# CHANGES IN FLOWER SIZE AND NUMBER UNDER HEAT STRESS IN ROSE (*ROSA×HYBRIDA*)

A Thesis

by

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## ABSTRACT

Roses (*Rosa×hybrida*) have been one of the most popular decorations for entertainment and ceremonies for the past 5,000 years, and have been used in the fragrance, medicinal, and food industry. Heat stress is one of the most significant abiotic stresses which negatively affects rose performance and reduces the market value of roses. This project examined the effect of heat on rose in diploid rose populations created by intercrossing heat tolerant and sensitive diploid parents.

Changes in flower size were examined in a heat shock (one hour at 44°C) experiment with potted plants and in field plots by comparing flower size in cool (spring and fall) versus warm (summer) seasons. As expected, the heat treatment decreased flower diameter, petal number, and flower dry weight. Flower size traits had moderately low narrow sense heritability (0.24 - 0. 35, 0.12 - 0.33, and 0.34 - 0.37) and moderately high to high broad sense heritability (0.62 - 0.67, 0.74 - 0.91, and 0.76 - 0.81) for flower diameter, petal number, and flower dry weight respectively. The G×E variance for flower diameter and flower dry weight accounted for 37% and 27% of the variance in the field experiment indicating that the heat stress had moderate differential genotypic effects as was indicated by the analysis of variance. However the genetic variance was several fold greater than the G×E variance indicating selection for flower size would be effective in any season but for the selection of a stable flower size (heat tolerant) rose genotype, selection would be required in both the cool and warm seasons.

The number of flowers per primary and secondary inflorescence had very low narrow sense (0.01 and 0.06) and moderate broad sense (0.43 and 0.34) heritability. The

G×E variance for the number of flowers per primary and secondary inflorescence accounted for 55.7% and 57.0% of the total variance in the field experiment indicating selection needs to be done for within each season. Only 26% of plants had tertiary inflorescences.

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# CHAPTER I

# INTRODUCTION

## 1.1 Introduction

Archeological records support that humans have cultivated roses for over ~5000 years in ancient China, Europe (Gudin, 2000; Krussmann, 1981), western Asia and northern Africa (Shepherd, 1954). It was in China that the ever blooming or recurrent flowering rose was first developed (Soules, 2009). In Chinese these roses were called 'yue ji hua' meaning flowering monthly and seasonally. Modern roses were developed by introgressing this ever blooming trait from Chinese roses into the European germplasm (Krussmann, 1981). The conversion of the rose into a continuous flowering bush and its floral diversity is key to its commercial importance. *Rosa* species are mainly distributed in the temperate region of the northern hemisphere (Wissemann and Ritz, 2007). As interbreeding among the species of *Rosa* is common especially within sections, the genus *Rosa* has been classified into morphospecies and evolutionary species (Wissemann and Ritz, 2007). The genus *Rosa* L. comprises about 150-200 morphospecies, that range in ploidy level (n = 7) from diploid to octoploid (Zlesak, 2006). Recently, a decaploid accession of *R. praelucens*, the highest naturally occurring ploidy in rose, was reported (Jian et al., 2010).The domesticated rose is an interspecific complex which has been classified into three major groups based on ornamental uses: garden roses, pot roses, and cut roses (Debener and Linde, 2009).

## 1.2 Heat effects on plants

High temperature and drought stress, are the major abiotic stresses affecting agriculture all over the world, especially in subtropical climates like Texas. Transitory or constant heat stress may result in irreversible damage to plant growth and development, and therefore reduces the economic yield (Wahid et al., 2007). For example, reduction in silage production and dry matter production have been observed in five temperate commercial maize hybrids when suffering high temperature stress (Giaveno and Ferrero, 2003). High temperature affects plant growth throughout its life cycle and the heat-threshold level varies with developmental stage (seed germination, reproductive, flowering, post-anthesis, etc) (Morrison and Stewart, 2002; Stone and Nicolas, 1994; Ur Rahman et al., 2004; Wahid et al., 2007).

The heat-stress threshold is defined as a critical temperature that causes yield reduction. In crop plants, yield is mainly defined as total seed weight. In flowering plants, yield can be defined as number of flowers per plant or flower intensity. The base threshold temperature for heat tolerance can vary from plant to plant and vary within different developmental stages (Wahid et al., 2007). The upper threshold that causes damage is now widely used in agronomic crops, such as maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) in calculating growing degree-days (McMaster and Wilhelm, 1997). High temperature stress can cause plant damage such as leaf senescence and abscission, leaf, branch and stem sunburn, leaf and twig scorch, growth inhibition, flower abscission and discoloration, reduce fruit set, and lead to yield reduction (Guilioni et al., 1997; Ismail and Hall, 1999; Vollenweider et al., 2005).

The plant responses under heat stress differ with the growing stage. For example, in maize, coleoptile growth is reduced or stopped by heat stress at the seedling establishment stage (Weaich et al., 1996), and starch, protein and oil contents are reduced at the anthesis and grain filling stages (Wilhelm et al., 1999). Heat stress may also negatively affect the process of photosynthesis, respiration, water relations, and membrane stability (Wahid et al., 2007). Cotton has a threshold temperature of 45 ℃ during the reproductive stage (Ur Rahman et al., 2004). In contrast, the threshold temperature for wheat during the post-anthesis stage is 26 ℃ (Stone and Nicolas, 1994).

Adaptation to heat stress has been an important abiotic stress in many crop breeding programs. Various approaches had been used in measuring heat-stress tolerance. Cell membrane thermostability (CMT) has been used to measure heat tolerance in soybean (Martineau et al., 1979), potato and tomato (Chen et al., 1982), wheat (Blum and Ebercon, 1981; Blum et al., 2001), cotton (Ashraf and Foolad, 2007), sorghum (Marcum, 1998), cowpea (Ismail and Hall, 1999), barley (Wahid and Shabbir, 2005), cabbage (Chauhan and Senboku, 1996), chrysanthemum (Yeh and Lin, 2003), pansy (Pearson et al., 2015), citrus (Ingram and Buchanan, 1984), *Cucumis melo* (Lester, 1985), and many other plant species (Wahid et al., 2007). And in rose, CMT was used in measuring heat stress tolerance, however, flower abscission and leaf necrosis after a heat shock treatment were better predictors of heat tolerance as measured by flower intensity under high heat conditions in the field (Greyvenstein, 2013; Greyvenstein et al., 2015).

### 1.3 Economic value of roses

Roses (*Rosa* spp.) are important ornamental crops which are commercially utilized as garden plants, cut flowers, and for food/medicinal/fragrance industrial use. All roses belong to the genus *Rosa L*. of the *Rosaceae* (Zlesak, 2006). Roses have a wide diversity of adaptations and plant/floral characteristics which makes it one of the world's favorite flowers (Cairns, 2001). Roses were originally domesticated in the northern hemisphere and have been spread throughout the world (Krussmann, 1981).

The rose is a major component of the ornamental flower market. More than twenty years ago, the rose was one of the top three cut flowers and worth more than US $11 billion per year (Short and Roberts, 1991; Duke, 1992). More recently, roses ranked in the top five most popular cut flowers in the U.S. and in the top five ornamental crops in the world (Debener and Linde, 2009; Hodges et al., 2015). It had been estimated that the production of rose was 18 billion cut stems, 60-80 million potted plants, and 220 million landscape plants (Blom and Tsujita, 2003; Pemberton et al., 2003; Roberts et al., 2003). The value of the world rose production was estimated at 24 billion Euros in 2008 (Heinrichs, 2008) and the Dutch rose cut flower market was estimated to be worth $10 billion (Ahmad et al., 2010). Recently, the annual value of the North American landscape rose industry was estimated at 1 billion dollars (Vineland Research and Innovation Centre, 2013).

For lack of well adapted cultivars, the sale of garden roses have decreased during the past 20 years (Byrne et al., 2010; Hutton, 2012). The market value of rose can be influenced by flower abscission and leaf damage, and decreased flower size which are

mainly caused by heat stress. So a rose with high temperature tolerance and consistent flowering during the warm season will contribute to maintaining a good landscape appearance (Greyvenstein et al., 2014).

**1.4 Effect of heat stress on roses**

Temperature and light play important roles in rose growth and development. Garden roses suffer from poor flower quality and decreasing flower yield due to high temperatures (Greyvenstein et al., 2014). The average daily maximum temperatures 8 – 14 days (about 2 weeks) before a flower opens affects flower dry weight significantly (Greyvenstein, 2013). Excessive heat stress may cause a negative effect on the longevity and quality of a cut rose (Marissen, 2001; Moe, 1975; Wahid et al., 2007) as well as on the flower size, petal number, flower color, flower number (by increasing flower abscission), the number of vegetative nodes before flowering, the time to flowering, and leaf appearance (Greyvenstein, 2013; Greyvenstein et al., 2014; Grossi et al., 2004; Shin et al., 2001). Besides affecting the appearance, high temperature also affects rose physiologically. In 'Samantha' roses, a reduction in photosynthesis and carbohydrate export was observed at 40 ℃ as compared to 15 ℃ (Jiao and Grodzinski, 1998).

Thus far, differences in heat tolerance have been detected among rose cultivars in their ability to maintain good flower size and good flower production under heat stress (Greyvenstein, 2013; Greyvenstein et al., 2014) but little is known about the genetic basis of these differences. Within the Texas A&M Rose Breeding Program, rose yield reduction caused by heat stress is quantified by rating flower intensity (% plant surface

covered by flowers) on a 0 - 9 scale and landscape performance on a 1 – 5 scale (Greyvenstein, 2013).

**1.5 Project goals**

The objective of this project was to document the effect of heat and assess the genetic basis of heat tolerance as expressed in the changes of flower diameter, petal number, flower dry weight and flower number per inflorescence.

The long term goal of this project is to develop high temperature tolerant garden rose cultivars that are well adapted to the climate in the southern U.S.A.

# CHAPTER II

# COMPARISON OF THE EFFECTS OF HEAT SHOCK BETWEEN POTTED PLANTS AND FIELD PERFORMANCE OF SELECTED ROSE PROGENIES

## 2.1 Synopsis

Compared to the rose market in the U.S. 20 years ago, the sales of the garden rose has decreased 25% to 30% (Byrne et al., 2010; Hutton, 2012). High temperature stress is one of the major limiting abiotic factors for plant growth throughout the world.

In this project, 10 diploid populations were used to measure the effect of heat shock (1 hour at 44°C) and assess the genetic variation for heat tolerance on flower size. All diploid populations were developed by intercrossing heat tolerant (M4-4, J06-20-14-3) and sensitive ('Sweet Chariot', 'Vineyard Song', 'Red Fairy', 'Little Chief', 'Old Blush', 97/7-2) diploid parents. Flower diameter, petal number, and flower dry weight were used to measure flower size. The families differed in their flower size and the heat shock treatment caused a 15.7%, 23.3%, and 16.9% decrease in flower diameter, petal number, and flower dry weight, respectively. The genetic analysis showed low to moderately low (0.12 - 0.34) narrow and moderate (0.62 - 0.76) broad sense heritability indicating a major non additive genetic component determining flower size. Flower size traits had sufficient variation to allow for improvement. If rose genotypes vary in heat tolerance, it would be expected that there is a differential response to heat among families and/or seedlings which would be detected statistically by a significant interaction effect. Among the flower size parameters, only flower diameter responded differentially on the family and progeny within family levels to the heat shock treatment.

In the genetic analysis, 6.3% of the G×E interaction variation indicating that there is a small opportunity for selection for heat tolerance as measured by flower diameter.

**2.2 Materials**

Five to 10 seedlings from each of ten diploid rose populations developed by crossing heat sensitive (*97/7-2* , 'Red Fairy', 'Sweet Chariot', 'Vineyard Song', 'Old Blush', and 'Little Chief') and tolerant (M4-4 and J06-20-14-3) roses (Table 1) were propagated during the fall of 2013 by rooting two node or ten centimeter long cuttings under mist in a peat and perlite mixture (Metro-Mix Professional Growing Mixes, Sun Gro Horticulture) in the greenhouse from mature shoots in 5 cm × 5 cm cells (Figure 1A). All rooted plants were potted into one gallon pots with same media and slow release fertilizer (Osmocote 14-14-14, Scotts Miracle-Gro) in the greenhouse (Figure 1B) when the roots were well established (~ 3 weeks after sticking the cuttings). These plants were grown in the greenhouse during the winter of 2013 and the spring of 2014, then moved outside to the outdoor nursery for plant establishment.

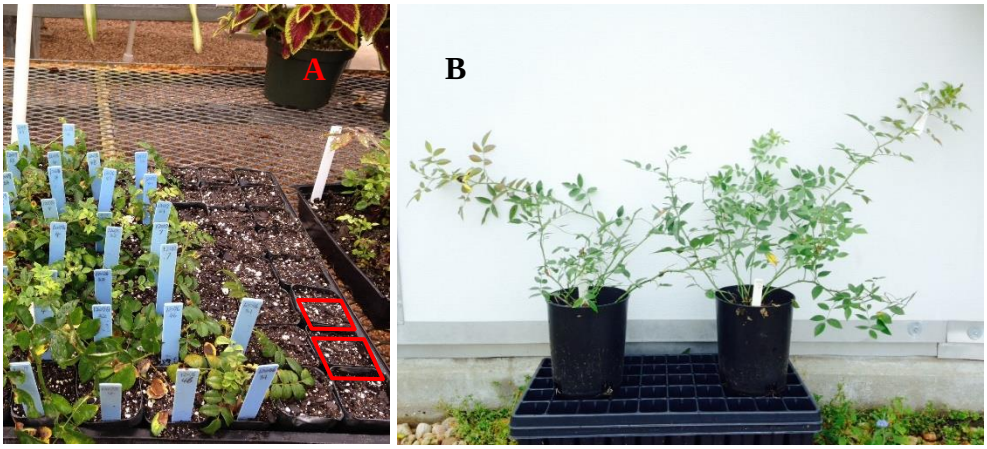



**Figure 1.** 5 cm × 5 cm cells for rooted and one gallon pot. A. 5 cm × 5 cm cells marked with red parallelogram; B. One gallon pot.

**Table 1.** The 10 diploid rose populations used for the heat shock experiment.

| Name | Female parent | Pollen parent | Numbers of seedlings available |
|---|---|---|---|
| OB × M4-4 | Old Blush | M4-4 | 9 |
| J14-3 × VS | J06-20-14-3 | Vineyard Song | 3 |
| J14-3 × SC | J06-20-14-3 | Sweet Chariot | 9 |
| SC × M4-4 | Sweet Chariot | M4-4 | 9 |
| J14-3 × LC | J06-20-14-3 | Little Chief | 8 |
| J14-3 × RF | J06-20-14-3 | Red Fairy | 8 |
| OB × RF | Old Blush | Red Fairy | 7 |
| SC × J14-3 | Sweet Chariot | J06-20-14-3 | 9 |
| M4-4 × 97/7-2 | M4-4 | 97/7-2 | 10 |
| SC × 97/7-2 | Sweet Chariot | 97/7-2 | 8 |
| Total | | | 80 |

## 2.3 Methods and treatments

Plants were pruned back to a standard size (3 nodes or a stem 10 cm long without flowers or visible flower buds on the plant) to synchronize their flowering cycles on April 15th, 2015 (Figure 2A). The plants were allowed to grow for 4 to 6 weeks until the plant had visible flower buds (Figure 2A and B) at which time they were given the heat shock treatment.

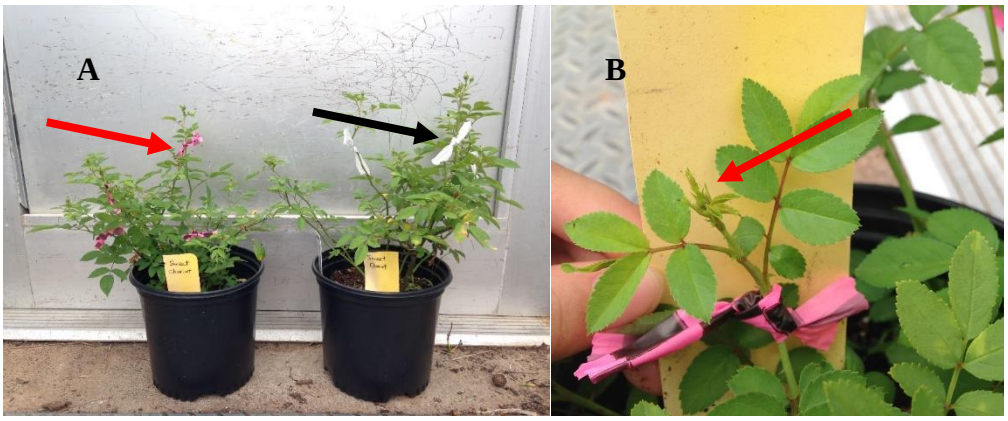



**Figure 2.** Synchronized plants with visible flower bud ready for heat shock. A. Visible flower bud labeled with white flagging (black arrow) and colored flagging (red arrow) indicate the control plant and the plant scheduled for heat shock treatment respectively; B. Visible flower bud marked with red arrow which is ready for the heat shock treatment.

The experiment was conducted using a randomized complete block design. Three plants of each genotype (80 propagated seedlings x 6 = 480 plants) were given a heat shock treatment (44℃ and 50% RH for 1 hour) and three plants were kept in a fan-and-pad cooled greenhouse (25/20℃ day/night) as untreated controls in College Station. All plants were well watered before being put into the heat chamber. After the treatment, all plants were kept in the outdoor nursery (25℃) and data were taken on flower petal number, flower diameter, and flower dry weight 1-2 weeks after treatment or when the labeled flower fully opened (May 13, 2015 – June 15, 2015).

The same flower parameters on these progenies were also assessed in the field trial during the spring, summer and fall seasons of 2015. Sixteen diploid hybrid populations (1 plant per seedling) and their parents including the 11 populations described in this chapter were planted in either A block in 2012 or D block in 2014.

At least 3 flowers were taken from each plant when the flower was fully open with dehiscing pollen (flower developmental stage 10 as described by Ma et al., 2015). Flower size was measured as flower diameter (cm), petal number and dry weight (mg). Flower dry weight was taken after the whole flower without the pedicel was dried for at least 3 days at 80℃. The flower petal number included both full size petals and petaloids.

## 2.4 Statistical analysis

All statistical analyses were performed using JMP software, Version 12.0.1 SAS Institute Inc. Student's *t* test were used to separate the population means between the two treatments. In roses, the flower type of single (5-8 petals) versus double flowers (petal number larger than 8) is controlled by a major gene with the double type conditioned by a dominant gene. Thus as the number of petals in single flowers does not vary throughout the year, only double flowers were considered in calculating the genetic variance of petal number.

A restricted estimated maximum likelihood (REML) model ($y = \mu + \sigma^2_{FP} + \sigma^2_{PP} + \sigma^2_{Prog[FP,PP]} + \sigma^2_{Season} + \sigma^2_{Season*FP} + \sigma^2_{Season*PP} + \sigma^2_{Season*Prog[FP,PP]} + + \sigma^2_{Error}$) was used to estimate genotypic and phenotypic variance and calculate narrow/broad sense heritability.

Heritability is the proportion of variance that is due to genetic components. It can be used to tell how well a certain trait can be improved, identify best genetic progenies or superior parents, and determine appropriate breeding method. Heritability varies widely for the same trait in the same crop because of statistical designs, different environments, different populations, and different estimation methods (Bernardo, 2010). In this study, parental variances were regarded as additive variance (Va), progeny variance was regarded as non-additive variance (Vd), variance due to the change of season was regarded as environmental variance (Ve), variance due to parents and progeny was regarded as genotypic variance (Vg), and the interaction of genotype and environment was treated as genetic-environmental variance (Vgxe) (Connor et al., 2005). The narrow sense heritability, $h^2$, was measured by additive variance (Va) divided by phenotypic variance (Vp) ($h^2=V_A/V_P$). The broad sense heritability, $H^2$, was measured by the sum of Va and dominance variance (Vd) divided by the Vp, where Vp=Va+Vd+Vgxe/e, e indicates the number of seasons used in the analysis (Hallauer et al., 2010; Holland et al., 2003).

## 2.5 Results

### *Normality analysis*

Normality is a fundamental assumption of many statistical models including the analysis of variance (ANOVA) (Razali and Wah, 2011). More normalized data improves the power of the statistical analysis. Therefore, transforming raw data is important if normality is improved. Raw data, $\log_{10}$ transformed data, $\log_e$ transformed data (data not shown), and square root transformed data (data not shown) were assessed for normality. The null hypothesis for the Shapiro-Wilk W test assumes the data is from the normal distribution. Therefore, non-significant result indicates normal distribution. According to the result, flower diameter showed good normality before transformation and no improvement after the $\log_{10}$ (Table 2), $\log_e$ or square root transformation (data not shown). In contrast, the normality of petal number and flower dry weight was improved after a $\log_{10}$ (Table 2), $\log_e$ and square root transformation (data not shown). Of these three transformations, the $\log_{10}$ and $\log_e$ transformation improved data normality better than the square root transformation for petal number and flower dry weight. The $\log_{10}$ and $\log_e$ transformation showed the same result. Thus, the raw data of flower diameter and $\log_{10}$ transformed data of petal number and flower dry weight were used in the statistical analyses. $\text{Log}_{10}$ data of flower diameter and raw data of petal number and flower dry weight were all used in the following analyses to assess whether results were changed after data transformed.

**Table 2.** Normality (Shapiro-Wilk W) test on the distribution of raw and transformed (log10) flower trait data for the 10 diploid rose populations in the heat shock experiment**.**

DM = flower diameter, PT = flower petal number, DW = flower dry weight

| | Shapiro-Wilk W test[z] | | | | | |
|---|---|---|---|---|---|---|
| Population | DM | $\log_{10}$ DM | PT | $\log_{10}$ PT | DW | $\log_{10}$ DW |
| OB × M4-4 | NS | NS | * | NS | * | NS |
| SC × M4-4 | NS | NS | NS | NS | * | * |
| J14-3 × VS | NS | NS | NS | NS | * | NS |
| J14-3 × SC | NS | NS | * | * | * | NS |
| J14-3 × LC | NS | NS | NS | NS | * | * |
| J14-3 × RF | NS | NS | * | * | * | * |
| OB × RF | NS | NS | * | NS | * | NS |
| SC × J14-3 | * | * | NS | * | * | NS |
| SC × 97/7-2 | NS | NS | | | * | * |
| M4-4 × 97/7-2 | NS | * | NS | NS | * | * |

[z]NS, * Nonsignificant or significant at $P \leq 0.05$.
$H_0$: The data is from the normal distribution. Small p-values reject $H_0$.

***General linear analyses of flower diameter, petal number, and flower dry weight***

Visible signs of stress were not seen until the plants were removed from the heat chamber. Two weeks after the heat shock treatment, the peduncles showed some browning (Figure 3).

The analysis of variance indicated that there were differences among populations and among the progeny within the families in flower size and that the heat shock caused a decrease in all three measures of flower size (Table 3). With respect to the family by heat shock and progeny [nested in family] by heat shock interactions, only flower diameter, but not petal number or flower dry weight, showed a significant interaction effect (Table 3). This interaction indicated that the families on average and the progeny within the families responded differentially to the heat stress which reflects differences among the materials in heat tolerance when measuring flower diameter.

