CONVENTIONAL BREEDING AND MOLECULAR TECHNIQUES TO IMPROVE PHYTOCHEMICAL CONCENTRATIONS IN PEPPER (*CAPSICUM* SPP.)

A Dissertation

by

JUSTIN DAVID BUTCHER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2011

Major Subject: Horticulture

Conventional Breeding and Molecular Techniques to Improve Phytochemical

Concentrations in Pepper (*Capsicum* spp.)

Copyright 2011 Justin David Butcher

CONVENTIONAL BREEDING AND MOLECULAR TECHNIQUES TO IMPROVE PHYTOCHEMICAL CONCENTRATIONS IN PEPPER (*CAPSICUM* SPP.)

A Dissertation

by

JUSTIN DAVID BUTCHER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Co-Chairs of Committee: Kevin M. Crosby
Bhimanagouda S. Patil
Committee Members: John L. Jifon
William L. Rooney
Head of Department: Leland S. Pierson III

December 2011

Major Subject: Horticulture

ABSTRACT

Conventional Breeding and Molecular Techniques to Improve Phytochemical Concentrations in Pepper (*Capsicum* spp.).

(December 2011)

Justin David Butcher, B.S., Southern Arkansas University;

M.S., University of Arkansas

Co-Chairs of Advisory Committee: Dr. Kevin M. Crosby
Dr. Bhimanagouda S. Patil

Five separate field experiments were conducted across different environmental locations in Texas for one of three purposes: for identification of the most superior individuals and optimum environmental locations to express elevated concentrations of different phytochemicals within various pepper species (first three experiments), to calculate broad sense heritability and % heterosis estimates for various fruit characteristics and phytochemical levels (fourth experiment), or for use in a specific biotechnology technique to potentially identify a molecular marker linked to elevated levels of ascorbic acid (AA) and flavonoids (quercetin and luteolin) (fifth experiment). In each of the three phytochemical experiments, significant differences were observed not only for fruit measurements but also for expression of the various phytochemicals in comparison to their respective commercial checks. From these experiments, we were able to confirm our hypotheses and identify different genotypes that were capable of expressing better traits for consumption. In addition, we were also able to identify an

optimum environmental location in each experiment that contributed to production of fruit with better traits. In the fourth experiment, results confirmed our hypotheses that paprika type material has higher AA and flavonoid concentrations than serrano peppers, while the opposite is true for capsaicinoid expression. From our correlation analyses, we were also able to identify the presence of several significant associations between the various characteristics we evaluated. In all, our results were able to reveal how effective certain combinations of parent material are towards production of offspring with improved traits expressing better fruit characteristics and elevated phytochemical concentrations. Finally, the quantitative measurements produced in our F_2 molecular marker experiment found significant amounts of variation for both flavonoids and AA expression. We also were able to identify a significant association between quercetin and luteolin, quercetin and total flavonoids (quercetin+luteolin), as well as, luteolin and total flavonoids. In addition, three candidate primers were eventually identified for their potential polymorphic expression. Although one of the three primers was identified as expressing a significant association, the value still represented a relatively low amount of variability with respect to luteolin. From our results, we were able to arguably conclude that an environmental component may serve a more essential role in activating the necessary physiological processes to produce specific secondary metabolites.

DEDICATION

My mother (Alexandria Wright), father (Randy Wright), brother (Kacey Butcher), and grandparents (Marvin and Ann Pike) for their unconditional support and inspiration.

ACKNOWLEDGEMENTS

First of all, I want to thank God for His continuous involvement and direction in my life. Without Him, I would not have been able to accomplish this difficult task. I also wish to thank my mother (Alexandria Wright) and dad (Randy Wright) for their continual support, always encouraging me when times seemed unbearable. Words on a page cannot declare how inspirational and valuable the two of you have been in my life. A special thanks also goes to my brother (Kacey Butcher) and late grandparents (Mr. and Mrs. Marvin Pike) who always encouraged me to do my best. Without my family's support and guidance, my goal of earning a Ph.D. would not have come true. I will forever be grateful for everything that each of them did for me.

To my adviser and committee members, I want to take this time to express my appreciation to each of you. I want to state my gratitude to Dr. Kevin Crosby for his support and input throughout my graduate career. I will always be thankful for everything you did for me. We spent several long hours together either in the greenhouse or traveling to various fields, and you were always willing to use that time to explain important breeding objectives to me that I will be able to take wherever I go. I only hope that I can become as successful a plant breeder as you have someday in the near future. I want to thank Dr. Bhimanagouda Patil for serving as my Co-adviser and allowing me to become a member of the Vegetable and Fruit Improvement Center (VFIC). I would also like to express my gratitude to Dr. Bill Rooney and Dr. John Jifon for their involvement as committee members. Both of you were valuable to the success

of my graduate career and assisted me beyond measure. To Dr. Daniel Leskovar, I want to express a sincere amount of appreciation to you for all of your assistance with growing material for several of my experiments in Uvalde and all the advice you bestowed upon me. In addition, I appreciate the input and assistance from Dr. Kilsun Yoo and Dr. Eun Jin Lee for their patience and training on all the HPLC analyses. A special thank you goes to Dr. Hajeen Bang for her assistance with respect to all the molecular procedures and techniques she so generously taught me. I am also very appreciative for the assistance with data analysis provided by Dr. Amir Ibrahim. Thanks to the greenhouse staff (Sixto Marquez) in College Station and all the student workers for their hard work. I also want to thank my fellow graduate students (Yan Ren, Jin Hee Kim, Haejin Bae, Noa Nomura, Priyanka Chaudhary, Kranthi Chebrolu, Ram Uckoo, and Amit Vikram) for their kind friendship. After we all graduate and go our separate ways, I hope we all keep in touch from time to time. I also want to thank Dr. Leonard and Roxy Pike for their encouragement and advice, which helped me to become a more, well-rounded individual. The opportunity to work alongside Dr. Pike on a few experiments was both an honor and a privilege that I really enjoyed. Finally, I wish to thank the Texas A&M Department of Horticultural Sciences and everyone at the VFIC for their support throughout my graduate career. To Charlotte and Connie, I want to express my appreciation and admiration for your tireless effort to make everything run smoothly. It has been an interesting endeavor and one that I will never forget. Thank you all for assisting me become an able scientist capable of applying the things I have learned to benefit others.

TABLE OF CONTENTS

	Page
ABSTRACT………………………………………………………………...	iii
DEDICATION……………………………………………………………...	v
ACKNOWLEDGEMENTS…………………………………………………...	vi
TABLE OF CONTENTS……………………………………………………...	viii
LIST OF FIGURES…………………………………………………………	xii
LIST OF TABLES………………………………………………………….	xiii
CHAPTER	
I INTRODUCTION AND LITERATURE REVIEW…………..	1
Pepper…………………………………………………	2
Taxonomy……………………………………..	3
Distribution…………………………………..	4
Economic Value……………………………….	5
Health Benefits……………………………………...	6
Ascorbic Acid………………………………….	6
Capsaicinoids…………………………………..	8
Flavonoids……………………………………...	12
Pepper Breeding………………………………………..	15
II ENVIRONMENTAL AND GENOTYPIC VARIATION OF CAPSAICINOID AND FLAVONOID CONCENTRATIONS IN HABANERO (*CAPSICUM CHINENSE*) PEPPERS...	22
Materials and Methods…………………………………	23
Plant Material…………………………………..	23
Capsaicin Extraction and Analysis……………..	24
Flavonoid Extraction and Analysis……………..	25
Statistical Analysis……………………………...	26

CHAPTER Page

Results and Discussion………………………………… 26
Fruit Weight………………………………….. 26
Capsaicin and Dihydrocapsaicin (DHC)……….. 27
Flavonoid Concentrations………………………. 28
Genotype by Location Impact on Phytochemical Concentrations and Quality Characteristics for Better Development of Improved Germplasm…. 29
Conclusions…………………………………………….. 31

III PHYTOCHEMICAL VARIABILITY AMONG *CAPSICUM ANNUUM* GENOTYPES SPATIALLY DISTRIBUTED ACROSS TEXAS……………………………………………... 33

Materials and Methods………………………………… 34
Plant Material………………………………….. 34
Ascorbic Acid Extraction and Analysis……….. 35
Capsaicin Extraction and Analysis…………….. 36
Flavonoid Extraction and Analysis……………. 36
Statistical Analysis…………………………….. 37
Results and Discussion………………………………… 37
Ascorbic Acid Concentrations in Different *C. annuum* Peppers…………………………….. 37
Capsaicin and DHC Concentrations in Different *C. annuum* Peppers…………………………….. 38
Quercetin and Luteolin Concentrations in Different *C. annuum* Peppers………………….. 39
Genotype by Environment Influence………….. 40
Conclusions……………………………………………. 41

IV PHYTOCHEMICAL EXPRESSION IN VARIOUS *CAPSICUM ANNUUM* HYBRIDS UNIQUE TO TEXAS CLIMATES DUE TO GENOTYPE AND ENVIRONMENT………………………………………………. 45

Materials and Methods…………………………………. 47
Plant Material…………………………………… 47
Ascorbic Acid Extraction and Analysis………… 47
Capsaicin Extraction and Analysis……………… 48
Flavonoid Extraction and Analysis……………... 49
Statistical Analysis……………………………… 49

CHAPTER Page

Results and Discussion…………………………………… 50
Conclusions…………………………………………… 53

V HERITABILITY INVESTIGATION IN DIFFERENT *CAPSICUM ANNUUM* GENOTYPES FOR FRUIT CHARACTERISTICS, ASCORBIC ACID, CAPSAICIN, AND FLAVONOIDS………………………………………… 55

Materials and Methods………………………………… 56
Plant Material………………………………….... 56
Ascorbic Acid Extraction and Analysis………... 57
Capsaicin Extraction and Analysis…………….. 57
Flavonoid Extraction and Analysis…………….. 58
Statistical Analysis…………………………….... 59
Results and Discussion…………………………………. 60
Fruit Measurements…………………………….. 60
Phytochemical Concentrations…………………. 61
Conclusions…………………………………………….. 62

VI GENETIC VARIATION AND MOLECULAR MARKER SCREENING IN A UNIQUE F_2 PEPPER (*CAPSICUM* SPP.) FAMILY FOR FLAVONOID (QUERCETIN AND LUTEOLIN) AND ASCORBIC ACID CONCENTRATIONS………………………………………… 65

Materials and Methods………………………………… 66
Plant Material………………………………….. 66
Flavonoid Extraction and Analysis……………. 67
Ascorbic Acid Extraction and Analysis……….. 69
DNA Extraction……………………………….. 71
Measuring DNA Concentration……………….. 72
RAPD Marker Screening Procedure…………... 72
Statistical Analysis…………………………….. 77
Results and Discussion………………………………… 82
Phytochemical Quantification…………………. 82
Molecular Marker Correlation Analysis……….. 82

CHAPTER Page

Applications for Researchers to Make Quick Returns and Develop Improved Lines for Human Consumption…………………………… 83
Conclusions…………………………………….……….. 84

VII FINAL CONCLUSIONS……………………………………… 87

Phytochemical Analyses……………………………….. 87
Heritability Experiment………………………………... 89
Molecular Marker Analysis……………………………. 92
Breeding Recommendations…………………………… 93

LITERATURE CITED…………………………………………………….. 95

APPENDIX…………………………………………………………………... 114

VITA………………………………………………………………………….. 155

LIST OF FIGURES

Page

Fig. 1. Picture of 'Ca377' (P_1) and 'B22' (P_2), respectively…………………… 67

Fig. 2. HPLC chromatograms depicting an example of quercetin standard (a), luteolin standard (b), flavonoid sample with both quercetin and luteolin (c), and overlay of all three (d)…………………………………………… 68

Fig. 3. HPLC chromatograms depicting an example of a 250 µg mL^{-1} ascorbic acid standard peak (e), selected F_2 ascorbic acid sample (f), and overlay of the two (g)……………………………………………………………. 70

Fig. 4. First image: Potential polymorphic segregation observed between both parents and high / low bulked DNA individuals with primer 2. Second image: Potential polymorphic segregation observed between each individual making up the high / low bulked ascorbic acid DNA groups with primer 2. Arrows indicate bands of interest……………………….. 75

Fig. 5. First image: P_1 = 'Ca377', P_2 = 'B22', F_2 individuals from 1-24, and 100 bp molecular marker ladder. Second image: F_2 individuals from 25-50. Third image: F_2 individuals from 51-76. Fourth image: F_2 individuals from 77-101. Fifth image: F_2 individuals from 102-125. Arrows indicate bands of interest……………………………………….. 76

Fig. 6. Frequency distribution table for quercetin concentrations in fruit tissue of all F_2 plants derived from the cross of 'Ca377' x 'B22'………. 78

Fig. 7. Frequency distribution table for luteolin concentrations in fruit tissue of all F_2 plants derived from the cross of 'Ca377' x 'B22'……………… 79

Fig. 8. Frequency distribution table for quercetin+luteolin concentrations in fruit tissue of all F_2 plants derived from the cross of 'Ca377' x 'B22'… 80

Fig. 9. Frequency distribution table for ascorbic acid concentrations in fruit tissue of all F_2 plants derived from the cross of 'Ca377' x 'B22'……… 81

LIST OF TABLES

Page

Table 1. Three preliminary studies where we used a penetrometer to measure the amount of pressure (lb) needed to puncture the outside wall of a few jalapeño (J) and serrano (S) peppers to better indicate their potential fruit firmness.. 114

Table 2. Average monthly environmental conditions during May to August 2009 for maximum and minimum temperatures, relative humidity, solar radiation, and precipitation... 115

Table 3. Average fruit number per plant, % dry matter, and correlation (r) analysis values between fruit yield (FY), fruit weight (FW), flavonoids (quercetin+luteolin), and capsaicinoids (capsaicin+DHC) for four select Habanero (*Capsicum chinense*) experimental hybrids grown in Uvalde, TX.. 116

Table 4. F-values and their significances when data from five fruit (*C. chinense*) characteristics were analyzed by the main effects (location, genotype) and their interactions... 117

Table 5. Fruit colors and fruit weights of mature Habanero peppers (*C. chinense*) grown in three Texas locations................................... 118

Table 6. Capsaicinoid (capsaicin and dihydrocapsaicin) concentrations in mature Habanero pepper fruits (*C. chinense*) grown in three Texas locations... 119

Table 7. Flavonoid (quercetin and luteolin) concentrations in mature Habanero pepper fruits (*C. chinense*) grown in three Texas locations.............. 120

Table 8. Genotype degree of freedom values, F-values, and their significances when six *C. chinense* genotypes were analyzed within three Texas locations (College Station-VFIC, Uvalde, and Weslaco) in 2009 for fruit weight and four different phytochemicals.............................. 121

Table 9. F-values and their significances when 2009 data for five fruit (*C. annuum*) characteristics were analyzed by the main effects (location, genotype) and their interactions... 122

Page

Table 10. Means of ascorbic acid concentrations (μg g^{-1} FW) in various pepper (*C. annuum*) fruits grown in three Texas locations in 2009…. 123

Table 11. Means of capsaicinoid (capsaicin and dihydrocapsaicin) concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in three Texas locations in 2009……………………………… 124

Table 12. Means of flavonoid (quercetin and luteolin) concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in three Texas locations in 2009…………………………………………….... 125

Table 13. Genotype degree of freedom values, F-values, and their significances when ten *C. annuum* genotypes were analyzed within each Texas location (Amarillo, College Station-VFIC, and Uvalde) in 2009 for five different fruit characteristics……………………………………. 126

Table 14. Location degree of freedom values, F-values, and their significances when ten *C. annuum* genotypes were analyzed across three Texas locations (Amarillo, College Station-VFIC, and Uvalde) in 2009 for five different fruit characteristics…………………….……………… 127

Table 15. F-values and their significances for 2010 *C. annuum* study when data for fruit diameter, fruit length, capsaicin, dihydrocapsaicin (DHC), quercetin, and luteolin were analyzed by the main effects (location, genotype) with their interactions………………………….. 128

Table 16. Genotype F-values, and their significances when 21 *C. annuum* genotypes were analyzed within two Texas locations (Uvalde and Weslaco) in 2010 for fruit weight, fruit diameter, fruit length, fruit wall thickness, and five different phytochemicals………………..… 129

Table 17. Mean fruit measurements of pepper (*C. annuum*) samples grown in two different Texas locations in spring 2010……………………….. 130

Table 18. Mean ascorbic acid concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in two different Texas locations in spring 2010………………………………………………………………. 131

Table 19. Mean capsaicin and dihydrocapsaicin concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in two different Texas locations in spring 2010…………………………………………….. 132

Page

Table 20. Mean quercetin and luteolin (flavonoid) concentrations (µg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in two different Texas locations in spring 2010………………………………….…………….. 133

Table 21. F-values and their significances when 21 *C. annuum* genotypes were analyzed across two Texas locations (Uvalde and Weslaco) in 2010 for eight different fruit characteristics………………………… 134

Table 22. Means of four fruit characteristics (weight, length, diameter, and wall thickness) in F_1 fruits of different pepper (*C. annuum*) crosses grown in Weslaco, Texas, in Spring 2008………………………….. 135

Table 23. Degrees of freedom, sum of square, mean square, F-value, and broad sense heritability values (h^2) for four fruit characteristics and five phytochemical groups when data were analyzed by main effects (genotype)…………………………………………………... 136

Table 24. Means of ascorbic acid, capsaicin, dihydrocapsaicin (DHC), quercetin, and luteolin concentrations (µg g^{-1} FW) in F_1 fruits of different pepper (*C. annuum*) crosses after grown in Weslaco, Texas, in Spring 2008……………………………………………………. 137

Table 25. Correlation (r) values for mid-parent and respective F_1 hybrids for all characteristics, as well as, r values between fruit characteristics and phytochemical groups………………………………………….. 138

Table 26. Amount of variability (R^2 values) for mid-parent and respective F_1 hybrids for all characteristics, as well as, R^2 values between fruit characteristics and phytochemical groups………………………….. 139

Table 27. Mid-parent heterosis (%) estimates for each fruit characteristic and phytochemical group…………………………………………….. 140

Table 28. High-parent (HP) heterosis (%) estimates for each fruit characteristic and phytochemical group……………………………. 141

Table 29. ANOVA table showing df, SS, MS, F, and CV values for quercetin concentrations across this F_2 family…………………………………. 142

Table 30. ANOVA table showing df, SS, MS, F, and CV values for luteolin concentrations across this F_2 family…………………………………. 143

Page

Table 31. ANOVA table showing df, SS, MS, F, and CV values for total flavonoid (quercetin+luteolin) concentrations across this F_2 family……………………………………………………………….. 144

Table 32. ANOVA table showing df, SS, MS, F, and broad sense heritability values (h^2) for quercetin concentrations across this F_2 family with two commercial jalapeño checks (Ixtapa and TMJ)………………..… 145

Table 33. ANOVA table showing df, SS, MS, F, and broad sense heritability values (h^2) for luteolin concentrations across this F_2 family with two commercial jalapeño checks (Ixtapa and TMJ)………………………. 146

Table 34. Means of quercetin, luteolin, and quercetin+luteolin concentrations (μg g^{-1} FW) in mature F_2 pepper fruits (*C. annuum*) grown in Uvalde, TX, in increasing concentrations………………………………….... 147

Table 35. ANOVA table showing df, SS, MS, F, and CV values for ascorbic acid concentrations across this F_2 family……………………………. 150

Table 36. ANOVA table showing df, SS, MS, F, and broad sense heritability values (h^2) for ascorbic acid concentrations across this F_2 family with two commercial jalapeño checks (Ixtapa and TMJ)…….................................. 151

Table 37. Means of ascorbic acid concentrations (μg g^{-1} FW) in mature F_2 pepper fruits (*C. annuum*) grown in Uvalde, TX, in increasing concentrations………………………………………………………… 152

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Fruits and vegetables offer a unique avenue for providing key nutrients required by our bodies to maintain a healthy life. As research continues to uncover factors which contribute to many of the diseases of our day, evidence continues to strengthen the assertion that consumption of more fruits and vegetables in one's diet can lead to a healthier life. In an effort to reduce the potential outbreak of harmful diseases or related problems, researchers must continue developing agricultural crops that contain both higher levels of health-promoting compounds and desirable fruit characteristics to better fulfill the needs of consumers.

Due to their assortment of positive attributes, peppers (*Capsicum* spp.) have become an important vegetable component in many cuisines (Greenleaf, 1986; Andrews, 1995; Crosby, 2008; Guzman et al., 2011) contributing to the color, flavor, aroma, and overall appearance of our meals (Greenleaf, 1986; Andrews, 1995; Crosby, 2008). Consumption trends indicate a growing importance for their use as a spice (Andrews, 1995), and even one source suggested the possibility of them overtaking *Piper nigrum* (black pepper) for this role (Vaughan and Geissler, 2009). Considering the vast amounts of phytochemicals present in different fruits and vegetables, peppers are unique for

---

This dissertation follows the style of HortScience.

contributing, among other things, various capsaicinoids, flavonoids, carotenoids, potassium, dietary fiber (Hanson, 1999), and even high levels of ascorbic acid (AA) (Greenleaf, 1986; Lee et al., 1995; Howard et al., 2000; Howard, 2001; Howard et al., 1994; Crosby et al., 2007a; Crosby, 2008; Antonious et al., 2009). Although genetic components are important for optimal expression of these phytochemicals within fruit tissue, reports indicate that environmental components can also serve influential roles and result in a significant amount of variation when the same genotype is grown in a different production area (Zewdie and Bosland, 2000; Lee et al., 2005; Lester, 2006; Leskovar et al., 2009). Reported levels of phytochemical variation is due in large part to various environmental conditions (abiotic and biotic stresses) acting on plants during their growth and development (Harvell and Bosland, 1997; Lee et al., 2005; Lester, 2006; Leskovar et al., 2009). Nonetheless, continual selection of material containing higher levels of these phytochemicals is a valuable component of a breeder's program and will undoubtedly result in creation of improved germplasm consumers can eat to benefit their well-being (Lee et al., 2005; Crosby et al., 2007a; Yoo et al., 2007; Crosby, 2008).

Pepper

Peppers (*Capsicum* spp.) are a group of prolific horticultural crops that have become increasingly important in diets of diverse cultures across the globe. Furthermore, peppers supply a degree of flavor, color, aroma, pungency, and various phytochemicals needed for protecting one's health (Greenleaf, 1986; Crosby et al.,

2007a; Yoo et al., 2007; Crosby, 2008; Guzman et al., 2011). As the Hispanic population continues to rise in this country, predictions postulate that an increase in consumption of various Mexican ethnic foods will likely continue (Andrews, 1995; Greenleaf, 1986). As more knowledge is acquired about the nutritional attributes of peppers, an increase in consumption and utilization in various recipes will also likely continue.

Taxonomy

As a member of the nightshade family (*Solanaceae*) (Eshbaugh, 1993), the *Capsicum* genus comprises between 20-30 species with five major cultivated species recognized: *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* (Basu and De, 2003; Andrews, 1995; Greenleaf, 1986). As a young seedling, peppers are considered herbaceous but can become woody as they age, having an erect, prostrate, or compact form (Andrews, 1995). Fruit shape and size varies widely—a characteristic, as reported by Andrews (1995), to be helpful in distinguishing different cultivars from one another. Selective breeding, which will be discussed in more detail later, has successfully produced such diverse fruit colors as white, green, yellow, orange, red, and purple (Howard et al., 2001). These diverse colors are due to the presence of specific compounds within fruit tissue and are a good indicator of the potential diversity of different phytochemicals that may be present (Guzman et al., 2011). Presence of α- and β-carotene, zeaxanthin, lutein, and β-cryptoxanthin provide yellow and orange colors (Howard, 2001). Carotenoid pigments such as capsanthin and capsorubin give

rise to mature red colors (Howard, 2001). Green fruits express this color because of chlorophyll and carotenoids typical of chloroplasts (Simon, 1997; Guzman et al., 2011). In general, green fruits are immature while red, orange, yellow, and chocolate fruits are fully mature.

