x Angle Face)	Unknown
Halo Today	MORtoday	Anytime X Gold Badge	Anytime X Lavender
Hi Ho	Hi Ho	Little Darling	Magic Wand
Homerun	WEKcisbako	City of San Francisco x Baby Love	Knock Out
Hoot Owl	MORhoot	Orangeade	Little Artist
Hope & Joy	MORhopjo	Show N Tell	Unknown
Iceberg	KORbin	Robin Hood	Virgo
Ice Tea	MORice	Sequoia Ruby	Sequoia Ruby
J06-20-14-3		DD	Unknown
J06-28-8-1		Anytime	91/100-5
J06,30-3-3		DD	M4-2

Name	Code	Seed Parent	Pollen Parent
J06-30-5-1		Lemon Meringue	M4-2
J06-32-4-1		Halo Fire2	M4-4
Jacquie Williams	MORwheels	Yellow Jewel X Tamango	Strawberry Ice
Jessica Rose	MORbahny	Lemon D	Red Fairy
Julie Link	Morlink	Peach Halo	Out of Yesteryear
Just for You	MORyou	Orangeade	Rainbows End
Kayla	MORkay	Sheri Anne	Violette
Lavender Delight	MORorcheri	Orangeade	Cherry Magic
Lavender Jewel	Lavender Jewel	Little Chief	Angel Face
Little Buckaroo	Little Buckaroo	0-47-19	Oakington Ruby x
Little Chief	Little Chief	Cotton Candy	Magic Wand
Little Emma	Moremma	1-72-1	Clytemnestra
Love and peace	MORlove	Peach Halo	44st
Lovely Lorrie	MORlaw	Sequoia Gold	Little Chief
Lucy	MORlucy	Anytime	Papa Gontier
M4-4	M4-4	WOB26	Unknown
Magseed	Red Rugostar	Anytime	Rugosa Magnifica
Make Believe	MORmake	Anytime	Angel Face
Mariposa Gem	MORmagem	Little Darling	Magic Wand
Max Colwell	Max Colwell	Red Flush	Little Darling x Seedling
Millie Walters	MORmilli	Little Darling	Galaxy
Moores Striped Rugosa	MORbeauty	9st	Rugosa Magnifica
My Stars	Mornothorns	Playboy	Basyes Legacy
Nurse Donna	MORfenn	Pink Petticoat	Rainbows End
O		Playboy	90-202
Old Blush		Unknown	Unknown
Ora Kelly	Morink	Peach Halo	Rise N Shine
Orange Frenzy	MORfrenzy	Joycie	Unknown
Orange Honey	Orange Honey	Rumba	Over the Rainbow

Orange Parfait	MORjoyart	Joycie	Work of Art
Out of Yesteryear	MORyears	Golden Angel	Muriel
Papoose	Papoose	R. wichurana	Zee
Patriot Song	MORfed	Orangeade	Sheridort
Persian Autumn	Morthirthree	Tigris	Anytime X Gold Badge
Pink Cameo	Pink Cameo	Soeur Therese X Skyrocket	Zee
Pink Elf	MORelfire	Ellen Poulsen	Fire Princess
Pinstripe	MORpints	Pinocchio (1940)	33st
Quietness	Quietness	Unknown	Unknown
Play Gold	MORplaygold	Sequoia Gold	Playboy
Rain Forest	MORforest	Sheri Anne	Scarlet Moss
Red Fairy	MORedfar	Simon Robinson	Simon Robinson
Renny	MORrenny	Anytime	Renae
Rise n Shine	Rise 'n' Shine	Little Darling	Yellow Magic
Rosa wichuriana		R. wichuraiana	R. wichurana
Rose Gilardi	MORose	Dortmund	33st
Roseberry Blanket	KORtwente	Not Reported	Not Reported
Roses are Red	Mornine	Tigris	Playboy
Ruby Princess	MORruby	Joycie	Red Fairy
Scarlet Moss	MORcarlet	Dort]XFairyMoss	(DortXFairyMoss)xGA822
Sequoia Gold	MORsegold	Lemon D	Gold Badge
Sheri Anne	MORsheri	Little Darling	New Penny
Southern Delight	MORdashin	Little Darling	Rise N Shine
Splish Splash	MORgoldart	Sequoia Gold	Little Artist
Spotlight	MORbrights	Orangeade	Little Artist
Star Delight	MORstar90	Yellow Jewel	Rugosa Magnifica
Stars n Stripes	Stars 'n' Stripes	Little Chief	26st
Strawberry Swirl	Strawberry Swirl	Little Darling	33st
Sweet Chariot	MORchari	Little Chief	Violette

Sweet Hannah	MORhan	Sequoia Gold	Little Chief
Tangerine Jewel	MORtange	Joycie	Out of Yesteryear
The Fairy	The Fairy	Paul Crampel	Lady Gay (1905)
Topaz Jewel	MORyelrug	Golden Angel	Belle Poitevine
Trinket	Trinket	0-47-19	Magic Wand
Twilight Skies	MORlight	Anytime	Vis Violet
Vineyard Song	MORgrapes	Little Chief	Violette
WOB26xOB		Old Blush	WOB26
Yellow Jewel	Yellow Jewel	Golden Glow	Little Darling