**Figure 3.** Browning of rose peduncles caused by heat shock.

**Table 3.** The effect of heat shock (one hour at 44°C) on the family and individual progenies from 10 diploid rose populations.

| | Flower diameter[z] | | | Petal number | | | Flower dry weight | | |
|---|---|---|---|---|---|---|---|---|---|
| Obs. | 447 | | | 190 | | | 447 | | |
| $r^2$ | 0.83 | | | 0.85 | | | 0.84 | | |
| | DF | Variance | MS[y] | DF | Variance | MS | DF | Variance | MS |
| Family | 9 | 35.50 | 3.94*** | 7 | 2.70 | 0.39*** | 9 | 7.90 | 0.88*** |
| Progeny[Family] | 70 | 62.08 | 0.89*** | 28 | 6.56 | 0.23*** | 70 | 8.34 | 0.12*** |
| HS[x] | 1 | 33.63 | 33.63*** | 1 | 0.31 | 0.31*** | 1 | 0.71 | 0.71*** |
| Family by HS | 9 | 2.06 | 0.23* | 7 | 0.21 | $0.03^{NS}$ | 9 | 0.07 | $0.01^{NS}$ |
| Progeny[Family] by HS | 70 | 12.37 | 0.18*** | 28 | 0.35 | $0.01^{NS}$ | 70 | 0.87 | $0.01^{NS}$ |

[z]Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight. Only petal number larger than 8 was considered.
[yNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[x]HS = Heat shock.

The flower diameter among the plants ranged from 1.70 cm to 5.30 cm and among the population means ranged from 4.06 cm to 3.03 cm (Table 4). The population mean flower diameter differed with the populations with OB × M4-4 and J14-3 × VS having the largest flower diameters and the populations M4-4 × 97/7-2 and J14-3 × LC having the smallest flower diameters (Table 4).

In rose, there is a major gene that determines whether a flower is single (8 or less petals) or double (9 or more petals) (Debener, 1999). Within the rose genotypes with single flowers, the number of petals does not vary due to heat stress whereas within those with double flowers, the petal number appeared to vary. Therefore this analysis focused on the petal number of only the double flowered rose genotypes within the population studied. This resulted in very few observations for the populations SC × M4-4 and M4-4 × 97/7-2 and none from the population SC × 97/7-2 and J14-3 × VS (Table 5). Among the double flowered genotypes in the 8 populations, the petal number ranged from 12 to 140 petals per flower (Table 5). The mean petal number of the populations differed and ranged from 29 (OB × M4-4) to 76 (SC × J14-3) (Table 5).

The flower dry weight ranged from 20 mg (J14-3 × LC, SC × 97/7-2, and M4-4 × 97/7-2) to 210 mg (OB × M4-4, J14-3 × SC, and OB × RF) (Table 6). The mean flower dry weight among the 10 populations differed and ranged from 41 mg (SC × 97/7-2 and M4-4 × 97/7-2) to 100 mg (SC × J14-3) (Table 6).

Over all populations, the average flower diameter for the heat stressed plants (3.22 cm) was 15.7% less than the control plants (3.82 cm) (Table 7). The average petal number was decreased by 23.3% due to the heat stress treatment (Table 7). The average flower dry weight of the heat stressed plants (64 mg) was 16.9% lower than the control plants (77 mg) (Table 7).

As expected, there were large differences among families, among the progeny within families (Tables 4, 5, and 6) as well as a substantial decrease (15.7-23.3%) in flower size due to the one hour heat shock treatment at 44°C (Table 7). Among the families the most heat tolerant was J14-3 × SC that only had a 9.9% decrease in flower diameter (Table 7).

**Table 4.** Mean flower diameter of 10 diploid rose populations in the heat shock experiment.

| Population[z] | Flower diameter (cm)[y] | | | |
|---|---|---|---|---|
| | Mean[x] | Max | Min | N |
| OB × M4-4 | 4.03a | 5.30 | 2.50 | 54 |
| SC × M4-4 | 3.58b | 4.83 | 2.30 | 51 |
| J14-3 × VS | 4.06a | 4.97 | 3.10 | 18 |
| J14-3 × SC | 3.54b | 4.65 | 2.60 | 45 |
| J14-3 × LC | 3.22cd | 4.80 | 1.77 | 46 |
| J14-3 × RF | 3.47b | 4.30 | 2.30 | 44 |
| OB × RF | 3.40bc | 5.00 | 1.80 | 37 |
| SC × J14-3 | 3.42b | 4.55 | 1.70 | 46 |
| SC × 97/7-2 | 3.43b | 4.60 | 2.30 | 60 |
| M4-4 × 97/7-2 | 3.03d | 4.30 | 1.90 | 46 |
| Overall | 3.49 | 5.30 | 1.70 | 447 |

[z] OB = 'Old Blush', J14-3=J06-20-14-3, VS = 'Vineyard Song', SC = 'Sweet Chariot', LC = 'Little Chief', RF = 'Red Fairy', N = number of observations.
[y]Raw data of flower diameter was used to calculate the differences among populations, average, maximum, and minimum flower diameter.
[x]Levels not connected by same letter are significantly different.

**Table 5.** Petal numbers for the control and heat shocked roses in 10 diploid rose populations for seedlings with double flowers.

| Parentage[z] | Petal number[y] | | | |
|---|---|---|---|---|
| | Mean[x] | Max | Min | N |
| OB × M4-4 | 29c | 58 | 15 | 24 |
| SC × M4-4 | 60ab | 107 | 12 | 6 |
| J14-3 × VS | - | - | - | - |
| J14-3 × SC | 55b | 126 | 21 | 29 |
| J14-3 × LC | 50b | 102 | 13 | 16 |
| J14-3 × RF | 49b | 100 | 12 | 38 |
| OB × RF | 48b | 98 | 17 | 25 |
| SC × J14-3 | 76a | 140 | 22 | 46 |
| SC × 97/7-2 | - | - | - | - |
| M4-4 × 97/7-2 | 57ab | 68 | 46 | 6 |
| Mean/total observations | 53 | 140 | 12 | 190 |

[z]OB = 'Old Blush', J14-3=J06-20-14-3, VS = 'Vineyard Song', SC = 'Sweet Chariot', LC = 'Little Chief', RF = 'Red Fairy', N = number of observations.
[y]$Log_{10}$ transformed data of petal number was used to calculate the differences among populations. Raw data of petal number were used to show the average, maximum, and minimum number of petal. Only petal number larger than 8 was considered.
[x]Levels not connected by same letter are significantly different.

**Table 6.** Mean flower dry weight of the 10 diploid rose populations in the heat shock experiment.

| Population[z] | Flower dry weight (mg)[y] | | | |
|---|---|---|---|---|
| | Mean[x] | Max | Min | N |
| OB × M4-4 | 79b | 210 | 30 | 54 |
| SC × M4-4 | 51d | 140 | 30 | 51 |
| J14-3 × VS | 67bc | 90 | 40 | 18 |
| J14-3 × SC | 82bc | 210 | 30 | 45 |
| J14-3 × LC | 62c | 200 | 20 | 46 |
| J14-3 × RF | 84b | 150 | 40 | 44 |
| OB × RF | 93ab | 210 | 30 | 37 |
| SC × J14-3 | 100a | 200 | 50 | 46 |
| SC × 97/7-2 | 41e | 70 | 20 | 60 |
| M4-4 × 97/7-2 | 41e | 110 | 20 | 46 |
| Overall | 67 | 210 | 20 | 447 |

[z]OB = 'Old Blush', J14-3=J06-20-14-3, VS = 'Vineyard Song', SC = 'Sweet Chariot', LC = 'Little Chief', RF = 'Red Fairy', N = number of observations.
[y]$Log_{10}$ transformed data of petal number was used to calculate the differences among populations. Raw data of petal number were used to show the average, maximum, and minimum number of petal. Only petal number larger than 8 was considered.
[x]Levels not connected by same letter are significantly different.

**Table 7.** Heat shock (one hour at 44°C) effect on 10 diploid rose progenies on flower size.

| | Flower diameter[y] | | | Petal number[z] | | | Flower dry weight | | |
|---|---|---|---|---|---|---|---|---|---|
| Parentage[x] | % of decrease | Heat stress | Control | % of decrease | Heat stress | Control | % of decrease | Heat stress | Control |
| OB ×M4-4 | 17.0%* | 3.66 | 4.41 | 30.0% | 24 | 34 | 15.1% | 73 | 86 |
| SC × M4-4 | 17.8%* | 3.23 | 3.93 | 1.4% | 60 | 61 | 11.2% | 48 | 54 |
| J14-3 × VS | 21.5%* | 3.57 | 4.55 | - | - | - | 32.0% | 54 | 80 |
| J14-3 × SC | 9.9%* | 3.36 | 3.73 | 16.6% | 50 | 60 | 20.4% | 73 | 92 |
| J14-3 × LC | 13.3%* | 2.99 | 3.45 | 41.4% | 37 | 62 | 12.7% | 58 | 66 |
| J14-3 × RF | 15.4%* | 3.18 | 3.76 | 28.0% | 41 | 57 | 12.5% | 79 | 90 |
| OB × RF | 12.2%* | 3.18 | 3.62 | 21.0% | 43 | 54 | 19.0% | 83 | 103 |
| SC × J14-3 | 15.9%* | 3.13 | 3.72 | 25.6% | 65 | 87 | 13.3% | 93 | 108 |
| M4-4 × 97/7-2 | 14.6%* | 3.16 | 3.7 | - | - | - | 19.3% | 36 | 45 |
| SC × 97/7-2 | 16.1%* | 2.76 | 3.29 | 17.6% | 52 | 63 | 19.1% | 37 | 46 |
| Mean | 15.7%* | 3.22 | 3.82 | 23.3%* | 46 | 60 | 16.9%* | 64 | 77 |

[z]Raw data of flower diameter, petal number and flower dry weight were used to compare family means in control vs. heat stressed. Only petal number larger than 8 was considered.
[y] NS, * Nonsignificant or significant between heat shock and control plants at $P \leq 0.05$.
[x]OB = 'Old Blush', J14-3=J06-20-14-3, VS = 'Vineyard Song', SC = 'Sweet Chariot', LC = 'Little Chief', RF = 'Red Fairy'.

On a progeny level, 45 showed a decrease in flower diameter due to heat shock treatment, 34 did not change, and one seedling had an increase of flower diameter (Table 8). Ideally, it is desirable that the flower size does not change throughout the year. This information indicates that this should be possible.

**Table 8.** The effect of heat shock on the flower diameter of individual seedlings.

| Heat shock < Control[z] | Heat shock > Control | Heat shock = Control |
|---|---|---|
| OB × M4-4-N002 | OB × RF-N041 | OB × M4-4-N040 |
| OB × M4-4-N004 | | OB × M4-4-N042 |
| OB × M4-4-N005 | | SC × M4-4-N044 |
| OB × M4-4-N021 | | J14-3 × SC-N001 |
| OB × M4-4-N035 | | J14-3 × SC-N003 |
| OB × M4-4-N038 | | J14-3 × SC-N004 |
| OB × M4-4-N049 | | J14-3 × SC-N010 |
| SC × M4-4-N041 | | J14-3 × SC-N017 |
| J14-3 × VS-N001 | | J14-3 × SC-N035 |
| J14-3 × VS-N046 | | J14-3 × SC-N047 |
| J14-3 × VS-N111 | | J14-3 × LC-N011 |
| J14-3 × SC-N007 | | J14-3 × LC-N014 |
| J14-3 × SC-N078 | | J14-3 × LC-N029 |
| J14-3 × LC-N031 | | J14-3 × LC-N049 |
| J14-3 × LC-N073 | | J14-3 × LC-N053 |
| SC × M4-4-N010 | | J14-3 × LC-N057 |

**Table 8.** Continued

| Heat shock < Control[z] | Heat shock > Control | Heat shock = Control |
|---|---|---|
| SC × M4-4-N020 | | SC × M4-4-N034 |
| SC × M4-4-N021 | | J14-3 × RF-85 |
| SC × M4-4-N027 | | J14-3 × RF-102 |
| SC × M4-4-N030 | | J14-3 × RF-117 |
| SC × M4-4-N032 | | OB × RF-N005 |
| J14-3 × RF-63 | | OB × RF-N079 |
| J14-3 × RF-78 | | SC × J14-3-N003 |
| J14-3 × RF-82 | | SC × J14-3-N008 |
| J14-3 × RF-84 | | SC × J14-3-N035 |
| J14-3 × RF-89 | | SC × J14-3-N048 |
| OB × RF-N029 | | M4-4 × 97/7-2-10 |
| OB × RF-N032 | | M4-4 × 97/7-2-11 |
| OB × RF-N043 | | M4-4 × 97/7-2-12 |
| OB × RF-N053 | | M4-4 × 97/7-2-8 |
| SC × J14-3-N004 | | M4-4 × 97/7-2-9 |

**Table 8.** Continued

| Heat shock < Control[z] | Heat shock > Control | Heat shock = Control |
|---|---|---|
| SC × J14-3-N028 | | SC × 97/7-2-2 |
| SC × J14-3-N032 | | SC × 97/7-2-5 |
| SC × J14-3-N044 | | SC × 97/7-2-9 |
| SC × J14-3-N046 | | |
| M4-4 × 97/7-2-1 | | |
| M4-4 × 97/7-2-2 | | |
| M4-4 × 97/7-2-3 | | |
| M4-4 × 97/7-2-4 | | |
| M4-4 × 97/7-2-7 | | |
| SC × 97/7-2-1 | | |
| SC × 97/7-2-3 | | |
| SC × 97/7-2-4 | | |
| SC × 97/7-2-6 | | |
| SC × 97/7-2-8 | | |

[z]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'.

### *Flower size correlation between field and nursery grown plants*

The flower size collected on plants grown in the field and on plants grown in pots in the nursery (heat shock experiment) was moderately to well correlated, with coefficients of 0.39-0.92, 0.76-0.38, and 0.49-0.34 for flower diameter, petal number and flower dry weight respectively (Table 9, Appendix 7, 8, and 9). All three flower traits data were correlated between heat shock and warm season and between control and cool season (Table 9, Appendix 10, 11, 12, 13, 14, and 15). The highest correlations were for the flower diameter between the control and cool season and heat shock and warm season data which indicates that the heat shock treatment was representative of what was occurring in the field. This indicated that the heat shock procedure would be a useful tool for the assessing the heat tolerance of rose genotypes as it relates to flower diameter.

### *Genetic analysis of flower diameter, petal number, and flower dry weight*

The total of interaction effect can be regarded as a genetic-environment interaction (G×E) effect. Since the genetic effect for treatment by female parent effect and the treatment by pollen parent in petal number and flower dry weight was less than 0.5%, these two effects were removed in the analysis. The estimated narrow sense heritability and broad sense heritability for flower diameter, petal number (only double flowered types) and petal dry weight (whole flower without the pedicel) were 0.24/0.62, 0.12/0.74, and 0.34/0.76, respectively (Table 10). Thus all the flower size traits had a moderately-small narrow sense heritability and a high broad sense heritability. The total interaction effect was relatively small with the flower diameter accounting for 6.3% of the variance (Table 10). The genetic variance component (G) was larger than the G×E

interaction for all three flower size diameter and the Vgxe/Vg ratios were small (0.15) (Table 10). The higher variance explained by the interaction for the flower diameter corresponds to a significant interaction in the analysis of variance that indicated better trait to select for flower size.

**Table 9.** Pairwise correlation coefficients of heat shock versus warm season flower size, control versus cool season, and field experiment versus heat shock experiment for flower size data from heat shock experiment and the field in 2015 and the average of control and heat stress from the nursery in the heat shock experiment.

| Variable | HS[z] vs. warm | Control vs. cool | Field vs. greenhouse heat shock experiment |
|---|---|---|---|
| Flower diameter | [y] 0.38*** | 0.59*** | 0.40*** |
| Petal number | 0.95* | 0.78*** | 0.80*** |
| Flower dry weight | 0.35* | 0.72*** | 0.67*** |

[z]HS: Heat shock treatment in heat shock experiment; warm: warm season in field experiment; Control: Control group in heat shock experiment; cool: cool season in field experiment.
[y]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

**Table 10.** Genetic variance calculated by REML for flower diameter, petal number, and flower dry weight with heat stress in 10 diploid populations in the heat shock experiment.

| | Flower diameter[y] | Petal number | Flower dry weight |
|---|---|---|---|
| $r^2$ | 0.71 | 0.77 | 0.79 |
| | Percentage of total variance | | |
| FP[z] | 2.3 | 7.9 | 3.4 |
| PP | 14.4 | 2.5 | 28.6 |
| Progeny[FP,PP] | 26.6 | 55.4 | 39.1 |
| Heat shock | 27.0 | 10.5 | 6.0 |
| HS×FP | 0 | - | - |
| HS×PP | 1.1 | - | - |
| P×HS | 5.2 | 0 | 0 |
| | Genetic variance | | |
| Va[x] | 0.081 | 0.009 | 0.017 |
| Vd | 0.129 | 0.048 | 0.020 |
| Vg | 0.210 | 0.057 | 0.037 |
| Vp | 0.338 | 0.078 | 0.049 |
| Vgxe | 0.031 | 0 | 0 |
| Vgxe/Vg | 0.145 | 0 | 0 |
| Verror | 0.113 | 0.021 | 0.012 |
| | Heritability | | |
| $h^2$ | 0.24 | 0.12 | 0.34 |
| $H^2$ | 0.62 | 0.74 | 0.76 |

[z]FP = female parent, PP = pollen parent, P×HS = progeny[FP,PP] by heat shock interaction. $H^2$ = broad sense heritability, $h^2$ = narrow sense heritability.
[y]Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight were used. Only petal number larger than 8 was considered.
[x]Va = Parental variances, Vd = progeny variance, Ve = variance due to the change of season, Vg = variance due to parents and progeny, Vgxe = variance due to the interaction of genotype and environment, Vp(phenotypic variance) = (Va+Vd+Vgxe/e+Verror), $h^2$ = Va/Vp, $H^2$ = (Va+Vd)/Vp, E = number of seasons.

## 2.5 Discussion

The heat stress caused by the greenhouse heat shock treatment caused a 15.7% to 23.3% decrease in flower size (Table 7). This agreed with previous studies of both cut flower roses and garden roses that flower diameter and flower dry weight were significantly decreased after heat stress (Shin et al., 2001; Greyvenstein, 2013; Greyvenstein et al., 2014). In addition, as expected, flower size differed among seedlings and populations (Tables 3 and 7). However, it is the interaction effect which indicates whether the genetic materials differ in heat tolerance. Previous work (Greyvenstein, 2013) showed that garden roses differed in their reaction to temperature in the field as measured by flower dry weight with higher temperatures leading to less flower dry weight. The % decrease in dry weight per 1℃ increase in temperature differed with the garden rose cultivar examined. However, in the heat shock experiment, no differential heat shock responses among populations or seedlings were observed for either petal number or flower dry weight. It was only flower diameter in which differences in heat stress response (heat tolerance) were detected both on the family and progeny within family basis.

All flower size parameters showed a large genotypic effect with moderately low narrow sense (0.12-0.34) and high broad sense (0.62 – 0.76) heritabilities indicating high non additive genetic effects (Table 10). This agreed with previous results reported on the genetic effects of petal number and flower diameter (Hibrand-Saint Oyant et al., 2008; Shupert, 2005; Gitonga et al., 2014).Only flower diameter showed a GxE interaction in the ANOVA and a positive GxE variance in the genetic analysis. Nevertheless, as this

variance is small (6.3%) compared to the variance attributed to the genetic effect (43.3%), the opportunity to select for heat tolerance using this approach is limited.

# CHAPTER III

# GENETIC AND ENVIRONMENTAL COMPONENTS OF FLOWER DIAMETER, PETAL NUMBER, DRY WEIGHT AND NUMBERS OF FLOWERS PER INFLORESCENCE OVER COOL AND WARM GROWING SEASONS

## 3.1 Synopsis

Heat stress is a major abiotic stress which reduces the flower size and value of the rose. The average flower size (petal number, flower diameter, and flower dry weight) was estimated by measuring three flowers per plant and the number of flowers produced were counted on 3 inflorescences per plant in the cool (spring and fall, mean temperature 17.8 – 21.7 C) and warm (August, mean temperature 29.4 - 31.1 C) season in plants of 15 diploid rose populations segregating for flower size, production and heat tolerance grown in the field from 2013 to 2015.

An analysis of variance indicated that the flower diameter, petal number, flower dry weight, and number of flowers per primary/secondary inflorescence varied over populations and seasons. The flower diameter, petal number and flower dry weight decreased in size during the warm season by 17.6%, 19.6-19.8%, and 30.8% respectively. Seasonal differences in flower productivity of new shoots did not appear related to heat stress. A population by environment interaction was observed for flower diameter, flower dry weight, and flowers per inflorescences but not for petal number.

A REML analysis indicated that flower diameter, petal number and flower dry weight have moderate narrow sense (0.33 - 0.37) and moderately high to high broad

sense (0.67-0.91) heritabilities in the populations and environments studied. The G×E interaction variances consisted of 15.3% to 37.0% of the total variance with flower diameter having the greatest G×E effect. Nevertheless good progress in increasing flower size should be possible from selection in any season. The narrow sense heritability for the heat tolerance index calculated with flower diameter data was 0.31.

Flower numbers in the primary and secondary inflorescences showed very low narrow sense (0.01-0.06) and moderate low broad sense (0.43-0.34) heritabilities. Very high genotype × environment interaction (55.7% and 57.0%) was observed in the number of flowers per primary/secondary inflorescence indicating selection needs to be done specific to each season.

**3.2 Introduction**

Roses, one of the most important ornamental crops domesticated in temperate regions, suffers in response to high temperatures. Various studies have shown that flower size (petal number, flower weight, flower diameter), flower color (intensity, anthocyanin concentration) and productivity (flower intensity, leaf area) decrease in response to higher temperatures in both cut flower (Shin et al., 2001; Gitonga et al., 2014; Dela et al., 2003) and garden roses (Greyvenstein et al., 2014). High temperature also reduced flowering stem length and plant height because the plant reaches the florogenesis and anthesis stage much earlier (Gitonga et al., 2014).

Therefore, flower size measured as flower diameter, petal number, and flower dry weight, and flower production measured as flower number per inflorescence were used to evaluate the heat effects on and the genetic variance of flower size and production.

### 3.3 Materials

Diploid rose populations for this study were planted in the field at Texas A&M University in 2012 and 2014 with one plant per seedling (Table 11 and 12). Each plant was pruned at the end of every winter to restrict the plant size and induce new growth.

To develop heat tolerance in rose cultivars, breeding lines were derived by introgressing heat tolerance from the wild rose species *Rosa wichurana* into ever blooming commercial rose germplasm. For this project, hybrid populations were created by crossing among heat tolerant (M4-4, J06-20-14-3, J06-30-3-6, J06-28-4-6, J06-30-3-3) and sensitive (‘Red Fairy’, ‘Sweet Chariot’, ‘Vineyard Song’, ‘Old Blush’, ‘Little Chief’, ‘The Fairy’, ‘97/7-2) diploid parents .