Distribution

Believed to have originated in the New World tropics and subtropics (Eshbaugh, 1993), peppers have been part of the human diet since about 7500 B.C. (Bosland, 1996; Howard, 2001), making them among the oldest cultivated crops of the Americas (Bosland, 1996). The greatest amount of genetic diversity of different pepper types appears to be from Mexico (Lee, 1996). Although official figures of world pepper production vary, estimates reveal that India, China, Pakistan, South Korea, and Mexico produce the most (Thampi, 2003). In the United States, production areas are mainly concentrated in the states of Florida, California, Texas, New Mexico, and Arizona, with the latter three serving as important producers for most of the hot types (Greenleaf, 1986). Most peppers grown in the United States are of the *Capsicum annuum* species, the exception being *C. frutescens* (Tabasco® pepper) (Huffman, 1977). Due to their environmental specifications, peppers require warm climates for effective growth and have been found to grow best in a medium-textured, well-drained sandy loam soil (Simon et al., 1984).

Economic Value

The economic value of peppers has arguably changed over the years in a positive direction. In 1973, reports indicated peppers covered approximately 8,000 acres of agricultural land in Texas (Huffman, 1977). From 1978 to 1980, reports from the United States Department of Agriculture (USDA) identified a significant increase from $60 million to over $300 million in grocery sales with an annual average production of 261,000 tons in the United States of green peppers for both the processing and fresh markets (Greenleaf, 1986). In worldwide production, old reports indicated China produced significantly more peppers on more acres than the United States (Greenleaf, 1986). According to 2002 statistics, approximately 17,000 acres were harvested in the state of New Mexico alone (Bosland and Walker, 2004) because of favorable environmental conditions needed for optimum growth. According to 2008 United Nations, Food and Agriculture Organization, *FAOStat*, world production of bell and chile pepper types was reported to exceed 850,000 metric tons in the U.S. alone. Reporting on the basis of a mixture of wet and dry-weights, estimates compiled from the USDA, National Agricultural Statistics Service (2000-05), and Economic Research Service (1980-99) suggested that 26,500 acres of chile peppers were planted in the U.S. in 2007, giving 24,700 acres of harvestable material, and resulting in a total of 170 cwt of yield. Provided worldwide pepper production continues to increase, it is very likely that an increase in the overall value of this particular crop will also continue to rise.

Health Benefits

Ascorbic Acid

Within pepper tissue, appreciable concentrations of ascorbic acid (AA) (Howard et al., 1994), flavonoids (Lee et al., 1995; Howard et al., 2000; Lee et al., 2005; Crosby et al., 2007a; Yoo et al., 2007), and capsaicinoids can be found (Howard, 2001; Crosby et al., 2005; Materska and Perucka, 2005; Crosby, 2008; Crosby et al., 2010). AA, for example, has been identified as an important water-soluble vitamin (Byers and Perry, 1992; Larson, 1988) involved in many of our bodily processes. Previous reports have identified that AA can act as an aqueous reducing agent in biological systems (Levine et al., 1999; Howard et al., 2000), having the capability to maintain healthy skin and facilitate the healing of wounds (Boyce et al., 2002), metabolize fats (Johnston et al., 2006), absorb inorganic iron, and form collagen giving structure to bones, cartilage, and blood vessels (Levine et al., 1999; Lee and Kader, 2000; Geleta and Labuschagne, 2006). Although evidence is still inconclusive with respect to coronary heart disease, AA has been found to be important in normal protein metabolism (Levine et al., 1999), serving an important role in photosynthesis and photoprotection (Conklin et al., 1996; Smirnoff and Wheeler, 2000). Evidence has also indicated AA's role in potentially neutralizing cancer causing free radicals, inhibiting the formation of cancer-causing nitrosamines in the digestive tract (Jacob and Sotoudeh, 2002), reducing the occurrence of different DNA mutations caused by various oxidative stresses (Lutsenko et al., 2002; Rodríguez-Burruezo et al., 2009), and may have an involvement in hormone synthesis and/or release from adrenal glands in response to stress. Over the years, researchers

have also suggested AA may be productive against the onset of scurvy and other various diseases (Oruña-Concha et al., 1998; Howard, 2001; Martinez et al., 2005). Other reports indicate that AA may serve as one of the first lines of defense to more effectively survive different stressful conditions. Conklin et al. (1996) strengthened this logic when they reported to have successfully isolated an *Arabidopsis* mutant lacking in L-AA to be hypersensitive and limited in its adaptation to sulfur dioxide and ultraviolet B irradiation. This idea may be helpful to better explain why AA degrades so rapidly.

Within plants, appreciable amounts of AA can often occur not only in chloroplasts but also in the majority of other cellular compartments including the cell wall (Smirnoff and Wheeler, 2000). Further scientific research has provided evidence that AA concentrations can increase during ripening stages with higher levels present in mature peppers due to light intensity and higher glucose levels (Howard, 2001; Lester, 2006). Variation in AA content can also be attributed to not only differences in environmental conditions while growing but also in fertilization and cultural practices, soil types, and genetics (Howard, 2001; Lester, 2006). Examining 17 hot pepper accessions from the *Capsicum* germplasm collection (four accessions of *C. chinense*, five accessions of *C. baccatum*; six accessions of *C. annuum*; and two of *C. frutescens*), Antonious et al. (2006) found that concentrations of AA and total phenolics were relatively higher in samples of *C. chinense* and *C. baccatum* groups in comparison to others. Evidence suggests three specific Plant Introductions (PI's) —(PI – 633757, PI – 387833, and PI – 633754) could possibly be useful as potential parents in a breeding program to produce new varieties having relatively higher levels of AA (Antonious et

al., 2006). Evidence also identified a strong correlation between AA and total phenols (Antonious et al., 2006). Examining different pepper varieties, Lee et al. (1995) discovered “Chile,” “yellow wax,” and “ancho” type peppers had higher AA contents than “jalapeño” peppers. Reports have indicated fully mature peppers contain higher levels of AA than immature fruit. A more detailed explanation on this topic will be discussed in a later section. According to Lee and Kader (2000), fruits and vegetables supply more than 90% of the AA present in human diets. The Recommended Daily Allowance (RDA) has been reported to be 75 mg day^{-1} for women and 90 mg day^{-1} for men (Jacob and Sotoudeh, 2002). Although AA content of peppers can vary depending on the particular cultivar examined, most are unique for contributing over 100% of the RDA (Howard, 2001). According to Lee (1996), AA was found to readily leach out of pepper fruit during brine equilibration and storage. As briefly mentioned earlier, it is important to note that this compound can degrade rather rapidly over time to dihydroascorbic acid (oxidation) and on to 2,3-diketogulonic acid (hydrolysis) in an aqueous solution (Gibbons et al., 2001); therefore, fruit samples must be stored in an environment that can be consistently maintained at -80 °C to avoid such problems.

Capsaicinoids

Among the several capsaicinoid molecules present in pepper fruit, capsaicin (N-vanillylnonanamide) has been identified as the predominant contributor to pepper pungency and spicyness (Andrews, 1995; Bennett and Kirby, 1968; Cooper et al., 1991; Howard, 2001; Iwai et al., 1979; Monforte-Gonzalez et al., 2010; Perucka and Oleszek,

2000; Poyrazoglu et al., 2005), followed by dihydrocapsaicin (DHC) (Antonious et al., 2009; Contreras-Padilla and Yahia, 1998; Quinones-Seglie et al., 1989). Activation of sensory receptors in the mouth sends signals to the brain and informs it of the pepper's "hotness" when eaten (Huffman, 1977). Capsaicinoid compounds having a similar structure, but contributing less to pungency expression, include nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin (Iwai et al., 1979; Poyrazoglu et al., 2005; Quinones-Seglie et al., 1989).

Steps involved in capsaicin production through the capsaicinoid biosynthetic pathway have been previously reported (Bennett and Kirby, 1968; Poyrazoglu et al., 2005; Ravishankar et al., 2003; Sukrasno and Yeoman, 1993). Within pepper fruits, an increase in peroxidase activity can result in a decrease in capsaicinoid concentration, indicating this enzyme's possible involvement in capsaicinoid degradation (Contreras-Padilla and Yahia, 1998). Depending on market preference and geographical location, it is vital that pepper breeders acknowledge this idea and understand how important the interaction is between a genotype and its appropriate environment to ensure the most desirable product is produced to better meet the demands of consumers.

Other factors influencing capsaicin content besides those just mentioned include ecological conditions, fruit maturity, and compartmentalization within fruit (Huffman, 1977; Monforte-Gonzalez et al., 2010). Within fruit tissue, capsaicin is unevenly distributed, concentrated in placental and cross wall regions (Huffman, 1977). Monforte-Gonzalez et al. (2010) reported that placental regions of fruit possessed an ability to channel inorganic forms of nitrogen (nitrate) ultimately into secondary

metabolites contributing to capsaicin content. In other reports, it has been dictated that capsaicinoid synthesis and content within fruit tissue more actively occurs between 20 and 40 days following anthesis as fruits increase in size and maturity (Sukrasno and Yeoman, 1993) with environmental stresses, such as water deficiency, potentially contributing to various levels (Howard, 2001).

In an effort to quantify the amount of capsaicin in pepper tissue more effectively, Wilbur Scoville developed the "Scoville Organoleptic Test" to measure the pungency of fruits in Scoville Heat Units (SHU) (Bosland, 1999). Values on the scale vary depending on the particular breed examined and can range from 0 SHU (Bell peppers) to as much as 1,000,000 SHU (Naga Jolokia); pure capsaicin is a hydrophobic, colorless, odorless, flavorless compound that measures 16,000,000 SHU (Huffman, 1977; Bosland, 1999). According to DeWitt (1999), capsaicin is seemingly unaffected by changes in temperature and is able to retain its potency despite cooking or freezing. Early work by Jones and Pyman (1925) reported the exact shape of the capsaicin molecule gives researchers a better means of determining pungency levels. Huffman (1977) found that variability in length associated with the acid portion side chain of the molecule contributed a remarkable effect to potential pungency. Using a High Performance Liquid Chromatography (HPLC) technique, scientists have determined a relative means of quantifying the amount of capsaicin present in fruit tissue (Cooper et al., 1991; DeWitt, 1999; Singh et al., 2009). Other groups have also previously described alternative techniques to accurately quantify capsaicinoid levels in pepper tissue (Iwai et al., 1979; Jarret et al., 2003).

Having the unique reputation of supplying a degree of 'hotness' to one's palate, pepper species can be potentially effective against indigestion, migraines, lowering blood cholesterol, boosting circulation, reducing blood pressure, boosting the immune system to fend off infections, toning the nervous system, relieving pain of arthritis, assisting to cauterize and heal ulcers (in some reports), and serving as a powerful catalyst for other herbs (Woodland Publishing, 1996). In Mayan and Aztec civilizations, wild fruits were once used for their medicinal properties associated with asthma, the common cold, coughing, and sore throats (Bosland, 1999). Conforti et al. (2007) examined different maturity stages (immature green, green, and red hot peppers) to determine their potential role against free radicals. Their results indicated peppers analyzed at the small green stage of maturity had the highest amount of radical-scavenging activity (IC_{50} of 129 μg mL^{-1}) while red pepper in a methanol extract had an even greater amount of antioxidative potency (IC_{50} of 3 μg mL^{-1}) (Conforti et al., 2007). This idea was verified by Howard et al. (2000) when they discovered concentrations of different antioxidant constituents can increase as the fruit becomes more mature. Capsaicin research has also been vital for indicating how productive these compounds are at displaying some chemoprotective effects (Surh et al., 1995; Teel, 1991) and inhibiting cellular growth of cancer cells by way of apoptosis induction (Jung et al., 2001; Lee et al., 2001; Richeux et al., 1999). Studies in an *in vivo* environment have shown capsaicin may inhibit tumor development (Ito et al., 2004; Jang et al., 1989), suppress the growth of leukemic cells, and induce apoptosis in mice without any toxic effects (Ito et al., 2004). Results from other studies indicate capsaicinoids may

contribute, in some degree, to a reduction in body fatness through stimulation of energy metabolism (Tremblay and Westerterp-Plantenga, 2007). These compounds may also possess some anti-inflammatory properties and an ability to alter enzymes of phase I status (Clevidence, 2010). Although these compounds provide various beneficial effects, caution should still be followed by those suffering from stomach abnormalities and ulcers (Surh and Lee, 1995).

Flavonoids

Flavonoids have been previously identified as a group of polyphenolic substances (Hertog et al., 1993) produced as a result of secondary metabolisms (Materska and Perucka, 2005; Ross and Kasum, 2002) and are found in the thylakoid membrane of chloroplasts (Havsteen, 1983). Between 4,000 and 5,000 different flavonoids have been described (Hollman et al., 1996; Hollman and Katan, 1999), providing color and flavor to fruits and vegetables. Factors affecting the amount of flavonoid variation include the specific cultivar, degree of maturity, processing methods, storage conditions (Ross and Kasum, 2002), light (Duthie and Crozier, 2000), and levels of nitrogen in soils (Amiot-Carlin et al., 2007). The biosynthesis of flavonoids begins and proceeds through the phenylpropanoid pathway (Lee, 1996; Jaakola, 2003). In a typical pepper flavonoid analysis, quercetin and luteolin are usually the two most prevalent compounds identified within fruit tissue as stated by Lee (1996), who found values ranging up to 800 mg kg^{-1} in different *C. annuum* genotypes. Howard (2001) found similar levels of variation among pepper types and cultivars from 1 to

852 mg kg^{-1}. Results from Lee (1996) seemed to provide evidence that *C. annuum* fruit generally contains higher flavonoid levels than those of *C. chinense*. Howard (2001) suggested a positive correlation generally exists between concentrations of quercetin and luteolin present in fruit tissue. Other reports from Lee (1996) and Howard (2001) support the idea that flavonoid content can decrease continuously during maturation, potentially affecting metabolic conversion to secondary phenolic compounds; yet, appreciable amounts of flavonoids can still exist when peppers are consumed. According to Pietta (2000), flavonoid intake by humans can vary between 50 and 800 mg per day. On the other hand, breeders are still interested in improving this component while maintaining the levels of other phytochemicals in a simultaneous manner, resulting in development of more superior genotypes having the capability to better protect the well-being of those who consume peppers on a regular basis.

Flavonoids have also been identified as an especially unique group of compounds for potentially affecting the lipid peroxidation process caused by reactive oxygen species (DiSilvestro, 2001) and may prevent progression of radical chain reactions by trapping chain-initiating radicals at membrane interfaces (Ross and Kasum, 2002). Flavonoids can also serve as antimicrobials (Cowan, 1999; Pietta, 2000; Howard, 2001; Cushnie and Lamb, 2005) and exhibit antiallergenic, antiviral (Cushnie and Lamb, 2005), immune-stimulating, anti-inflammatory, anti-oxidative (Duthie and Crozier, 2000; DiSilvestro, 2001), and anti-platelet properties (Miean and Mohamed, 2001; Howard, 2001; Havsteen, 2002; Ren et al., 2003; Kim et al., 2004; Yao et al., 2004; Cushnie and Lamb, 2005). Flavonoids may be linked to a reduced risk of coronary heart disease (Hertog et

al., 1993; Hertog et al., 1995; Yao et al., 2004), cancer (Pietta, 2000; Ross and Kasum, 2002; Yoshida et al., 1990), and may serve a role in inducing detoxifying enzyme systems such as glutathione S-transferase (Smith and Yang, 1994; DiSilvestro, 2001). In addition, many of these flavonoid compounds are unique for contributing to various pigments in different plant tissues (Havsteen, 2002; Jaakola, 2003; Yao et al., 2004). One to two grams have been reported (Havsteen, 2002) to be the daily intake from normal food. Of the flavonoids present within peppers, reports indicate the conjugate forms of quercetin and luteolin are generally the most prominent (Lee et al., 1995; Howard, 2001; Miean and Mohamed, 2001). Both of these compounds may exhibit some antioxidant properties and assist in the process of free radical scavenging (Larson, 1988; Miean and Mohamed, 2001). Quercetin, a flavonol, may also protect against coronary heart disease (Hollman et al., 1996), cancer (Yoshida et al., 1990), and cardiovascular disease (Yao et al., 2004). Luteolin, a flavone, has been shown to be a potent enzyme inhibitor (Larson, 1988) and has been reported to serve an important role in inhibition of Lipopolysaccharide (LPS)-induced transcriptional activity in various cell experiment studies (Kotanidou et al., 2002; Jang et al., 2008). Other flavonoids that have been previously reported in variable levels in different pepper genotypes include apigenin, myrcetin, and possibly even kaempferol (Miean and Mohamed, 2001; Sampson et al., 2002; Bahorun et al., 2004).

Because our bodies cannot produce these different phytochemicals, we must consume them on a regular basis in the food we eat. Therefore, development of improved pepper material containing elevated levels of these phytochemicals through

the use of traditional breeding techniques will provide consumers with higher quality products to better sustain their health. As Hertog et al. (1992) discussed, more reliable studies are needed, especially on flavonoids, to determine their potential role in combating human cancer and occurrence in different foods. As Crosby et al. (2007a) presented, creation of genotypes having higher levels of various phytochemicals, appreciable yield, flavor, and appearance remain important objectives to pepper breeders.

Pepper Breeding

Development of improved pepper germplasm by way of cross-breeding two superior individuals can often result in creation of a desirable offspring (Greenleaf, 1986; Pickersgill, 1997; Zatykó, 2006). In most cases, reports indicate a successful achievement is due to an advertent scheme by breeders exploiting a worthwhile amount of heterosis present when two pepper individuals are brought together (Allard, 1960; Pickersgill, 1997; Geleta and Labuschagne, 2004; Zatykó, 2006). To date, various selection methods (mass, pedigree, backcross, and single plant) and techniques have been deployed that have achieved relative success for the pepper industry (Greenleaf, 1986; Bosland, 1996; Crosby and Villalon, 2002; Crosby et al., 2005; Zatykó, 2006; Crosby et al., 2007b; Paran et al., 2007; Crosby et al., 2010). Considered a self-pollinated crop (Allard, 1960), peppers generally self-pollinate but can out-cross (7 to 91%) when grown in field conditions due to the presence of natural insect pollinators

(Bosland, 1996). Most *Capsicum* species have 2n=24 chromosomes (Bosland, 1996); the basic chromosome number is 12 (Greenleaf, 1986; Pickersgill, 1997).

In addition to the aforementioned phytochemicals, various characteristics are beginning to become more valued within different fruits and vegetables for increased consumer acceptability (Rico et al., 2007). For peppers, large fruit having thick walls and a relative degree of firmness have gained favor, especially in jalapeño and serrano markets (Crosby, personal communication). This particular characteristic is especially valuable if products have to be exported over long distances before reaching consumers (Showalter, 1973). As one might expect, firmer fruit would have more potential in maintaining its integrity over a longer period of time, resulting in less product being lost due to damage or quality deterioration (Hall and Stephens, 1999). Other equally important characteristics that have become of more value to breeders and consumers are: color, shape, bluntness / pointiness, and aroma (Weisenfelder et al., 1978). As reported by Watada (1995), Abbott (1999), and Rico et al. (2007), several techniques can be used to measure the various quality aspects of fruits that breeders can, in turn, use to more accurately characterize fruits. Using a penetrometer, we were able to conduct some preliminary experiments to gain a better idea of fruit firmness after genotypes were grown in different locations across the US (Table 1). These preliminary measurements can be potentially examined in future studies and possibly exploited in various breeding methods to maximize their potential superiority as parent material for development of several improved specimens.

Often times, the exact explanation as to how two specific individuals were able to create such a superior offspring is not always completely evident. In certain situations, it is apparent that a combination of different genes acting in a synergistic manner can lead to a preferred result (Allard, 1960; Poehlman and Sleper, 1995; Bernardo, 2002; Liu, 2003). Reports from Geleta and Labuschagne (2004) verified this when they identified some hybrids that expressed a significant amount of improvement with respect to, among other characteristics, fruit length, plant height, yield, and earliness in comparison to their parents. Expression of these different characteristics of interest can also be indicative of the specific gene interaction involved in controlling the trait, as in an additive or non-additive fashion (dominance, overdominance, or epistasis) (Allard, 1960; Poehlman and Sleper, 1995; Bernardo, 2002; Zatykó, 2006), which may be beyond the breeder's control in some instances. Focusing on yield components, Zatykó (2006) reported that noticeable variation can also exist due to some functionality on the part of the specific trait within the cultivar and / or different environmental factors acting on the material while growing. Crossing two distantly related individuals expressing opposing characteristics of interest is also a well-known technique to increase the potential genetic variation. As reported by Greenleaf (1986), a continuous range of variation was observed in fruit size and shape in an F_2 family developed by crossing an oblate and elongate type, suggesting the presence of polygenes. Currently, these two characteristics have become more important as the trend has shifted to production of cultivars with larger fruit, especially for the fresh market industry (Crosby, 2008). Several additional examples are also available verifying that a significant amount of

positive heterosis can be exploited for such traits as average fruit weight, fruit width, and fruit length after various pepper crosses were made (Gopalakrishnan et al., 1987; Bhagyalakshmi et al., 1991; Reddy et al., 2008; Prasath and Ponnuswami, 2008). High heritability values have also been reported for fruit length, fruit weight (Manju and Sreelathakumary, 2002; Sreelathakumary and Rajamony, 2004), and pericarp thickness (Jabeen et al., 1999; Sood et al., 2009; Yadeta et al., 2011). Despite the success achieved in these areas, it is also possible that specific deficiencies may result in offspring developed from elite parents. We have observed this phenomenon firsthand in several advanced lines when they were used in different crossing schemes and believe that it may indicate their reduced combining ability potential (unpublished). Although each parent has the potential to contribute to a desirable outcome, most pepper breeders want to have a more detailed, scientific understanding of the inheritance or heritability of these specific characteristics so that they may effectively exploit and produce superior hybrids expressing enhanced attributes in a more timely fashion. Successful extrapolation of these estimates could then lead to improved germplasm options that could become of high value to both producers and consumers.

As reported by Crosby et al. (2007a), expression of various phytochemicals within pepper fruit tissue may be due to polygenic inheritance after an occurrence of continuous phenotypic observations become evident. For AA inheritance, a closer examination by Sharma et al. (2010) found heritability values exhibiting an additive gene action. In contrast, Sood et al. (2009) reported AA inheritance exhibited nonadditive gene activity. Reports by Geleta and Labuschagne (2006) indicated

inheritance of AA was impacted less by environmental factors when a controlled environment was used. On the other hand, most pepper germplasm is grown in an uncontrolled field environment where exposure to different durations of light intensities and other environmental stresses are common. For capsaicin inheritance, reports by Greenleaf (1986) discuss the notion of a single dominant gene being responsible, mapped to chromosome 2, exhibiting quantitative inheritance (Guzman et al., 2011). Other reports by Ribeiro and da Costa (1990) mentioned capsaicin expression in *C. chinense* material was controlled by many genes acting in an additive fashion. Narrow sense heritability estimates from Riberiro and da Costa (1990) also postulated pungency improvements can potentially occur through the use of simple selection procedures. As with all of these phytochemicals, verification of pungency expression can then be confirmed in fruit tissue using a quantitative High Performance Liquid Chromatography (HPLC) or related technique (Poyrazoglu et al., 2005) to measure concentrations in comparison to a popular, commercial check (Bosland, 1996). Genotypes expressing appreciable levels for a specific target market can then be examined in more detail in more advanced trials. In contrast, six genes were reported to encode for flavonol synthase (FLS), an important enzyme involved in the flavonoid biosynthetic pathway to produce, among other things, succinate and flavonol (Winkel-Shirley, 2001). Arguably, this hypothesis may be helpful to explain reports from Lee et al. (1995), Howard (2001), Crosby et al. (2007a), and Sun et al. (2007), who all indicated a significant amount of variation in flavonoid (quercetin and luteolin) expression when different pepper genotypes were evaluated. Because flavonoid expression can vary so widely among

different pepper genotypes both within and across different locations (Howard et al., 2000; Lee et al., 2005), scientific understanding is still arguably unable to effectively explain how complex interactions occur with respect to other various phytochemicals present within a typical pepper fruit. Therefore, one can arguably postulate that flavonoid synthesis and production within pepper fruit tissue is dictated by quantitative inheritance.