**Table 11.** Diploid rose populations used for flower size characterization in the field in College Station, TX.

| Population | Female parent[z] | Pollen parent | Population size | Cross year |
|---|---|---|---|---|
| OB × J3-6 | **Old Blush** | J06-30-3-6 | 63 | 2010 |
| TF × J3-6 | **The Fairy** | J06-30-3-6 | 4 | 2010 |
| OB ×M4-4 | **Old Blush** | M4-4 | 11 | 2010 |
| SC × M4-4 | **Sweet Chariot** | M4-4 | 52 | 2010/2012 |
| J4-6 × RF | J06-28-4-6 | **Red Fairy** | 45 | 2010 |
| J3-3 × RF | J06-30-3-3 | **Red Fairy** | 6 | 2010 |
| VS × J14-3 | **Vineyard Song** | J06-20-14-3 | 5 | 2010 |
| J14-3 × VS | J06-20-14-3 | **Vineyard Song** | 65 | 2010 |
| J14-3 × SC | J06-20-14-3 | **Sweet Chariot** | 22 | 2010 |
| M4-4 × SC | M4-4 | **Sweet Chariot** | 11 | 2010 |
| J14-3 × LC | J06-20-14-3 | **Little Chief** | 17 | 2012 |
| J14-3 × RF | J06-20-14-3 | **Red Fairy** | 53 | 2012 |
| OB × RF | **Old Blush** | **Red Fairy** | 13 | 2012 |
| SC × J14-3 | **Sweet Chariot** | J06-20-14-3 | 5 | 2012 |
| M4-4 × 97/7-2 | M4-4 | **97/7-2** | 10 | 2012 |

[z]Heat sensitive parents are in bold, heat tolerant parents are in normal font.

**Table 12.** Diploid rose populations used for flower production characterization in the field in College Station, TX.

| Population | Female Parent[z] | Pollen parent | Population size | Cross year |
|---|---|---|---|---|
| OB × J3-6 | **Old Blush** | J06-30-3-6 | 112 | 2010 |
| TF × J3-6 | **The Fairy** | J06-30-3-6 | 6 | 2010 |
| OB ×M4-4 | **Old Blush** | M4-4 | 20 | 2010 |
| SC × M4-4 | **Sweet Chariot** | M4-4 | 119 | 2010/2012 |
| J4-6 × RF | J06-28-4-6 | **Red Fairy** | 192 | 2010 |
| J3-3 × RF | J06-30-3-3 | **Red Fairy** | 9 | 2010 |
| VS × J14-3 | **Vineyard Song** | J06-20-14-3 | 12 | 2010 |
| J14-3 × VS | J06-20-14-3 | **Vineyard Song** | 93 | 2010 |
| J14-3 × SC | J06-20-14-3 | **Sweet Chariot** | 55 | 2010 |
| M4-4 × SC | M4-4 | **Sweet Chariot** | 20 | 2010 |
| J14-3 × LC | J06-20-14-3 | **Little Chief** | 50 | 2012 |
| J14-3 × RF | J06-20-14-3 | **Red Fairy** | 130 | 2012 |
| OB × RF | **Old Blush** | **Red Fairy** | 158 | 2012 |
| SC × J14-3 | **Sweet Chariot** | J06-20-14-3 | 27 | 2012 |
| M4-4 × 97/7-2 | M4-4 | **97/7-2** | 20 | 2012 |
| SC × 97/7-2 | **Sweet Chariot** | **97/7-2** | 5 | 2012 |

[z]Heat sensitive parents are in bold, heat tolerant parents are in normal font.

### 3.4 Weather

The climate data used in this study were obtained from NOAA in College Station Easterwood Field (station code: GHCND:USW00003904) (Figure 4). The weather station was about 2 kilometers away from the field location. Spring data was taken during April, or before the average temperature reached 20℃. Summer data was taken during August, or two weeks after average temperature surpassed 30℃. Fall data was taken during November or two weeks after average temperature fell below 20℃.

In 2014, the number of days with the average temperature above 30℃ was less than in 2013 and 2015 (Figure 4). The summer maximum daily temperature in 2014 was around 36℃ in August (Figure 4). However, the maximum daily temperature in 2013 and 2015 was as high as or higher than 40℃ in August (Figure 4). The temperature of the period of 0 – 14 days or 15 – 28 days before flowering is critical in the development of the flower size (Greyvenstein, 2013). Average temperature of 4 weeks before flowering in summer of 2013 (SM13), fall of 2013 (FL13), summer of 2014 (SM14), fall of 2014 (FL14), spring of 2015 (SP15), summer of 2015 (SM15), and fall of 2015 (FL15) was 31.1 ℃, 17.8 ℃, 29.4 ℃, 16.4 ℃, 21.7 ℃, 29.5 ℃, and 20.2 ℃, respectively (NOAA, 2015) (Table 13).

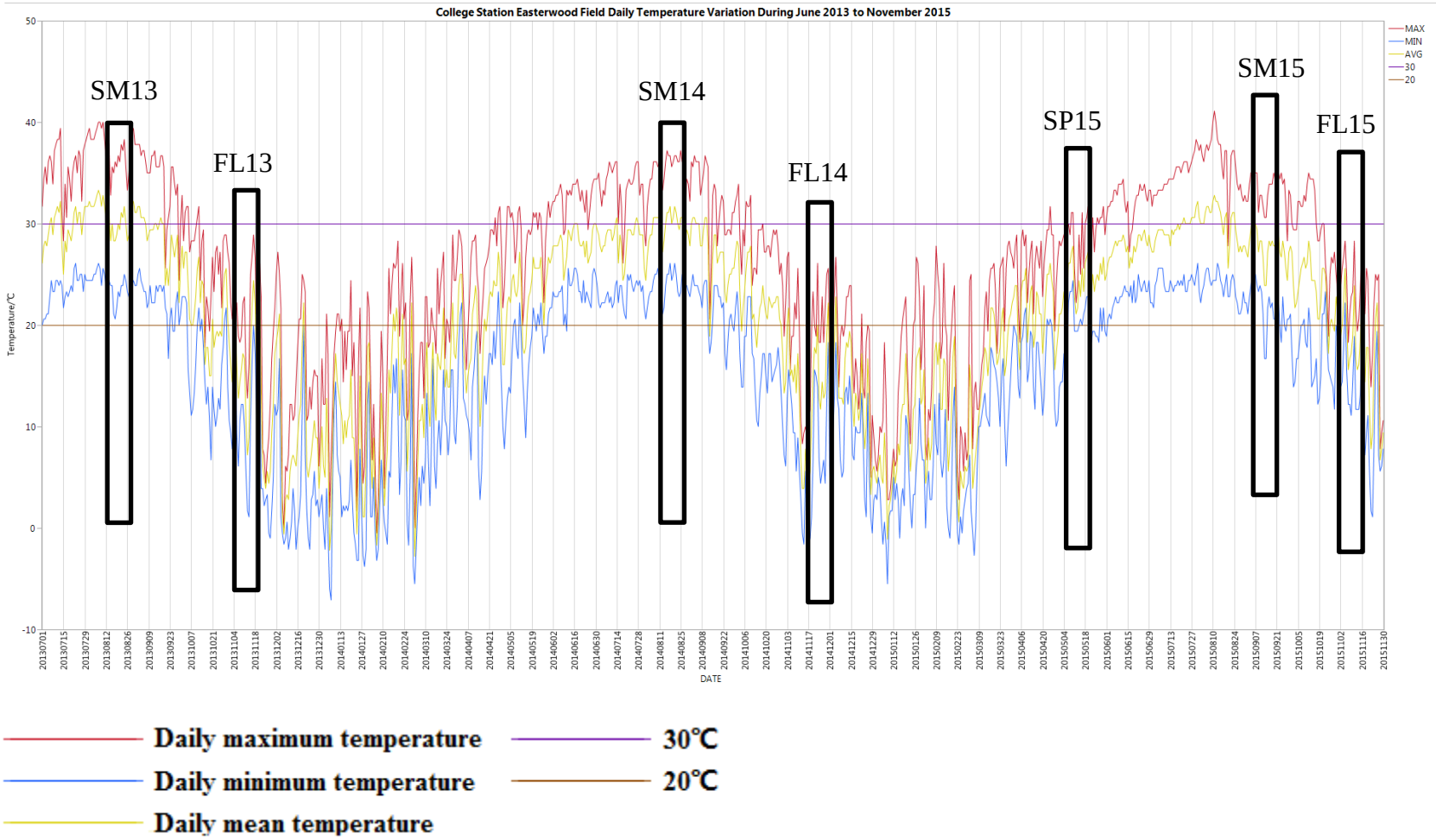



**Figure 4.** College Station Easterwood Field daily temperature variation during June 2013 to November 2015 (NOAA).

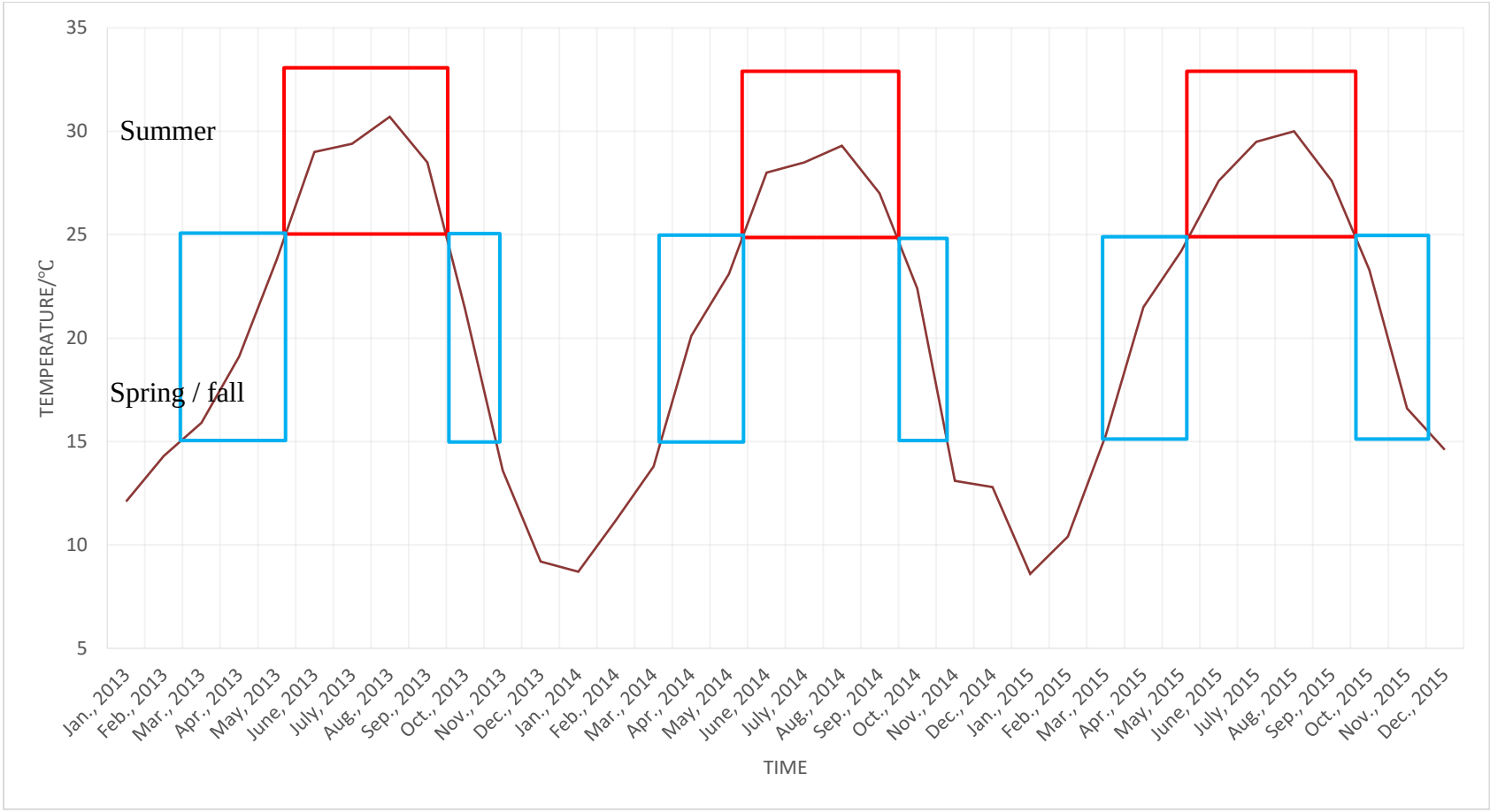



**Figure 5.** Cool (spring/fall) and warm (summer) season mean monthly temperatures for College Station, Texas. January 2013 to December 2015 (NOAA)

**Table 13.** Temperature data for 1 – 14 days, 15 – 28 days, and 4 week period, and precipitation data for the 4 week period prior to taking data for College Station Easterwood Field for summer 2013 (August), fall 2013 (November), summer 2014 (August), fall 2014 (November), spring 2015 (April), summer 2015 (August), and fall 2015 (November) (NOAA[z]).

| Time | Temperature/℃ | | | | | Precipitation/mm |
|---|---|---|---|---|---|---|
| | 1 - 14 days avg. | 15 – 28 days avg. | 4 weeks avg. | 4 weeks max | 4 weeks min | 4 weeks/year |
| August 2013 | 32.1 | 30.0 | 31.1 | 39.4 | 20.6 | 20/1000 |
| November 2013 | 19.8 | 15.8 | 17.8 | 28.9 | 1.1 | 115/1000 |
| August 2014 | 28.7 | 30.2 | 29.4 | 37.2 | 20.6 | 33/1007 |
| November 2014 | 20.3 | 12.5 | 16.4 | 29.4 | -1.6 | 81/1007 |
| April 2015 | 22.1 | 21.4 | 21.7 | 31.7 | 10.0 | 121/1479 |
| August 2015 | 30.6 | 28.5 | 29.5 | 41.1 | 21.1 | 70/1479 |
| November 2015 | 21.4 | 19.1 | 20.2 | 30.0 | 11.1 | 260/1479 |
| [z] http://www.ncdc.noaa.gov/cdo-web/search, date range from July 1, 2013 to December 20, 2015. | | | | | | |

### 3.5 Methods

The flower size traits including: flower diameter, flower petal number, and flower dry weight, and the flower production traits, number of flowers per primary/secondary/tertiary inflorescence were measured during the spring, summer, and fall when the average temperatures went up to 20 ℃, up to 30 ℃, and down to 20 ℃, respectively. The flower size data were collected from at least three fully open flowers randomly chosen from the primary inflorescence in each plant. Based on observation, a fully open (flower development stage 10 of Ma et al., 2015) flower with dehiscing pollen tends to have more stable petal number and size. Flower diameter (cm) was measured in the field whereas petal number and flower dry weight were determined from flowers (without pedicel) collected and put into paper bags (9 cm x 16.5 cm). Flower dry weight data was taken in the lab after samples were dried at 80℃ for at least 3 days. The petal count (done in the lab) represents the number of normal petals and petaloids (irregular shaped petals). The flower production traits were collected from three shoots randomly chosen from each plant. All these shoots were grown since they were pruned to a standard size in February/March, June, and September. Plants have 3 nodes or 20 cm long shoots are regarded as standard size plant. Each parameter was compared between seasons to check for changes in warmer summer season versus cooler spring and fall season.

### 3.6 Statistical analysis

All statistical analyses were performed using JMP software, Version 12.0.1 SAS Institute Inc. Flower petal number, flower diameter, flower dry weight, number of flowers per primary inflorescence, and number of flower per secondary inflorescence were performed on $\log_{10}$ and root transformation to improve normality and fit. A Shapiro-Wilk W test was performed to test the normality of raw and transformed data (Razali and Wah, 2011). In rose, flower petal numbers are controlled by a major dominant gene for double flower (>8 petals). Therefore, only double/semi-double (>8 petals) flowers were considered in petal number data because petal number in single flowers (5 - 8 petals) change very little. In order to directly estimate the heritability of heat tolerance in rose, a heat tolerance index was calculated by dividing flower size (flower diameter, petal number, and flower dry weight) in warm season by flower size in cool season. Correlation coefficients of all six components were generated from Pearson product-moment correlation analysis. Two-way factorial analysis of variance was conducted to compare among the population and seasonal means as well as to assess the interaction effect.

The genetic variation of the measured flower traits under cool and heat stressed conditions was estimated by using a restricted maximum likelihood estimation (REML) model ($y = \mu + \sigma^2_{FP} + \sigma^2_{PP} + \sigma^2_{Prog[FP,PP]} + \sigma^2_{Season} + \sigma^2_{Season*FP} + \sigma^2_{Season*PP} + \sigma^2_{Season*Prog[FP,PP]} + \sigma^2_{Error}$) (Dieters et al., 1995; Littell et al, 2006) with JMP software, Version 12.0.1 SAS Institute Inc. Parental variances were regarded as additive variance (Va), progeny variance was regarded as non-additive variance (Vd), variance due to the change of season was regarded as environmental variance (Ve), variance due to parents and progeny was regarded as genotypic variance (Vg), and the interaction of genotype and environment was treated as genetic-environmental variance (Vgxe) (Connor et al., 2005). Heritability were calculated by this REML model. The narrow sense heritability, $h^2$, was measured by additive variance (Va) divided by phenotypic variance (Vp) ($h^2$=Va/Vp), $H^2$ was measured by the sum of Va and dominance variance (Vd) divided by the Vp, where Vp=(Va+Vd+Vgxe/e), e indicates the number of seasons used in the analysis (Hallauer et al., 2010; Holland et al., 2003).

## 3.7 Results

### *Normality analysis*

Normality was tested by the Shapiro-Wilk W test (Ott and Longnecker, 2008; Roman et al., 2015). Based on the result of the year of 2015, the normality of petal number, flower dry weight, and number of flowers per primary/secondary inflorescence but not flower diameter was improved after either the $\log_{10}$ or square root transformation (data not shown), although the $\log_{10}$ transformation was better (Table 14). The normality of petal number data collected during summer of 2013, fall of 2013, and summer of 2014 was also improved after a $\log_{10}$ transformation (Table 15). The normality of heat tolerance index calculated by flower diameter, petal number, and flower dry weight were checked by using the Shapiro-Wilk W Test. The result showed that raw data of heat tolerance index calculated by flower diameter and $\log_{10}$ transformed data of heat tolerance index calculated by petal number and flower dry weight had better normality (Table 16). Thus subsequent statistical analyses were conducted with raw flower diameter data and $\log_{10}$ transformed petal number, flower dry weight, and number of flowers per primary/secondary inflorescence data. Conclusions from the transformed data and the untransformed data were not different.

**Table 14.** Normality (Shapiro-Wilk W) test on the distribution of raw and transformed ($log_{10}$) flower trait data for 16 diploid rose populations assessed over three seasons in the field in 2015.

| | Shapiro-Wilk W test[z] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Population[y] | DM[x] | log DM | PT | log PT | DW | log DW | PM | log PM | SE | log SE |
| OB × J3-6 | NS | NS | NS | NS | * | * | * | * | * | * |
| TF × J3-6 | NS | NS | - | - | NS | NS | NS | NS | * | * |
| OB ×M4-4 | NS | NS | NS | NS | NS | NS | NS | NS | * | * |
| SC × M4-4 | NS | * | NS | * | * | NS | * | * | * | * |
| J4-6 × RF | NS | * | * | NS | * | * | * | * | * | * |
| J3-3 × RF | NS | NS | NS | NS | NS | NS | NS | NS | * | NS |
| VS × J14-3 | NS | * | NS | NS | NS | NS | NS | NS | * | NS |
| J14-3 × VS | NS | * | * | NS | * | * | * | * | * | * |
| J14-3 × SC | NS | * | * | * | * | * | * | NS | * | * |
| M4-4 × SC | NS | NS | NS | NS | NS | * | * | NS | * | NS |
| J14-3 × LC | * | * | * | * | * | NS | * | * | * | * |
| J14-3 × RF | NS | * | * | * | * | * | * | * | * | * |
| OB × RF | NS | * | * | NS | * | NS | * | * | * | * |
| SC × J14-3 | NS | NS | NS | NS | NS | NS | * | NS | * | NS |
| M4-4 × 97/7-2 | NS | * | - | - | NS | * | * | NS | * | * |
| SC × 97/7-2 | NS | NS | - | - | NS | NS | NS | NS | NS | NS |

[zNS, *] Nonsignificant or significant at $P \leq 0.05$. $H_0$: The data is from the normal distribution. Small p-values reject $H_0$.

[y]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'

[x]DM = flower diameter, PT = flower petal number, DW = flower dry weight, PM = number of flowers per primary inflorescence, SE = number of flowers per secondary inflorescence.

**Table 15.** Normality (Shapiro-Wilk W) test on the distribution of raw and transformed ($log_{10}$) petal number data for 9 diploid rose populations assessed over summer of 2013, fall of 2013, and summer of 2014 in the field of College Station.

| | Shapiro-Wilk W Test [z] | |
|---|---|---|
| Population | Petal number | log Petal number |
| OB × J3-6 | * | NS |
| TF × J3-6 | * | * |
| OB ×M4-4 | * | NS |
| SC × M4-4 | NS | NS |
| J4-6 × RF | * | NS |
| J3-3 × RF | NS | NS |
| VS × J14-3 | NS | NS |
| J14-3 × VS | * | NS |
| J14-3 × SC | * | NS |
| M4-4 × SC | NS | NS |

[zNS, *] Nonsignificant or significant at $P \leq 0.05$. $H_0$: The data is from the normal distribution. Small p-values reject $H_0$.

**Table 16.** Normality (Shapiro-Wilk W) test on the distribution of raw and transformed ($log_{10}$) heat tolerance index for the 15 diploid rose populations in the field. College Station, 2015.

| | Shapiro-Wilk W Test[z] | | | | | |
|---|---|---|---|---|---|---|
| Population[y] | DM | log DM | PT | log PT | DW | log DW |
| OB × J3-6 | NS | * | NS | * | NS | * |
| TF × J3-6 | NS | NS | NS | NS | NS | NS |
| OB × M4-4 | NS | NS | NS | NS | NS | NS |
| SC × M4-4 | NS | NS | * | NS | * | * |
| J4-6 × RF | NS | NS | * | NS | * | NS |
| J3-3 × RF | NS | NS | NS | NS | NS | NS |
| VS × J14-3 | NS | NS | NS | NS | NS | NS |
| J14-3 × VS | * | * | * | * | * | NS |
| J14-3 × SC | * | * | NS | * | NS | NS |
| M4-4 × SC | NS | NS | NS | NS | NS | NS |
| J14-3 × LC | * | * | * | NS | NS | NS |
| J14-3 × RF | NS | * | NS | * | * | * |
| SC × J14-3 | NS | NS | * | NS | NS | * |
| M4-4 × 97/7-2 | NS | NS | NS | NS | NS | NS |
| SC × 97/7-2 | NS | NS | - | - | NS | NS |

[zNS, *] Non-significant or significant at $P \leq 0.05$.
$H_0$: The data is from the normal distribution. Small p-values reject $H_0$.
[y]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'
[x]DM = heat tolerance index flower diameter, PT = heat tolerance index flower petal number, DW = heat tolerance index flower dry weight

***Correlation analysis***

Flower dry weight was positively correlated with flower diameter and petal number (R = 0.46 and 0.44 respectively) whereas flower diameter was negatively correlated to petal number (R = -0.11) reflecting a weak tendency of the larger flowers to have fewer petals in this rose germplasm and vice versa. The flower production data (flowers per primary/secondary/tertiary inflorescence) were well correlated among each other (R=0.85, 0.86, and 0.74). However, there was little correlation between flower size and flower number (Table 17, Appendix 1, 2, 3, 4, 5, and 6).