Evidence from all of these studies provide proof that improvement in both fruit characteristics as well as phytochemical expression using traditional breeding methods is achievable and has a promising role for breeders to exploit for the purpose of developing materials with improved traits of interest. On the other hand, several reports have demonstrated how different components can be expressed when grown in a particular environment, resulting in a significant amount of variability after material has been exposed to various environmental stresses (Harvell and Bosland, 1997; Hoffmann and Merilä, 1999; Zewdie and Bosland, 2000; Howard, 2001; Lee et al., 2005; Materska and Perucka, 2005; Lester, 2006; Crosby, 2008; Guzman et al., 2011). As previously reported, different soil types, irrigation strategies (Leskovar, 2009), humidity, day and nighttime temperatures, solar radiation, precipitation (Lee et al., 2005), altitudes, insect and weed pressure, and even neighboring plants all exert a form of stress on a particular cultivar (Hill et al., 1998; Lester, 2006), which often affects the genotype's performance to produce a precise characteristic of interest. With respect to AA synthesis and production within fruit tissue, scientific evidence indicates a significant contribution of sunlight to AA accumulation and the ultimate creation of material expressing elevated

levels can occur (Crosby et al., 2003; Lester, 2006). Our group has observed this firsthand in previous studies in which significant amounts of variation in AA concentrations were found when other *C. annuum* material were grown in different environments where sunlight intensities were expected to be variable (unpublished). In regards to flavonoids, Havsteen (1983) reported their probable involvement as catalysts of the electron transport process in light phase steps of photosynthesis. Arguably, higher qualities (Simkin et al., 2003) and intensities of solar radiation within a particular environment may allow for more of its capture and thus increased synthesis within fruit tissue (Lester, 2006). Nonetheless, continual selection of material expressing appreciable fruit characteristics and higher levels of these phytochemicals when evaluated in a favorable and / or unfavorable environment can still contribute to the vitality many pepper breeders are pursuing for the purpose of developing an improved cultivar for human consumption (Hill et al., 1998; Hoffmann and Merilä, 1999; Zatykó, 2006).

CHAPTER II

ENVIRONMENTAL AND GENOTYPIC VARIATION OF CAPSAICINOID AND FLAVONOID CONCENTRATIONS IN HABANEO (*CAPSICUM CHINENSE*) PEPPERS

Habanero peppers have become increasingly popular in the U.S. for supplying unique flavors and high levels of pungency. As consumption of this product increases, development of improved cultivars with elevated phytochemicals will likely result in additional demand from consumers. This study evaluated fruit size, capsaicinoid, and flavonoid concentrations in six Habanero (*Capsicum chinense*) genotypes grown at three different Texas locations: College Station-Vegetable and Fruit Improvement Center (VFIC), Uvalde, and Weslaco. Five of these Habanero experimental hybrids (H1-red, H2-orange, H3-orange, H5-dark orange, and H6-yellow) were developed at Texas A&M University with genetic improvement in numerous traits of interest, and Kukulkan F_1 (Kuk-orange) was included as a commercial check.

Although many studies have been conducted analyzing capsaicin and flavonoid concentrations in fruits (Contreras-Padilla and Yahia, 1998; Cooper et al., 1991; Harvell and Bosland, 1997; Hertog et al., 1992; Howard et al., 2000; Kurian and Starks, 2002; Lee et al., 1995; Lee et al., 2005; Poyrazoglu et al., 2005; Sanatombi and Sharma, 2008; Singh et al., 2009; Zewdie and Bosland, 2000), this study was performed for the purpose of analyzing Habanero peppers for capsaicin and flavonoid concentrations when grown in different environments.

Because each of these Habanero experimental hybrids was developed from different pedigrees, quantitative analysis was expected to reveal differences in phytochemical concentrations among them and in comparison to a popular commercial check. Before this study, researchers had limited data for these experimental hybrids with respect to their phytochemical concentrations. To date, there is little or no evidence of fruit color being correlated with capsaicin or flavonoid concentrations, so researchers were more interested in determining the best genotype capable of expressing elevated concentrations of these phytochemicals across different locations. The objective of this experiment was to determine the degree of variability in phytochemical expression in these six Habanero genotypes, as well as, to determine the best environment that would enhance the concentrations of these phytochemicals within fruit tissue. Our purpose was also to identify good candidates for introduction as new Habanero cultivars.

Materials and Methods

Plant Material

Five advanced Habanero experimental hybrids fixed for various traits of importance to the seed industry, and one commercial check were all chosen for evaluation. The diverse pedigrees of these genotypes have resulted in variation for fruit color, size, shape, yield, disease resistance, and days to maturity. H1 is a large-fruited, early maturing, red type with a small plant. H2, H3, and H5 are orange-fruited types, with larger plants and express resistance to PepMoV and TSWV, derived from Plant

Introduction (PI) 152225. H6 produces heavy yield of golden-yellow fruit with no pungency, on vigorous plants.

Habanero transplants were set out between March 1 and April 15, 2009, at three Texas A&M AgriLife Research Centers: College Station-VFIC (30.61° N; 96.32° W), Uvalde (29.22° N; 99.78° W), and Weslaco (26.16° N; 97.98° W) (Table 2). Fruit harvest took place between late June and August 2009. A sub-surface drip irrigation method was utilized in each location, and commercial agricultural practices typical for Habanero peppers were followed. Fully colored, matured fruits were harvested from six separate plots. All fruit specimens were selected that appeared healthy, completely colored, and turgid at the time of harvest. After fruit weights were measured on each genotype, all samples were stored at -80 °C until analysis could ensue.

Capsaicin Extraction and Analysis

Capsaicinoids (capsaicin and DHC) were extracted as described by Singh et al. (2009) with some modifications. All extraction procedures used smashed pepper tissue with seeds from five fruits combined from each plant. These frozen fruits were pulverized using a mallet, and approximately 5 g of mixed fruit tissue with seeds was taken. The sample was homogenized in 20 mL of 100% methanol using a Polytron PT 10-35 Homogenizer (Kinematica Inc., Bohemia, NY), and final volumes were adjusted to 30 mL. The fruit tissue extract was allowed to precipitate in a -20 °C freezer before a sample of supernatant was collected and injected into a High Performance Liquid Chromatography (HPLC) system. The HPLC system includes a Perkin Elmer Model

200 pump, autosampler, and LC 295 UV/Vis detector. Forty microliters (μL) from each sample was injected, and the peak area was calculated to determine capsaicin and DHC concentrations on a fresh weight basis. Capsaicin and DHC levels were detected at 280 nm using a Nova-Pak C_{18} (4.6 × 150 mm, 4 μm) column with a guard cartridge, and the flow rate was 1.0 mL per min. of 45% Acetonitrile (ACN) with 0.5% H_3PO_4. External standards of capsaicin (23 μg mL^{-1}) and DHC (14 μg mL^{-1}) were used to quantify the samples.

Flavonoid Extraction and Analysis

The flavonoid extraction method was similar to that of Lee et al. (1995; 2005) with some modifications. Four microliters of each extract used in the capsaicinoid analysis was mixed with 2 mL of 3N hydrochloric acid and hydrolyzed at 90 °C for 60 min. Flavonoids (quercetin and luteolin) were also analyzed by an HPLC system and quantified at 360 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4 μm) column with a guard cartridge at a flow rate of 0.8 mL min^{-1}. Mobile phase program conditions employed solvent A (0.5% H_3PO_4 in water) and solvent B (0.5% H_3PO_4 in methanol) to increase from 40% B to 100% B in 20 min. The injection volume was 20 μL, and external standards of quercetin (45.65 μg mL^{-1}) and luteolin (28.82 μg mL^{-1}) were used to quantify the samples.

Statistical Analysis

In each location, Habanero genotypes were planted in a completely randomized design. Statistical analyses used a General Linear Model (GLM) program in SAS (SAS Institute, 2008) to analyze for differences in locations (L), genotypes (G), and location by genotype (L x G) interactions when considering each source as a fixed effect. Separations were also made by LSD at the 0.05 level of calculated mean values for genotypes both within and across locations to compare differences in fruit weight, capsaicin, DHC, quercetin, and luteolin. Hartley's Homogeneity of Variance (HOV) test was also conducted as described by Hoshmand (2006). Correlation analyses were also conducted between total capsaicinoids (capsaicin+DHC) and total flavonoids (quercetin+luteolin), as well as, fruit yield and the different phytochemicals (Table 3). Finally, % dry matter was calculated on four of the genotypes grown in the Uvalde location (Table 3) using the formula:

$$\%\ \text{Dry Matter} = \frac{\text{Weight of Dry Product}}{\text{Weight of Fresh Product}} * 100$$

Results and Discussion

Fruit Weight

Results of the Analysis of Variance (ANOVA) revealed significant L and L x G interactions (Table 4). The Weslaco location produced larger fruit than the other locations (Table 5), and H1-red had the highest overall mean fruit weight value. H1-red had the largest fruit weights at both College Station-VFIC and Weslaco but not in Uvalde (Table 5); however, H6-yellow was not significantly different than H1-red.

Although Weslaco produced the second largest mean fruit weights for both H2-orange (7.71 g versus 8.61 g in College Station-VFIC) and H5-dark orange (6.70 g versus 7.22 g in Uvalde), their values were not significantly different from the highest value obtained from their alternate location (Table 5). Based on market demands for larger fruit, Weslaco may be an optimum location to grow high quality Habanero peppers, and H1-red may have some potential for further studies and potential release due to its appealing phenotypic attributes.

Capsaicin and Dihydrocapsaicin (DHC)

The capsaicin data from the ANOVA found significant F-values for L, G, and the L x G interaction (Table 4), while DHC data showed significant F-values for G and the L x G interaction (Table 4). Previous reports from Antonious et al. (2009) observed significant differences existing in fresh fruit of different *C. chinense* PI's they examined (highest PI reached levels of 2.7 mg g^{-1} of capsaicin plus dihydrocapsaicin). In general, concentrations were higher in fruit tissue grown at the Weslaco location (Table 6). In nearly all cases, Kuk-orange produced the highest amount of capsaicin and DHC, followed by H5-dark orange and H2-orange. Results also indicated H6-yellow as a potential candidate for mild markets due to its significantly lower expression comparable to previous levels found in TMH (Table 6). However, at both College Station-VFIC and Weslaco, Kuk-orange yielded significantly less fruit per plant than the other hybrids (data not shown). It is possible that the content of phenolics was influenced by the fruit load, as more photosynthates would be available per fruit. Also, previous investigations

(Milerue and Nikornpun, 2000; de Sousa and Maluf, 2003) demonstrated a role of heterosis in different peppers for both fruit yield and pungency. Specific hybrid combinations might lead to more elevated phenolics compared to others. Alternatively, other studies (Ben-Chaim et al., 2006) have indicated a moderately negative correlation ($r = -0.33$) between fruit weight and capsaicinoid content across environments. We have, however, observed this phenomenon firsthand in some non-pungent sibling jalapeño lines that yielded significantly better than some pungent lines with other plant traits being very similar (unpublished data).

Flavonoid Concentrations

The ANOVA for the quercetin data found significant F-values for L, G, and their L x G interaction (Table 4). College Station-VFIC was generally the best environment (other than for H1-red) for producing fruit with higher levels of quercetin (Table 7). In this location, Kuk-orange produced the highest amount of quercetin followed by H6-yellow and H3-orange while H1-red produced the lowest amount. In Uvalde, H1-red produced the highest levels of quercetin followed by H2-orange and H3-orange while H5-dark orange produced the lowest amount. In Weslaco, Kuk-orange produced the highest amount of quercetin followed by H3-orange and H2-orange while the remaining genotypes were all comparably low in their respective concentrations (Table 7). For the luteolin data, inconsistent expression by genotypes were found across locations making it difficult to conclude which location was the best (Table 7). The ANOVA showed a significant F-value only for the L x G component of variance (Table 4). In College

Station-VFIC, H5-dark orange produced the highest amount of luteolin followed by H3-orange and H2-orange while H1-red produced the lowest amount. In Uvalde, H2-orange produced the highest levels of luteolin followed by H3-orange and H6-yellow while H1-red produced the lowest amount. In Weslaco, Kuk-orange produced the highest amount followed by H3-orange and H2-orange while H6-yellow produced the lowest (Table 7).

Genotype by Location Impact on Phytochemical Concentrations and Quality Characteristics for Better Development of Improved Germplasm

Correlation analysis between capsaicinoids and flavonoids produced a correlation (r) value of 0.3634 and identified 13.2% of the variability of total capsaicinoids (capsaicin+DHC) to be explained by total flavonoids (quercetin+luteolin). Therefore, results indicated that total capsaicinoids (capsaicin+DHC) are not significantly associated with total flavonoids (quercetin+luteolin). Correlation analyses conducted between fruit yield, fruit weight, and these two phytochemical groups did not produce any significant associations either (Table 3). According to Hartley's HOV test, data analyzed within individual locations found the variances of each measurement to be significant and therefore heterogeneous. When data were analyzed across locations, only the variance of the fruit weight measurement was non-significant and therefore homogeneous. These results may be due to significantly variable values produced for each characteristic. Fruit harvested from the Weslaco location was larger in size than fruit from the other two locations. Significant improvement in fruit size for these different genotypes may have been the result of the material's genetic potential, the

specific environment, and improved cultural practices available in Weslaco that actually promoted fruit development more successfully than the other two locations. Results from this experiment also identified the Weslaco location as being the most optimum for producing Habanero fruit expressing higher levels of capsaicinoids. Therefore, exposing Habanero peppers to an environment similar to Weslaco would potentially produce fruit with higher levels of capsaicinoids, provided all other factors (pepper genotype, stage of maturity, and generation stage) were fixed. In contrast, an environmental location similar to College Station-VFIC may be an optimum environment to produce Habanero fruit with higher levels of flavonoids. Although these assumptions may be difficult to meet due to the unpredictability of the weather from year to year, they can serve as guidance for producers interested in maximizing Habanero fruit quality. As reported by previous researchers (Harvell and Bosland, 1997; Lee et al., 2005; Zewdie and Bosland, 2000), a significant genotype by environment (G x E) interaction can potentially exist with respect to concentrations of different phytochemicals present in pepper fruit tissue when planted in different environmental locations. Pungency levels in excess of 6,000 Scoville Heat Units were reported by Harvell and Bosland (1997), signifying the relative contribution a particular environment can have on variation observed in phytochemical expression. Previous reports by Lee et al. (2005) and Leskovar et al. (2009) suggest that variations in phytochemical expression are due to environmental differences and can be the result of changes in daytime and nighttime temperatures, soil type, elevation, cultural practices, solar radiation, and precipitation. Therefore, choosing the appropriate

combination of environment and genotype will potentially assist in production of the highest quality pepper fruit for consumers.

Conclusions

In an effort to develop improved Habanero genotypes that address current and future trends of the industry, breeders need to focus on creation of material with larger fruit, elevated phytochemicals, and disease resistance. From our results, we were able to identify genotypes that produce larger fruit than the commercial check. Our conclusions also confirmed previous reports by Lee et al. (2005) indicating that the Weslaco environment produces larger fruit with higher amounts of capsaicinoids. H5-dark orange was the most stable genotype and produced capsaicinoid levels comparable to Kuk-orange (standard) while H6-yellow produced the lowest comparable to the standard TMH (Crosby et al., 2005). These observations could, therefore, lead to H5-dark orange being a potential candidate for markets where hot, pungent Habanero peppers are valued and H6-yellow being another mild option for consumers who desire a product low in "heat". In regards to flavonoids, results from this experiment found levels in Habanero fruit tissue to be relatively low as previously mentioned (Howard et al., 2000). This outcome may be due to the convergence of the phenylpropanoid and capsaicinoid biosynthetic pathways during fruit maturation (Materska and Perucka, 2005; Sukrasno and Yeoman, 1993). If flavonoids are produced further downstream in comparison to capsaicinoids, this may explain why a possible inverse relationship exists and why a decrease in flavonoid concentrations are found in fruit tissue of material generally

having higher capsaicinoid levels. As previously mentioned by Howard (2001), we hypothesized that Habanero peppers with higher capsaicinoid levels will potentially have more phenylalanine being diverted toward the production of capsaicinoids within fruit tissue at the expense of flavonoid production. However, further studies are needed to confirm this speculation.

This experiment also complements results from previous studies (Lee et al., 2005; Harvell and Bosland, 1997; Zewdie and Bosland, 2000) showing both genotype and genotype x environment components impact phytochemical expression in peppers. Identification of the appropriate environmental location to grow a specific pepper genotype is an important factor to produce the highest quality product. Changing the environmental location can affect not only the size of marketable fruit, but also levels of different phytochemicals present within fruit tissue. Therefore, we conclude that the new Habanero material described herein can potentially compete against commercial cultivars for fruit weight, capsaicinoid and flavonoid levels, as well as, disease resistance.

CHAPTER III

PHYTOCHEMICAL VARIABILITY AMONG *CAPSICUM ANNUUM* GENOTYPES SPATIALLY DISTRIBUTED ACROSS TEXAS

Depending on the genetic potential of an entry, variation in ascorbic acid (AA), capsaicinoids (capsaicin and dihydrocapsaicin), and flavonoids (quercetin and luteolin) can be observed when material is grown in different environmental locations. To better address this topic and identify the phytochemical concentrations present in fruit tissue of different *Capsicum annuum* hybrids within the Vegetable and Fruit Improvement Center's (VFIC) breeding program, quantification of the aforementioned phytochemicals was conducted in ten different genotypes after growing in three Texas locations: Amarillo, College Station-VFIC, and Uvalde over the spring 2009 season. This experiment examined the effects of genotype and environment on levels of health-promoting phytochemicals in peppers. The goal is to provide consumers with a choice of pepper products with improved health benefits.

Although several previous studies have reported on evaluating material (*C. annuum*) for different phytochemicals (Howard et al., 2000; Lee et al., 1995; Perucka and Oleszek, 2000; Poyrazoglu et al., 2005; Zewdie and Bosland, 2000; Lee et al., 2005; Materska and Perucka, 2005; Sanatombi and Sharma, 2008; Singh et al., 2009), this experiment examined concentrations of AA, capsaicin, and flavonoids in several advanced *C. annuum* hybrids, which are not currently present in the industry, after being grown in multiple Texas locations. The objectives of this experiment were to accurately

quantify the phytochemical concentrations in these select genotypes and report on their genotypic potential in comparison to current commercial checks. Our goal was to determine the phytochemical potential of these select hybrids in an attempt to gain more scientific evidence to identify the most favorable genotypes and determine if a justification exists for them to ultimately replace current material in the industry.

Materials and Methods

Plant Material

In this experiment, four advanced jalapeño hybrids and three commercial checks (Dragon, Ixtapa, and J1845) were evaluated, as well as, two advanced cayenne hybrids with a known commercial check (Mesilla) for a total of ten genotypes. Each genotype was developed from different pedigrees, resulting in mature fruit varying in fruit size, days to maturity, and potentially, phytochemical levels. These genotypes were transplanted into the field in the spring of 2009 at three Texas A&M AgriLife Research and / or Extension locations: Amarillo (latitude: 35.22° N; longitude: 101.82° W), College Station-VFIC (latitude: 30.61° N; longitude: 96.32° W), and Uvalde (latitude: 29.22° N; longitude: 99.78° W). A sub-surface drip irrigation method was utilized in the College Station-VFIC and Uvalde locations while an overhead method was utilized in the Amarillo location. In Amarillo, transplants were established in a Pullman silty clay loam soil; in College Station-VFIC, transplants were established in a sandy clay loam textured soil; and in Uvalde, transplants were established in a silty clay loam (fine-silty, mixed, hyperthermic aridic calciustoll) textured soil. Full-sized green jalapeño and

red cayenne fruits were randomly harvested from three to five individual plants per plot from each location. Harvested fruits were selected that appeared healthy, turgid, and were of an appropriate size before they were transported to College Station-VFIC and stored at -80 °C to avoid phytochemical degradation.

Ascorbic Acid Extraction and Analysis

The AA extraction method was similar to that followed by Wimalasiri and Wills (1983) with some modifications. All extraction procedures used three separate sub-samples of frozen, fresh pepper tissue smashed with seeds (~ 5 grams) to serve as replications, and each sample was homogenized in 20 mL with 3% meta-phosphoric acid before being adjusted to 30 mL. Each extraction tube was then thoroughly shaken, filtered, and centrifuged at 6,000 rpm for 10 min. before being injected into a High Performance Liquid Chromatography (HPLC) machine using a Perkin Elmer LC 295 UV/Vis detector. AA concentrations were quantified at 254 nm using a µBondapak NH_2 125A (3.9 x 300 mm, 10µm) column with a guard cartridge at a flow rate of 1.0 mL per min. for 10 min. Mobile phase conditions employed 70% Acetonitrile (ACN) in nanopure water with ammonium dihydrogen phosphate (1.158 g L^{-1}). Using a pure concentration of AA (Sigma Aldrich Chemical Co.), a standard curve (31.25, 62.5, 125, 250, and 500 µg g^{-1}) was prepared to quantify levels within fresh fruit tissue.

Capsaicin Extraction and Analysis

Capsaicin and DHC levels were extracted as described by Singh et al. (2009) with some modifications. All extraction procedures, again, used three separate sub-samples of frozen, fresh pepper tissue smashed with seeds (~ 5 grams) to serve as replications, but each sample was homogenized in 20 mL of 100% methanol using a Polytron PT 10-35 Homogenizer (Kinematica Inc., Bohemia, NY) before being adjusted to 30 mL. Fruit tissue were allowed to precipitate in a -20 °C freezer before a sample of supernatant was collected and placed into an HPLC system where a Perkin Elmer LC 295 UV/Vis detector was used. Capsaicin and DHC levels were quantified at 280 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4µm) column with a guard cartridge at a flow rate of 1.0 mL per min. for 20 min. using of combination of 45% ACN in water + 0.5% H_3PO_4. The final step included injecting a volume of 40 µL from each sample, and the area under the curve was calculated using external standards of capsaicin and DHC to determine concentrations present on a fresh weight basis.

Flavonoid Extraction and Analysis

The flavonoid extraction method was similar to that followed by Lee et al. (1995; 2005) with some modifications. A sample of supernatant from each methanol extraction tube was collected and hydrolyzed with 3N hydrochloric acid at 90 °C for 60 minutes before being placed into an HPLC system to detect quercetin and luteolin concentrations using a Perkin Elmer LC 295 UV/Vis detector. Flavonoids were quantified at 360 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4µm) column with a guard cartridge at a flow rate

of 0.8 mL per min. for 20 min. Mobile phase conditions employed solvent A (0.5% H_3PO_4 in water) and solvent B (0.5% H_3PO_4 in methanol) to increase from 40% B to 100% B in 20 min. An injection volume of 20 µL from each sample was injected into the HPLC system and, as mentioned previously, quantified using known external standards of quercetin and luteolin.

Statistical Analysis

All plant material were planted in completely randomized designs. A SAS (2008) program employing a General Linear Model (GLM) procedure, and Least Significant mean comparisons by LSD ($P \leq 0.05$) were used to analyze for differences in Locations (L), Genotypes (G), and Location by Genotype (L x G) interactions for these phytochemicals across different locations and in each individual location when considering each source as a fixed effect. Hartley's Homogeneity of Variance (HOV) test was also conducted as described by Hoshmand (2006). In addition, a correlation analysis was conducted between total AA, total capsaicinoids (capsaicin+DHC), and total flavonoids (quercetin+luteolin).