**Table 17.** Pairwise correlations among flower diameter, petal number, flower dry weight, number of flowers per primary/secondary/tertiary inflorescence in the field in 2015.

| Variable | by Variable | Obs. | R | Significance[z] |
|---|---|---|---|---|
| Flower diameter | Petal number | 1652 | -0.11 | *** |
| Flower dry weight | Petal number | 1501 | 0.47 | *** |
| Flower dry weight | Flower diameter | 1497 | 0.44 | *** |
| Primary | Petal number | 92 | 0.03 | NS |
| Primary | Flower diameter | 91 | -0.09 | NS |
| Primary | Flower dry weight | 92 | 0.22 | * |
| Secondary | Petal number | 75 | -0.01 | NS |
| Secondary | Flower diameter | 74 | -0.09 | NS |

**Table 17.** Continued

| Variable | by Variable | Obs. | R | Significance[z] |
|---|---|---|---|---|
| Secondary | Flower dry weight | 74 | -0.19 | NS |
| Secondary | Primary | 207 | 0.85 | *** |
| Tertiary | Petal number | 17 | 0.39 | NS |
| Tertiary | Flower diameter | 17 | -0.27 | NS |
| Tertiary | Flower dry weight | 17 | -0.20 | NS |
| Tertiary | Primary | 69 | 0.74 | *** |
| Tertiary | Secondary | 74 | 0.86 | *** |
| [z]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. | | | | |

***Flower size***

**General linear analysis of flower petal number for 10 populations for 2013 and 8 populations for 2014 field data**

The preliminary experiment focused on the number of petals. Two way factorial analysis of petal count data for three seasons (Summer 2013, Fall 2013 and Summer 2014) indicated that petal numbers varied among populations and seasons but that there was no interaction (Table 18). Examination of the seasonal effect indicated that there were no differences between the petal counts for the Summers of 2013 and 2014 but that these differed from the counts from the Fall of 2013. Therefore, data collected from summers of 2013 and 2014 were combined as warm season data. Data collected from the fall of 2013 was regarded as cool season data.

**Table 18.** Analyses of variance (ANOVA) of population, season, and population by season interaction effects among summer of 2013 (13 Summer), fall of 2013 (13 Fall), and summer of 2014 (14 Summer) in College Station for petal number. $Log_{10}$ transformed of petal number data was used. Only a petal number larger than 8 was considered.

| | 13 Summer vs. 13 Fall[z] | | 13 Fall vs. 14 Summer | | 13 Summer vs. 14 Summer | |
|---|---|---|---|---|---|---|
| | DF | MS | DF | MS | DF | MS |
| Population | 9 | 1.09*** | 7 | 0.92*** | 7 | 0.67*** |
| Season | 1 | 0.37*** | 1 | 0.44*** | 1 | $0.00^{NS}$ |
| Population × Season | 9 | $0.05^{NS}$ | 7 | $0.04^{NS}$ | 7 | $0.06^{NS}$ |

[zNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

**Cool versus warm season analysis**

Data collected from summers of 2013 and 2014 were combined by taking the average as warm season data. Data collected from the fall of 2013 was regarded as cool season data. The analysis with the combined data confirmed the differences among rose populations and the seasons and the lack of an interaction effect for petal counts (Tables 19 and 20). Over all seasons, population VS × J14-3 and J14-3 × VS had the highest petal number (78.5 and 62.4) and population OB × J3-6 and OB ×M4-4 had the lowest petal number (27.3, and 26.5) (Table 20). There was a 19.6% decrease in petal number in the warm season as compared to the cool season (Table 20).

**Table 19.** Cool and warm season effect on the petal number of 10 diploid rose populations during 2013 and 2014 in College Station. $Log_{10}$ transformation of petal number was used. Only a petal number larger than 8 was considered.

| | DF | Variance | Mean Square[z] |
|---|---|---|---|
| Population | 9 | 10.34 | 1.15*** |
| Season (cool vs. warm) | 1 | 0.72 | 0.72*** |
| Population × Season | 9 | 0.26 | $0.03^{NS}$ |
| [zNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. | | | |

**Table 20.** Mean petal number for 10 diploid rose populations grown in College Station during 2013 and 2014. $Log_{10}$ transformed data was used. Only petal number larger than 8 was considered.

| Population[z] | Mean[y] | Warm | Cool | % of decrease[x] |
|---|---|---|---|---|
| OB × J3-6 | 27f | 25 | 29 | 14.7 |
| TF × J3-6 | 39def | 32 | 47 | 30.2 |
| OB ×M4-4 | 271f | 22 | 31 | 30.6 |
| SC × M4-4 | 57bc | 47 | 67 | 30.5 |
| J4-6 × RF | 33ef | 27 | 40 | 33.0 |
| J3-3 × RF | 46cde | 42 | 49 | 13.9 |
| VS × J14-3 | 78a | 86 | 71 | -21.0 |
| J14-3 × VS | 62ab | 50 | 75 | 33.0 |
| J14-3 × SC | 54cd | 50 | 58 | 12.9 |
| M4-4 × SC | 35ef | 27 | 43 | 36.4 |
| Mean | 45 | 41 | 51 | 19.6* |

[z] J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'

[yz]Levels not connected by same letter are significantly different at $P \leq 0.05$.

[x]NS, * Nonsignificant or significant at $P \leq 0.05$.

**General linear analysis of flower diameter, petal number, and flower dry weight of 15 populations for 2015 data**

In 2015, all three flower size traits varied among populations and two (flower diameter and flower dry weight) of the three varied depending on the season the data was taken. The population by season effect was only detected for flower diameter (Table 21). The spring and the fall data did not show any difference in flower diameter, petal number, or flower dry weight (Table 22), so these seasons were averaged to create the cool season data and the analysis run to compare cool versus warm season effects.

**Table 21.** Spring, summer, and fall season effects on 15 diploid rose populations grown in College Station, 2015.

| | Diameter[z] | | Petal | | Dry weight | |
|---|---|---|---|---|---|---|
| | DF | MS[y] | DF | MS | DF | MS |
| Population | 13 | 4.60*** | 10 | 0.88*** | 13 | 0.55*** |
| Season | 2 | 4.82*** | 2 | $0.02^{NS}$ | 2 | 0.26*** |
| Population × Season | 26 | 0.45* | 20 | $0.03^{NS}$ | 26 | $0.02^{NS}$ |

[z]Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight. Only petal number larger than 8 was considered.
[yNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

**Table 22.** Changes of flower diameter, petal number, and flower dry weight in 14 populations among spring, summer, and fall in College Station, 2015.

| | Spring[z] | Summer | Fall |
|---|---|---|---|
| Flower diameter[y] | 3.9a | 3.3b | 3.9a |
| Petal number | 46a | 42a | 49a |
| Flower dry weight | 81.4a | 63.5b | 82.5a |

[z]LSMeans within the components connected by the same letter are not significantly different among seasons at $P \leq 0.05$, with LSD adjustment.
[y]Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight. Only petal number larger than 8 was considered.

**Cool versus warm season analysis**

The combined analysis indicated that all three measures of flower size varied with the population and the season (warm vs. cool) and that there was a significant interaction effect for flower diameter and flower dry weight but not for petal number (Table 23).

**Table 23.** Cool and warm season effects on 15 diploid rose populations grown in College Station, TX in 2015.

| | Flower diameter[z] | | Petal number | | Flower dry weight | |
|---|---|---|---|---|---|---|
| | DF | MS[y] | DF | MS | DF | MS |
| Population | 14 | 6.40*** | 13 | 0.75*** | 14 | 0.64*** |
| Season | 1 | 37.07*** | 1 | 0.44*** | 1 | 2.37*** |
| Population × Season | 14 | 0.69*** | 13 | $0.01^{NS}$ | 14 | 0.06* |

[z]Data from spring and fall were combined into the cool season data. Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight. Only petal number larger than 8 was considered.
[yNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

The population OB × J3-6 had the largest flower diameter (4.2 cm) and large flower dry weight (88 mg), but smallest petal number (22) (Table 24). Population SC × J14-3 had the largest petal number (94) and large flower dry weight (109 mg), but low flower diameter (3.2 cm) (Table 24). Results indicated negative correlation between flower diameter and petal number that flowers with large flower diameter usually had a small petal number. The flower dry weight reflected the combination of flower diameter and petal number.

**Table 24.** Mean flower diameter, petal number, and flower dry weight for 15 diploid rose populations grown in College Station, 2015.

| | LSMeans[z] | | |
|---|---|---|---|
| Population[y] | Flower diameter/cm[x] | Petal number | Flower dry weight/mg |
| OB × J3-6 | 4.2a | 22h | 88bc |
| TF × J3-6 | 3.4cdefg | 56bcde | 81abcde |
| OB × M4-4 | 4.0ab | 23gh | 69def |
| SC × M4-4 | 3.5c | 50cd | 58fgh |
| J4-6 × RF | 3.4deg | 31fg | 52gh |
| J3-3 × RF | 3.5cdeg | 39cdefg | 70cdefg |
| VS × J14-3 | 3.6cdg | 72b | 102ab |
| J14-3 × VS | 3.6c | 53c | 77d |
| J14-3 × SC | 3.5cd | 47cde | 64ef |
| M4-4 × SC | 3.7bc | 43cdef | 46hi |
| J14-3 × LC | 3.2ef | 46cde | 64efg |
| J14-3 × RF | 3.2f | 42de | 74de |
| OB × RF | 3.6c | 38efg | 106a |
| SC × J14-3 | 3.2efgh | 94a | 109a |
| M4-4 × 97/7-2 | 2.8h | | 27i |

[z]LSMeans within the components connected by the same letter are not significantly different among populations at $P \leq 0.05$, with LSD adjustment.
[y]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'
[x]Data from spring and fall were combined as cool season's data. Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight. Only petal number larger than 8 was considered.

The interaction effect was significant for flower diameter and flower dry weight. Over all populations, there were 17.6% loss in flower diameter, 19.8% loss in petal number, and 30.8% loss in flower dry weight in the warm season as compared to the cool season (Table 25). The flower diameter of 12 among 15 populations was decreased due to high temperature (Table 25, Appendix 16). Eleven populations among 15 had flower dry weight decrease during warm season (Table 25, Appendix 17). Population SC × J14-3 was affected the most by temperature in both flower diameter (29.2% decrease) and flower dry weight (60.3% decrease). (Table 25). The range of the flower diameter differences within a population ranged from a low of 0.31 cm for the TF × J3-6 population to a high of 1.08 cm for the SC × J14-3 population. This differential response among the populations indicate that they respond differently to heat stress and consequently differ in their tolerance to heat stress. Those populations which have a smaller decrease in flower diameter due to heat stress would be considered more heat tolerant.

**Table 25.** Mean flower diameter, petal number, and flower dry weight for the cool and warm seasons over 15 diploid rose populations grown in College Station, 2015.

| | Flower diameter[y] | | | Petal number | | | Flower dry weight | | |
|---|---|---|---|---|---|---|---|---|---|
| Population[z] | % of decrease[x] | warm | cool | % of decrease | warm | cool | % of decrease | warm | cool |
| OB × J3-6 | 11.9* | 3.92 | 4.45 | 19.8 | 20 | 25 | 26.8* | 75 | 102 |
| TF × J3-6 | 8.7$^{NS}$ | 3.29 | 3.60 | 36.3 | 44 | 68 | -11.4$^{NS}$ | 86 | 77 |
| OB × M4-4 | 21.8* | 3.50 | 4.48 | 10.1 | 21 | 24 | 46.7* | 48 | 90 |
| SC × M4-4 | 12.3* | 3.31 | 3.78 | 10.1 | 47 | 53 | 19.2* | 53 | 65 |
| J4-6 × RF | 10.7* | 3.19 | 3.57 | 5.8 | 30 | 32 | 32.8* | 41 | 62 |
| J3-3 × RF | 14.8$^{NS}$ | 3.19 | 3.74 | -2.1 | 40 | 39 | 13.3$^{NS}$ | 65 | 75 |
| VS × J14-3 | 10.4$^{NS}$ | 3.38 | 3.77 | 10.3 | 68 | 75 | 3.8$^{NS}$ | 100 | 104 |
| J14-3 × VS | 16.7* | 3.25 | 3.90 | 16.3 | 48 | 58 | 34.8* | 61 | 93 |
| J14-3 × SC | 11.6* | 3.31 | 3.74 | 20.8 | 42 | 53 | 45.6* | 45 | 83 |
| M4-4 × SC | 18.7* | 3.33 | 4.10 | 20.8 | 38 | 48 | 35.7* | 36 | 56 |
| J14-3 × LC | 19.7* | 2.86 | 3.56 | 22.5 | 40 | 51 | 15.2$^{NS}$ | 59 | 69 |
| J14-3 × RF | 28.0* | 2.66 | 3.69 | 19.9 | 38 | 47 | 31.7* | 60 | 88 |
| OB × RF | 23.1* | 3.14 | 4.08 | 21.5 | 33 | 42 | 41.4* | 79 | 134 |
| SC × J14-3 | 29.2* | 2.61 | 3.69 | 33.4 | 75 | 112 | 60.3* | 62 | 156 |
| M4-4 × 97/7-2 | 26.5* | 2.41 | 3.28 | - | - | - | 34.3* | 22 | 33 |
| Overall | 17.6* | 3.17 | 3.83 | 19.8* | 42 | 52 | 30.8* | 59 | 86 |

[z]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'.

[y]Data from spring and fall were combined as cool season's data. Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight. Only petal number larger than 8 was considered.

[xNS, *] Nonsignificant or significant between seasons at $P \leq 0.05$.

**General linear analysis of the heat tolerance index for flower diameter, petal number, and flower dry weight for 15 populations for 2015 data**

In order to directly estimate the heritability of heat tolerance in rose, heat tolerance index was created by dividing flower size (flower diameter, petal number, and flower dry weight) in warm season by flower size in cool season. The analysis of variance indicated that heat tolerance index calculated with flower diameter and flower dry weight varied with the population (Table 26).

The population TF × J3-6 had the highest heat tolerance index (0.92 and 1.10) when calculated on flower diameter and flower dry weight (Table 27). The population SC × J14-3 was the most heat sensitive as indicated by its low heat tolerance index (0.71 and 0.43 for flower diameter and dry weight respectively) (Table 27). The high heat tolerance index of the populations J3-3 × RF (0.83-1.04) and VS × J14-3 (0.90-1.05) indicated these two populations were heat tolerant.

**Table 26.** Analysis of population variance on heat tolerance index on 15 diploid rose populations grown in College Station, TX in 2015.

| | Flower diameter[z] | | | Petal number | | | Flower dry weight | | |
|---|---|---|---|---|---|---|---|---|---|
| | DF | Sum of Square | F Ratio[y] | DF | Sum of Square | F Ratio | DF | Sum of Square | F Ratio |
| Population | 14 | 1.46 | 6.03*** | 13 | 0.37 | 1.29[NS] | 14 | 1.71 | 4.49*** |
| Error | 367 | 6.35 | | 243 | 5.35 | | 364 | 9.87 | |
| Total | 381 | 7.82 | | 256 | 5.72 | | 378 | 11.58 | |

[z]Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight were used. Only petal number larger than 8 was considered.
[y]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

**Table 27.** Mean heat tolerance index for 15 diploid rose populations grown in College Station, 2015.

| Population[z] | Flower diameter[y] | Petal number[w] | Flower dry weight |
|---|---|---|---|
| OB × J3-6 | 0.89a | 0.85 | 0.77bcd |
| TF × J3-6 | 0.92abc | 0.69 | 1.10a |
| OB × M4-4 | 0.81abcde | 1.05 | 0.63def |
| SC × M4-4 | 0.88a | 0.94 | 0.89abc |
| J4-6 × RF | 0.89a | 0.96 | 0.67ef |
| J3-3 × RF | 0.85abcd | 1.04 | 0.83abcde |
| VS × J14-3 | 0.91abc | 0.90 | 1.05ab |
| J14-3 × VS | 0.84bc | 0.90 | 0.66ef |
| J14-3 × SC | 0.90ab | 0.84 | 0.59f |
| M4-4 × SC | 0.82abcd | 0.77 | 0.62def |
| J14-3 × LC | 0.81cd | 0.86 | 0.86abc |
| J14-3 × RF | 0.72e | 0.83 | 0.69def |
| OB × RF | 0.77cde | 0.88 | 0.65def |
| SC × J14-3 | 0.71de | 0.76 | 0.43g |
| M4-4 × 97/7-2 | 0.74de | - | 0.68cdef |
| Mean[x] | 0.84* | $0.88^{NS}$ | 0.73* |

[z]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'

[y]LSMeans within the components connected by the same letter are not significantly different among populations at $P \leq 0.05$, with LSD adjustment.

[x]NS, * Nonsignificant or significant among populations at $P \leq 0.05$, respectively.

[w]Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight were used. Only petal number larger than 8 was considered.

**Genetic analysis of flower petal number between cool and warm season during 2013 to 2014**

Petal counts collected during summer of 2013, fall of 2013, and summer of 2014 were used to calculate the genetic variance and heritability with diploid rose populations. Since there was no seasonal effect between summer of 2013 and summer of 2014, data collected from summer of 2013 and summer of 2014 were combined as warm season data. The narrow and broad sense heritability estimates were 0.33 and 0.85 respectively. The sum of interaction effects (season by female parent effect, season by pollen parent, and season by progeny within family effect) is regarded as the G×E effect. Therefore, G×E effect was moderately low accounting for about 22% of the genetic variance indicating selection throughout the year should be effective. (Table 28).

**Table 28.** Genetic variance for petal number in 10 diploid populations in College Station between cool and warm season in the field (2013-2014).

| | |
|---|---|
| obs. | 422 |
| $r^2$ | 0.87 |
| Percentage of total variance | |
| FP[z] | 22.49 |
| PP | 3.11 |
| Progeny[FP, PP] | 40.86 |
| Season | 10.52 |
| Season × FP | 0.23 |
| Season × PP | 0.65 |
| Environment × Progeny[FP, PP] | 22.15 |
| Genetic variance | |
| Va[y] | 0.019 |
| Vd | 0.030 |
| Vg | 0.049 |
| Vp | 0.058 |
| Vgxe | 0.017 |
| Vgxe/Vg | 0.347 |
| Heritability | |
| $h^2$ | 0.33 |
| $H^2$ | 0.85 |

[z]FP = female parent, PP = pollen parent, $h^2$ = narrow sense heritability, $H^2$ = broad sense heritability. $Log_{10}$ transformed data of petal number was used. Only petal number larger than 8 was considered.
[y]Va = Parental variances, Vd = progeny variance, Ve = variance due to the change of season, Vg = variance due to parents and progeny, Vgxe = variance due to the interaction of genotype and environment, Vp(phenotypic variance) = (Va+Vd+Vgxe/E), $h^2$ = Va/Vp, $H^2$ = (Va+Vd)/Vp, E = number of seasons.

**Genetic analysis of flower diameter, petal number and flower dry weight between cool and warm season in 2015**

Flower diameter, petal number, and flower dry weight were analyzed in a restricted estimated maximum likelihood (REML) model. Since the genetic effect for treatment by female parent effect and the treatment by pollen parent in petal number was less than 0.5%, these two effects were removed in the analysis. The sum of interaction effects was regarded as the G×E effect.

All flower size measurements had a low to moderate narrow sense (0.33 to 0.37) and a moderately high to high broad sense heritability (0.67 to 0.91) indicating substantial non additive genetic variation. The G×E effect varied from a high of 37.0% for flower diameter, to 27.0% for flower dry weight to a low of 15.3% for the number of petals. In all cases, the total genetic variance was larger than the G×E variance, indicating that selection over various seasons could be effective for these traits. Petal number had the highest broad sense heritability of 0.91 and the lowest Vgxe/Vg ratio of 0.191 (Table 29). Flower diameter had the lowest broad sense heritability (0.67) also had the highest Vgxe/Vg ratio (0.981) which indicated that flower diameter was differentially affected by heat stress more than the other traits (Table 29).

**Table 29.** Genetic variance for flower diameter, petal number, and flower dry weight for cool and warm seasons in 15 diploid populations in College Station field plots (2015) calculated by REML (restricted estimated maximum likelihood).

| | Flower diameter | Petal number | Flower dry weight |
|---|---|---|---|
| Obs. | 764 | 514 | 759 |
| $r^2$ | 0.78 | 0.91 | 0.85 |
| | Percentage of total variance | | |
| FP[z] | 4.1 | 18.2 | 16.9 |
| PP | 15.7 | 11.0 | 9.7 |
| Progeny[FP, PP] | 17.9 | 51.3 | 32.0 |
| Season | 25.3 | 4.1 | 14.4 |
| Season × FP | 6.5 | - | 3.3 |
| Season × PP | 3.1 | - | 3.8 |
| Environment × Progeny[FP, PP] | 27.4 | 15.3 | 20.0 |
| | Genetic variance | | |
| Va[y] | 0.105 | 0.021 | 0.019 |
| Vd | 0.095 | 0.037 | 0.022 |
| Vg | 0.200 | 0.059 | 0.041 |
| Vp | 0.298 | 0.064 | 0.051 |
| Vgxe | 0.196 | 0.011 | 0.019 |
| Vgxe/Vg | 0.981 | 0.191 | 0.461 |
| | Heritability | | |
| $h^2$ | 0.35 | 0.33 | 0.37 |
| $H^2$ | 0.67 | 0.91 | 0.81 |

[z]FP = female parent, PP = pollen parent, $h^2$ = narrow sense heritability, $H^2$ = broad sense heritability. Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight were used. Only petal number larger than 8 was considered.

[y]Va = Parental variances, Vd = progeny variance, Ve = variance due to the change of season, Vg = variance due to parents and progeny, Vgxe = variance due to the interaction of genotype and environment, Vp(phenotypic variance) = (Va+Vd+Vgxe/E), $h^2$ = Va/Vp, $H^2$ = (Va+Vd)/Vp, E = number of seasons.

**Genetic analysis of the Heat Tolerance Index using flower diameter, petal number and flower dry weight between cool and warm season in 2015**

A restricted estimated maximum likelihood (REML) model was used to estimate the heritability of heat tolerance. The season component did not make any variance contribution in this analysis. Therefore, broad sense heritability was not calculated. The $r^2$ of the REML model analysis for heat tolerance index calculated with petal number was -0.02 indicating petal number was not appropriate to calculate heat tolerance index (Table 30). Narrow sense heritability for heat tolerance index calculated with flower diameter and flower dry weight were moderately low to low at 0.31 and 0.24 respectively (Table 30). Unfortunately, given the low $r^2$ of the analyses the reliability of these estimates is suspect. Given suspect results, heat tolerance index might be not appropriate in this model.

**Table 30.** Genetic variance for the heat tolerance index in 15 diploid populations in College Station, 2015 calculated with REML (restricted estimated maximum likelihood).

| | Flower diameter | Petal number | Flower dry weight |
|---|---|---|---|
| obs. | 382 | 257 | 379 |
| $r^2$ | 0.17 | -0.02 | 0.11 |
| Percentage of total variance | | | |
| FP[z] | 19.1 | 0.3 | 12.0 |
| PP | 11.4 | 0.0 | 12.1 |
| Progeny[FP, PP] | 69.5 | 99.7 | 75.9 |
| Genetic variance | | | |
| Va[y] | 0.008 | 0.000 | 0.009 |
| Vd | 0.017 | 0.023 | 0.028 |
| Vp | 0.025 | 0.023 | 0.037 |
| Heritability | | | |
| $h^2$ | 0.31 | 0.00 | 0.24 |

[z]FP = female parent, PP = pollen parent, $h^2$ = narrow sense heritability. Raw data of flower diameter, $\log_{10}$ transformed data of petal number and flower dry weight were used. Only petal number larger than 8 was considered. Heat tolerance index = warm season flower size /cool season flower size.
[y]Va = Parental variances, Vd = progeny variance, Vg = variance due to parents and progeny, Vp(phenotypic variance) = Vg, $h^2$ = Va/Vp.