Results and Discussion

Ascorbic Acid Concentrations in Different C. annuum *Peppers*

All values in the AA Analysis of Variance (ANOVA) table revealed significant L, G, and L x G interactions (Table 9). After separating mean values of each genotype across locations by $LSD_{(0.05)}$, only three jalapeños (J1, Dragon, and Ixtapa) and two

cayennes (C2 and Mesilla) produced significant differences (Table 10). In general, material grown in the College Station-VFIC location produced fruit with higher levels of AA. As expected, cayenne samples contained higher levels of AA than jalapeños. Of the four jalapeño hybrids examined, J1 showed the most promise to compete against these three commercial checks with respect to their AA concentrations. In comparison to the commercial cultivar, Mesilla, both C1 and C2 expressed higher AA levels at each location (Table 10).

Capsaicin and DHC Concentrations in Different C. annuum *Peppers*

All components in the capsaicin ANOVA table were significant except the L source of variance (Table 9). When each entry was analyzed across locations, significant differences in mean values were observed except for J2, J3, J4, and C1 when separated by LSD $_{(0.05)}$ (Table 11). With respect to the DHC ANOVA table, all components were significant except the G source of variance (Table 9). When each entry was analyzed across locations, significant differences in mean values were again observed except for J3 and C2 when separated by LSD $_{(0.05)}$ (Table 11). In most instances, Amarillo produced fruit with higher capsaicinoids. In some cases, jalapeño genotypes expressed significantly higher capsaicinoids than some cayenne genotypes (J4-101.13 μg g^{-1} versus C2-33.09 μg g^{-1} in the College Station-VFIC location) while in other comparisons, certain cayenne genotypes expressed significantly higher capsaicinoids than other jalapeño genotypes (Mesilla-241.56 μg g^{-1} versus J2-60.05 μg g^{-1} in the Amarillo location). This evidence further supports the idea that it is important

for plant breeders to match the genotype with its appropriate environment to maximize expression of these specific phytochemicals. Interestingly, both jalapeño and cayenne hybrids generally expressed appreciably lower levels of capsaicinoid concentrations than their respective commercial checks. These hybrids may, therefore, give farmers an opportunity to produce an improved pepper product for markets interested in milder genotypes.

Quercetin and Luteolin Concentrations in Different C. annuum *Peppers*

All components in the quercetin ANOVA table were significant except the L x G interaction source of variance (Table 9). When each entry was analyzed across locations, significant differences in mean values were observed except for J2, C1, C2, and Mesilla when separated by $LSD_{(0.05)}$ (Table 12). With respect to the luteolin ANOVA table, all components were significant (Table 9). When each entry was again analyzed across locations, significant differences in mean values were observed for each entry except for Mesilla when separated by $LSD_{(0.05)}$ (Table 12). In general, College Station-VFIC produced pepper fruit with higher flavonoid concentrations. As expected, cayenne genotypes expressed higher flavonoid values than jalapeños. In a direct comparison to the commercial checks, J1 and J3 produced similar levels. In a direct comparison to Mesilla, C2 may hold some potential especially if grown in an environment that mimics that of College Station-VFIC or Uvalde.

Genotype by Environment Influence

Results from our correlation analyses produced sample correlation (r) values of 0.0866, 0.0683, and 0.713 for total capsaicinoids (capsaicin+DHC) versus total flavonoids (quercetin+luteolin), total capsaicinoids (capsaicin+DHC) versus total AA, and total flavonoids (quercetin+luteolin) versus total AA, respectively. These respective R^2 values (r value^2*100), therefore, identified 0.75% of the variability of total capsaicinoids (capsaicin+DHC) to be explained by total flavonoids (quercetin+luteolin); 0.47% of the variability of total capsaicinoids (capsaicin+DHC) to be explained by total AA; and 50.77% of the variability of total flavonoids (quercetin+luteolin) to be explained by total AA. These results signified that total capsaicinoids (capsaicin+DHC) and total flavonoids (quercetin+luteolin), as well as, total capsaicinoids (capsaicin+DHC) and total AA are not significantly associated. It did, however, provide some evidence that total flavonoids (quercetin+luteolin) can be associated to total AA to some degree. Although this is one of the first reports of this association, to our knowledge, more research is needed before it can be confirmed. According to Hartley's HOV test, data analyzed both within and across locations produced significant and therefore heterogeneous variance values for each measurement.

Development of improved pepper material containing elevated phytochemical levels through the use of traditional breeding techniques is valuable for researchers to provide consumers with higher quality products to better sustain their health. Results from this experiment provide evidence that elite pepper materials exist for these characteristics of interest. It can be concluded that differences in phytochemical

expression observed in fruit tissue of these genotypes are due in large part to not only genetics but also maturity stage of fruit (Howard et al., 2000), environmental conditions, cultural practices, altitude (Lee et al., 2005; Leskovar et al., 2009), and other various post-harvest conditions that arise (Howard, 2001; Lee et al., 1995; Amiot-Carlin et al., 2007). Although their experiment included an examination on both phenolic content and phenolic acid in hard spring wheat (Mpofu et al., 2006), variation due to both genotypic and environmental components were identified. As with other groups (Mpofu et al., 2006), we acknowledge the limited amount of evidence with respect to studies evaluating phytochemical expression in germplasm after being grown in different locations. However, if we apply similar reasoning to peppers, it can be assumed that testing new cultivars over years and locations is crucial to characterizing their potential as sources of elevated human wellness phytochemicals. Therefore, it is possible that development of pepper material with increased levels of these beneficial phytochemicals will garner more interest by consumers concerned with maintaining a healthy lifestyle, resulting in more of these products being consumed on a regular basis.

Conclusions

Consistent performance by one or all of these hybrids grown in different locations across different years may support future release as a commercial cultivar. Depending on market preference, J1 and C2 may provide farmers with an improved option they can produce to deliver enhanced flavonoids and AA, respectively. If lower pungency is an important trait of interest in a particular market, results indicate J3 could

be a potential heatless option, while J1 and J2 could be utilized as potential mild pungency cultivars. With respect to cayenne markets, C1 expressed higher capsaicinoid values than C2, but other traits such as yield and dry matter might determine where these two genotypes would be most useful. As we previously mentioned, College Station-VFIC produced fruit with higher levels of both AA and flavonoids. Therefore, producers desiring to generate comparable phytochemical concentrations using these genotypes would probably be most successful if an environmental location mimicking that of College Station-VFIC was chosen. One explanation for the reason why the Amarillo location produced fruit with higher capsaicinoids may have been due, in some part, to both the higher altitude and overhead delivery system of the irrigation. Higher altitudes can potentially assist in developing material expressing elevated phytochemical concentrations, as in the case dictated by Kurz and Constabel (1998) who revealed a common observation of anthocyanin formation by plants grown in high altitudes in response to UV irradiation. With respect to the different irrigation systems, a sub-surface drip method, as in the College Station-VFIC and Uvalde locations, would have been able to apply a more direct amount of moisture for better plant uptake, thereby, reducing the amount of stress experienced by the plant and potentially reducing their need to synthesize capsaicinoids. Although the use of an overhead irrigation system, as in the Amarillo location, would be relatively effective at cooling leaf temperatures below that of the air as discussed by Lomas et al. (1972), it is possible that this method was not as accurate in its delivery to the plants as the drip irrigation methods. Included in Shashidhara (2006) are a few examples of different groups who examined variable

irrigation practices to gain insight into their potential productivity. Arguably, plants not receiving an adequate supply of moisture would potentially be subjected to more stress, which would increase their production of capsaicinoids, as reported by Estrada et al. (1999) who evaluated Padrón peppers in a similar experiment. We also postulate that the Amarillo site has the potential to expose material to different amounts of drought stress caused by the presence of variable amounts of dry winds than when compared to the other two locations. It is also possible that both lower humidity and heat could impact to either dry the soil in an irregular fashion or too much between irrigation schedules. Because these different locations are positioned at different altitudes across Texas, it is possible that different amounts of solar radiation were present, which could have arguably contributed to variable amounts of capsaicinoid synthesis. One hypothesis may be that this cultural practice assisted in stimulation of genotypes to produce higher levels than when grown at the alternate locations where the drip irrigation method was used. In lieu of this hypothesis, it still leaves the explanation open as to how increased AA concentrations were present in fruit tissue grown in College Station-VFIC more than in Amarillo. One obvious explanation, as mentioned earlier, may be due to the presence of different soil types in each location and possibly the amount of cloud cover present during the experiment. From our results, it appeared as though the use of an overhead irrigation system in the Amarillo location contributed to production of different stressful conditions that may have assisted in producing fruit with higher capsaicinoid levels; however, it should be mentioned that this cultural practice on peppers can result in a potential increase in disease pressure. On the other hand, we

have provided evidence of phytochemical variation in new hybrid pepper material that may serve the needs of producers in various markets.

CHAPTER IV

PHYTOCHEMICAL EXPRESSION IN VARIOUS *CAPSICUM ANNUUM* HYBRIDS UNIQUE TO TEXAS CLIMATES DUE TO GENOTYPE AND ENVIRONMENT

Significant variation in phytochemical expression within pepper fruit tissue is dependant upon several factors. Genotypic, as well as, environmental differences have both contributed to material of variable phenotypic expression. The ultimate goal of pepper breeders is, therefore, to use this knowledge and apply it in a manner to more effectively match the best genotype with its optimum environment to achieve the most desirable output. For this experiment, fruit measurements (fruit diameters, lengths, and wall thicknesses) and quantification of three different phytochemical groups (ascorbic acid, capsaicinoids, and flavonoids) were compared in 21 different *Capsicum annuum* genotypes after each were grown in two diverse environmental locations in Texas during the spring 2010 season. Ideally, evidence from this experiment will further suggest the potential benefit this material could have for growers interested in replacing current material in the industry to more successfully provide consumers with a healthier product.

Design of this experiment was meant to quantify the concentrations of different phytochemicals (ascorbic acid, capsaicin, and flavonoids) and report on variation in fruit characteristics (fruit length, fruit diameter, and wall thickness) on 16 new pepper (jalapeño, serrano, and cayenne) hybrids developed by researchers at Texas A&M University in comparison to 5 commercial checks. Comparison of a total of 21 different

genotypes was crucial to distinguish the most favorable lines for potential release in the future. Although several previous studies have reported on evaluating material (*C. annuum*) for different phytochemicals (Lee et al., 1995; Perucka and Oleszek, 2000; Howard et al., 2000; Zewdie and Bosland, 2000; Materska and Perucka, 2005; Lee et al., 2005; Poyrazoglu et al., 2005; Sanatombi and Sharma, 2008; Singh et al., 2009), this experiment was meant to complement those results and introduce new advancements in the area of plant breeding by examining a combination of these three phytochemical groups, as well as, fruit characteristics in recently developed pepper material not currently present in the marketplace after being grown in two diverse Texas locations. Our objectives were to identify the best genotype(s) across these locations and in each location, with regards to consistent phytochemical levels, and to select visually appealing fruit characteristics in an effort to give farmers an improved option that might perform well in their markets. Ultimately, our goal was to identify the most elite hybrids having the capacity and potential to out perform current genotypes present in the industry. In the near future, material from this experiment, expressing improved characteristics of interest, can be evaluated on a large scale in a one-on-one comparison by farmers to provide further evidence of their superior qualities. This comparison could then result in some of this material being used to replace current commercial hybrids.

Materials and Methods

Plant Material

All pepper material for this experiment were grown at two Texas A&M AgriLife Research and Extension locations: Uvalde (latitude: 29.22° N; longitude: 99.78° W) and Weslaco (latitude: 26.16° N; longitude: 97.98° W) in the spring of 2010. A sub-surface drip irrigation method was utilized in each location and as close to commercial agricultural practices as possible were followed. At Uvalde, transplants were established in a silty clay loam (fine-silty, mixed, hyperthermic aridic calciustoll) textured soil; and in Weslaco, transplants were established in a Hidalgo fine sandy loam textured soil. All fruit harvested were selected that were of an appropriate size, appeared healthy, and turgid at the time of harvest before all were held at -80 °C to avoid phytochemical degradation.

Ascorbic Acid Extraction and Analysis

The AA extraction method was similar to that followed by Wimalasiri and Wills (1983) with some modifications. All extraction procedures used multiple sub-samples of frozen, fresh pepper material smashed with seeds (~ 5 grams) to serve as replications, and each sample was homogenized in 20 mL with 3% meta-phosphoric acid before being adjusted to 30 mL. Each extraction tube was then thoroughly shaken, filtered, and centrifuged at 6,000 rpm for 10 min. before being injected into an HPLC system using a Perkin Elmer LC 295 UV/Vis detector. AA concentrations were quantified at 254 nm using a µBondapak NH_2 125A (3.9 x 300 mm, 10µm) column with a guard cartridge at a

flow rate of 1.0 mL per min. for 10 min. Mobile phase conditions employed 70% ACN in nanopure water with ammonium dihydrogen phosphate (1.158 g L^{-1}). Using a pure concentration of AA (Sigma Aldrich Chemical Co.), a standard curve (31.25, 62.5, 125, 250, and 500 µg g^{-1}) was prepared to quantify levels within fresh fruit tissue.

Capsaicin Extraction and Analysis

Capsaicin and dihydrocapsaicin (DHC) levels were extracted similar to that described by Singh et al. (2009) with some modifications. All extraction procedures used multiple sub-samples of frozen, fresh pepper material smashed with seeds (~ 5 grams) to serve as replications, and each sample was homogenized in 20 mL of 100% methanol using a Polytron PT 10-35 Homogenizer (made by Kinematica AG, Switzerland) before being adjusted to 30 mL. Fruit tissue was allowed to precipitate before a sample of supernatant was collected and inserted into an HPLC system where a Perkin Elmer LC 295 UV/Vis detector was used to complete the analysis. Capsaicin and DHC levels were quantified at 280 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4µm) column with a guard cartridge at a flow rate of 1.0 mL per min. for 20 min. using a combination of 45% Acetonitrile (ACN) in water + 0.5% H_3PO_4. The final step included injecting a volume of 40 µL from each sample into the HPLC system, and the area under the curve was calculated to determine capsaicin and DHC levels present on a fresh weight basis. External capsaicin and DHC standards were then used to quantify the concentrations of these compounds within fruit tissue.

Flavonoid Extraction and Analysis

The flavonoid extraction method was similar to that followed by Lee et al. (1995; 2005) with some modifications. A sample of supernatant from each methanol extraction tube was collected and hydrolyzed with 3N hydrochloric acid at 90 °C for 60 minutes before being placed into an HPLC system to detect flavonoids (quercetin and luteolin) using a Perkin Elmer LC 295 UV/Vis detector. Flavonoids were quantified at 360 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4μm) column with a guard cartridge at a flow rate of 0.8 mL per min. for 20 min. Mobile phase conditions employed solvent A (0.5% H_3PO_4 in water) and solvent B (0.5% H_3PO_4 in methanol) to increase from 40% B to 100% B in 20 min. An injection volume of 40 μL from each sample was injected into the HPLC system and, as mentioned previously, quantified using known external standards of quercetin and luteolin.

Statistical Analysis

All experiments were planted in completely randomized designs. A SAS program employing a General Linear Model (GLM) procedure, and least significant mean comparisons by LSD ($P \leq 0.05$) were used to analyze for differences in Locations (L), Genotypes (G), and Location by Genotype (L x G) interactions for these phytochemicals and fruit characteristics across locations and in each individual location (SAS Institute, 2008) when considering each source as a fixed effect. Hartley's Homogeneity of Variance (HOV) test was also conducted as described by Hoshmand

(2006). In addition, a correlation analysis was conducted between total capsaicinoids (capsaicin+DHC), total ascorbic acid, and total flavonoids (quercetin+luteolin)

Results and Discussion

For this experiment, different jalapeño, serrano, and cayenne hybrids were grown and evaluated for comparison to current popular, commercial genotypes. After statistically analyzing the data across these locations for the different fruit characteristics, all the F-values were significant except the L x G parameter for the wall thickness characteristic (Table 15). Within each location, all the F-values were significant (Table 16). When each entry was analyzed across locations, significant differences in mean values were observed except for J1, J2, J6, J8, and Dragon for fruit diameter and J2 for wall thickness when separated by $LSD_{(0.05)}$ (Table 17). Results also identified the Uvalde location as contributing the necessary conditions to effectively produce larger fruit in terms of not only weight (data not shown) and length, but also, larger diameters and thicker walls than those grown at Weslaco (Table 17). In general, each hybrid had comparable if not better fruit characteristics than their respective hybrid counterparts (Table 17).

For AA, significant variation in expression was observed both within and across these two locations (Table 18). In general, Weslaco produced material with significantly higher concentrations of AA than when the same genotype was grown in Uvalde. In a closer examination, results seemed to identify J-9 as being the most consistent jalapeño hybrid at each location (500.81 and 681.46 $\mu g\ g^{-1}$ FW, respectively, versus 476.46 and

441.46 µg g^{-1} FW for Dragon). When examining the serrano hybrids, the S-3 hybrid (178.53 µg g^{-1} FW) performed better in the Uvalde location than the other hybrids but still expressed a lower concentration of AA than both Halcon (265.85 µg g^{-1} FW) and Magnum45 (390.13 µg g^{-1} FW). In the Weslaco location, the S-1 hybrid (722.55 µg g^{-1} FW) out-performed all others including the commercial checks. For the cayenne hybrids, C-1 (1272.12 and 2167.59 µg g^{-1} FW, respectively) significantly outperformed C-2 (1214.74 and 1557.65 µg/g FW, respectively) and Mesilla (610.93 and 865.60 µg g^{-1} FW, respectively).

For capsaicinoids, Weslaco again seemed to produce material with higher concentrations than those from Uvalde (Table 19). Results found J-10 (104.59 and 208.50 µg g^{-1} FW, respectively) as being the most optimum jalapeño hybrid in the two locations for its potential use in hot markets and for growers desiring a product to compete against Dragon (62.82 and 281.41 µg g^{-1} FW, respectively) or Tormenta (139.35 and 125.85 µg g^{-1} FW, respectively). For mild markets, J-1 (0.00 µg g^{-1} FW) and J-3 (0.00 µg g^{-1} FW) may hold some promise for their ability to express little to no heat in either location. For the cayennes, both hybrids expressed lower concentrations of capsaicinoids than Mesilla. This evidence could be useful for consumers interested in a cayenne genotype having no heat (C-1) or a cayenne genotype having only a moderate level of heat (C-2).

For flavonoids, Uvalde seemed to be better at producing material with elevated quercetin and luteolin concentrations (Table 20). In a closer examination, results found J-5, J-6, and J-9 as all being superior to the two commercial checks in each location. In

nearly all cases, each serrano and cayenne hybrid outperformed its respective commercial check with respect to flavonoid expression (Table 20).

Results from our correlation analyses produced sample correlation (r) values of -0.3658, -0.1098, and 0.6495 for total capsaicinoids (capsaicin+DHC) versus total flavonoids (quercetin+luteolin), total capsaicinoids (capsaicin+DHC) versus total AA, and total flavonoids (quercetin+luteolin) versus total AA, respectively. These respective values, therefore, identified 13.38% of the variability of total capsaicinoids (capsaicin+DHC) to be explained by total flavonoids (quercetin+luteolin); 1.21% of the variability of total capsaicinoids (capsaicin+DHC) to be explained by total AA; and 42.18% of the variability of total flavonoids (quercetin+luteolin) to be explained by total AA. These results signified that total capsaicinoids (capsaicin+DHC) and total flavonoids (quercetin+luteolin) had a very minimal association, total capsaicinoids (capsaicin+DHC) and total AA to not be significantly associated, and total flavonoids (quercetin+luteolin) and total AA to, in fact, be associated to some degree. We have previously observed and identified this association between total flavonoids (quercetin+luteolin) and total AA in other similar studies, however, more research is needed before it can be completely confirmed. According to Hartley's HOV test, data analyzed within individual locations found the variance values of only fruit diameter and quercetin to be non-significant and therefore homogeneous. However, when data were analyzed across locations, all the variance values were significant and therefore heterogeneous.

After an improvement is made in the genotypic potential of a new genotype, the next step is to identify the most optimum environmental location that will effectively complement its performance and result in the most desirable product. Ultimately, development of new germplasm expressing an assortment of unique characteristics of interest that are also adaptable and consistent across multiple locations are all objectives of most plant breeders. Therefore, it is important to evaluate one's genotypes in different locations to gain more insight into their potential performance, which will provide further evidence of the various inputs that are critical to achieve the most desirable quality.

Conclusions

All of these results provide further evidence and strengthen previous work provided by our group (Lee et al., 2005; Leskovar et al., 2009) indicating significant variation in different characteristics of interest for a particular genotype after being grown in different environmental locations within the same season. Evidence from this experiment also suggests how important it is for plant breeders to make careful and detailed observations of their material when making field / greenhouse selections to more successfully identify the best location for a particular genotype. Furthermore, this experiment strengthens the imperative reasoning that a particular location should contain all the essential parameters to more effectively maximize the potential output of the product. As more consumers become aware of the potential health benefits that different fruits and vegetables can contribute to the human body, development of improved

material having both desirable fruit characteristics, as well as, elevated concentrations of phytochemicals will likely prompt breeders to continue this trend (Yoo et al., 2007; Rodríguez-Burruezo et al., 2009). In this experiment, results are provided that farmers and fellow scientists can use to gage how this material will perform in a similar location. From these results, one can arguably state the genotypes J-9, S-1, and C-1 possess many of the quality attributes that growers are searching for, and will likely result in their commercialization in the near future.

CHAPTER V

HERITABILITY INVESTIGATION IN DIFFERENT *CAPSICUM ANNUUM* GENOTYPES FOR FRUIT CHARACTERISTICS, ASCORBIC ACID, CAPSAICIN, AND FLAVONOIDS

Pepper diversity is present in great detail across the *Capsicum* genus with respect to fruit size, shape, color, length, firmness, flavor, and even concentrations of different phytochemicals. When these species are consumed on a regular basis, they have the potential to benefit one's health and protect against deadly diseases. In an effort to determine the relative ease of incorporating these particular traits of interest into an improved specimen, a combination of 29 F_1 paprika and serrano pepper (*Capsicum annuum*) hybrids along with 19 of their respective parents were chosen for evaluation after being grown at the Texas AgriLife Research and Extension Station in Weslaco in the spring of 2008. Our idea, therefore, is that these results will be used as potential guidelines to inform breeders of the relative feasibility to develop improved lines for these characteristics.

For this experiment, our goal was to identify the elite F_1 material for specific characteristics while assessing parent lines for capacity to transmit useful traits to progeny. Although several studies have reported on the inheritance of these fruit characteristics and phytochemicals in different pepper (*C. annuum*) cultivars (Gopalakrishnan et al., 1987; Jabeen et al., 1999; Manju and Sreelathakumary, 2002; Geleta and Labuschagne, 2004; Sreelathakumary and Rajamony, 2004; Prasath and

Ponnuswami, 2008; Reddy et al., 2008; Yadeta et al., 2011), we also wanted to provide genetic evidence from our families to compare with these results, and gauge how effectively a particular characteristic can be passed from two pepper parents to their offspring. In this experiment, broad sense heritability estimates and various correlations were calculated in advanced material not currently present in the marketplace.

Materials and Methods

Plant Material

Seed were developed by controlled pollinations for 29 select F_1 paprika and serrano pepper hybrids developed from a combination of 19 parental lines. After germinating, all transplants were set out and grown during the spring 2008 season in a Hidalgo fine sandy loam textured soil at the Texas A&M AgriLife Research and Extension Station in Weslaco (latitude: 26.16° N; longitude: 97.98° W). A sub-surface drip irrigation method was utilized, and commercial agricultural practices were followed. All fruit harvested were selected at an appropriate maturity stage and size, and were healthy and turgid before being relocated to College Station-VFIC, TX. Fruit measurements (fruit weight, fruit length, fruit diameter, and pericarp wall thickness) were conducted on fruits to gain insight into their potential variation (Table 22), and all fruits were then stored at -80 °C until phytochemical analysis (AA, capsaicin, DHC, quercetin, and luteolin) could ensue.

Ascorbic Acid Extraction and Analysis

The AA extraction method was similar to that followed by Wimalasiri and Wills (1983) with some modifications. All extraction procedures used multiple sub-samples of frozen, fresh pepper material smashed with seeds (~ 5 grams) to serve as replications, and each sample was homogenized in 20 mL with 3% meta-phosphoric acid using a Polytron PT 10-35 Homogenizer (Kinematica AG, Switzerland) before being adjusted to 30 mL. Each extraction tube was then thoroughly shaken, filtered, and centrifuged at 6,000 rpm for 10 min. before being injected into an HPLC system to detect AA levels using a Perkin Elmer LC 295 UV/Vis detector. AA concentrations were quantified at 254 nm using a μBondapak NH_2 125A (3.9 x 300 mm, 10μm) column with a guard cartridge at a flow rate of 1.0 mL per min. for 10 min. Mobile phase conditions employed 70% Acetonitrile (ACN) in nanopure water with ammonium dihydrogen phosphate (1.158 g L^{-1}). Pure concentrations of AA were obtained (Sigma Aldrich Chemical Co.) to construct a standard curve (31.25, 62.5, 125, 250, and 500 μg g^{-1}) to quantify levels within fruit tissue on a fresh weight basis.

Capsaicin Extraction and Analysis

Capsaicin and dihydrocapsaicin (DHC) levels were extracted similar to that described by Singh et al. (2009) with some modifications. All extraction procedures used multiple sub-samples of frozen, fresh pepper material smashed with seeds (~ 5 grams) to serve as replications, and each sample was homogenized in 20 mL of 100% methanol using a Polytron PT 10-35 Homogenizer (Kinematica AG, Switzerland) before

being adjusted to 30 mL. Fruit tissue was allowed to precipitate before a sample of supernatant was collected and placed into an HPLC system where a Perkin Elmer LC 295 UV/Vis detector was used. Capsaicin and DHC levels were quantified at 280 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4μm) column with a guard cartridge at a flow rate of 1.0 mL per min. for 20 min. using of combination of 45% ACN in water and 0.5% H_3PO_4. External standards of capsaicin and DHC (Sigma Aldrich Chemical Co.) were also used to quantify these compounds within fruit tissue. The final step of this process was to inject a volume of 40 μL from each sample into the HPLC system, and the area under the curve was calculated to determine capsaicin and DHC levels present on a fresh weight basis.

Flavonoid Extraction and Analysis

The flavonoid extraction method was similar to that followed by Lee (1995; 2005) with some modifications. A sample of supernatant from each methanol extraction tube was collected and hydrolyzed with 3N hydrochloric acid at 90 °C for 60 minutes before being placed into an HPLC system to detect flavonoid (quercetin and luteolin) concentrations using a Perkin Elmer LC 295 UV/Vis detector. Flavonoids were quantified at 360 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4μm) column with a guard cartridge at a flow rate of 0.8 mL per min. for 20 min. Mobile phase conditions employed solvent A (0.5% H_3PO_4 in water) and solvent B (0.5% H_3PO_4 in methanol) to increase from 40% B to 100% B. An injection volume of 40 μL from each sample was

injected into the HPLC system and, as mentioned previously, quantified using known external standards of quercetin and luteolin.

Statistical Analysis

Plant material for this experiment were planted in a completely randomized design. A SAS program employing a General Linear Model (GLM) procedure and least significant mean comparisons by Duncan ($P \leq 0.05$) were used to analyze for differences in genotypes (G) for four fruit characteristics and the aforementioned phytochemicals (SAS Institute, 2008) when considering the genotype source as a fixed effect. Although the overall family structure of these genotypes were limited to only a few consistent ones, broad sense heritability estimates (h^2_B) (Table 23) were calculated via our ANOVA tables, allowing us to gain more of an idea with respect to their repeatability potential. In an effort to calculate the genotypic variance (σ^2_G), for example, we used the Mean Square (MS) values from our respective ANOVA table and calculated using the formula:

$$(MS_{Entry} - MS_{Error}) / \text{\# of replications}$$

To calculate the error variance (σ^2_e), we obtained the MS_{Error} value from the ANOVA table and were then able to insert all the components into the formula as shown below:

$$h^2_B = \sigma^2_G / \sigma^2_P, \text{ or more specifically, } [\sigma^2_G / (\sigma^2_G + \sigma^2_e)]$$

In addition, a correlation analysis was conducted in all combinations between total AA, total capsaicinoids (capsaicin+DHC), and total flavonoids (quercetin+luteolin), as well as, the fruit characteristics mentioned earlier (fruit weight, length, diameter, and wall thickness). In addition, a correlation analysis was conducted between F_1 offspring and

the mid-parent value from their respective parents for each characteristic. Mid-parent heterosis (MPH) values were also calculated in an effort to potentially identify the % increase of different F_1 hybrids in comparison to their respective parents for these different traits using the formula as described by Geleta and Labuschagne (2006) (Table 27).

$$MPH = \frac{(F_1 - MP)}{MP} * 100$$

And finally, high-parent heterosis (HPH) values were also calculated in a similar manner as above using the mean of the high-parent instead of the mid-parent (Table 28).

$$HPH = \frac{(F_1 - HP)}{HP} * 100$$

Results and Discussion

Fruit Measurements

Results in the Analysis of Variance (ANOVA) table revealed significant F-values for each fruit measurement, and relatively high heritability values were produced (Table 23). Although its mean value may not have been statistically different from all the other paprika parents for these different fruit measurements, results implied that PapP27 may be more likely to exhibit appreciable genes of interest for use as parent material to improve fruit weights and fruit diameters (Table 22). In contrast, PapP30 expressed some potential to possibly assist with improving fruit length, and PapP26 may assist more with improving fruit wall thickness. Hybrid Pap4 expressed the highest mean value for fruit weight (62.95 g), fruit diameter (42.00 mm), and wall thickness (3.20

mm), signifying its high Specific Combining Ability (SCA) potential for these characteristics, while hybrid Pap2 expressed the highest mean value for fruit length (188.80 mm). For the serrano material, SP50 was identified as having the highest mean value for both fruit weight (16.20 g) and fruit length (116.20 mm), while SP128 had the highest mean value for both fruit diameter (24.90 mm) and fruit wall thickness (3.90 mm) (Table 22). Evaluation of material for their potential combining ability revealed the highest mean value to be expressed in hybrid S28 for fruit weight (15.39 g), hybrid S107 for fruit length (109.80 mm), and hybrid S14 for both fruit diameter (20.00 mm) and fruit wall thickness (4.20 mm).

Phytochemical Concentrations

Results in the ANOVA table also revealed significant F-values for the different phytochemical groups and produced relatively high heritability values as well (Table 23). AA results found PapP26 to express the highest mean value (1781.36), while hybrid Pap5 expressed the highest SCA potential (Table 24). In the serrano material, SP2 expressed the highest mean value for AA (1599.78), while hybrid S48 expressed the highest mean value and SCA potential, respectively. PapP67 expressed the highest mean value for quercetin (211.70), while PapP30 had the highest mean value for luteolin expression (37.44). The Pap4 hybrid expressed the highest SCA potential for quercetin, and Pap5 expressed the highest respective potential for luteolin. With respect to capsaicinoid concentrations, all paprika material expressed very little to no capsaicin or DHC in their fruit tissue. For the serrano material, SP79 expressed the highest mean

value for capsaicin (285.71), SP71 for DHC (199.39), SP16 for quercetin (71.97), and SP2 for luteolin (15.89), respectively (Table 24). Hybrids expressing the highest mean values and SCA potential were S48, S68, and S12 for quercetin, capsaicin and DHC, and luteolin, respectively.

Conclusions

Results from this experiment were intended to provide evidence with respect to both the ease and / or difficulty facing pepper breeders interested in improving these related characteristics of interest in their germplasm through traditional breeding methods. Successful development of improved cultivars containing these characteristics of interest could lead to their eventual widespread acceptance and demand by consumers (Gepts and Hancock, 1986). Consistent performance by one or all of these varieties when grown in different locations across different years may allow for that cultivar becoming a new, unique option for producers to replace cultivars currently in the industry (Greenleaf, 1986). The ultimate goal of this experiment was to report on the heritability of a select number of characteristics to help breeders gain a more thorough idea of their relative ease in passing them from parent to offspring. In some instances, we strengthened previous reports by Geleta and Labuschagne (2006), indicating a few hybrids can in fact express an improvement for a particular characteristic of interest with respect to their parents (Tables 22 and 24). For example, hybrid Pap4 expressed a higher mean value for both fruit weight (62.95 g) and fruit length (169.60 mm) than either of its parents (PapP27 and PapP67), while hybrid S90 expressed higher concentrations of both quercetin (19.37 $\mu g\ g^{-1}$) and luteolin (7.86 $\mu g\ g^{-1}$) than either of its parents (SP41 and

SP95). These results could very well indicate positive non-additive gene action between these different parents and a useful specific combining ability potential. Interestingly, we were also able to calculate correlation values for each characteristic (Table 25), allowing us to expand on the potential variability that was present and identify a significant amount of association between various characteristics (Table 26). For example, correlation values of 90.6% (fruit weight and fruit diameter) and 96.8% (total capsaicinoids and capsaicin) were calculated. As expected, fruits expressing higher weights will more than likely have the necessary potential to produce fruits with larger diameters to compensate for the increased fruit size. In addition, those genotypes expressing elevated capsaicin content will, as expected, more than likely produce higher amounts of total capsaicinoids. Correlation analyses between F_1 offsprings and their mid-parent values indicated, among others, an association of 91.4 and 81.2 % for fruit length and fruit diameter, respectively (Table 25). This particular evidence can, therefore, serve as a general idea to verify how possible it is for breeders to express these different characteristics in an F_1 offspring and gauge how productive they might be in comparison to their respective parents. We were also able to identify a significant amount of positive heterosis (% increase) in some material with respect to their F_1 performance and respective parents (Table 27). In some comparisons, we were able to identify a significantly high % increase value as seen in hybrid S27 for capsaicin expression (1289.23 %) and hybrid S32 for total capsaicinoid expression (902.32 %). This evidence may suggest the potential existence of an ideal amount of specific combining ability between these individuals' parents. On the other hand, we were also

able to identify some negative heterosis estimates as seen in S43 for both quercetin and luteolin expression (-74.93 and -55.92 %, respectively). These negative values could, therefore, serve as an indicator of the potential existence of a reduced amount of specific combining ability when crossing ensued. Due to the significantly high heterosis values we obtained for these particular characteristics of interest, we postulate that this could explain why our heritability estimates were relatively higher than expected in this experiment. On the other hand, all of this information will arguably reiterate how important it is for breeders to maximize the amount of potential heterosis and achieve more success when two particular genotypes are crossed in a unique direction for the purpose of developing an ideal offspring the agricultural community will more likely accept. These trends and germplasm can then be exploited on a larger scale for use in future hybrid production practices.

CHAPTER VI

GENETIC VARIATION AND MOLECULAR MARKER SCREENING IN A UNIQUE F_2 PEPPER (*CAPSICUM* SPP.) FAMILY FOR FLAVONOID (QUERCETIN AND LUTEOLIN) AND ASCORBIC ACID CONCENTRATIONS

As pepper consumption continues to increase throughout the world, development of improved varieties expressing elevated phytochemicals will likely continue as a widespread objective in many breeders' programs. One important goal is to create new germplasm that exhibits the genetic capacity to consistently meet the nutritional needs of those who consume them on a regular basis. In this experiment, the main objectives were to quantify the genetic potential of a unique F_2 pepper family developed from a cross ('Ca377' x 'B22') to identify whether a useful amount of phytochemical variation could be found in fruit tissue, and then use that knowledge in an attempt to find a reproducible candidate molecular marker for both flavonoids (quercetin and luteolin) and ascorbic acid (AA).

Although phytochemical expression is dependent upon several parameters outside the biology of the plant (Lester, 2006; Leskovar et al., 2009; Oh, et al., 2009a; Oh et al., 2009b), our goal was also to potentially identify reliable molecular markers that pepper breeders could use to more successfully identify superior genotypes for future germplasm development linked to elevated levels of quercetin, luteolin, or AA. To our knowledge, no molecular markers have been previously identified in pepper linked to elevated expression of these compounds. Our hypotheses were that a useful amount of genetic variation would be observed in the amount of quercetin, luteolin, and

AA concentrations present within fruit tissue harvested from this family, and this information would provide scientific evidence to better assist us in determining how potentially productive one or both of these parents might be in future projects. Our focus also included attempting to identify a molecular marker linked to elevated levels of these three phytochemicals present within pepper fruit tissue.

Materials and Methods

Plant Material

Plant material for this experiment were developed from a unique cross between two pepper individuals—'Ca377' (a paprika type pepper) and 'B22' (a bell pepper) (Fig. 1). Approximately 125 F_1 seeds were collected and planted. Seedlings were then set out and transplanted into the field (fine-silty, mixed, hyperthermic aridic calciustoll clay loam soil) in the spring of 2008 at the Texas AgriLife Research and Extension Center in Uvalde, TX, (29.22° N; 99.78° W). A sub-surface drip irrigation method was utilized and commercial agricultural practices were followed. F_2 pepper fruits were harvested separately from each plant and bulked together, as well as young, disease free leaf tissue. Due to some individuals dying or not producing any fruit, fruit tissue was collected from 115 separate individuals. All fruits harvested were selected at a fully mature stage of development with appropriate size. Fruit tissue were stored at -80 °C to avoid phytochemical degradation until analytical quantification could ensue, and leaf tissue were stored at -20 °C until DNA extraction could ensue.

Fig. 1. Pictures of ‘Ca377’ (P_1) and ‘B22’ (P_2), respectively.

Flavonoid Extraction and Analysis

The flavonoid extraction method was similar to that followed by Lee et al. (1995; 2005) with some modifications. All extraction procedures used multiple sub-samples of frozen, fresh pepper material smashed with seeds to serve as replications. The frozen fruits were pulverized using a mallet, and approximately 5 grams of mixed fruit tissue was taken. Each sample was homogenized with 20 mL of 100% methanol using a Polytron PT 10-35 Homogenizer (Kinematica Inc., Bohemia, NY), and final volumes were adjusted to 30 mL. Each extracted sample was thoroughly shaken and allowed to precipitate completely in a -20 °C freezer. Four mL of each extract was mixed with 2 mL of 3N hydrochloric acid (HCl) and hydrolyzed at 90 °C for 60 min. Each hydrolyzed sample was then placed into an HPLC sample vial for analysis before being injected into the HPLC system. The HPLC system consisted of a Perkin Elmer Model 200 pump, autosampler, and LC 295 UV / Vis detector. Flavonoid (quercetin and luteolin) concentrations were quantified at 360 nm using a Nova-Pak C_{18} (4.6 x 150 mm, 4μm) column with a guard cartridge, and the solvent flow rate was 0.8 mL per min. using a combination of solvent A (0.5% H_3PO_4 in water) and solvent B (0.5% H_3PO_4 in

methanol) to increase from 40% B to 100% B in 20 min. External standards of quercetin (46.6 μg mL^{-1}) and luteolin (67.67 μg mL^{-1}) were used to quantify samples on a fresh weight basis (Fig. 2).

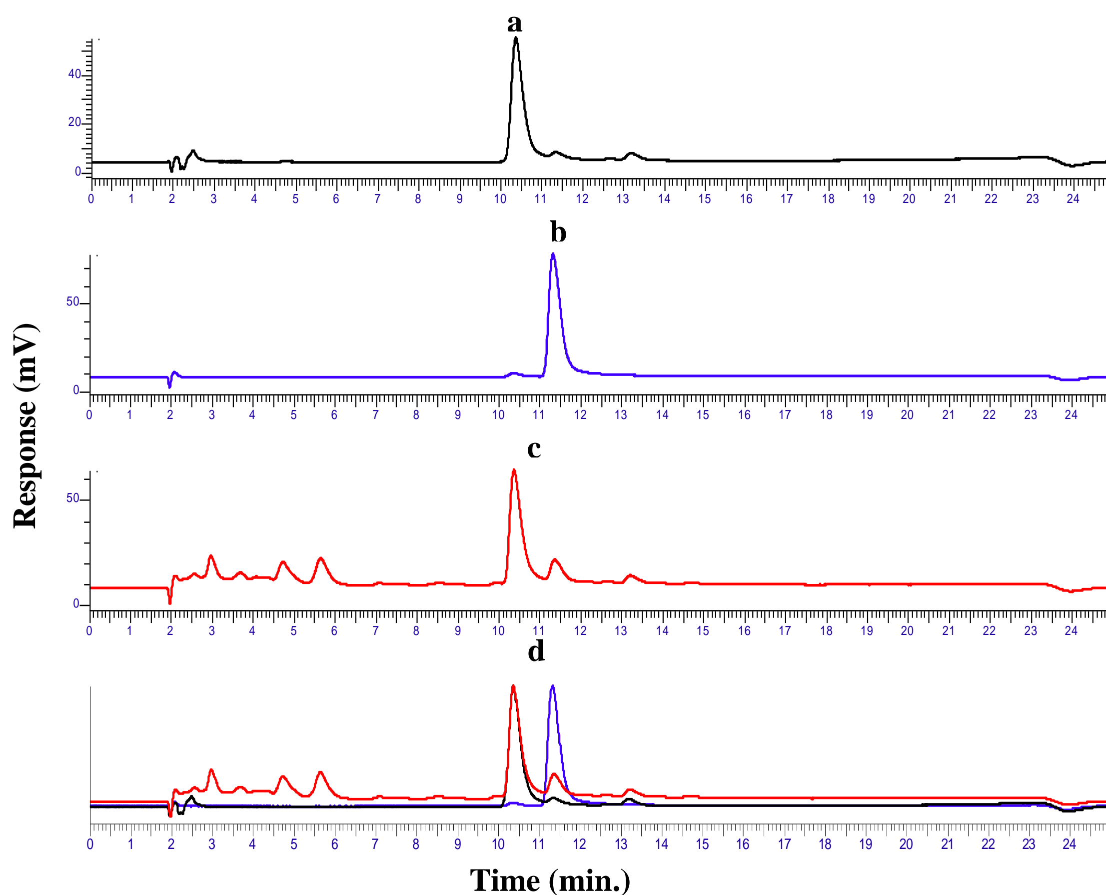

Fig. 2. HPLC chromatograms depicting an example of quercetin standard (a), luteolin standard (b), flavonoid sample with both quercetin and luteolin (c), and overlay of all three (d).

Ascorbic Acid Extraction and Analysis

The AA analysis method was similar to that of Wimalasiri and Wills (1983) with some modifications. The frozen fruits were again pulverized using a mallet, and approximately 5 grams of smashed fruit tissue was taken. Each sample was homogenized with 20 mL of 3% meta-phosphoric acid using a Polytron PT 10-35 Homogenizer (Kinematica AG, Switzerland). Final volumes were then adjusted to 30 mL. Each extraction tube was thoroughly shaken, filtered with P8 coarse filter paper (Fisher Scientific, Pittsburgh, PA), and centrifuged at 6,000 rpm for 10 min. before being injected into an HPLC system using a Perkin Elmer LC 295 UV / Vis detector. AA concentrations were quantified at 254 nm using a μBondapak NH_2 125A (3.9 x 300 mm 10μm) column with a guard cartridge at a flow rate of 1.0 mL per min. for 10 min. Mobile phase conditions employed 70% Acetonitrile (ACN) in nanopure water with ammonium dihydrogen phosphate (1.158 g L^{-1}). Using a pure concentration of ascorbic acid (Sigma Aldrich Chemical Co.), a standard curve (31.25, 62.5, 125, 250, and 500 μg g^{-1}) was prepared to quantify ascorbic acid levels within fresh fruit tissue (Fig. 3).

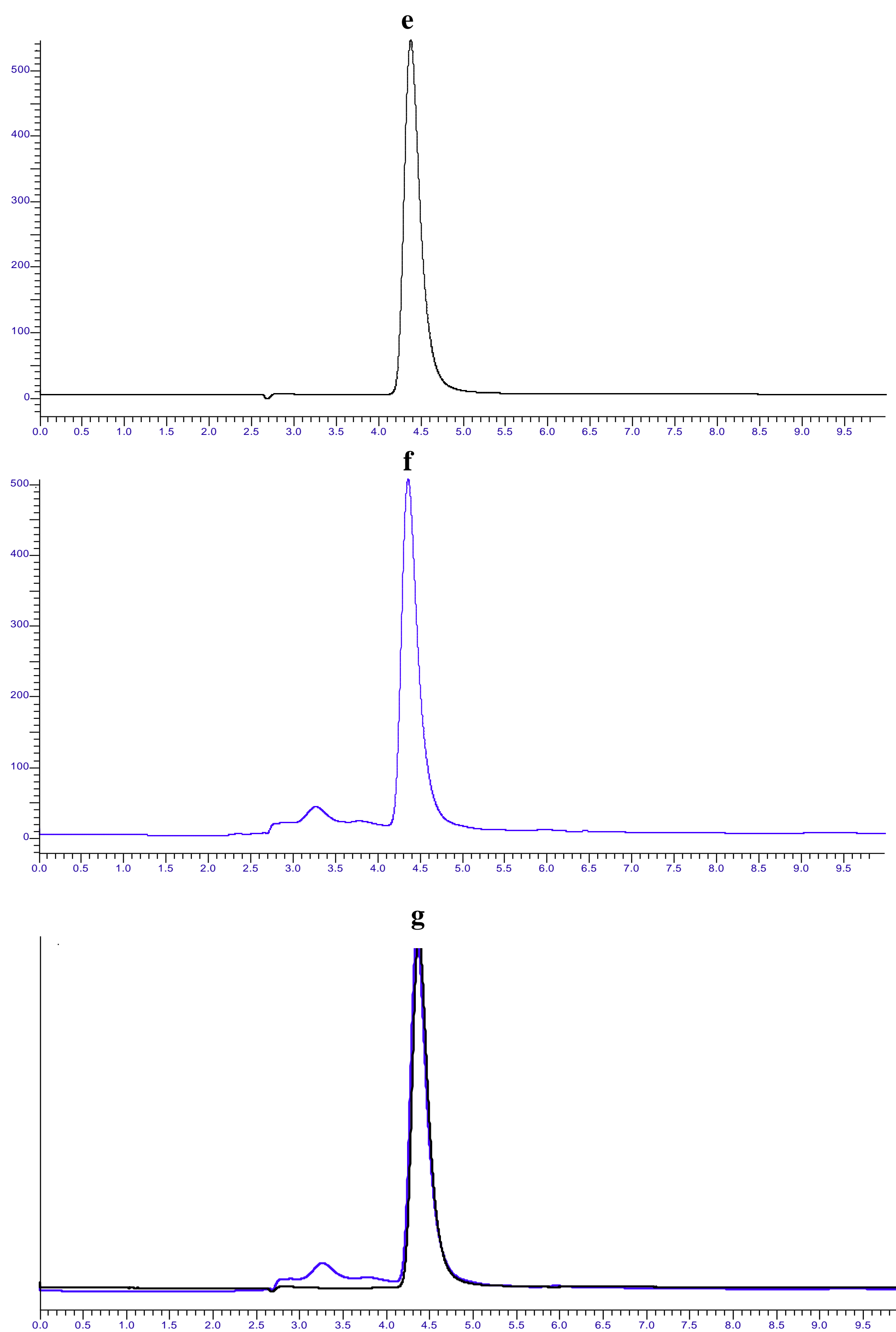

Fig. 3. HPLC chromatograms depicting an example of a 250 µg mL^{-1} ascorbic acid standard peak (e), selected F_2 ascorbic acid sample (f), and overlay of the two (g).