***Flower production***

**General linear analysis of number of flowers per primary/secondary inflorescence.**

Both components, the number of flowers per primary inflorescence and the number of flowers per secondary inflorescence varied among the populations and between seasons, but in a population specific fashion (significant population x season effect) (Table 31).

**Table 31.** Analyses of variance (ANOVA) of population, season, and population by season interaction effect in College Station, 2015 during spring, summer, and fall for the number of flowers per primary and secondary inflorescence.

| | Number of flowers per primary inflorescence[z] | | Number of flowers per secondary inflorescence | |
|---|---|---|---|---|
| | DF | MS[y] | DF | MS |
| Population | 15 | 0.81*** | 14 | 0.26*** |
| Season | 2 | 1.29*** | 2 | 1.71*** |
| Population × Season | 30 | 0.32*** | 28 | 0.27*** |

[z]$Log_{10}$ transformed data of number of flowers per primary and secondary inflorescence was used.
[yNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Over all seasons, population SC × M4-4 and M4-4 × 97/7-2 had the largest number of flowers in both components (14.8 and 6.7, 14.3 and 5.7) which indicates that these two populations had the highest flower density (Table 32). Population OB × J3-6 had the least flowers per primary/secondary inflorescence (7.7 and 3.5) (Table 32).

Over all plants, the number of flowers per primary and secondary inflorescence was highest in the spring and decreased as the season progressed (Table 33). These two flower production traits started high and decreased most rapidly in the population SC × M4-4 (Table 33). Since the number of flowers per primary and secondary inflorescence were not consistently less in the summer as compared to the Spring and the Fall, the change in flower production was not clearly caused by heat stress.

**Table 32.** Least square means (LSMeans) of number of flowers per primary and secondary inflorescence over 3 seasons (spring, summer, and fall) with 16 diploid rose populations in College Station, 2015. Raw data was used to calculate the LSMeans.

| | | LSMeans[y] | |
|---|---|---|---|
| Population[z] | Obs. | Number of flowers/ primary inflorescence | Number of flowers/ secondary inflorescence |
| OB × J3-6 | 129 | 7.7d | 3.5d |
| TF × J3-6 | 3 | 8.7abcde | 2.0bcd |
| OB ×M4-4 | 3 | 7.0abcde | 2.7bcd |
| SC × M4-4 | 105 | 14.8a | 6.7a |
| J4-6 × RF | 30 | 13.3abce | 6.4abc |
| J3-3 × RF | 15 | 10.4ce | 4.1abcd |
| VS × J14-3 | 6 | 11.0abce | 2.7abcd |
| J14-3 × VS | 126 | 13.6a | 4.3bc |
| J14-3 × SC | 63 | 13.0ab | 5.1ab |
| M4-4 × SC | 24 | 11.9abce | 4.0abcd |
| J14-3 × LC | 138 | 9.7c | 3.8cd |
| J14-3 × RF | 186 | 13.4a | 4.7b |
| OB × RF | 3 | 8.0abcde | 0.0 |
| SC × J14-3 | 9 | 9.1bcd | 2.8bcd |
| M4-4 × 97/7-2 | 66 | 14.3a | 5.7ab |
| SC × 97/7-2 | 9 | 15.7a | 4.6abcd |

[z]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'.
[y]LSMeans within the components connected by the same letter are not significantly different among populations at $P \leq 0.05$, with LSD adjustment.

**Table 33.** Changes of number of flowers per primary/secondary inflorescence in 16 populations among spring, summer, and fall in College Station, 2015. $Log_{10}$ transformed data was used.

| | No. Flowers/primary[y] | | | | No. Flowers/secondary | | | |
|---|---|---|---|---|---|---|---|---|
| Population[z] | Obs. | Spring | Summer | Fall | Obs. | Spring | Summer | Fall |
| OB × J3-6 | 129 | 10.74a | 8.79a | 3.51b | 63 | 5.29a | 3.67a | 1.57b |
| TF × J3-6 | 3 | 8.00a | 8.00a | 10.00a | 3 | 2.00a | 2.00a | 2.00a |
| OB × M4-4 | 3 | 10.00a | 7.00a | 4.00a | 3 | 5.00a | 2.00a | 1.00a |
| SC × M4-4 | 105 | 22.57a | 13.23b | 8.54c | 81 | 10.89a | 5.44b | 3.67c |
| J4-6 × RF | 30 | 20.20a | 15.30a | 4.50b | 12 | 10.75a | 7.50a | 1.00b |
| J3-3 × RF | 15 | 14.60a | 12.60a | 4.00b | 12 | 5.75a | 5.00a | 1.50a |
| VS × J14-3 | 6 | 16.00a | 9.50a | 7.50a | 3 | 3.00a | 3.00a | 2.00a |
| J14-3 × VS | 126 | 17.98a | 14.00b | 8.69c | 72 | 6.25a | 3.96b | 2.75b |
| J14-3 × SC | 63 | 16.62a | 12.43b | 10.00b | 45 | 7.00a | 4.47b | 3.87b |
| M4-4 × SC | 24 | 18.00a | 11.88ab | 5.88b | 12 | 6.25a | 3.25a | 2.50a |
| J14-3 × LC | 138 | 12.89a | 8.89b | 7.39b | 93 | 5.42a | 3.48b | 2.61b |
| J14-3 × RF | 186 | 15.84a | 9.63b | 14.79a | 156 | 6.08a | 2.92b | 5.19a |
| OB × RF | 3 | 14.00a | 4.00a | 6.00a | 0 | - | - | - |
| SC × J14-3 | 9 | 16.67a | 6.33b | 4.33b | 6 | 2.00a | 2.00a | 4.50a |
| M4-4 × 97/7-2 | 66 | 17.68a | 14.50a | 10.64b | 51 | 7.29a | 5.76ab | 4.00b |
| SC × 97/7-2 | 9 | 25.00a | 16.67a | 5.33b | 9 | 7.67a | 4.67ab | 1.33b |
| Overall | 915 | 15.28a | 10.80b | 7.96c | 621 | 6.04a | 3.94b | 2.63c |

[z]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'.

[y]LSMeans within the components connected by the same letter are not significantly different among seasons at $P \leq 0.05$, with LSD adjustment.

**Genetic analysis of number of flowers per primary/secondary inflorescence**

The number of flowers per primary inflorescence (primary flowers) and the number of flowers per secondary inflorescence (secondary flowers) had a very low narrow sense heritability (0.01 and 0.06 respectively) and a low broad sense heritability (0.43 and 0.34 respectively (Table 34). Narrow (0.01 – 0.06 vs 0.33 - 0.37) and broad (0.34 – 0.43 vs 0.67 – 0.91) sense heritabilities of the primary and secondary flowers were lower than that of flower diameter, petal number, and flower dry weight (Tables 10, 28, 29, and 34) collected on the same populations and in the same environments. The non-additive genetic effect of the number of flowers per primary/secondary inflorescence (0.022/0.014) was larger than additive effect (0.001/0.003) (Table 34). The G×E effect for the number of primary and secondary flowers accounted for 55.7% and 57.0% of total variance, respectively (Table 34). Consequently the Vgxe/Vg ratio for primary and secondary flowers was 3.94 and 5.83 which are larger than the Vgxe/Vg ratio for the flower size trait (Table 29 and 34).

**Table 34.** Restricted estimated maximum likelihood (REML) model used to calculate genetic variance for number of flowers per primary and secondary inflorescence in 16 diploid populations among spring, summer, and fall in College Station, 2015 in the field experiment.

| | Number of flowers/primary[y] | Number of flowers/secondary |
|---|---|---|
| obs. | 924 | 621 |
| $r^2$ | 0.65 | 0.53 |
| | Percentage of total variance | |
| FP[z] | 0 | 0 |
| PP | 0.3 | 1.9 |
| Progeny[FP, PP] | 13.8 | 7.9 |
| Season | 30.1 | 33.2 |
| Season × FP | 11.3 | 5.6 |
| Season × PP | 15.1 | 16.9 |
| Environment × Progeny[FP, PP] | 29.3 | 34.5 |
| | Genetic variance | |
| Va[x] | 0.001 | 0.003 |
| Vd | 0.022 | 0.014 |
| Vg | 0.022 | 0.017 |
| Vp | 0.051 | 0.057 |
| Vgxe | 0.087 | 0.050 |
| Vgxe/Vg | 3.941 | 5.834 |
| | Heritability | |
| $h^2$ | 0.01 | 0.06 |
| $H^2$ | 0.43 | 0.34 |

[z]FP = female parent, PP = pollen parent, $h^2$ = narrow sense heritability, $H^2$ = broad sense heritability. $Log_{10}$ transformed data were used.
[y]$Log_{10}$ transformed data were used.
[x]Va = Parental variances, Vd = progeny variance, Ve = variance due to the change of season, Vg = variance due to parents and progeny, Vgxe = variance due to the interaction of genotype and environment, Vp(phenotypic variance) = (Va+Vd+Vgxe/E), $h^2$ = Va/Vp, $H^2$ = (Va+Vd)/Vp, E = number of seasons.

**Percentage analysis of number of flowers per tertiary inflorescence**

Number of flowers per tertiary inflorescence was analyzed as a Boolean data type because very few plants have tertiary inflorescences. Therefore, 0 and 1 were used to indicate whether a certain bush had a tertiary inflorescence or not.

The percentage of plants that have tertiary inflorescences varied among 16 populations and 3 seasons (Table 35). The population by season effect was significant (Table 35). Among the populations, M4-4 × 97/7-2, J14-3 × RF, and SC × 97/7-2 had the highest percentage of tertiary inflorescence at 70%, 62% and 60%, respectively (Table 36). Three of the 16 populations (TF × J3-6, OB × M4-4, and J3-3 × RF) did not have any tertiary inflorescences (Table 36).

Five populations (OB ×J3-6, VS ×J14-3, OB ×RF, SC × J14-3, and SC × 97/7-2) had tertiary flower shoots during the spring or fall but did not have any during summer (Table 36). The percentage of tertiary inflorescences ranged from 0% to 70% among the 16 populations (Table 36). Over all plants, only about 26% of the plants had tertiary inflorescences (Table 36). The seasonal effect over all plants was calculated by using the percentage of tertiary inflorescence of different populations in different seasons. Therefore, the overall percentage was different from the LSMeans percentage. There was no average seasonal effect over all populations although the populations produced different numbers of tertiary inflorescences in the various seasons as evidenced by a highly significant interaction effect (Tables 35 and 36).

**Table 35.** Nominal logistic fit of population, season, and population by season interaction effect in College Station, 2015 during spring, summer, and fall for number of flowers per tertiary inflorescence.

| | DF | Chi Square[z] |
|---|---|---|
| Population | 15 | 110.47*** |
| Season | 2 | 7.22[NS] |
| Population × Season | 30 | 48.19*** |

[zNS, *, **, ***] Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

**Table 36.** Percent of plants with tertiary inflorescences in 16 diploid rose populations over 3 seasons (spring, summer, and fall) in College Station field plots (2015).

| Population[z] | Obs. | # of tertiary[y] | Overall/ % | Spring/% | Summer/% | Fall/% |
|---|---|---|---|---|---|---|
| OB × J3-6 | 112 | 7 | 6.25 | 6.25a | 0.00a | 0.00a |
| TF × J3-6 | 6 | 0 | 0.00 | 0.00a | 0.00a | 0.00a |
| OB ×M4-4 | 20 | 0 | 0.00 | 0.00a | 0.00a | 0.00a |
| SC × M4-4 | 119 | 43 | 36.13 | 30.25a | 7.56b | 9.24b |
| J4-6 × RF | 192 | 32 | 16.67 | 14.06a | 5.73b | 1.56b |
| J3-3 × RF | 9 | 0 | 0.00 | 0.00a | 0.00a | 0.00a |
| VS × J14-3 | 12 | 2 | 16.67 | 16.67a | 0.00b | 0.00b |
| J14-3 × VS | 93 | 21 | 22.58 | 18.28a | 3.23b | 5.38b |
| J14-3 × SC | 55 | 22 | 40.00 | 38.18a | 1.82b | 7.27b |
| M4-4 × SC | 20 | 6 | 30.00 | 30.00a | 5.00b | 5.00b |
| J14-3 × LC | 50 | 22 | 44.00 | 42.00a | 8.00b | 2.00b |
| J14-3 × RF | 130 | 81 | 62.31 | 54.62a | 2.31c | 15.38b |
| OB × RF | 158 | 6 | 3.80 | 2.53a | 0.00a | 1.27a |
| SC × J14-3 | 27 | 4 | 14.81 | 11.11a | 0.00a | 3.70a |
| M4-4 × 97/7-2 | 20 | 14 | 70.00 | 50.00a | 35.00a | 10.00b |
| SC × 97/7-2 | 5 | 3 | 60.00 | 60.00a | 0.00b | 0.00b |
| Overall | 1028 | 263 | 25.58 | 23.37 | 4.29 | 3.80 |

[z]J14-3 = J06-20-14-3, J4-6 = J06-28-4-6, J3-6 = J06-30-3-6, J3-3 = J06-30-3-3, OB = 'Old Blush ', LC = 'Little Chief', RF = 'Red Fairy', TF = 'The Fairy', SC = 'Sweet Chariot', VS = 'Vineyard Song'.
[y]LSMeans within the components connected by the same letter are not significantly different among seasons at $P \leq 0.05$, with LSD adjustment.

### 3.8 Discussion

As expected, all flower size traits differed among populations (Table 20 and 24). Heat stress caused by either a heat shock treatment or by summer temperatures caused the flower size to decrease whether measured in terms of flower diameter (~16-18%), petal number (~20-23%), or flower dry weight (~17-31%) (Tables 7, 20 and 25). Previous reports of the decrease flower size of both cut flower roses and garden roses agree with the results in this study (Shin et al., 2001; Greyvenstein, 2013; Greyvenstein, 2014).

The interaction effect indicated whether the genetic materials differed in heat tolerance. In a previous study, the decrease in flower dry weight of garden roses in response to increasing temperature was shown to differ among the rose varieties studied (Greyvenstein, 2013). In this field study, flower diameter and dry weight showed a differential population response to the summer heat stress as indicated by significant population by season interaction. When this differential response to warm (summer) versus cool (spring/fall) was examined as a heat tolerance index (flower size in warm season/flower size in cool season) there were population differences in heat tolerance as measured by flower diameter and flower dry weight. In contrast, although petal number decreased under higher temperature conditions, there is little evidence of a differential population response which is consistent with a previous study with cut rose germplasm (Gitonga et al., 2014).

Data were correlated among flower size traits and among flower production traits but not between flower size and production traits. Gitonga et al. (2014) also did not find correlations between flower size and productivity traits.

In this study, flower size showed moderate narrow sense heritability (0.35, 0.33/0.33, and 0.37 for flower diameter, petal number, and flower dry weight, respectively) and moderately high to high broad sense heritability (0.67, 0.91/0.85, and 0.81 for flower diameter, petal number, and flower dry weight, respectively) indicating important additive and non-additive genetic effects (Table 28 and 29). The G×E effect for flower diameter, petal number, and flower dry weight was about 37.0%, 15.3 – 23.0%, and 27.0%, respectively indicating a moderate G×E effect (Tables 28 and 29). The genetic analysis of the heat tolerance index indicated a low narrow sense heritability for the heat tolerance calculated with flower diameter ($h^2 = 0.31$) and flower dry weight ($h^2 = 0.24$) data although in both cases given the low $r^2$ of the analysis, the reliability of the estimates was low (Table 30).

High genetic variance and moderately low G×E interaction variance indicated that good genetic progress can be made by selecting for flower size in either cool and warm seasons. Nevertheless, as seen in the analysis of variance, there were differences in how the populations responded to warm temperatures with respect to flower size so among elite materials it would be best to evaluate them during the warm season as well. Among three flower size traits we used in this study, flower diameter would be the easiest for selection in the field and from a consumer point of view it is the most visible trait as well.

The number of flowers per primary, secondary and tertiary inflorescence were different among populations and seasons. Among the 16 populations, the population SC × M4-4 had the highest flower density. Flower production was highest in the spring and decreased as the season progressed. However, we cannot conclude that flower production per inflorescence was directly affected by heat stress as with the flower size traits as the flowers per primary/secondary/tertiary inflorescences in the spring were higher than that seen in the summer and fall. In this study, the flower number in the warm summer season was not less than cool season, and spring was not equal to fall which indicates the changes were not only due to the heat effect (Table 33). As the data was taken on only new growth since the last pruning (done February/March, June, and September for spring, summer, and fall), the time for growth as well as the diameter from which this growth emerged was different between seasons and consequently were important confounding factors. In future work, the time from pruning to data collection should be standardized.

The restricted estimated maximum likelihood indicated about 55.7% and 57.0% of the variance was due to the G×E interaction for number of flowers per primary and secondary inflorescence, respectively (Table 34). Therefore, selection needs to be conducted within the season for progress.

# CHAPTER IV

# CONCLUSIONS

The work in this thesis assesses the effect of heat stress and the inheritance of heat tolerance as measured by petal number, flower diameter, and flower dry weight in diploid roses. Heat stress was applied artificially with a one hour heat shock (44°C) in the heat chamber experiment in Chapter II, and by natural heat stress in the field experiment in Chapter III.

## 4.1 Flower size

Heat stress caused a 15.7% - 30.8% loss in flower size (Tables 7, 20, and 25). In addition, all three flower size traits (flower diameter, petal number, and flower dry weight) differed among populations in the field and in the heat shock experiment. In the heat shock experiment, there were differences among populations and among the progeny within the families in flower size. The populations that have the largest flower diameter were OB × M4-4 and J14-3 × VS, and the largest flower dry weight populations were SC × J14-3 and OB × RF. The populations SC × J14-3 and VS × J14-3 had the highest petal number.

The high non-additive variance of petal number in double flowers (40.9% - 55.4% of total genetic variance) indicating major gene effects in petal number determination among double flowered rose genotypes. The variance analysis indicated that 16.7% - 19.8% and 26.6% - 32.0% of the genetic variance was additive variance for flower diameter and flower dry weight, respectively. Flower size traits had moderately low narrow sense (0.24-0.35, 0.12-0.33, and 0.34-0.37 for flower diameter, petal

number, and flower dry weight respectively) and moderately high to high broad sense (0.62-0.67, 0.74-0.91, and 0.76-0.81 for flower diameter, petal number, and flower dry weight respectively) heritability. This indicates the importance of additive and non-additive genetic effects. The two flower size traits (flower diameter and flower dry weight) that showed a significant genotype by heat stress effect in the analysis of variance showed a moderate G×E effect (37.0% and 27.0% of variance respectively) in the REML analysis.

As flower diameter shows the most consistent population based differential response to heat stress, the highest G×E genetic variance (37.0% of total variance), is easy to measure in the field and is the most visible trait from a consumer perspective, it is the best flower size trait to use for the selection of heat tolerant roses.

When the data is transformed into a heat tolerance index (stressed flower diameter/non stressed flower diameter), population based differences in heat tolerance were visible and there is a moderate narrow sense heritability (0.30) indicating an ability to improve on this trait. Given that the genetic variation is several fold higher than the G×E variance, selection for flower diameter would be effective irrespective of the season of selection but to develop a stable flower diameter over cool and warm seasons, selection for flower diameter during the both the cool and warm seasons would be required.

**4.2 Flower production**

Flower productivity as measured by the number of flowers per primary/secondary inflorescence and percentage of plants that have tertiary flowers

varies by population and the season. The number of flowers among populations in the field ranged from 7.7 to 14.8 in primary shoots and from 3.5 to 6.7 in secondary shoots. The percentage of plants that had tertiary flowers ranged from 0% to 70%. The population that has the highest flower production is SC × M4-4. The population OB × J3-6 had the fewest flowers per primary/secondary inflorescence. Over all plants, the number of flowers per primary and secondary inflorescence and percentage of plants that have tertiary flowers was highest in the spring and decreased as the season progressed. Therefore, the seasonal changes in the flower production traits were not considered strictly as heat related but rather were strongly affected by the stem diameter from which the regrowth was derived (thicker in spring versus summer and fall) as well as the time from pruning to data collection. In the future it is suggested to standardize the time from pruning to data collection at 2 months.

The analysis of variance indicated that the number of flowers per primary/secondary inflorescence showed significant population by season effect. Both additive variance and dominant variance for these two components were relatively low (0.3% – 1.9% and 13.8% – 7.9%) as compared to flower diameter, petal number, and flower dry weight. This results in low narrow sense heritability (0.01 – 0.06) and moderate broad sense heritability (0.43 – 0.34). The covariance between genetic and the environmental effects (55.7% - 57.0%) were higher than the 3 flower size traits (15.3% - 37.0%). The large G×E effect indicates that selection should be made within each season.

## REFERENCES


Ahmad, I., J.M. Dole, M.A. Khan, M. Qasim, T. Ahmad, and A. S. Khan. 2010. Present status and future prospects of cut rose production in Punjab, Pakistan. HortTechn. 20:1010–1015.

Ashraf, M., and M. R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. and Exp. Botany 59:206–216.

Bernardo, R. 2010. Breeding for quantitative traits in plants. Stemma Press.

Blom, T. J., and M. J. Tsujita. 2003. Cut rose production, p. 594-600. In: A. V. Roberts, T. Debener, and S. Gudin (eds.). Encyclopedia of Rose Science. Elsevier Academic Press, Oxford.

Blum, A., and A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21:43-47.

Blum, A., N. Klueva, and H. T. Nguyen. 2001. Wheat cellular thermotolerance is related to yield under heat stress. Euphytica 117:117–123.

Byrne, D. H., N. Anderson, M. Orwat, and V. Soules. 2010. Field assessment of black spot resistance in roses in a hot humid climate. Acta Hort. 870:115–120.

Cairns, T., 2001. The geography and history of the rose. Amer. Rose Ann. 18–29.

Chauhan, Yashvir S., and T. Senboku. 1996. Thermostabilities of cell-membrane and photosynthesis in cabbage cultivars differing in heat tolerance. J. Plant Physiol. 6:729-734.

Chen, H., Z. Shen, and P. H. Li. 1982. Adaptability of crop plants to high temperatures stress. Crop Sci. 22:719-725

Connor, A. M., M. J. Stephens, H. K. Hall, and P. A. Alspach. 2005. Variation and heritabilities of antioxidant activity and total phenolic content estimated from a red raspberry factorial experiment. J. Amer. Soc. for Hort. Sci. 130:403–411.

Debener, T. 1999. Genetic analysis of horticulturally important morphological and physiological characters in diploid roses. Gartenbauwissenschaft. 64:14-19.

Debener, T., and M. Linde. 2009. Exploring complex ornamental genomes: The rose as a model plant. Critical Reviews in Plant Sci. 28:267–280.

Dela, G., E. Or, R. Ovadia, A. Nissim-Levi, D. Weiss, and M. Oren-Shamir. 2003. Changes in anthocyanin concentration and composition in “Jaguar” rose flowers due to transient high-temperature conditions. Plant Sci. 164:333–340.