DNA Extraction

Total genomic DNA was extracted from leaf tissue as described by Skroch and Nienhuis (1995). Between 0.5 and 0.75 grams of leaf tissue were ground to homogenization with a sterilized mortar and pestle to successfully break the cells. Ground tissue was then transferred into a 1.5 mL microcentrifuge tube, and 500 μL of potassium ethyl xanthagenate (PEX) extraction buffer containing 1 M Tris (pH = 7.5), NaCl, PEX, and 0.5M EDTA (pH = 8.0) was added and vortexed. Each tube was placed into a heating block, so the mixture could incubate at 65 °C for at least 1 hour, vortexing every 10 minutes for the first 30 minutes and again at the end of 60 minutes. Each tube was then centrifuged at 14,000 RPM for 10 minutes. The supernatant was transferred to a clean 1.5 mL microcentrifuge tube, and a 6:1 mixture of ethanol and 7.5 M ammonium acetate was added to the top of each tube. Each tube was then carefully inverted at least 10 times and allowed to sit at room temperature for 30 minutes to precipitate the nucleic acids. Samples were centrifuged at 7,000 RPM for 5 minutes to pellet the precipitated nucleic acids. The supernatant liquid was discarded, and each tube was blotted on paper towels. Then, 300 μL of dilute TE (0.1X) buffer ((1mM Tris (ph = 7.5) and 0.1 mM EDTA (pH = 8.0)) was added to each tube along with 5 μL of RNAse A (10mg mL^{-1} solution). Each tube was then vortexed and incubated at 37 °C for 60 minutes, vortexing every 10 minutes for the first 30 minutes and then again at the end of 60 minutes. Each tube was centrifuged for 14,000 RPM for approximately 30 seconds to pellet any remaining plant debris. The supernatant was then transferred to a clean microcentrifuge tube by decanting. A 20:1 mixture of ethanol and 3 M sodium acetate was added to the

top of each tube, mixed by inverting at least 10 times, and allowed to sit at room temperature for 30 minutes to precipitate the nucleic acids. Each tube was then centrifuged at 7,000 RPM for 5 minutes to pellet the DNA. The ethanol was discarded from each tube and dried by blotting on paper towels. Each DNA pellet was washed by adding 1 mL of 70% ethanol to each tube and vortexed. Each tube was then centrifuged for approximately 30 seconds at 14,000 RPM to collect each DNA pellet. The ethanol from each tube was discarded, the tubes dried by blotting on paper towels, and then placed inverted for approximately 30 minutes to dry each pellet completely. Each DNA pellet was rehydrated by adding 300 μL of dilute TE (0.1X) buffer and then incubated for 1 hour at 37 °C. Each tube was vortexed every 10 minutes to completely resuspend the pellet. At the conclusion of this process, each tube was stored at -20 °C.

Measuring DNA Concentration

DNA concentrations were measured on each sample using a nanodrop spectrophotometer (DU 530 Lifescience; Beckman, Fullerton, CA). According to their specific concentrations, each sample was diluted to ensure a concentration of 50 ng μL^{-1} was achieved.

RAPD Marker Screening Procedure

In an effort to use a bulked segregant analysis procedure, DNA of the five highest and the five lowest F_2 individuals were combined or bulked together for both

flavonoids (quercetin and luteolin) and AA. DNA of each parent ('Ca377' and 'B22') were also extracted and included for analysis.

Using a Polymerase Chain Reaction (PCR) based Random Amplified Polymorphic DNA (RAPD) technique, a total of 600 primers were tested with diluted DNA in 6 separate groups (both parents, high bulked flavonoid group, low bulked flavonoid group, high bulked AA group, and low bulked AA group) (Fig. 4 A). Using a thermalcycler machine (PTC-100 Programmable Thermal Controller; MJ Research, Waltham, MA), each primer master mix solution consisted of PCR grade water, 5x buffer, individual RAPD primer, MgdNTP, and Taq DNA polymerase. Eight µL of each master mix solution and two µL of each diluted DNA group was added to each well, and a sticky Microseal 'A' Film (MJ Research, Waltham, MA) was applied to completely seal the top of each PCR plate. Each sealed PCR plate was then placed into the PCR machine. The PCR program consisted of two cycles at 91 °C for 60 seconds, 42 °C for 7 seconds, and 72 °C for 70 seconds. Denaturation, annealing, and elongation steps used 38 cycles of: 1 second at 91 °C, 7 seconds at 42 °C, and 70 seconds at 72 °C. The final step consisted of 4 minutes at 72 °C before cooling and storing at 4 °C (Dr. Soon Park, Weslaco, TX).

For construction of the agarose gels, approximately 4.5 g of ultra pure agarose powder (Invitrogen Corporation, Carlsbad, CA) was obtained and combined with 300 mL of 0.5X TBE buffer. The solution was mixed by swirling in a 1,000 mL flask and placed in a microwave for 2 minutes. The solution was taken out, swirled again, and placed back into the microwave for an additional 30 seconds to ensure all the particles

were completely dissolved. The flask was then placed into a water bath, and the liquid was stirred at a slow speed using a stir bar until the agarose solution had cooled to 65 °C. The solution was then carefully poured into an electrophoresis gel tray. Three combs were placed into the gel at equal distances from each other, and the solution was allowed to solidify. Each end was carefully removed, and the plate containing the gel was placed into the electrophoresis box (Submarine/Horizontal Gel Unit; C.B.S. Scientific Co., Del Mar, CA). If needed, additional 0.5X TBE buffer was added to cover the top of the gel. Wells in the gel were filled by transferring the liquid solution from each PCR plate. With the gel filled, the cover was placed on top of the electrophoresis box, and the power source was switched to the on position. The voltage was set at approximately 217 amps, and the electrophoresis was allowed to run for 1 hour and 20 minutes. Upon completion, the power source was turned off; the gel was removed from the electrophoresis box, and carefully placed into a staining solution containing 20 µL of ethidium bromide with water. The gel was then cut into three equal sections using a razor blade and remained there for 1 hour. Afterwards, the gels were transferred to a destaining container for 15 minutes. After 15 minutes, an ultraviolet illuminator (T1202; Sigma, St. Louis, MO.) and digital camera (EDAS 290; Eastman Kodak Company, Rochester, NY) was used to take a photograph of the different bands. Primers showing potential polymorphisms were then screened with each individual making up each bulked group to identify the segregation pattern for each candidate band (Fig. 4 B). Primers that expressed the most consistent potential with respect to their segregations

between high and low groups were then screened with both parents and the entire F_2 population of 115 individuals (Fig. 5).

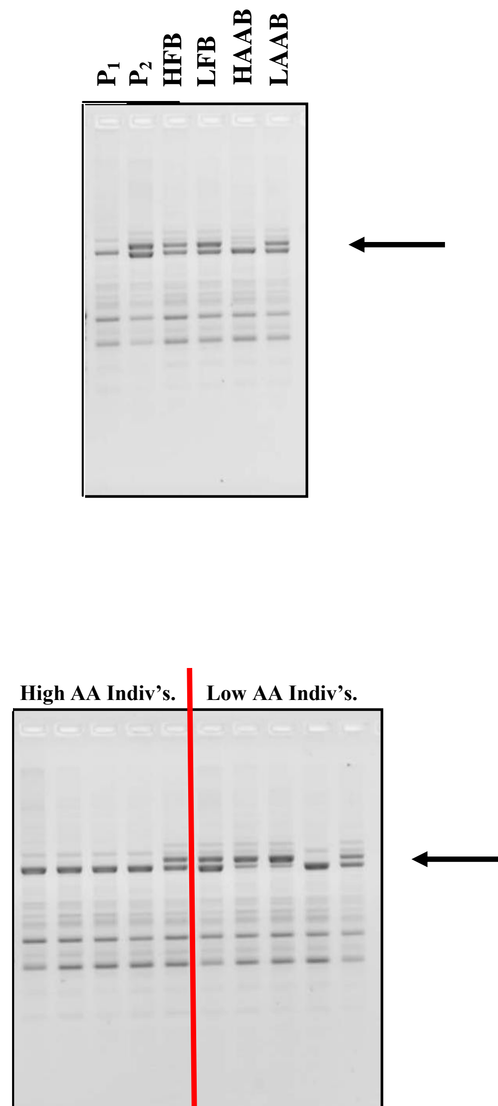

Fig. 4. First image: Potential polymorphic segregation observed between both parents and high / low bulked DNA individuals with primer 2. Second image: Potential polymorphic segregation observed between each individual making up the high / low bulked ascorbic acid DNA groups with primer 2. Arrows indicate bands of interest.

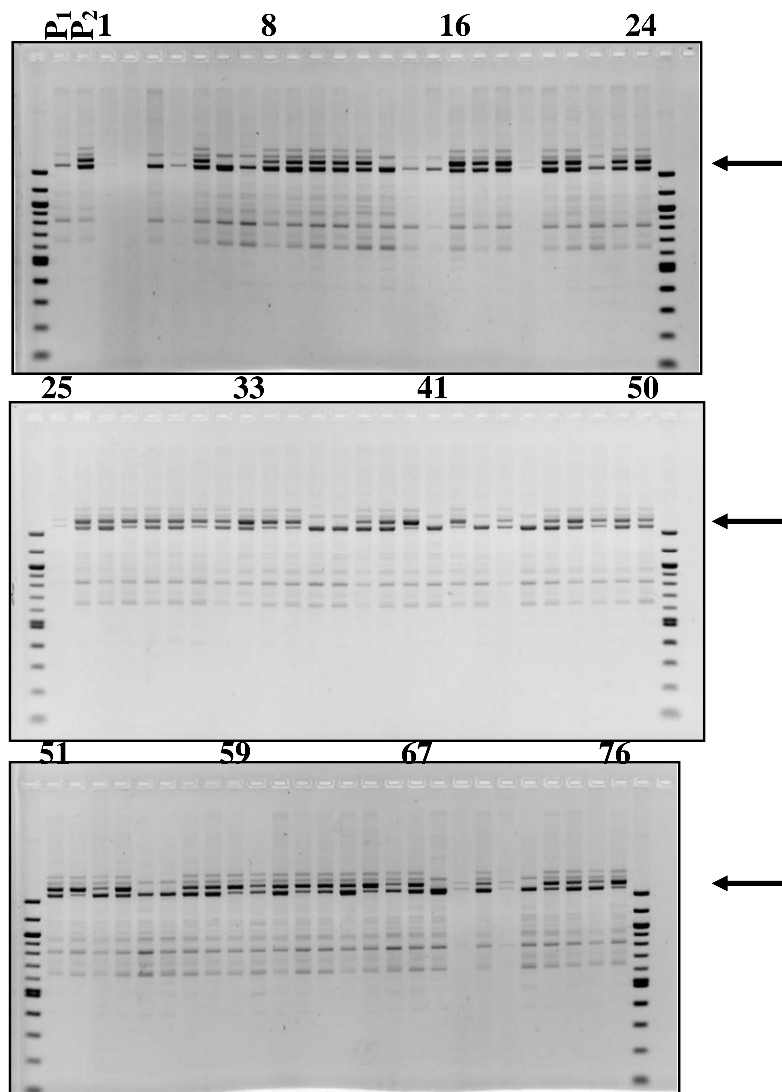

Fig. 5. First image: P_1 = 'Ca377', P_2 = 'B22', F_2 individuals from 1-24, and 100 bp molecular marker ladder. Second image: F_2 individuals from 25-50. Third image: F_2 individuals from 51-76. Fourth image: F_2 individuals from 77-101. Fifth image: F_2 individuals from 102-125. Arrows indicate bands of interest.

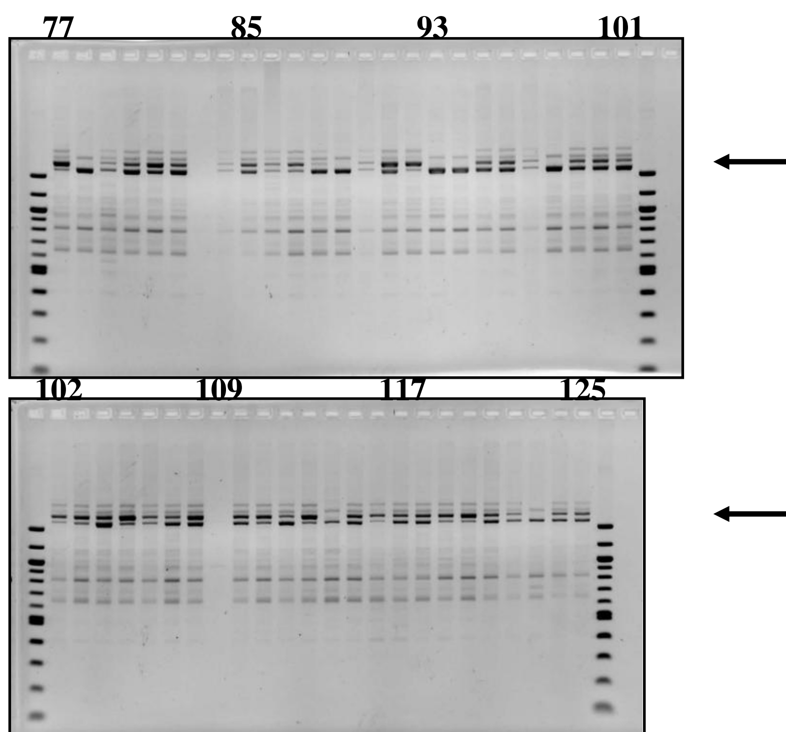

Fig. 5. Continued.

Statistical Analysis

Pepper transplants for this experiment were planted in a completely randomized design. Using SAS (SAS Institute, 2008), a General Linear Model (GLM) procedure was used to test for differences in genotypes (G) for these phytochemicals (quercetin, luteolin, quercetin+luteolin, and AA) so that analysis of variance (ANOVA) tables (Tables 28-32 and 34-35) could be constructed, and mean comparisons separated by Duncan ($P \leq 0.05$) (Tables 33 and 36) when considering the genotype source as a fixed effect. Frequency distribution tables were also constructed for quercetin (Fig. 6), luteolin (Fig. 7), quercetin+luteolin (Fig. 8), and AA (Fig. 9) concentrations expressed in offspring of this F_2 family. After "scoring" the best candidate primers with both parents

and the whole F_2 family, a correlation analysis was conducted using SAS to identify whether a significant association existed between the best candidate primers and levels of these three phytochemical groups, as well as, comparisons between AA and quercetin, AA and luteolin, quercetin and luteolin, quercetin and total flavonoids (quercetin+luteolin), luteolin and total flavonoids (quercetin+luteolin), and AA and total flavonoids (quercetin+luteolin). In an effort to calculate heritability estimates, additional data from two commercial jalapeño checks (Ixtapa and TMJ) were also included to account for the environmental component (Tables 31-32, and 35).

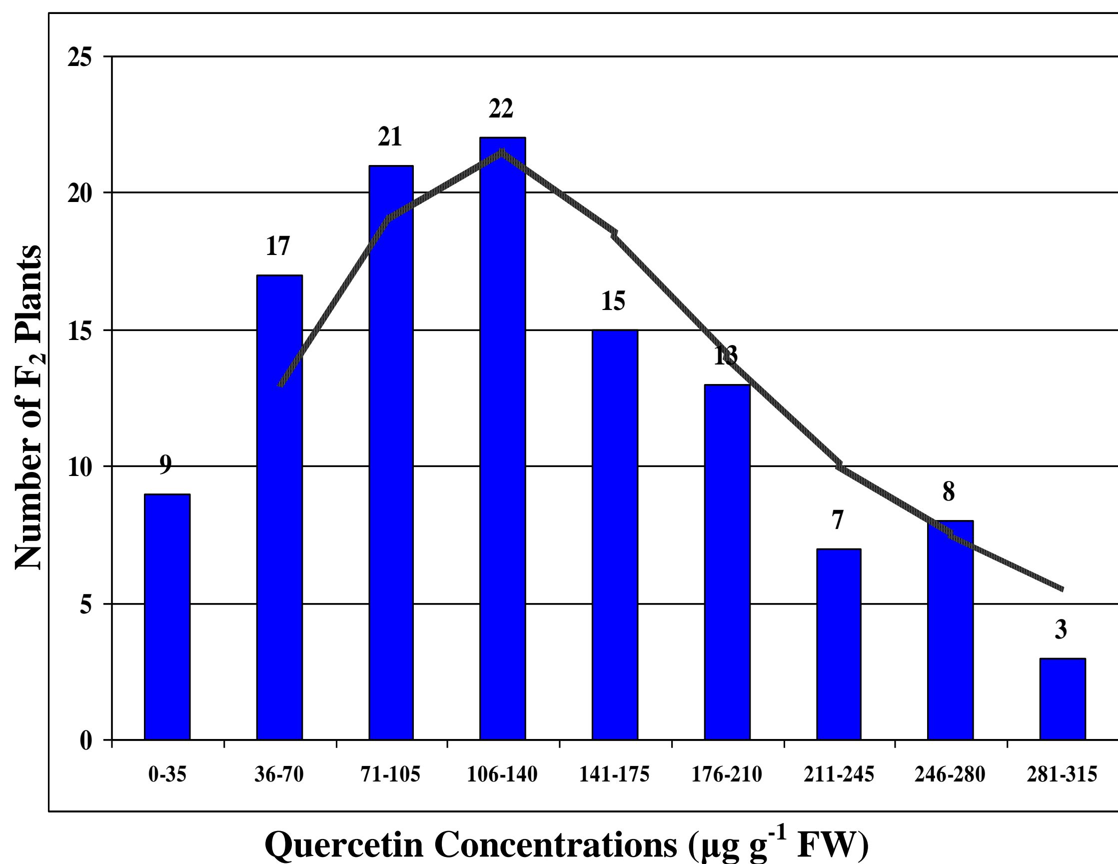

Fig. 6. Frequency distribution table for quercetin concentrations in fruit tissue of all F_2 plants derived from the cross of ‘Ca377’ x ‘B22’.

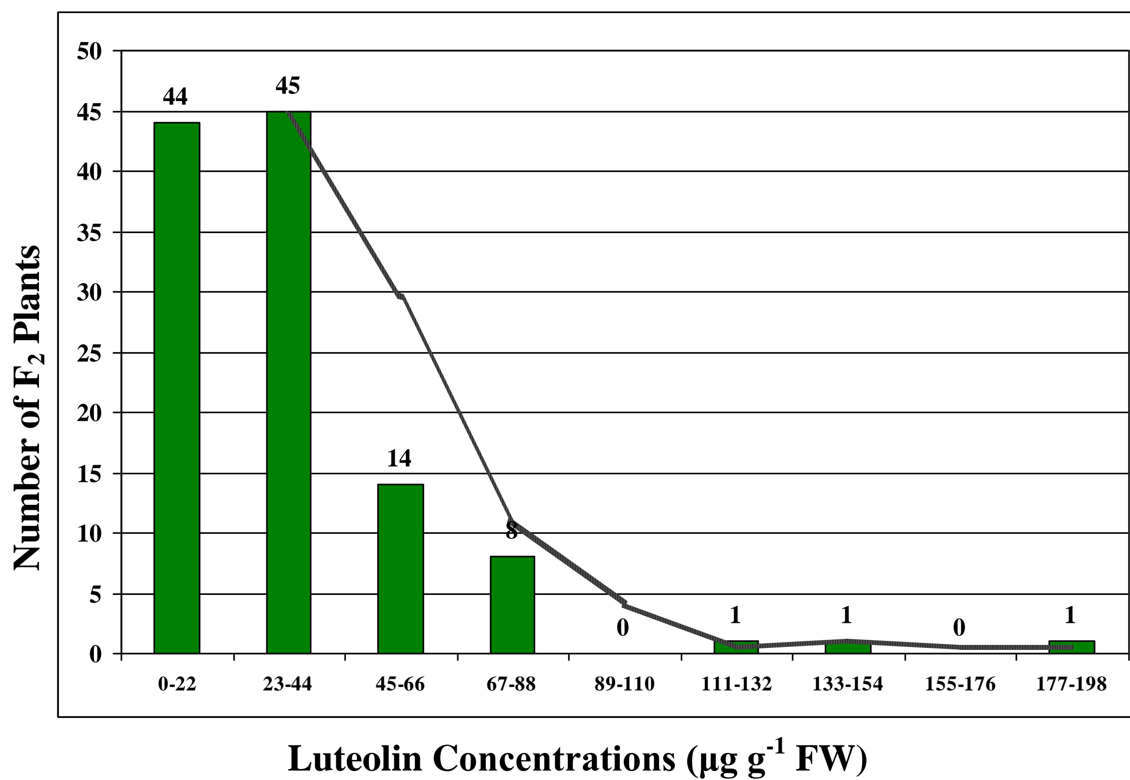

Fig. 7. Frequency distribution table for luteolin concentrations in fruit tissue of all F_2 plants derived from the cross of ‘Ca377’ x ‘B22’.

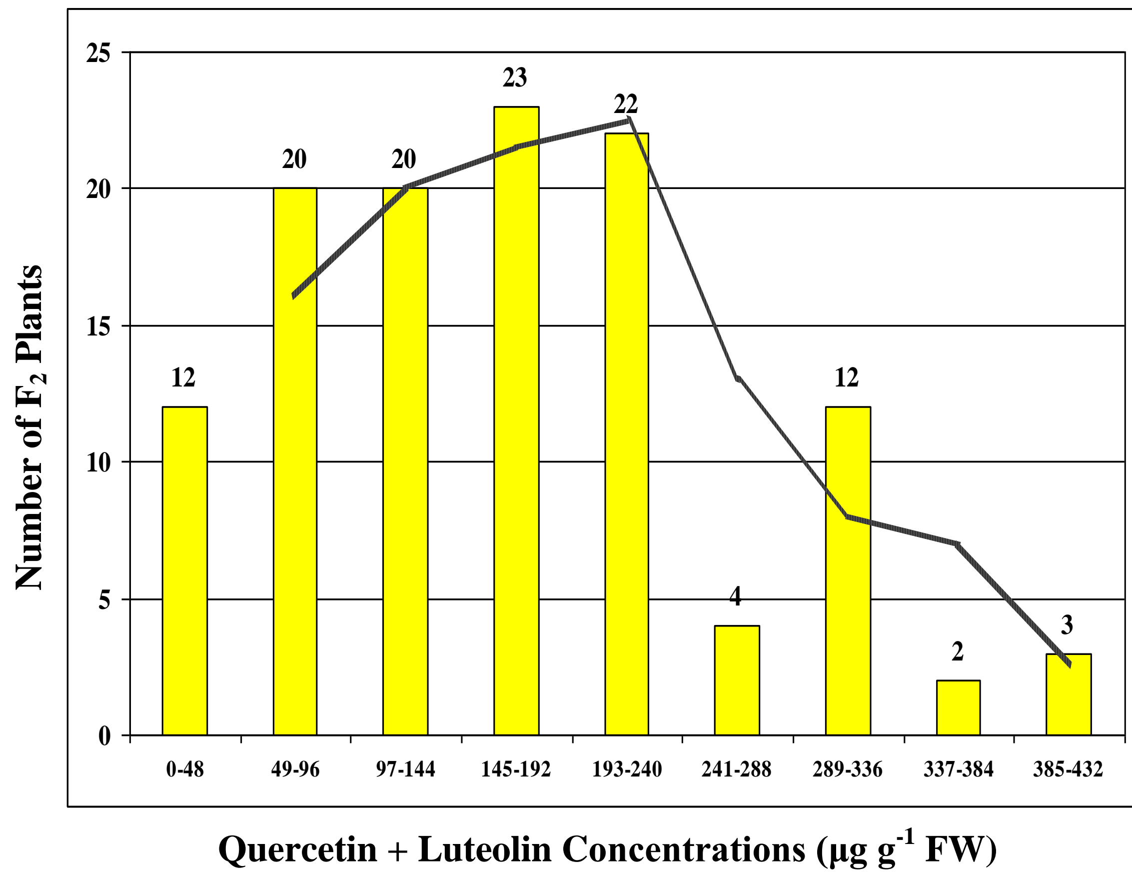

Fig. 8. Frequency distribution table for quercetin+luteolin concentrations in fruit tissue of all F_2 plants derived from the cross of ‘Ca377’ x ‘B22’.