Dieters, M. J., T. L. White, R. C. Littell, and G. R. Hodge, 1995. Application of approximate variances of variance components and their ratios in genetic tests. Theor. Appl. Gen. 91:15–24.

Duke, J. A., 1992. Tropical botanical extractives, p. 53-62. In: Plotkin, M., and L. Famolare (eds.). Sustainable Harvest and Marketing of Rain Forest Products. Island Press.

Giaveno, C., and J. Ferrero. 2003. Introduction of tropical maize genotypes to increase silage production in the central area of Santa Fe, Argentina. Crop Breeding and Appl. Biotechn. 3:89–94.

Gitonga, V. W., Koning-Boucoiran, C. F., Verlinden, K., Dolstra, O., Visser, R. G., Maliepaard, C., and Krens, F. A. 2014. Genetic variation, heritability and genotype by environment interaction of morphological traits in a tetraploid rose

population. BMC Genetics 15: 146-159.

Greyvenstein, O. F. C., T. W. Starman, H. B. Pemberton, G. Niu, and D. H. Byrne. 2015. Development of a rapid screening method for selection against high temperature susceptibility in garden roses. HortSci. 50:1-8.

Greyvenstein, O. F. C., H. B. Pemberton, T. W. Starman, G. Niu, and D. H. Byrne. 2014. Effect of two-week high-temperature treatment on flower quality and abscission of Rosa L. "Belinda"s dream' and "RADrazz" (KnockOut®) under controlled growing environments. HortSci. 49:701–705.

Greyvenstein, O. F. C., 2013. Phenotyping of high temperature susceptibility in garden roses (*Rosa* × *Hybrida*). Texas A&M Univ., College Station, PhD Diss.

Grossi, S. J. A., H. B. Pemberton, and H. J. Lang. 2004. Influence of cultivar and seasonal growing environment on growth and postharvest characteristics of single-shoot pot rose plants. HortSci. 39:138–141.

Gudin S. 2000. Rose: genetics and breeding, p. 159-190. In: J. Janick (eds.). Plant Breeding Reviews. Purdue Univ. Press, West Lafayette, Ind.

Guilioni, L., J. Wery, and F. Tardieu. 1997. Heat stress-induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs? Ann. Botany 80:159–168.

Hallauer, A. R., M. J. Carena, M. J., and J. B. Miranda Filho. 2010. Means and variances, p. 33-67. In: A. R. Hallauer, M. J. Carena, and J. B. Miranda Filho (eds.). Quantitative Genetics in Maize Breeding. Springer Science Business Media, New York.

Heinrichs, F. 2008. International statistics flower and plants. AIPH, 56:16-90.

Hibrand-Saint Oyant, L., L. Crespel, S. Rajapakse, L. Zhang, and F. Foucher. 2008. Genetic linkage maps of rose constructed with new microsatellite markers and locating QTL controlling flowering traits. Tree Genet. Genom. 4:11–23.

Hodges, Alan W., C. R. Hall, and M. A. Palma. 2015. Economic contributions of the green industry in the United States in 2013. HortTechn. 25:805-814.

Holland, J. B., W. E. Nyquist, and C. T. Cervantes-Martínez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breeding Reviews 22: 9-112.

Hutton, S. 2012. The future of the rose industry. Amer. Rose 41:36–37.

Ingram, D. L., and D. W. Buchanan. 1984. Lethal high temperatures for roots of three citrus rootstocks. J. Amer. Soc. for Hort. Sci. 2: 189-193.

Ismail, A. M., and A. E. Hall. 1999. Reproductive-stage tolerance, leaf membrane thermostability and plant morphology in cowpea. Crop Sci. 39:1762–1768.

Jian, H., H. Zhang, K. Tang, S. Li, Q. Wang, T. Zhang, X. Qiu, and H. Yan. 2010. Decaploidy in *Rosa praelucens* Byhouwer (Rosaceae) endemic to Zhongdian Plateau, Yunnan, China. Caryologia 63:162–167.

Jiao, J., and B. Grodzinski. 1998. Environmental influences on photosynthesis and carbon export in greenhouse roses during development of the flowering shoot. J. Amer. Soc. for Hort. Sci. 123:1081–1088.

Krussmann G. 1981. The Complete Book of Roses. Timber Press, Portland.

Lester, G. E. 1985. Leaf cell membrane thermostabilities of *Cucumis melo*. J. Amer. Soc.

for Hort. Sci. 110:506-509.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 2006. SAS system for mixed models Cary. NC SAS Inst. 2:88-90.

Ma, N., W. Chen, T. Fan, Y. Tian, S. Zhang, D. Zeng, and Y. Li. 2015. Low temperature-induced DNA hypermethylation attenuates expression of RhAG, an AGAMOUS homolog, and increases petal number in rose (*Rosa hybrida*). BMC Plant Biology. 15: 237-249.

Marcum, K. B. 1998. Cell membrane thermostability and whole-plant heat tolerance of Kentucky bluegrass. Crop Sci. 38:1214–1218.

Marissen, N. 2001. Effects of pre-harvest light intensity and temperature on carbohydrate levels and vase life of cut roses. Acta Hort. 543:331–336.

Martineau, J. R., J. E. Specht, J. H. Williams, and C. Y. Sullivan. 1979. Temperature tolerance in soybeans. I. evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. 19:75-78.

McMaster, G. S., and W. Wilhelm. 1997. Growing degree-days: One equation, two interpretations. Agr. For. Meteorol. 87:291–300

Moe, R. 1975. The effect of growing temperature on keeping quality of cut roses. Acta Hort. 41:77–92.

Morrison, M. J., and D. W. Stewart. 2002. Heat stress during flowering in summer brassica. Crop Sci. 42:797–803.

NOAA. 2015. Climate data, date range 1 July, 2013 to 20 Dec., 2015. National Centers for Environmental Information. 30 Dec., 2015. <http://www.ncdc.noaa.gov/cdo-

web/search>

Ott, R., and M. Longnecker. 2008. An Introduction to Statistical Methods and Data Analysis. Cengage Learning.

Pearson, S., A. Parker, S. R. Adams, P. Hadley, and D. R. May. 2015. The effects of temperature on the flower size of pansy (*Viola X Wittrockiana* Gams.). J. Hort. Sci. 70:183-190.

Pemberton, H. B., J. W. Kelly, and J. Ferare. 2003. Pot Rose Production, p. 587–593. In: A. V. Roberts, T. Debener, and S. Gudin (eds.). Encyclopedia of Rose Science. Elsevier Academic Press, Oxford.

Razali, N. M., and Y. B. Wah. 2011. Power comparisons of Shapiro-Wilk , Kolmogorov-Smirnov , Lilliefors and Anderson-Darling tests. J. Stat. Modeling and Analytics. 2:21–33.

Roberts, A. V., T. Debener, and S. Gudin. 2003. Introduction, p. vi-vii. In: A. V. Roberts, T. Debener, and S. Gudin (eds.). Encyclopedia of Rose Science. Elsevier Academic Press, Oxford.

Roman, H., M. Rapicault, A. S. Miclot, M. Larenaudie, K. Kawamura, T. Thouroude, A. Chastellier, A Lemarquand, F. Dupuis, F. Foucher, S. Loustau, and L. Hibrand-Saint Oyant. 2015. Genetic analysis of the flowering date and number of petals in rose. Tree Gen. Genom. 11:1-13.

Shepherd, R. E. 1954. History of the Rose. Macmillan, New York.

Shin, H. K., J. H. Lieth, S. H. Kim. 2001. Effects of temperature on leaf area and flower size in rose. Acta Hort. 547:185–191.

Short, K. C., and A. V. Roberts. 1991. Rosa spp.(roses): In vitro culture, micropropagation, and the production of secondary products. Biotechn. Agric. Forestry 15:376–397.

Shupert, D. 2005. The inheritance of several traits in three diploid interspecific rose populations. Texas A&M University, College Station, MS Thesis.

Soules, A. S. 2009. Analysis of genetic diversity and relationships in the China rose group. Texas A&M University, College Station, MS Thesis.

Stone, P., and M. Nicolas. 1994. Wheat cultivars vary widely in their responses of grain-yield and quality to short periods of postanthesis heat-stress. Australian J. Plant Physiol. 21:887–900.

Ur Rahman, H., S. A. Malik, and M. Saleem. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. Field Crop Res. 85:149–158.

Vineland research and innovation centre. 2013. The innovation report. VRIC. Vineland Station, Ontario, Canada. pp. 1-23.

Vollenweider, P., and M. S. Günthardt-Goerg. 2005. Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. Environ. Pollution 137:455–65.

Wahid, A., S. Gelani, M. Ashraf, and M. Foolad. 2007. Heat tolerance in plants: An overview. Environ. Exp. Botany 61:199–223.

Wahid, A., and A. Shabbir. 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. Plant Growth Regulation

46:133–141.

Weaich, K., K. L. Bristow, and A. Cass. 1996. Modeling preemergent maize shoot growth: II. high temperature stress conditions. Agronomy J. 88:398-403.

Wilhelm, E. P., R. E. Mullen, P. L. Keeling, and G. W. Singletary. 1999. Heat stress during grain filling in maize. Crop Sci. 39:1733-1741.

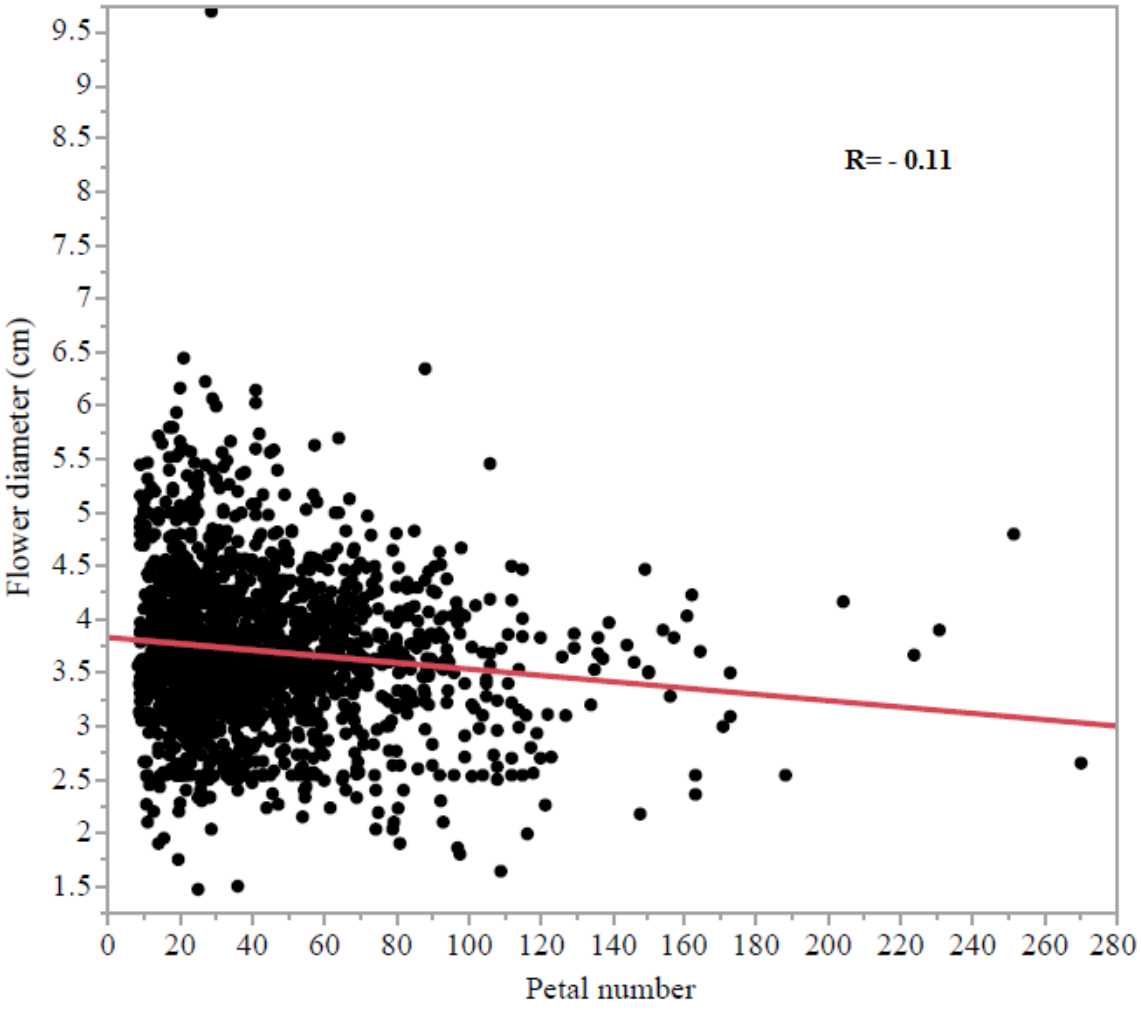
Wissemann, V., and C. M. Ritz. 2007. Evolutionary patterns and processes in the genus *Rosa* (Rosaceae) and their implications for host-parasite co-evolution. Plant Systematics and Evolution 266:79–89.

Yeh, D. M., and H. F. Lin. 2003. Thermostability of cell membranes as a measure of heat tolerance and relationship to flowering delay in chrysanthemum. J. Amer. Soc. for Hort. Sci. 5:656-660.

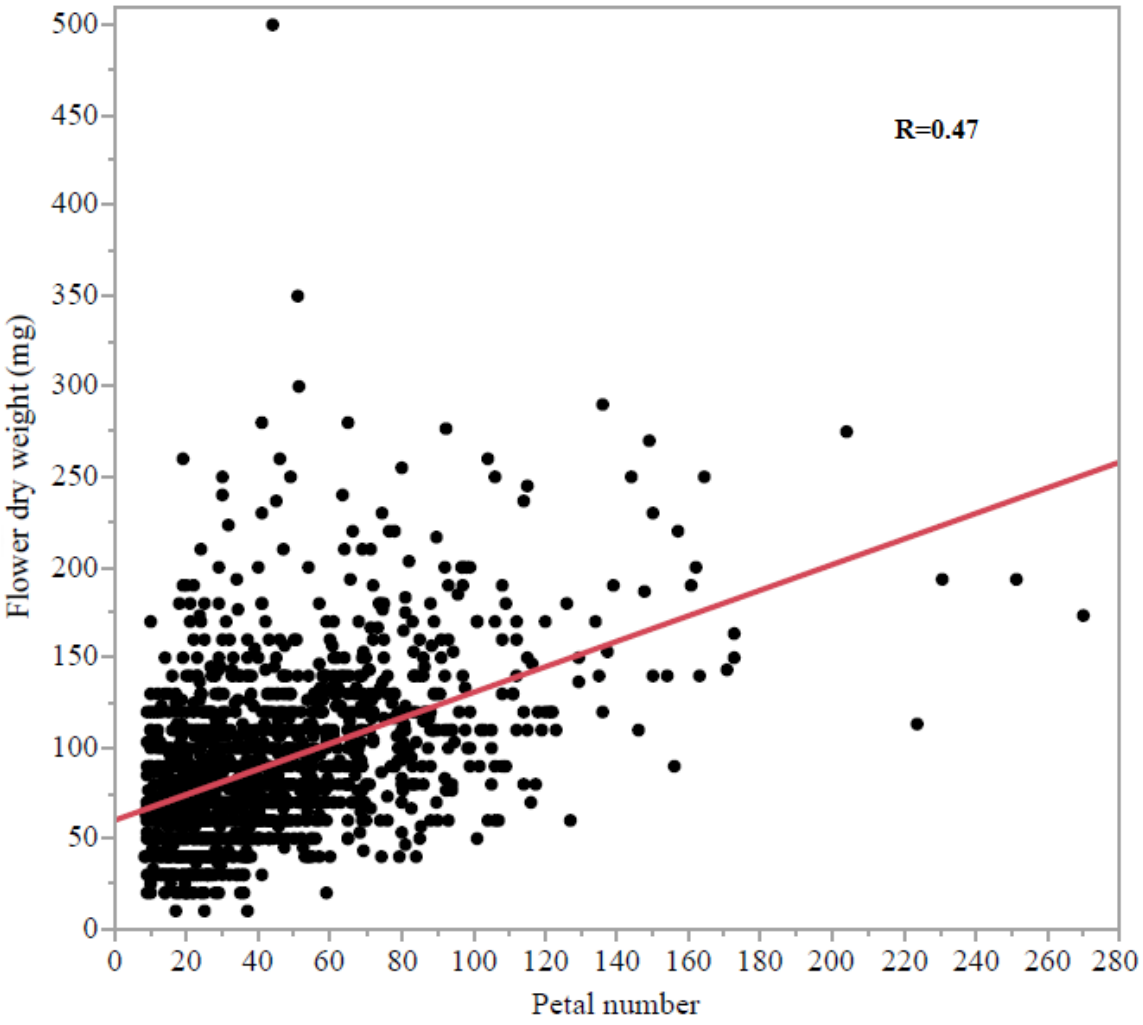
Zlesak, D. C. 2006. Rosa, p. 695–740. In: N. Anderson (eds.). Flower Breeding and Genetics. Springer Netherlands.

## APPENDICES

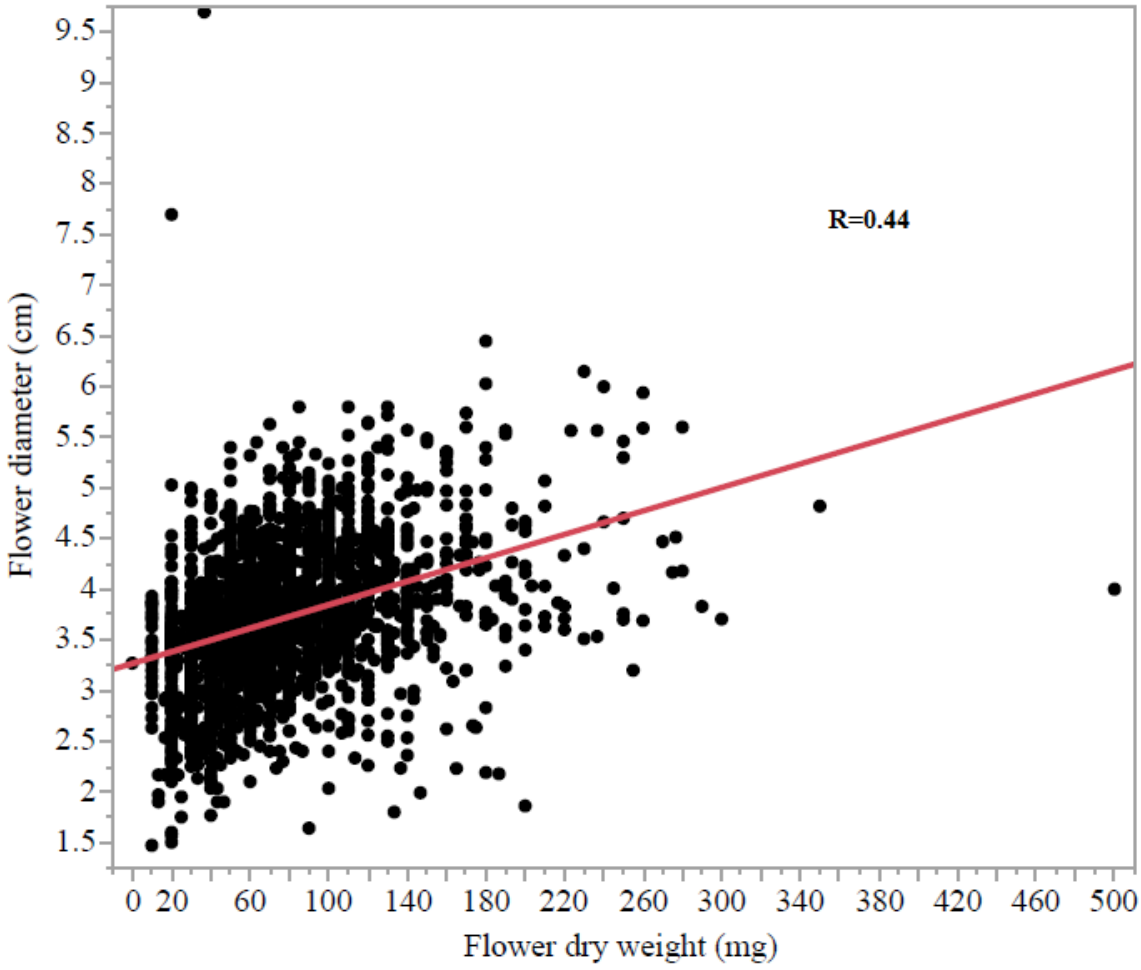
**Appendix 1.** Correlation scatterplot for the petal number and the flower diameter, among 16 populations in College Station, 2015.



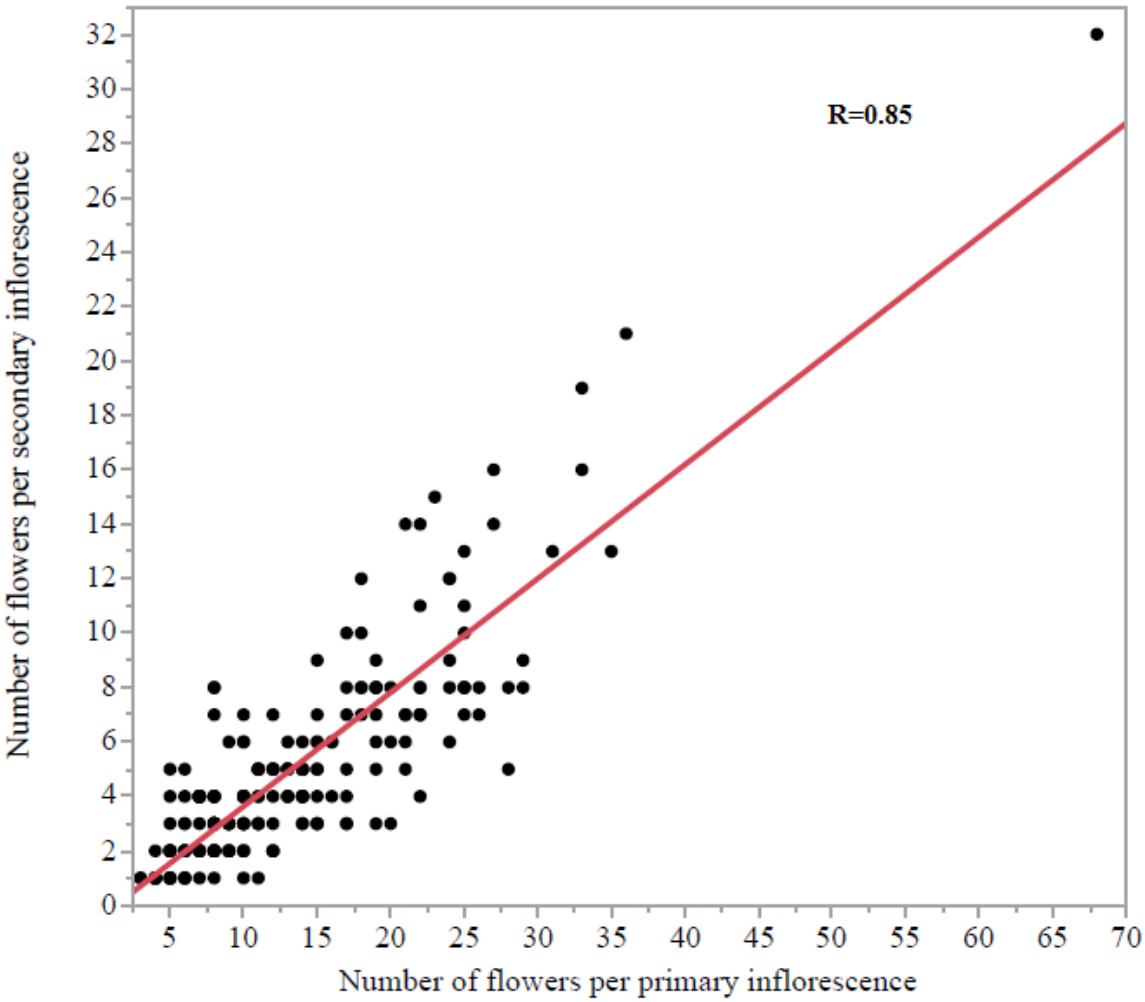
**Appendix 2.** Correlation scatterplot for the petal number and the flower dry weight among 16 populations in College Station, 2015.



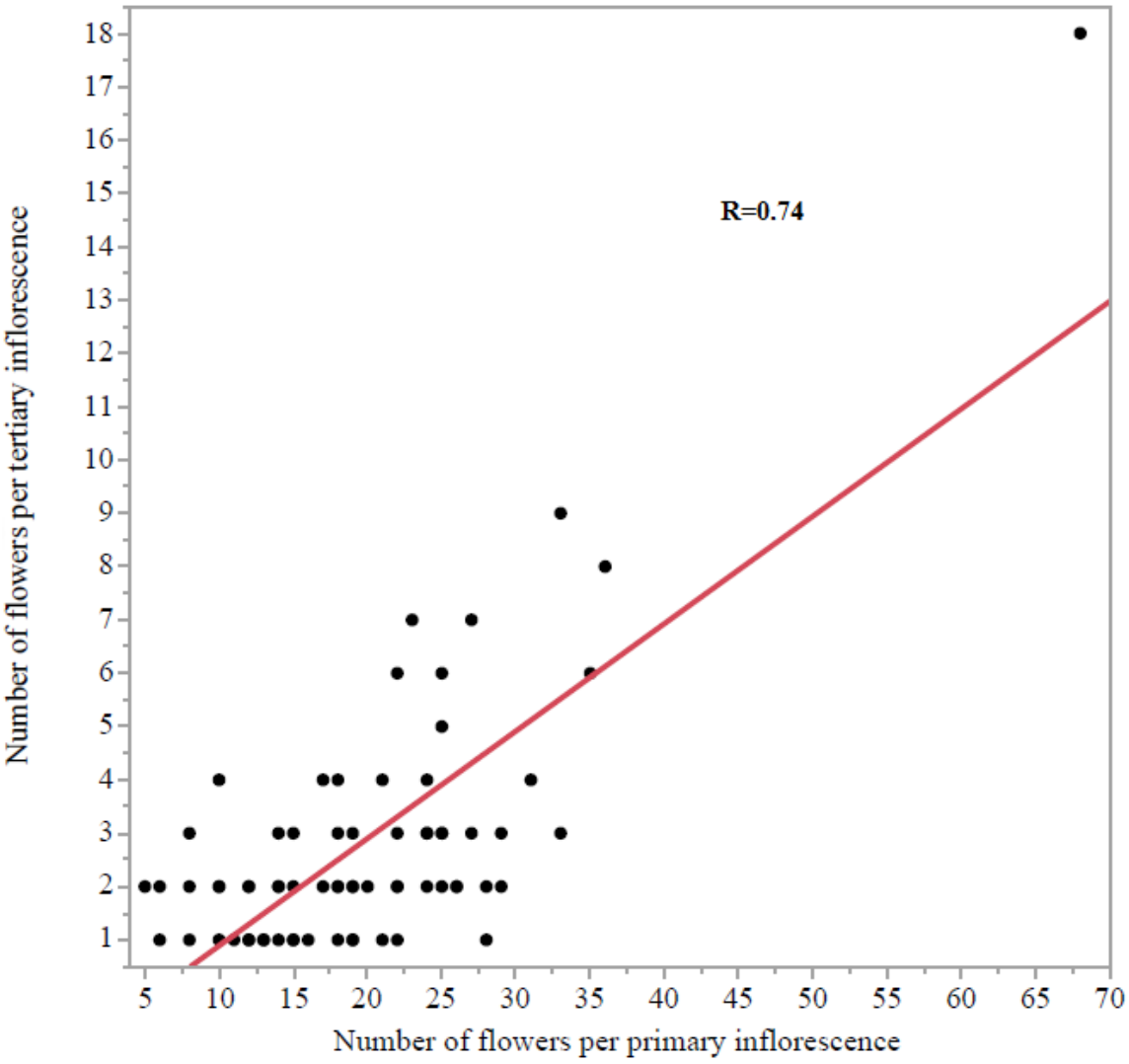
**Appendix 3.** Correlation scatterplot for the flower diameter and the flower dry weight among 16 populations in College Station, 2015.



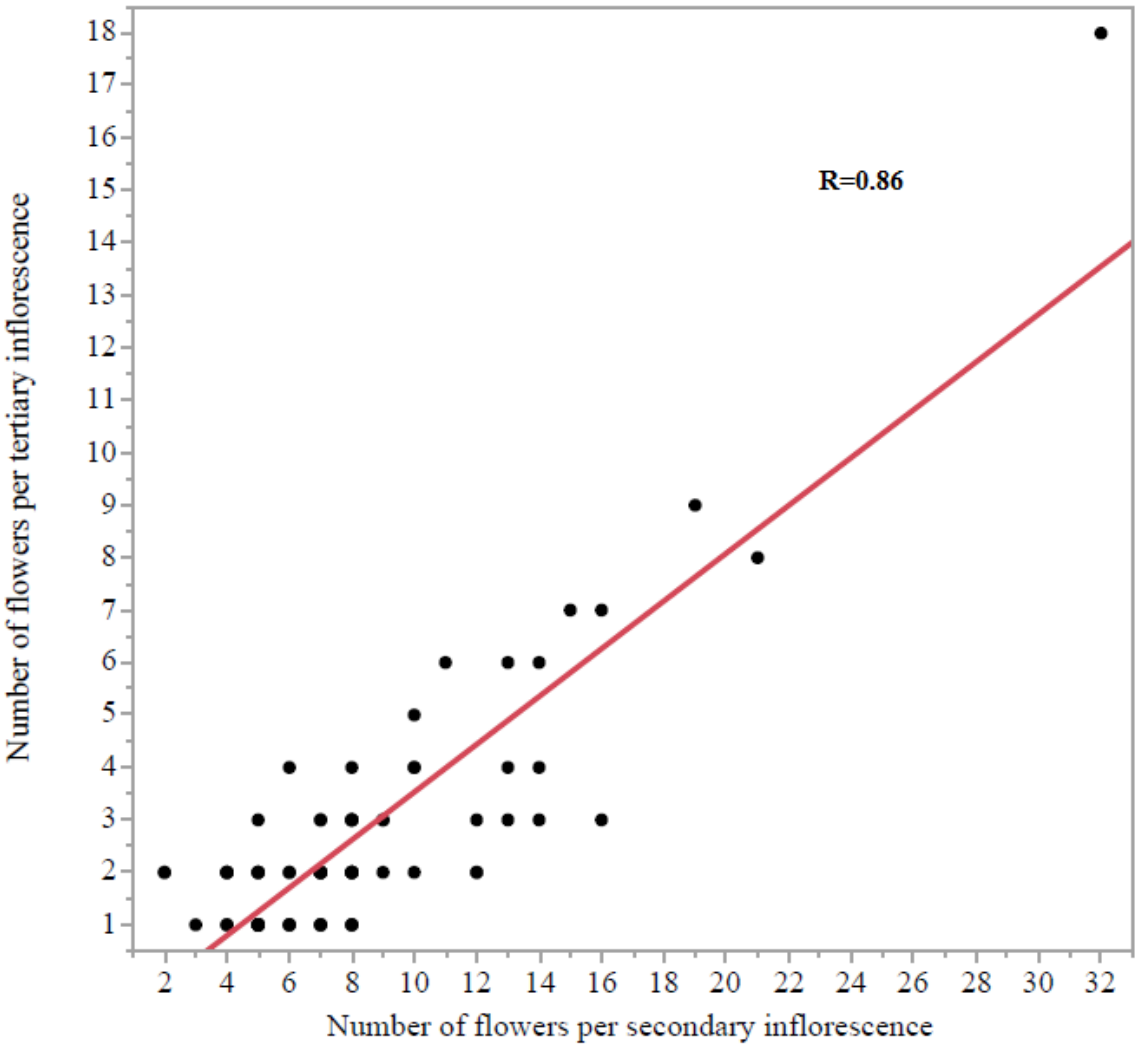
**Appendix 4.** Correlation scatterplot for the number of flowers per primary and secondary inflorescence among 16 populations in College Station, 2015.



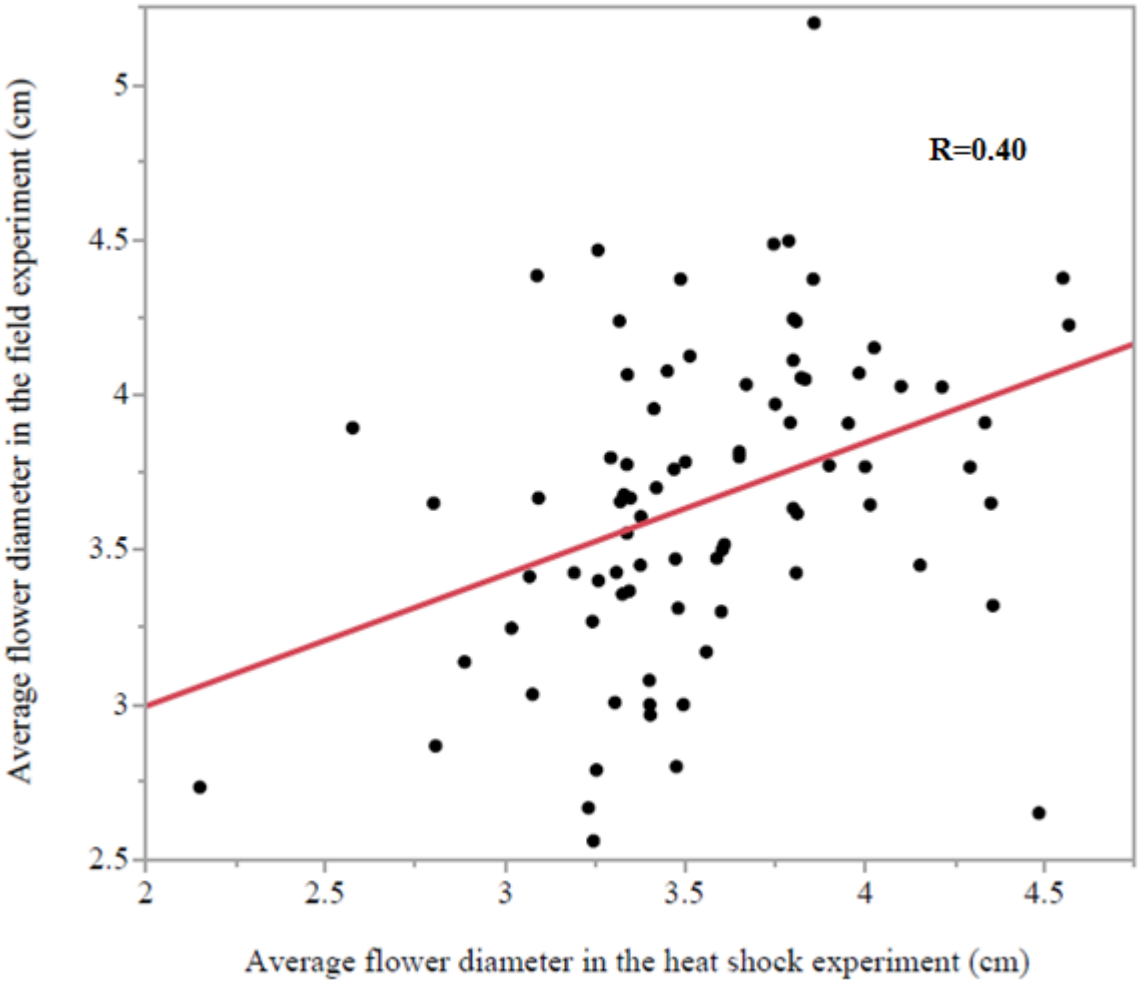
**Appendix 5.** Correlation scatterplot for the number of flowers per primary and tertiary inflorescence among 16 populations in College Station, 2015.



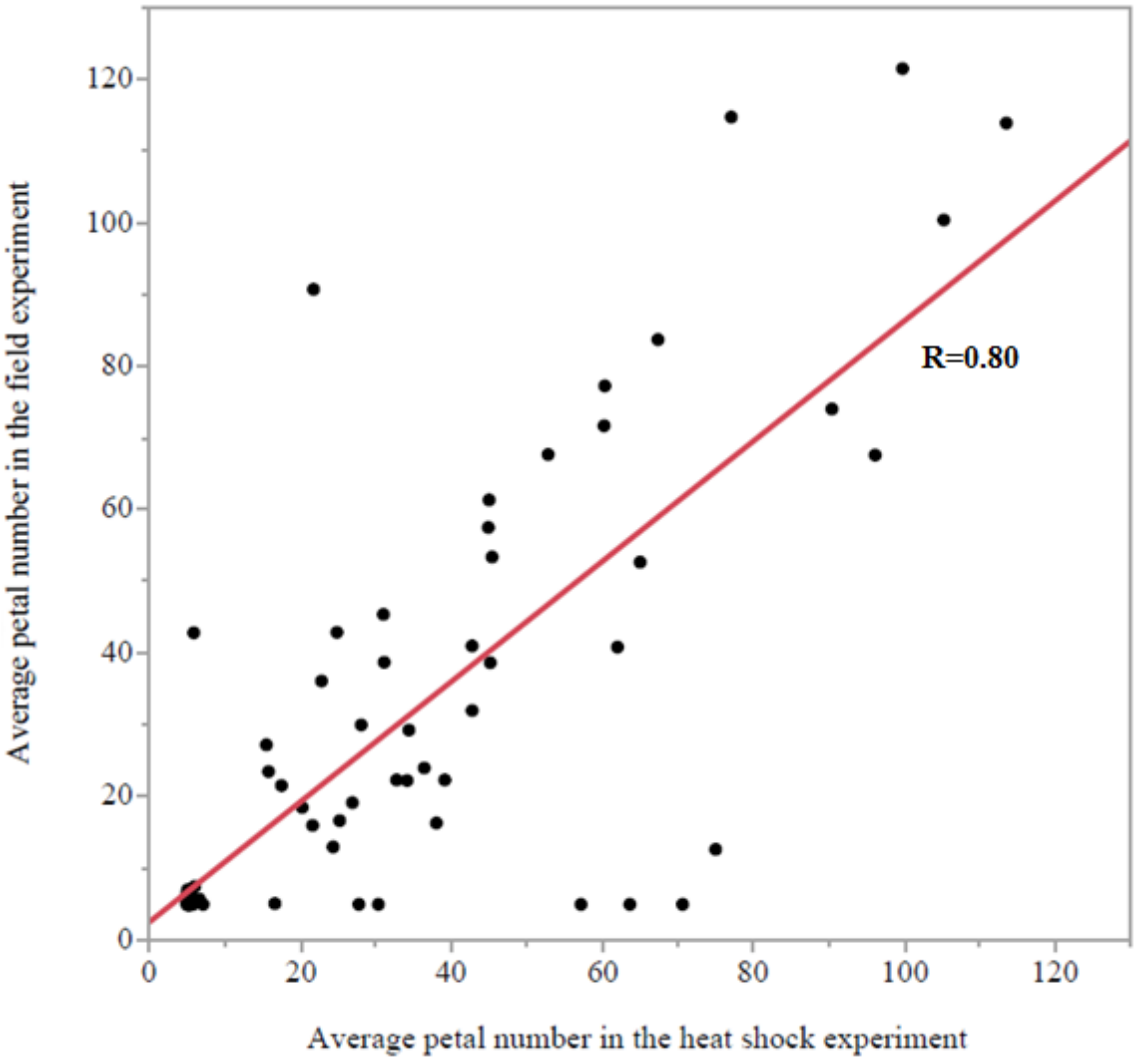
**Appendix 6.** Correlation scatterplot for the number of flowers per secondary and tertiary inflorescence among 16 populations in College Station, 2015.



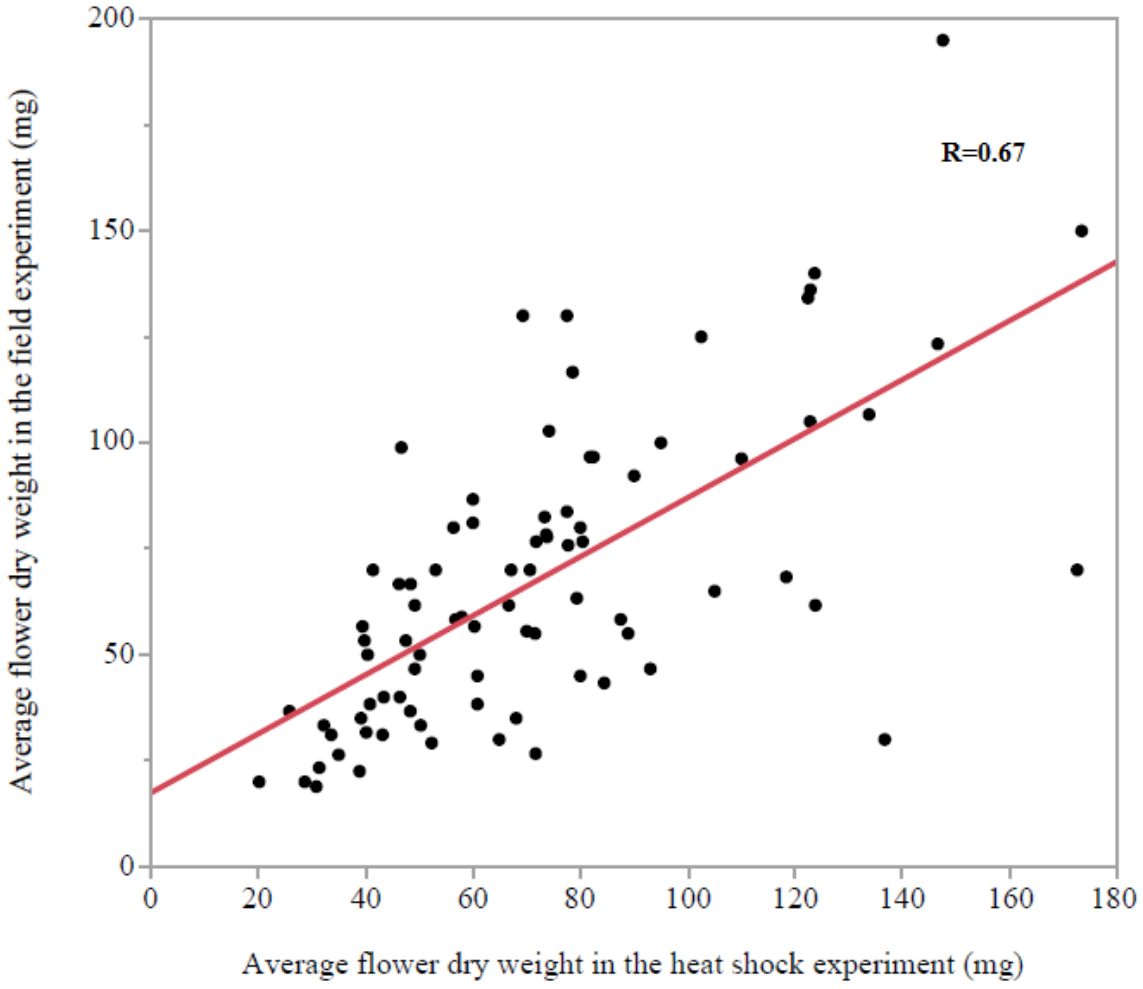
**Appendix 7.** Correlation scatterplot for the average flower diameter between the heat stress experiment and the field experiment among 10 populations in College Station, 2015.



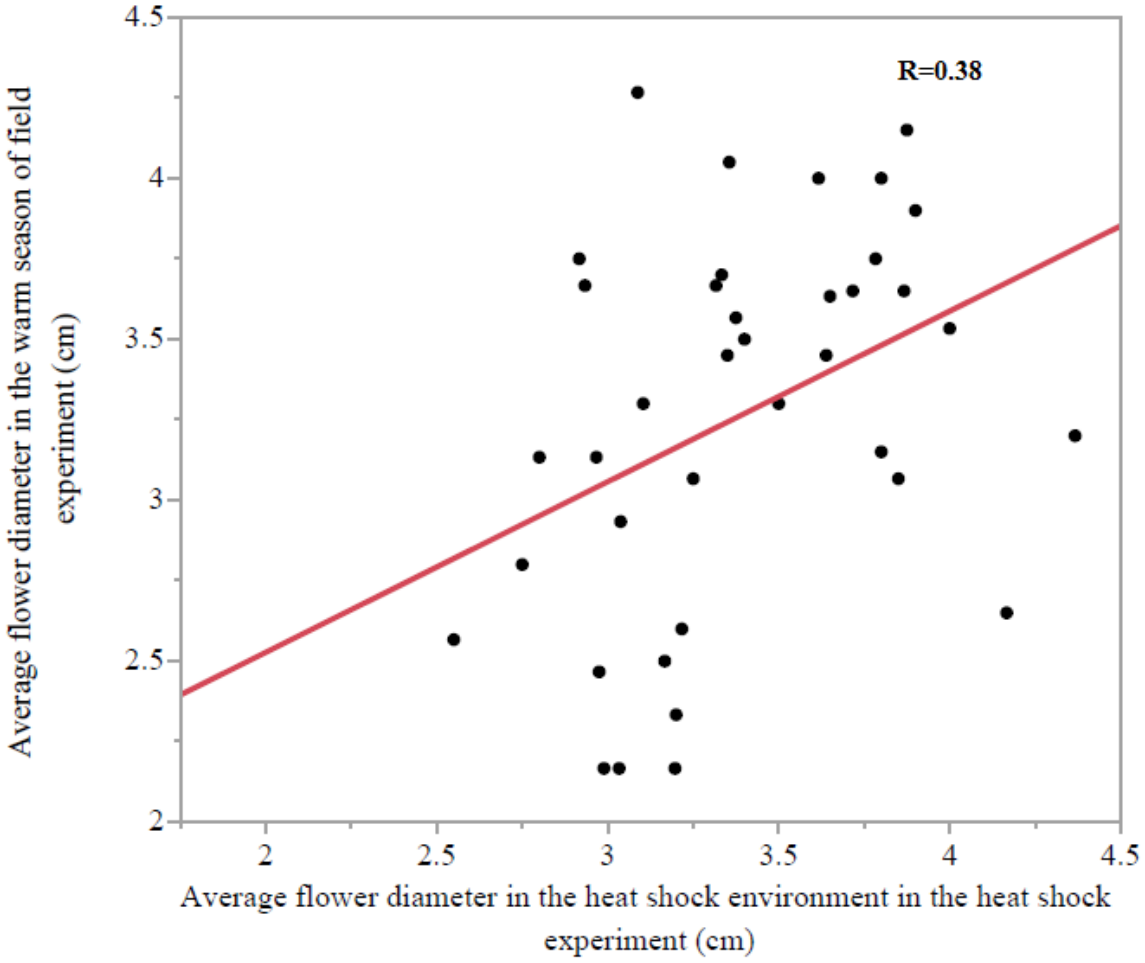
**Appendix 8.** Correlation scatterplot for the petal number between the heat stress experiment and the field experiment among 10 populations in College Station, 2015.