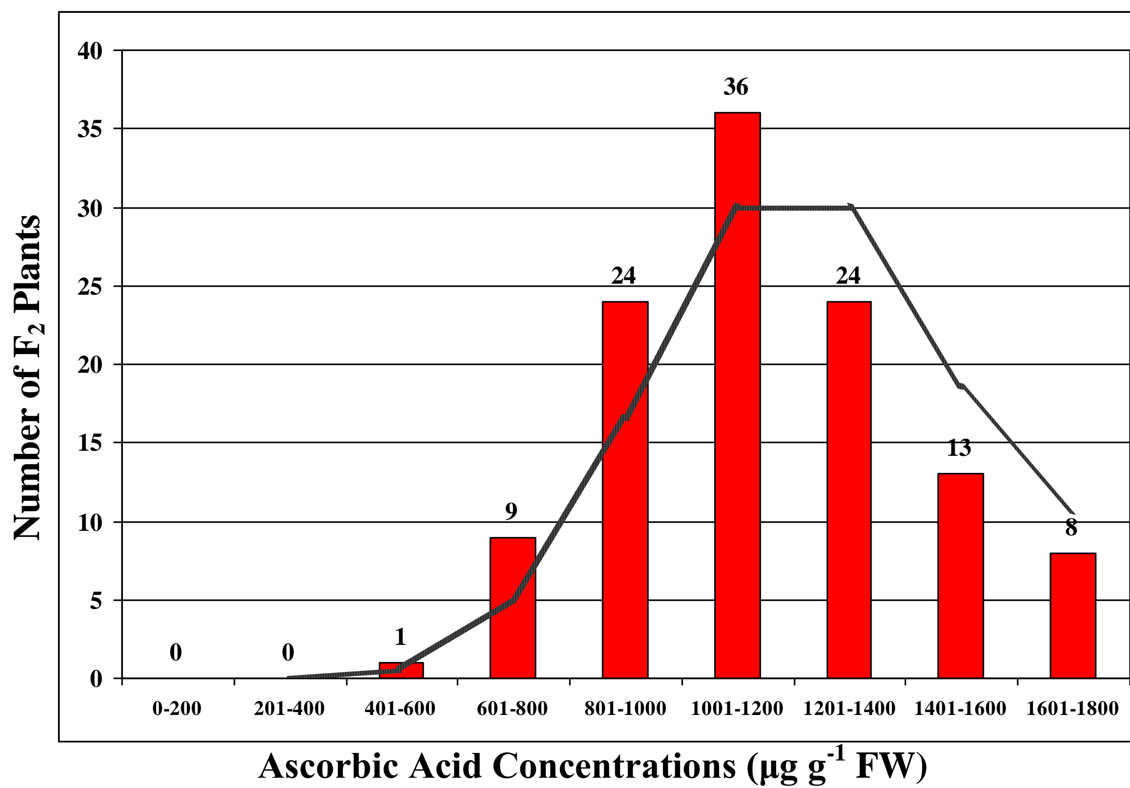

Fig. 9. Frequency distribution table for ascorbic acid concentrations in fruit tissue of all F_2 plants derived from the cross of 'Ca377' x 'B22'.

Results and Discussion

Phytochemical Quantification

HPLC quantification of these three phytochemicals found significant variation, which allowed us to differentiate phytochemical concentrations into three main groups (high, moderate, and low). From this analysis, we were able to identify the highest and lowest individuals for each bioactive compound. During the process, we observed that those individuals expressing the highest levels of flavonoids did not necessarily express the highest levels of AA and vice versa. Nonetheless, 'Ca377' was found to produce significantly higher levels of quercetin, luteolin, and AA than 'B22', which may have contributed to higher phytochemical expression in several of these F_2 individuals.

Molecular Marker Correlation Analysis

Of the candidate primers expressing the most consistent polymorphic results, primer 1 produced correlation (r) values of -0.18343, -0.07704, and 0.12480 for AA, quercetin, and luteolin, respectively, which identified 3.36, 0.59, and 1.56% of the variability to be explained by AA, quercetin, and luteolin. Primer 2 produced r values of -0.15605, 0.11047, and 0.21861 for AA, quercetin, and luteolin, respectively, which identified 2.44, 1.22, and 4.78% of the variability to be explained by AA, quercetin, and luteolin. Primer 3 produced r values of -0.00042, 0.02243, and 0.03684 for AA, quercetin, and luteolin, respectively, which identified 0.00000018, 0.05, and 0.14% of the variability to be explained by AA, quercetin, and luteolin. The only significant association here was found between primer 2 and luteolin.

In the second part of our correlation analysis, we calculated r values of 0.10053, -0.14526, 0.67096, 0.97739, 0.81257, and 0.03760 between AA and quercetin, AA and luteolin, quercetin and luteolin, quercetin and total flavonoids (quercetin+luteolin), luteolin and total flavonoids (quercetin+luteolin), and AA and total flavonoids (quercetin+luteolin), respectively, which explained 1.01, 2.11, 45.02, 95.53, 66.03, and 0.14% of the variability. From these results, we confirmed reports from several of our previous studies identifying a significant association between quercetin and luteolin, quercetin and total flavonoids (quercetin+luteolin), as well as, luteolin and total flavonoids (quercetin+luteolin).

In the end, we are one of the first groups, to our knowledge, to be able to report on finding a significant r value with respect to any of these phytochemicals. However, we still need to find a more reliable and reproducible marker that expresses a significantly higher correlation value before we can apply it in a pepper breeding program and screen an entire segregating population for this particular characteristic of interest.

Applications for Researchers to Make Quick Returns and Develop Improved Lines for Human Consumption

In an effort to better inform the public of the importance fruits and vegetables can have on maintaining a healthy lifestyle, results from Liu (2003) proposed the idea that consumption of food containing more phytochemicals will supply consumers with a potent combination of additive and synergistic health-promoting effects. Development of pepper material with increased levels of different phytochemicals would then

ultimately garner more interest by consumers concerned with protecting their bodies from various degenerative diseases to better maintain their well-being (Byers and Perry, 1992; Temple and Gladwin, 2003; Kader, 2008; Crosby et al., 2007a; Yoo et al., 2007; Crosby et al., 2009). Broad sense heritability calculations for quercetin, luteolin, and AA expression produced values of 96.91, 94.04, and 85.29, respectively. The next goal will be to use the information obtained from this experiment and apply it in a different manner to more successfully identify a molecular marker linked to elevated phytochemical concentrations. Identification of molecular markers linked to either elevated or reduced phytochemical expression will provide researchers with the means to identify those exceptional individuals at an earlier stage of development, accelerating the process of developing an improved genotype containing these traits.

Conclusions

Results from previous studies are continuing to provide evidence that breeders are successfully increasing the levels of different phytochemicals in many of the fruits and vegetables we consume on a regular basis through traditional breeding (Crosby et al., 2005; Crosby et al., 2006; Crosby et al., 2007a; Crosby et al., 2007b; Crosby et al., 2009). Breeders can take the knowledge they gain from these experiments and apply it in their own programs to ultimately develop material expressing elevated levels of these desirable traits of interest. As we previously mentioned, our results provided evidence that ‘Ca377’ contains some potential as being a useful candidate in the development of new material capable of expressing appreciable levels of flavonoids (quercetin and

luteolin) and AA. Five potential transgressive segregates for AA expression (plant numbers 116, 45, 93, 100, and 3), five for quercetin expression (plant numbers 87, 7, 93, 13, and 89), and forty three for luteolin expression were identified. However, if quercetin and luteolin values were combined for total flavonoids, eleven plants expressed higher values than either parent. Therefore, consistent performance of 'Ca377' as a parent with other material when grown in different locations across multiple years may allow for the potential release of better germplasm to the public.

A detailed report on the synthesis of flavonoids and AA in plants has already been previously mentioned by Creasy (1968) and Smirnoff (1996), respectively. Although the heritability values of these phytochemicals seem relatively high, expression of them has also been proven to be highly impacted by the environment. Therefore, we are still left with the conclusion that a minimal opportunity exists here for pepper breeders to successfully identify a molecular marker that is tightly linked to quercetin, luteolin, or AA concentrations due to some potentially limiting or restricting factors. These observations, therefore, leave us with the hypothesis that the intensity in quantitative expression with respect to these compounds is more indicative of environmental influences acting on the genotypes while growing in the field (Hoffmann and Merllä, 1999). It is certain that a particular genotype needs to have the genetic capacity to produce an elevated concentration of these phytochemicals, but the environmental exposure acting on that genotype may serve a more essential role in activating the necessary physiological processes to produce a specific secondary metabolite. Although the RAPD technique we used is fairly straightforward and useful, some may argue that a

more reliable technique would have been better. Therefore, we postulate that success may be possible in the near future if a different molecular marker technique which generates more data is used.

CHAPTER VII

FINAL CONCLUSIONS

Collectively, all of the results that have been described in the preceding chapters provide sufficient proof of the diversity that is present within the *Capsicum* genus with respect to both fruit characteristics and phytochemical expression. In addition, we have successfully provided a substantial amount of evidence verifying reports from previous groups dictating how influential the interaction is between a particular genotype and its surrounding environment. Diverse screening of various other genotypes in future studies will potentially unravel even further evidence related to the degree of variability that can be found within these plant species. The evidence provided in this document will be potentially valuable to future scientists interested in observing related traits in variable environmental locations, in an effort to maximize the potential output of a particular genotype.

Phytochemical Analyses

The initial hypothesis became evident relatively quickly in Chapters II, III, and IV when it was proposed that a useful amount of phytochemical expression could be found after examining different genotypes in various environmental locations. Significant differences were observed in phytochemical expression for both *C. chinense* and *C. annuum* genotypes, which could be used to guide both farmers and scientists in a particular direction for proper identification of superior individuals and the most

optimum environment to more effectively produce a high quality crop. The various characteristics making up a particular environment can collectively either benefit or impede the potential productivity of a particular genotype. The key is to successfully identify the optimum synergistic combination between these two factors in a timely and cost-effective fashion. If this is successfully achieved, consumers will be more apt to reap the benefits of a higher quality product they can eat that will better protect their bodies from various diseases. For example, results across these various experiments provide evidence identifying Weslaco's ability to more consistently produce fruit with higher concentrations of both capsaicin and dihydrocapsaicin. These results can, therefore, indicate that an environment, as the one in Weslaco, can exert a high amount of influence with respect to expression of this particular secondary metabolite. It is also very possible that similar or related species would be able to perform equally well if grown in a similar location. Moreover, depending on market preference, results from Chapter II indicate various Habanero options that producers could potentially pursue to satisfy additional clientele (Hab5-dark orange for hot markets, Hab6-yellow for mild markets, and Hab3-orange for flavonoid expression). Results from this experiment are especially unique due to the lack of research being conducted on this species and the number of available, active breeding programs that currently exist (Crosby, personal communication). Therefore, this evidence could potentially gain more popularity with commercial representatives interested in pursuing a high valued, niche market. Similarly, significant variation was also found in both ascorbic acid (AA) and flavonoid expression (quercetin and luteolin) within various *C. annuum* genotypes, as seen in

Chapters III and IV. Depending on the particular experiment, elevated AA concentrations were found more in fruit tissue grown in College Station-VFIC when data were evaluated in the 2009 *C. annuum* study (Chapter III), while Weslaco produced fruit with higher levels in the 2010 *C. annuum* study (Chapter IV). Our results provide evidence of J-1's, S-1's, and C-1's ability to express attributes that could result in their widespread acceptability by producers in the near future. For flavonoids, an environmental location similar to either Uvalde (Chapters II and IV) or College Station-VFIC (Chapter III) may hold promise for fruit development expressing higher concentrations. Evaluating these results can also be explained in a similar manner as to the performance of the previous phytochemical groups. For application purposes, producers interested in any of these genotypes should carefully evaluate their priorities first to determine the best location that will allow them to produce fruit with comparable phytochemical levels without jeopardizing the opportunity to produce fruit with visually appealing fruit characteristics. This idea supports the importance of testing a particular genotype in multiple locations before deciding which location is optimum. This practice will potentially help avoid major setbacks related to monocropping in one location year after year.

Heritability Experiment

The hypothesis that high heritability estimates exist for many of these characteristics was also found to be true, as reported by previous groups. These results verify the relative degree of certainty that plant breeders can create improved genotypes

expressing related characteristics that both consumers and producers find important. This does not imply that every new genotype that is created will be accepted in a timely fashion or even at all by the industry. It implies that breeders interested in moving the mean value for one of these related characteristics into a particular direction can succeed to an extent, within the confines of the specific pepper population. On the other hand, it is possible that use of different breeding (backcross method) or selection strategies could result in variable heritability expression for a particular trait. Therefore, breeders will need to continually examine their outputs to ensure they have not inadvertently selected against their intended target. If so, more time will obviously be required of them to go back and incorporate those traits of interest into their specimen. Furthermore, although a particular genotype may express an elevated concentration of ascorbic acid, for example, producers still demand that the product has high yield, disease resistance, desirable fruit attributes, and other characteristics having a higher caliber than what they are currently growing. Nonetheless, it is possible that incorporation of superior individuals using an appropriate breeding strategy will give researchers a higher probability of success and will possibly result in more attention being paid by interested parties. Repeating this experiment in different locations across different years will ultimately reveal the potential performance of these genotypes, and may give researchers a better idea of how they may perform in different production areas. Ideally, identification of a superior specimen having a stable performance when evaluated in different environmental locations, while also being able to continuously produce those particular traits of importance, can result in an opportunity for that genotype to become more unique than

those currently being marketed (Becker and Léon, 1988). Interestingly, identification of several hybrids that perform better than their parents lends itself to the idea of a positive degree of potential heterosis involved and the fact that either one or both parents has / have good combining ability for the particular trait in question. As provided in Table 22, paprika hybrid Pap4, developed from the cross between PapP27 and PapP67, produced a significantly heavier fruit (62.95 g) in comparison to either parent (38.45 and 38.12 g, respectively). As a result, this hybrid's performance could possibly result in the produce industry accepting it over either parent. Likewise, this evidence could possibly attract further interest from the seed industry desiring to use one or both of these parents in different crossing schemes with their material in an effort to produce a similar output. This idea, however, does not imply that using this parent in different crossing schemes will result in as favorable a hybrid as we have identified. It implies that there are several factors (both genetic and environmental) involved that can all contribute to variable expression and represent the sheer amount of genetic variation that can result. In another comparison, hybrid Pap4 displayed what appeared to be the characteristics similar to that of a transgressive segregate due to its ability to express fruit having larger fruit diameters (42.00 mm) than either of its parents (35.90 and 27.50 mm, respectively). Various other examples were found and can be explained in a similar manner with respect to the other characteristics, as seen in Tables 22 and 24. Also, identification of highly significant heterosis estimates could lead to the conclusion of potential hybrid vigor present as in the performance by S27 that produced a capsaicin percentage value of 1289.23 (Table 27). Hybrids expressing a negative heterosis value could indicate the reduced

performance with respect to that particular characteristic in comparison to their parents (Table 27). This evidence, therefore, shows that different amounts of hybrid vigor can result when two genotypes are brought together. From this, one can more easily understand and gain a better idea of the truth behind the phrase often spoken in a typical plant breeding class that “plant breeding is both an art as well as a science of improving the heredity of plants for the benefit of mankind” (Crosby, plant breeding lecture).

Molecular Marker Analysis

Results from this experiment were rather disappointing in the fact that a molecular marker expressing a highly significant correlation and respective variability (R^2) value (something over 50%), as well as, tight linkage with respect to these particular characteristics of interest was not found. On the other hand, it is possible that the observations discussed in Chapter VI could spur future interest and lend itself to more elaborate ideas for the ultimate identification of a reproducible marker that can be deployed in a segregating population to more accurately distinguish between individuals for concentrations of ascorbic acid, quercetin, or luteolin. As we previously discussed, it is possible that other groups may experience a variable amount of success provided a different biotechnology technique is implemented. At that moment, examination of a similar or alternate segregating family for these phytochemicals can ensue, and the goal of identifying a molecular marker can be examined in more detail. Only time will tell if a scientific breakthrough is possible in this particular area of biotechnology. On a positive note, we were able to identify a continuous range of variation in this segregating

F_2 population for these two phytochemical groups and were able to identify the existence of a few transgressive segregants that could be useful in future studies. The amount of gain from selection that is observed in the next generation with respect to these characteristics will dictate to breeders how stringent their selection procedures should be to more effectively achieve a desirable outcome.

Breeding Recommendations

A collective examination of all these results verifies the opportunity for a vast amount of future breeding projects. Potentially, all of the information provided in these preceding chapters will lend itself to a detailed list of germplasm that breeders within the Texas A&M University pepper program will be able to explore in more detail and exploit for future development. For the purpose of developing an assortment of improved specimens, introgressing several of these characteristics into an improved specimen can commence with several controlled pollinations. In an effort to recover the traditional commercial characteristics of importance, successive backcrosses and implementation of a recurrent selection or related procedure could provide the necessary means to achieve success (Crosby, 2008). Advanced testing of these genotypes in multiple locations could then ensue to determine their relative degree of performance and would assist to identify their optimum production environment. With respect to Habanero germplasm, we previously dictated in Chapter II of Hab1-red's ability to possibly serve a role in future studies designed to increase fruit size or Hab5-dark orange's ability to express elevated capsaicin concentrations. Fruit size and elevated

capsaicin expression are both valuable traits that nearly all pepper farmers demand, especially in markets where hot peppers are a priority (Crosby, personal communication). Depending on market preference, Habanero genotypes expressing the preferred color (orange or yellow as opposed to red or chocolate), as well as capsaicin or flavonoid content, will more effectively influence how some of these genotypes may be used in future practices to achieve a particular outcome. Use as parents to create variable families followed by appropriate selection procedures in these diverse families could lend itself to production of improved lines for future release. In an effort to verify the concentration of a particular phytochemical compound, routine analyses could be employed, as discussed in Chapters III and IV, to more accurately quantify levels within fruit tissue. This information would then be able to assist the breeder to maintain or shift the course of breeding and selection. As practiced over many years now, use of a greenhouse facility and available field space in multiple locations provides the best opportunity for pepper breeders to move their goals from that of ideas into realities. As with any profession, plant breeding is designed to create a product that is capable of leaving a lasting impression on both the minds of those who consume them on a regular basis, as well as, in the stomachs of those interested in maintaining a healthy life.

LITERATURE CITED

Abbott, J.A. 1999. Quality measurement of fruits and vegetables. Postharvest Biol. and Technol. 15: 207-225.

Allard, R.W. 1960. Principles of plant breeding. Wiley, New York.

Amiot-Carlin, M.J., Margotat, A., and Tourniaire, F. 2007. Flavonoids in food and wine. In: Desjardins, Y. (ed.). Proc. 1st IS on Hum. Health Effects of F&V. Acta Hort. 744, ISHS: 107-116.

Andrews, J. 1995. Peppers: the domesticated *Capsicums*. University of Texas Press, Austin.

Antonious, G.F., Kochhar, T.S., Jarret, R.L., and Snyder, J.C. 2006. Antioxidants in hot pepper: variation among accessions. J. of Environmental Science & Health, Part B – Pesticides, Food Contaminants, & Agricultural Wastes. 41 (7): 1237-1243.

Antonious, G.F., Lobel, L., Kochhar, T., Berke, T., and Jarret, R.L. 2009. Antioxidants in *Capsicum chinense*: variation among countries of origin. J. Environ. Sci. Health Part B. 44: 621-626.

Bahorun, T., Luximon-Ramma, A., Crozier, A., and Aruoma, O. 2004. Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J. Sci. Food Agric. 84: 1553-1561.

Basu, S.K. and De A.K. 2003. *Capsicum*: historical and botanical perspectives. In: De, A.K. (ed.), *Capsicum*: the genus *Capsicum*. Taylor & Francis Inc., New York: 1-15.

Becker, H.C. and Léon, J. 1988. Review: stability analysis in plant breeding. Plant Breeding. 101: 1-23.

Ben-Chaim, A., Borovsky, Y., Falise, M., Mazourek, M., Kang, B.C., Paran, I., and Jahn, M. 2006. QTL analysis for capsaicinoid content in *Capsicum*. Theor. Appl. Genet. 113 (8): 1481–1490.

Bennett, D. J. and Kirby, G. W. 1968. Constitution and biosynthesis of capsaicin. J. Chem. Soc.: 442-446.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Bhagyalakshmi, P.V., Shankar, C.R., Subramanyam, D., and Babu, V.G. 1991. Heterosis and combining ability studies in chillies. Ind. J. Gen. Pl. Breed. 51: 420-423.

Bosland, P. 1999. The history of the chile pepper. In: Hanson, Beth. Chile peppers: hot tips and tasty picks for gardeners and gourmets. 21st-Century Gardening Series. Handbook #161. Brooklyn Botanic Garden, Inc. Brooklyn, NY: 7-21.

Bosland, P.W. 1996. Capsicums: innovative uses of an ancient crop. In: Janick, J. (ed.), Progress in new crops. ASHS Press, Arlington, VA. 479-487. Available at: http://www.hort.purdue.edu/newcrop/proceedings1996/v3-479.html.

Bosland, P.W. and Walker, S. 2004. Growing chiles in New Mexico. Cooperative Extension Service. Guide H-230. Available at: http://aces.nmsu.edu/pubs/_h/h-230.pdf .

Boyce, S.T., Supp, A.P., Swope, V.B., and Warden, G.D. 2002. Vitamin C regulates keratinocyte viability, epidermal barrier, and basement membrane *in vitro*, and reduces wound contraction after grafting of cultured skin substitutes. J. Investigative Dermatology. 118 (4): 565-572.

Byers, T. and Perry G. 1992. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. Annu. Rev. Nutr. 12: 139-159.

Clevidence, B.A. 2010. Tropical and subtropical fruits: phytonutrients and anticipated health benefits. In: Souza, M. and Drew, R. (eds.). Proc. IIIrd IS on Trop. and Subtrop. Fruits. *Acta Hort*. 864, ISHS: 485-498.

Cooper, T.H., Guzinski, J.A., and Fisher, C. 1991. Improved high-performance liquid chromatography method for the determination of major capsaicinoids in *Capsicum* oleoresins. J. Agric. Food Chem. 39: 2253-2256.

Conforti, F., Statti, G.A., and Menichini, F. 2007. Chemical and biological variability of hot pepper fruits (*Capsicum annuum* var. *acuminatum* L.) in relation to maturity stage. Food Chem. 102: 1096-1104.

Conklin, P.L., Williams, E.H., and Last, R.L. 1996. Environmental stress sensitivity of an ascorbic acid-deficient Arabidopsis mutant. PNAS, USA. 93: 9970-9974.

Contreras-Padilla, M. and Yahia, E.M. 1998. Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity. J. Agric. Food Chem. 46: 2075-2079.

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Rev. 12 (4): 564–582.

Creasy, L.L. 1968. The increase in phenylalanine ammonia-lyase activity in strawberry leaf disks and its correlation with flavonoid synthesis. Phytochemistry. 7 (3): 441-446.

Crosby, K.M. and Villalon, B. 2002. 'TAM Mild Jalapeño II': a new, multiple-virus-resistant, mild jalapeño pepper. HortScience. 37: 999-1000.

Crosby, K., Yoo, K.S., Leskovar, D., and Pike, L. 2003. Impact of environment, irrigation and maturity on ascorbic acid concentrations of diverse pepper (*Capsicum* spp.) germplasm lines grown at two Texas locations. ASHS-Providence. HortScience. 38: 664.

Crosby, K.M., Leskovar, D.I., and Yoo, K.S. 2005. 'TAM mild habanero,' a low-pungency habanero pepper. HortScience. 40 (2): 490-491.

Crosby, K., Leskovar, D.I., Jifon, J., and Yoo, K. 2006. 'TAM Ben Villalón' and 'TAM Valley Hot': two new pepper cultivars from the Texas Agricultural Experiment Station at Weslaco. HortScience. 41 (4): 1072.

Crosby, K.M., Jifon, J., Pike, L., and Yoo, K.S. 2007a. Breeding vegetables for optimum levels of phytochemicals. In: Desjardins, Y. (ed.). Proc. 1st IS on Hum. Health Effects of F&V. Acta Hort. 744, ISHS: 219-224.