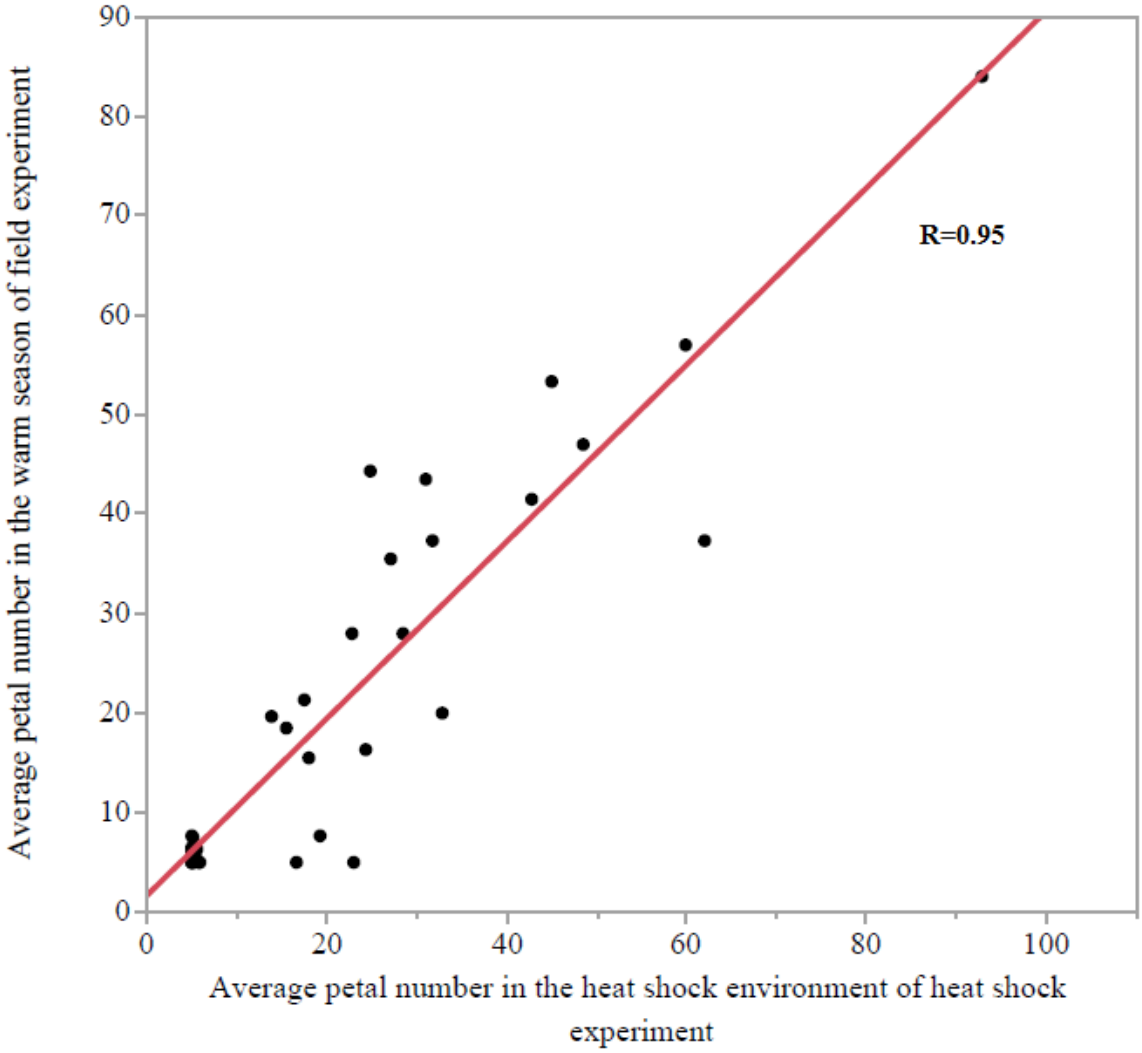
**Appendix 9.** Correlation scatterplot for the flower dry weight between the heat stress experiment and the field experiment among 10 populations in College Station, 2015.



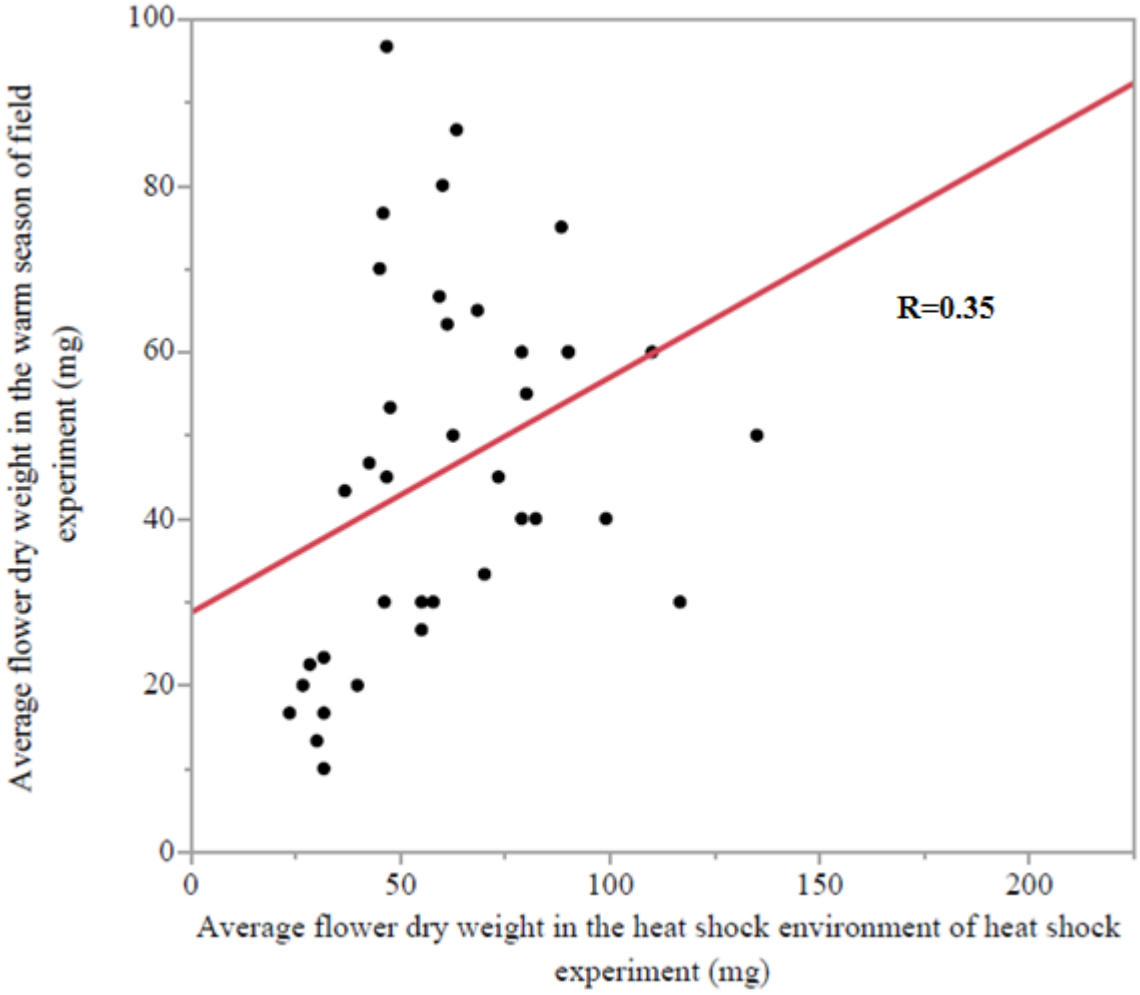
**Appendix 10.** Correlation scatterplot for the flower diameter between the heat stress treatment in the heat stress experiment and the warm season in the field experiment among 10 populations in College Station, 2015.



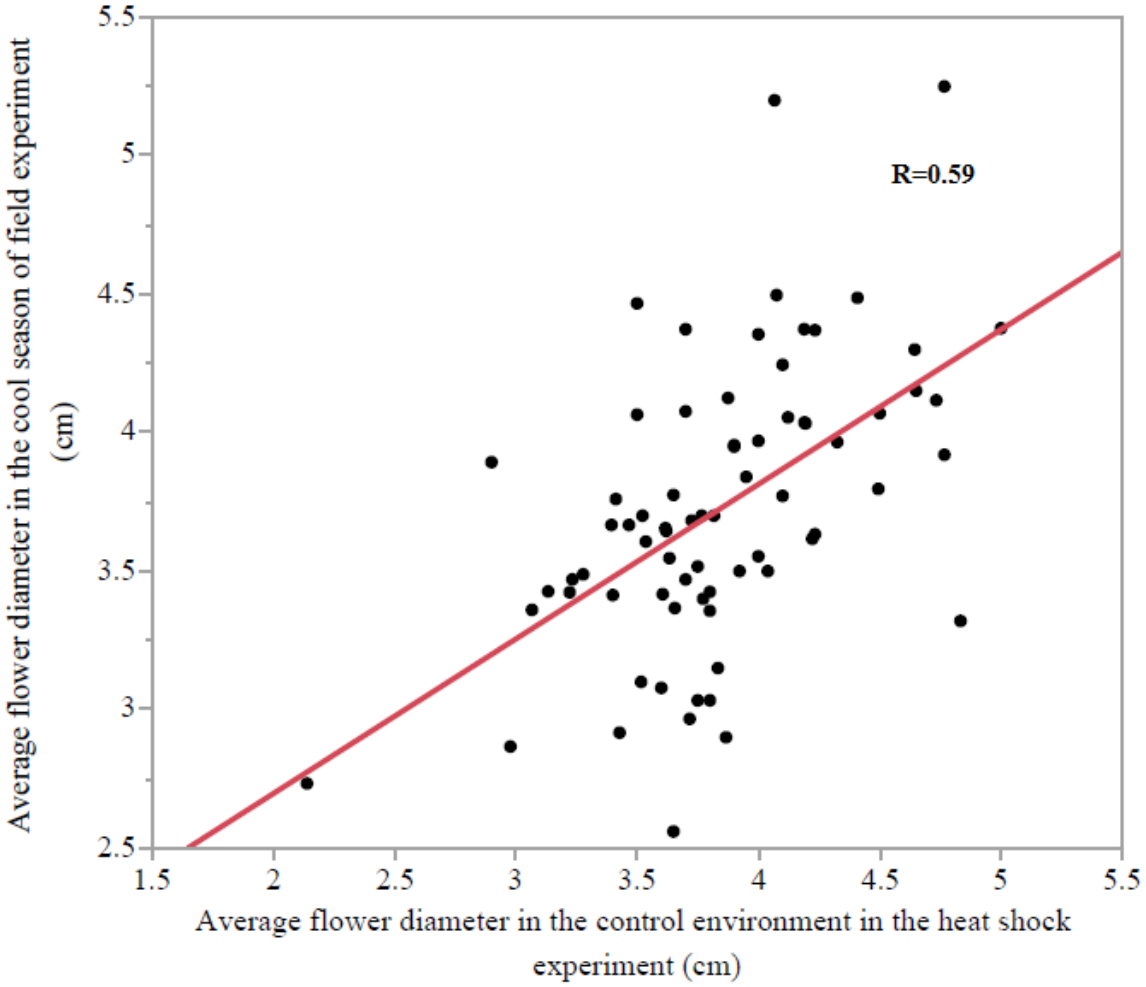
**Appendix 11.** Correlation scatterplot for the petal number between the heat stress treatment in the heat stress experiment and the warm season in the field experiment among 10 populations in College Station, 2015.



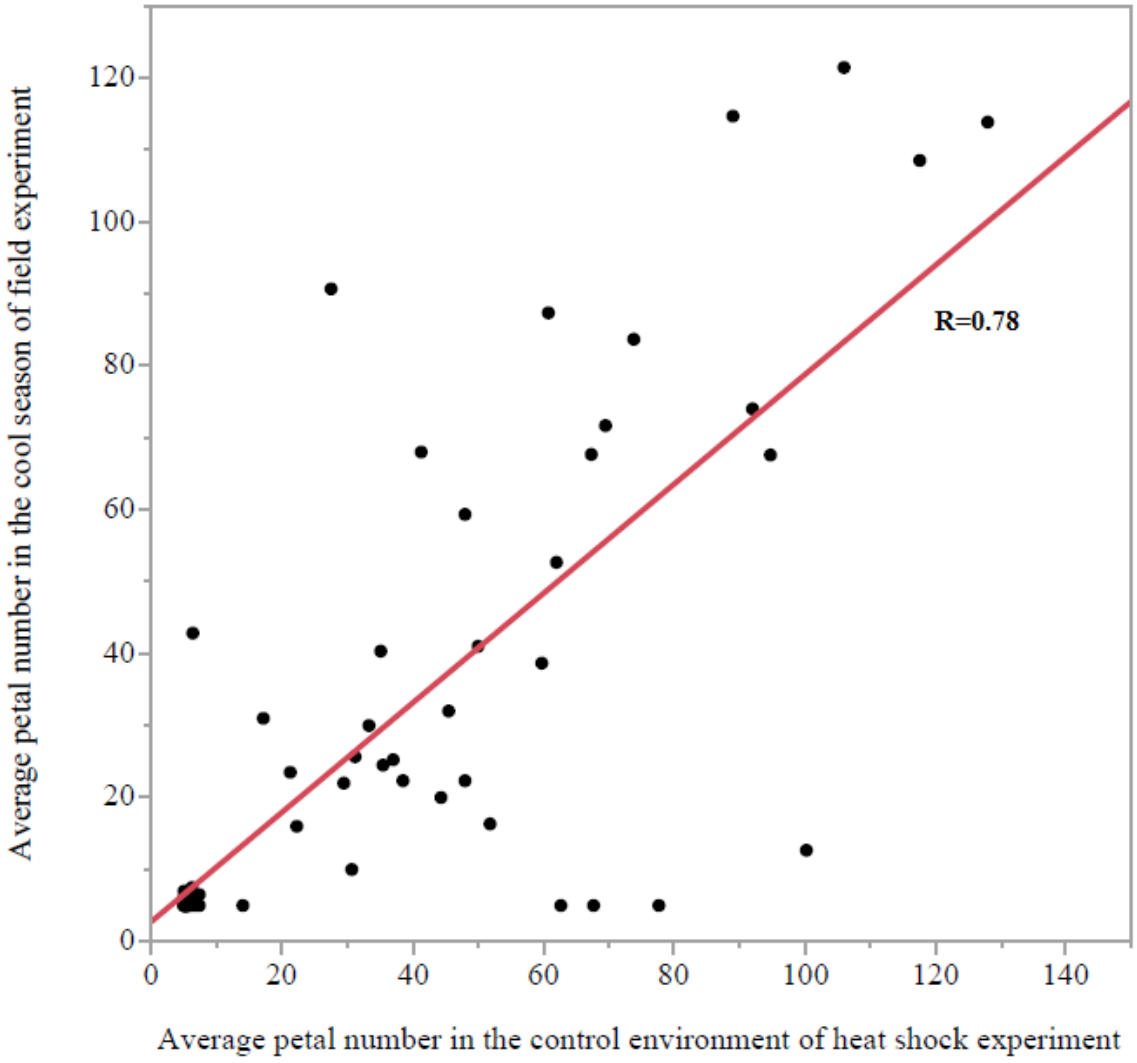
**Appendix 12.** Correlation scatterplot for the flower dry weight between the heat stress treatment in the heat stress experiment and the warm season in the field experiment among 10 populations in College Station, 2015.



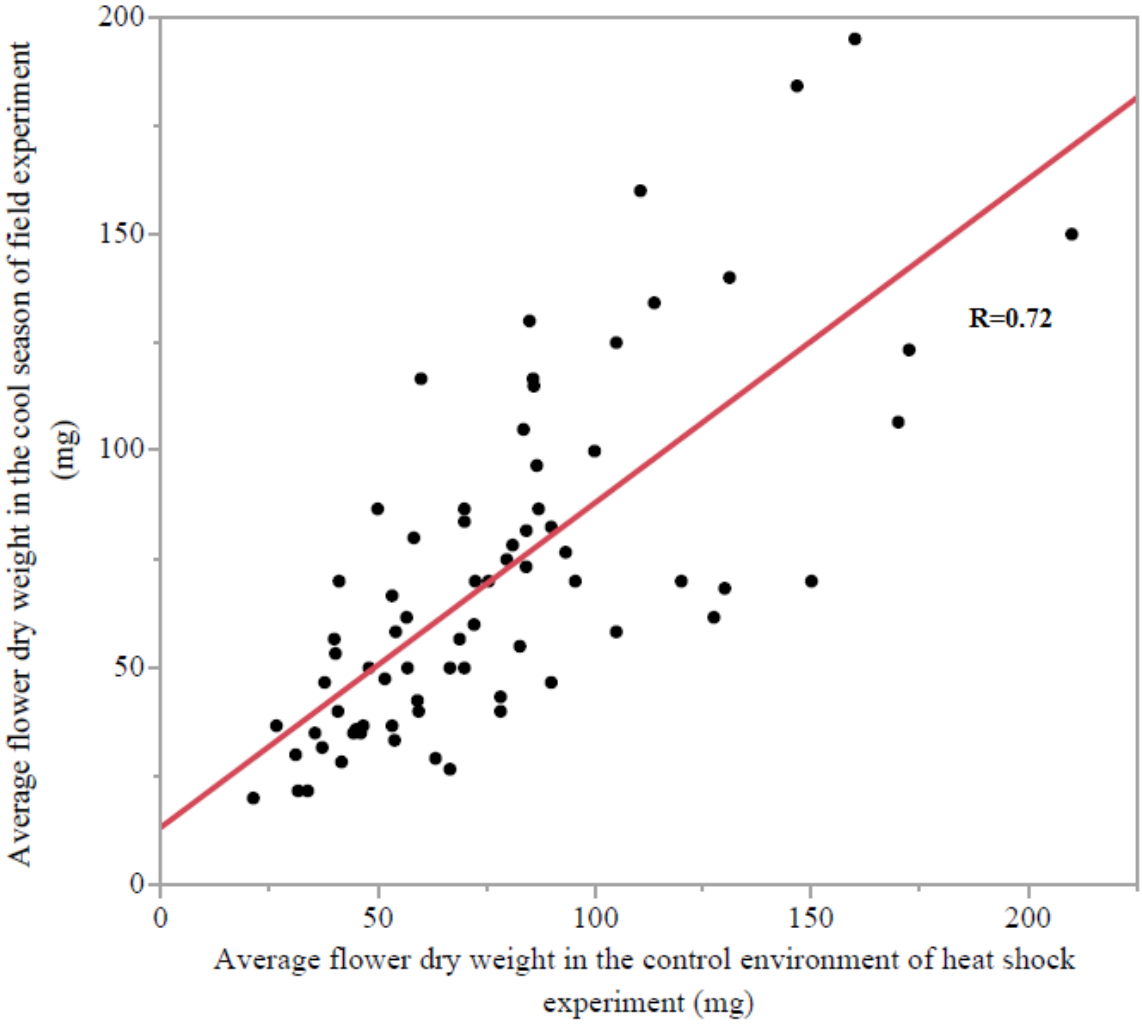
**Appendix 13.** Correlation scatterplot for the flower diameter between the control group in the heat stress experiment and the cool season in the field experiment among 10 populations in College Station, 2015.



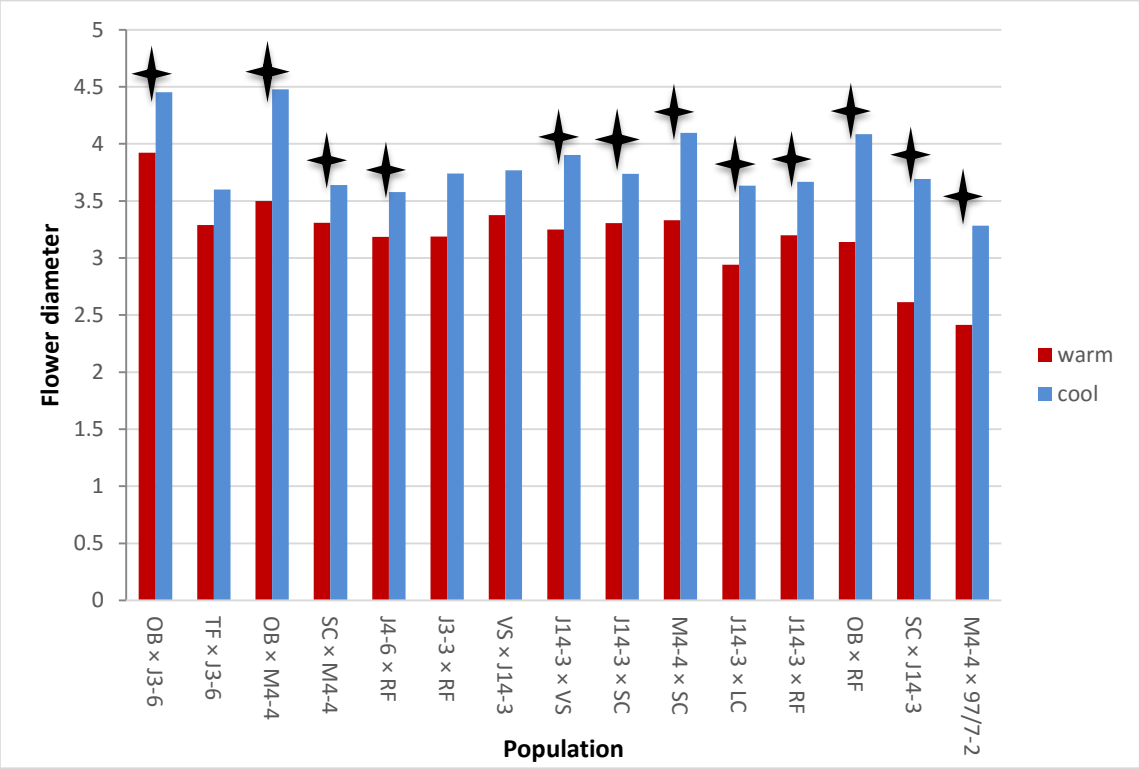
**Appendix 14.** Correlation scatterplot for the petal number between the control group in the heat stress experiment and the cool season in the field experiment among 10 populations in College Station, 2015.



**Appendix 15.** Correlation scatterplot for the flower dry weight between the control group in the heat stress experiment and the cool season in the field experiment among 10 populations in College Station, 2015.



**Appendix 16.** Changes of flower diameter among 15 populations between the cool and warm season in College Station, 2015. Data from spring and fall were combined as one cool season. Raw data of flower diameter was used. Stars indicated flower diameter in warm season significantly different from flower diameter in cool season at $P \leq 0.05$.



**Appendix 17.** Changes of the flower dry weight among 15 populations between the cool and warm season in College Station, 2015. Data from spring and fall were combined as one cool season. Raw data of flower dry weight was used. Stars indicated flower dry weight in warm season significantly different from flower dry weight in cool season at $P \leq 0.05$.