Crosby, K.M., Jifon, J.L., and Villalon, B. 2007b. 'TAM Dulcito,' a new, multiple virus-resistant sweet jalapeño pepper. HortScience. 42 (6): 1488-1489.

Crosby, K.M. 2008. Pepper. In: Prohens, J. and Nuez, F. (eds.). Vegetables II: Fabaceae, Liliaceae, Umbelliferae, and Solanaceae. Springer, New York: 221-248.

Crosby, K.M., Jifon, J.L., Leskovar, D.I., and Yoo, K.S. 2009. Novel vegetable cultivars from TAMU – improving human health benefits, flavor and productivity. In: Patil, B. (ed.). Proc. IInd IS on Human Health Effects of F&V. Acta Hort. 841, ISHS: 499-501.

Crosby, K.M., Butcher, J., Yoo, K.S., and Leskovar, D.I. 2010. 'TAM Ben Villalon', a new, multiple-virus resistant, mild, green-chile pepper. HortScience. 45 (11): 1756-1758.

Cushnie, T.P.T. and Lamb, A.J. 2005. Antimicrobial activity of flavonoids. International J. of Antimicrobial Agents. 26: 343-356.

de Sousa, J.A. and Maluf, W.R. 2003. Diallel analyses and estimation of genetic parameters of hot pepper (*Capsicum chinense* Jacq.). Scientia Agrícola. 60 (1): 105-113.

DeWitt, D. 1999. The nature of capsaicin. Online. 25 Apr. 2008. Internet. Available at: http://www.fiery-foods.com/Dave/capsaicin.asp.

DiSilvestro, R.A. 2001. Flavonoids as antioxidants. In: Wildman, R.C., ed. Handbook of nutraceuticals and functional foods. Baca Raton, FL: CRC Press: 127-142.

Duthie, G. and Crozier, A. 2000. Plant-derived phenolic antioxidants. Curr. Opin. Lipidol. 11: 43-47.

Eshbaugh, W.H. 1993. History and exploitation of a serendipitous new crop discovery. In: Janick, J. and Simon, J.E. (eds.). New crops. Wiley, New York: 132-139.

Estrada, B., Pomar, F., Díaz, J., Merino, F. and Bernal, M.A. 1999. Pungency level in fruits of the Padrón pepper with different water supply. Scientia Hort. 81 (4): 385-396.

Geleta, L.F. and Labuschagne, M.T. 2006. Combining ability and heritability for vitamin C and total soluble solids in pepper (*Capsicum annuum* L.). J. Sci. Food Agric. 86: 1317-1320.

Gepts, P. and Hancock, J. 2006. The future of plant breeding. Crop Sci. 46: 1630-1634.

Gibbons, E., Allwood, M.C., Neal, T., and Hardy, G. 2001. Degradation of dehydroascorbic acid in parental nutrition mixtures. J. Pharmaceutical Biomedical Analysis. 25: 605–611.

Gopalakrishnan, T.R., Gopalakrishnan, P.K., and Peter, K.V. 1987. Heterosis and combining ability in chilli. The Indian Journal of Genetics and Plant Breeding. 47 (2): 205-209.

Greenleaf, W.H. 1986. Pepper breeding. In: Bassett, M.J. (ed.). Breeding vegetable crops. AVI Publishing Co. Westport, CT: 67-134.

Guzman, I., Bosland, P.W., and O'Connell, M.A. 2011. Heat, color, and flavor compounds in *Capsicum* fruit. In: Gang, D.R. (ed.). The biological activity of phytochemicals, recent advances in phytochemistry. Springer Science. New York: 109-126.

Hall, H.K. and Stephens, M.J. 1999. Fruit firmness in raspberries. In: McGregor, G., Hall, H., and Langford, G. Proc. VII Int. Rubus-Bibes Symp. Acta Hort. ISHS: 505.

Hanson, B. 1999. Chile peppers: hot tips and tasty picks for gardeners and gourmets. 21st-Century Gardening Series. Handbook #161. Brooklyn Botanic Garden, Inc. Brooklyn, NY.

Harvell, K.P. and Bosland, P.W. 1997. The environment produces a significant effect on pungency of chiles. HortScience. 32 (7): 1292.

Havsteen, B. 1983. A class of natural products of high pharmacological potency. Biochem. Pharmacology. 32 (7): 1141-1148.

Havsteen, B.H. 2002. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics. 96: 67– 202.

Hertog, M.G.L., Hollman, P.C.H., and Venema, D.P. 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agricultural and Food Chem. 40: 1591-1598.

Hertog, M.G., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., Menotti, A., and Nedeljkovic, S., et al. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch-Intern-Med. 155 (4): 381-386.

Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., and Kromhout, D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The Lancet. 342: 1007-1011.

Hill, J., Becker, H.C., and Tigerstedt, P.M.A. 1998. Quantitative and ecological aspects of plant breeding. Chapman & Hall, New York.

Hoffmann, A.A. and Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol Evol. 14 (3): 96-101.

Hollman, P.C.H., Hertog, M.G.L., and Katan, M.B. 1996. Analysis and health effects of flavonoids. Food Chem. 57: 43-46.

Hollman, P.C. and Katan, M.B. 1999. Dietary flavonoids: intake, health effects and bioavailability. Food Chem. Toxicol. 37 (9-10): 937-942.

Hoshmand, A.R. 2006. Single-factor experimental designs. In: Design of experiments for agriculture and the natural sciences. 2nd ed. Taylor & Francis Group, LLC. Boca Raton, FL: 13-36.

Howard, L.R., Smith, R.T., Wagner, A.B., Villalon, B., and Burns, E.E. 1994. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapenos. J. Food Sci. 59: 362-365.

Howard, L.R., Talcott, S.T., Brenes, C.H., and Villalon, B. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. J. Agric. Food Chem. 48: 1713-1720.

Howard, L.R. 2001. Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum annuum*). In: Wildman, R.C., ed. Handbook of nutraceuticals and functional foods. Baca Raton, FL: CRC Press: 209-233.

Huffman, V.L. 1977. Volatile components and pungency in fresh and processed jalapeño peppers (*Capsicum annuum*). Diss. Texas A&M University, College Station, TX.

Ito, K., Nakazato, T., Yamato, K., Miyakawa, Y., Yamada, T., Hozumi, N., Segawa, K., Ikeda, Y., and Kizaki, M. 2004. Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. Cancer Research. 64: 1071-1078.

Iwai, K., Suzuki, T., and Fujiwake, H. 1979. Simultaneous microdetermination of capsaicin and its four analogues by using high-performance liquid chromatography and gas chromatography-mass spectrometry. J. Chromatography. 172: 303-311.

Jaakola, L. 2003. Flavonoid biosynthesis in bilberry (*Vaccinium myrtillus* L.). Diss. University of Oulu, Finland.

Jabeen, N., Ahmad, N., and Tanki, M.I. 1999. Genetic variability in hot pepper (Capsicum annum L.). Applied Biological Research. 1 (1): 87-89.

Jacob, R.A. and Sotoudeh, G. 2002. Vitamin C function and status in chronic disease. Nutr. in Clinical Care. 5 (2): 66-74.

Jang, J.J., Kim, S.H., and Yun, T.K. 1989. Inhibitory effect of capsaicin on mouse lung tumor development, *in vivo*. J. Korean Med. Sci. 3: 49-53.

Jang, S., Kelley, K.W., and Johnson, R.W. 2008. Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. PNAS. 105 (21): 7534–7539.

Jarret, R.L., Perkins, B., Fan, T., Prince, A., Guthrie, K., and Skoczenski, B. 2003. Using EIA to screen *Capsicum* spp. germplasm for capsaicinoid content. J. Food Composition and Analysis. 16: 189-194.

Johnston, C.S., Corte, C., and Swan, P.D. 2006. Marginal vitamin C status is associated with reduced fat oxidation during submaximal exercise in young adults. Nutr. & Metabolism. 3 (35): 1-5.

Jones, E.C.S. and Pyman, F.L. 1925. The relation between chemical constitution and pungency in acid amines. J. Chem. Soc., Trans. 127: 2588-2598.

Jung, M.Y., Kang, H.J., and Moon, A. 2001. Caspase-induced apoptosis in Sk-Hep-1 hepatocarcinoma cells involves bel-2 downregulation and caspase-3 activation. Cancer Lett. 165: 139-145.

Kader, A.A. 2008. Perspective flavor quality of fruits and vegetables. J. Sci. Food Agric. 88: 1863-1868.

Kim, H.P., Son, K.H., Chang, H.W., and Kang, S.S. 2004. Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacological Sci. 96: 229-245.

Kotanidou, A., Xagorari, A., Bagli, E., Kitsanta, P., Fotsis, T., Papapetropoulos, A., and Roussos, C. 2002. Luteolin reduces lipopolysaccharide-induced lethal toxicity and expression of proinflammatory molecules in mice. Am. J. Respir. Crit. Care Med. 165: 818-823.

Kurian, A.L. and Starks, A.N. 2002. HPLC analysis of capsaicinoids extracted from whole orange Habanero chili peppers. J. Food Sci. 67 (3): 956-962.

Kurz, W.G.W. and Constabel, F. 1998. Production of secondary metabolites. In: Altman, A. and Colwell, R.R. (eds.). Agricultural biotechnology. Marcel Dekker, Inc. CRC Press, New York: 183-224.

Larson, R.A. 1988. The antioxidants of higher plants. Phytochemistry. 27 (4): 969-978.

Lee, J.J., Crosby, K.M., Pike, L.M., Yoo, K.S., and Leskovar, D.I. 2005. Impact of genetic and environmental variation on development of flavonoids and carotenoids in pepper (*Capsicum* spp.). Scientia Hort. 106: 341-352.

Lee, S.K. and Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20: 207-220.

Lee, S.Y., Nam, D.H., and Kim, J.A. 2001. Induction of apoptosis by capsaicin in A172 human glioblastoma cell. Cancer Lett. 161: 121-130.

Lee, Y., Howard, L.R., and Villalon, B. 1995. Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. J. Food Sci. 60 (2): 1-4.

Lee, Y. 1996. Antioxidant compounds in peppers (*Capsicum annuum* L.) as affected by genotype, maturation, processing, and storage. Diss. Texas A&M University, College Station, TX.

Leskovar, D.I., Crosby, K., and Jifon, J.L. 2009. Impact of agronomic practices on phytochemicals and quality of vegetable crops. In: Patil, B. (ed.). Proc. IInd IS on Human Health Effects of F&V. Acta Hort. 841, ISHS: 317-322.

Lester, G.E. 2006. Environmental regulation of human health nutrients (ascorbic acid, β-carotene, and folic acid) in fruits and vegetables. HortScience. 41 (1): 59-64.

Levine M., Rumsey, S.C., Daruwala, R., Park, J.B., and Wang, Y. 1999. Criteria and recommendations for vitamin C intake. JAMA. 281: 1415-1423.

Liu, R.H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nutr. 78: 517-520.

Lutsenko, E.A., Cárcamo, J.M., and Golde, D.W. 2002. Vitamin C prevents DNA mutation induced by oxidative stress. J. Biological Chem. 277 (19): 16895-16899.

Manju, P.R. and Sreelathakumary, I. 2002. Genetic variability, heritability, and genetic advance in hot chilli (*Capsicum chinense* Jacq.). J. Trop. Agr. 40: 4-6.

Martinez, S., Lopez, M., Gonzalez-Raurich, M., and Alvarez, A.B. 2005. The effects of ripening stage and processing systems on vitamin C content in sweet peppers (*Capsicum annuum* L.). Intl. J. Food Sci. Nutr. 56 (1): 45-51.

Materska, M. and Perucka, I. 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). J. Agric. Food Chem. 53: 1750-1756.

Miean, K.H. and Mohamed, S. 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J. Agric. Food Chem. 49: 3106-3112.

Milerue, N. and Nikornpun, M. 2000. Studies on heterosis of chili (*Capsicum annuum* L.). Kasetsart J. (Nat. Sci.) 34: 190-196.

Monforte-Gonzalez, M., Guzman-Antonio, A., Uuh-Chim, F, and Vazquez-Flota, F. 2010. Capsaicin accumulation is related to nitrate content in placentas of Habanero peppers (*Capsicum chinense* Jacq.). J. Sci. Food Agr. 90: 764-768.

Mpofu, A., Sapirstein, H.D., and Beta, T. 2006. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J. Agric. Food Chem. 54: 1265-1270.

Oh, M.M., Carey, E.E., and Rajashekar, C.B. 2009a. Environmental stresses induce health-promoting phytochemicals in lettuce. Plant Physiology and Biochemistry. 47: 578-583.

Oh, M.M., Trick, H.N., and Rajashekar, C.B. 2009b. Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. J. of Plant Physiology. 166: 180-191.

Oruña-Concha, M.J., Gonzalez-Castro, M.J., and Lopez-Hernandez, J. 1998. Monitoring of the vitamin C content of frozen green beans and pardon peppers by HPLC. J. Sci. Food Agric. 76: 477-480.

Paran, I., Ben-Chaim, A., Kang, B.C., and Jahn, M. 2007. Capsicums. In: Kole, C., (ed.). Genome mapping and molecular breeding in plants. Volume 5. Springer-Verlag, Berlin, Heidelberg: 209-226.

Perucka, I. and Oleszek, W. 2000. Extraction and determination of capsaicinoids in fruit of hot pepper *Capsicum annuum* L. by spectrophotometry and high-performance liquid chromatography. Food Chem. 71: 287-291.

Pietta, P.G. 2000. Flavonoids as antioxidants. J. Nat. Prod. 63: 1035-1042.

Pickersgill, B. 1997. Genetic resources and breeding of *Capsicum* spp. Euphytica. 96: 129-133.

Poehlman, J.M. and Sleper, D.A. 1995. Breeding field crops. 4th Ed. A Blackwell Publishing Company, Iowa State Press, Ames.

Poyrazoglu, E.S., Yemis, O., Kadakal, C., and Artik, N. 2005. Determination of capsaicinoid profile of different chilli peppers grown in Turkey. J. Sci. Food Agric. 85: 1435-1438.

Prasath, D. and Ponnuswami, V. 2008. Heterosis and combining ability for morphological, yield and quality characters in paprika type chilli hybrids. Indian J. Hort. 65 (4): 441-445.

Quinones-Seglie, C.R., Burns, E.E., and Villalon, B. 1989. Capsaicinoids and pungency in various capsicums. Lebensm.-Wiss.-u.-Technol.-Food Sci. Technol. 22: 196–198.

Ravishankar, G.A., Suresh, B., Giridhar, P., Rao, S.R., and Johnson, T.S. 2003. Biotechnological studies on *Capsicum* for metabolite production and plant improvement. In: De, A.K. (ed.). *Capsicum*: The genus *Capsicum*. Harwood Academic Publishers, UK: 96-128.

Reddy, M.G., Kumar, H.D.M., and Salimath, P.M. 2008. Heterosis studies in chillies (*Capsicum annuum* L.). Karnataka J. Agric. Sci. 21 (4): 570-571.

Ren, W., Qiao, Z., Wang, H., Zhu, L., and Zhang, L. 2003. Flavonoids: promising anticancer agents. Medicinal Res. Rev. 23 4: 519-534.

Ribeiro, A. and da Costa, C.P. 1990. Inheritance of pungency in *Capsicum chinense* Jacq. (Solanaceae). Rev. Brasil. Genet. 13 (4): 815-823.

Richeux, F., Cascante, M., Ennamany, R., Saboureau, D., and Creppy, E.E. 1999. Cytotoxicity and genotoxicity of capsaicin in human neuroblastoma cells SHSY-5Y. Arch. Toxicol. 73: 403-409.

Rico, D., Martín-Diana, A.B., Barat, J.M., and Barry-Ryan, C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends in Food Sci. & Technol. 18: 373-386.

Rodríguez-Burruezo, A., Prohens, J., Raigón, M.D., and Nuez, F. 2009. Variation for bioactive compounds in ají (*Capsicum baccatum* L.) and rocoto (*C. pubescens* R. & P.) and implications for breeding. Euphytica. 170: 169-181.

Ross, J.A. and Kasum, C.M. 2002. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22: 19-34.

Sampson, L., Rimm, E., Hollman, P.C.H., de Vries, J.H.M., and Katan, M.B. 2002. Flavonol and flavone intakes in US health professionals. J. Amer. Dietetic Assn. 102 (10): 1414-1420.

Sanatombi, K. and Sharma, G.J. 2008. Capsaicin content and pungency of different *Capsicum* spp. cultivars. Not. Bot. Hort. Agrobot. Cluj. 36 2: 89-90.

SAS Institute Inc. 2008. SAS 9.2 user's guide, statistics, 2nd ed. SAS Institute Inc., Cary, NC.

Sharma, V.K., Semwal, C.S., and Uniyal, S.P. 2010. Genetic variability and character association analysis in bell pepper (*Capsicum annuum* L.). J. of Horticulture and Forestry. 2 (3): 58-65.

Shashidhara, K.G. 2006. Response of chilli to drip irrigation and fertigation on a vertisol of Malaprabha Command Area. Thesis. University of Agricultural Sciences, Dharwad, India.

Showalter, R.K. 1973. Factors affecting pepper firmness. Florida State Horticultural Society. Handling and Processing Section. 230-232. Available at: http://www.fshs.org/Proceedings/Password%20Protected/1973%20Vol.%2086/230-232%20(SHOWALTER).pdf.

Simkin, A., Zhu-Changfu, J., Kuntz, M., and Sandman, G. 2003. Light-dark regulation of carotenoids biosynthesis in pepper (*Capsicum annuum*). J. Plant Physiol. 160: 439-443.

Simon, P.W. 1997. Plant pigments for color and nutrition. HortScience. 32 1: 12-13.

Simon, J.E., Chadwick, A.F., and Craker, L.E. 1984. Herbs: an indexed bibliography. 1971-1980. The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books, 770 pp., Hamden, CT. Available at: http://www.hort.purdue.edu/newcrop/med-aro/factsheets/CAPSICUM_PEPPER.html.

Singh S., Jarret, R., Russo, V., Majetich, G., Shimkus, J., Bushway, R., and Perkins, B. 2009. Determination of capsinoids by HPLC-DAD in *Capsicum* species. J. Agric. Food Chem. 57: 3452-3457.

Skroch, P.W. and Nienhuis, J. 1995. Qualitative and quantitative characterization of RAPD variation among snap bean genotypes. Theoretical and Applied Genetics. 91: 1078-1085.

Smirnoff, N. 1996. The function and metabolism of ascorbic acid in plants. Annals of Botany. 78: 661-669.

Smirnoff, N. and Wheeler, G.L. 2000. Ascorbic acid in plants: biosynthesis and function. Crit. Rev. Biochem. Mol. Biol. 35 (4): 291-314.

Smith, T.J. and Yang, C.S. 1994. Effect of food phytochemicals on xenobiotic metabolism and tumorigenesis. In: Haung, M.T., Osawa, T., Ho, C.T., and Rosen, R.T. (ed.). Food phytochemicals for cancer prevention. American Chemical Society, Washington D.C. 17-48.

Sood, S., Sood, R., Sagar, V., and Sharma, K.C. 2009. Genetic variation and association analysis for fruit yield, agronomic and quality characters in bell pepper. International J. of Vegetable Science. 15: 272-284.

Sreelathakumary, I. and Rajamony, L. 2004. Variability, heritability and genetic advance in chilli (*Capsicum annuum* L.). J. Tropical Agriculture. 42(1-2): 35-37.

Sukrasno, N. and Yeoman, M.M. 1993. Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. Phytochemistry, 32 (4): 839-844.

Sun, T., Xu, Z., Wu, C.T., Janes, M., Prinyawiwatkul, W., and No, H.K. 2007. Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). J. Food Sci. 72: 98-102.

Surh, Y.J. and Lee, S.S. 1995. Capsaicin, a double-edged sword: toxicity, metabolism, and chemopreventive potential. Life Sci. 22: 1845-1855.

Surh, Y.J., Lee, B.C., Park, K.K., Mayne, S.T., Liem, A., and Miller, J.A. 1995. Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and *N*-nitrosodimethylamine. Carcinogenesis (Lond.). 16: 2467-2471.

Teel, R.W. 1991. Effects of capsaicin on rat liver S9-mediated metabolism and DNA binding of aflatoxin. Nutr. Cancer. 15: 27-32.

Temple, N.J. and Gladwin, K.K. 2003. Fruit, vegetables, and the prevention of cancer: research challenges. Nutrition. 19: 467-470.

Thampi, P.S.S. 2003. A glimpse of the world trade in *Capsicum*. In: De, A.K. (ed.). *Capsicum*: the genus *Capsicum*. Taylor & Francis Inc., New York: 16-24.

Tremblay, A. and Westerterp-Plantenga, M. 2007. Capsaicin and energy balance. In: Desjardins, Y. (ed.). Proc. 1st IS on Hum. Health Effects of F&V. Acta Hort. 744, ISHS: 149-153.

United Nations, Food and Agriculture Organization, *FAOStat*. 2008. Available at: http://usda01.library.cornell.edu/usda/ers/BellAndChile/Table64.xls.

U.S. Department of Agriculture, National Agricultural Statistics Service (2000-05) and Economic Research Service, USDA (1980-99). Available at http://usda01.library.cornell.edu/usda/ers/BellAndChile/Table48.xls.

Vaughan, J.G. and Geissler, C.A. 2009. Chillies and peppers. The new Oxford book of food plants. Oxford University Press Inc., New York.

Watada, A.E. 1995. Methods for determining quality of fruits and vegetables. Acta Horticulturae. 379: 559-567.

Weisenfelder, A.E., Huffman, V.L., Villalon, B., and Burns, E.E. 1978. Quality and processing attributes of selected jalapeño pepper cultivars. J. Food Sci. 43: 885-887, 891.

Wimalasiri, P. and Wills, R.B.H. 1983. Simultaneous analysis of ascorbic acid and dehydroascorbic acid in fruit and vegetables by high-performance liquid chromatography. J. of Chromatography. 256: 368-371.

Winkel-Shirley, B. 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiology. 126: 485-493.

Woodland Publishing. 1996. *Capsicum*: therapeutic powerhouse and herbal catalyst. Pleasant Grove, UT. Online. 20 Mar. 2008. Internet. Available at: http://www.nutraceutical.com/educate/pdf/capsicum.pdf.

Yadeta, B., Belew, D., Gebreselassie, W., and Marame, F. 2011. Variability, heritability and genetic advance in hot pepper (*Capsicum annuum* L.) genotypes in West Shoa, Ethiopia. American-Eurasian J. Agric. & Environ. Sci. 10 (4): 587-592.

Yao, L.H., Jiang, Y.M., Shi, J., Tomás-Barberán, F.A., Datta, N., Singanusong, R., and Chen, S.S. 2004. Flavonoids in food and their health benefits. Plant Foods for Human Nutr. 59: 113–122.

Yoo, K.S., Pike, L., Patil, B.S., Leskovar, D., Crosby, K., and King, S. 2007. Challenges of phytochemical analysis and its application in developing new fruits and vegetables with improved health benefits. In: Desjardins, Y. (ed.). Proc. 1st IS on Hum. Health Effects of F&V. Acta Hort. 744, ISHS: 101-106.

Yoshida, M., Sakai, T., Hosokawa, N., Marui, N., Matsumoto, K., Fugioka, A., Nishino, H., and Aoike, A. 1990. The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. FEBS Letter. 260: 10-13.

Zatykó, L. 2006. Pepper (*Capsicum annuum* L.) breeding methods at the turn of the century. Acta Agronomica Hungarica. 54 (2): 179-202.

Zewdie, Y. and Bosland, P.W. 2000. Evaluation of genotype, environment, and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. Euphytica. 111: 185-190.

APPENDIX

Table 1. Three preliminary studies where we used a penetrometer to measure the amount of pressure (lb) needed to puncture the outside wall of a few jalapeño (J) and serrano (S) peppers to better indicate their potential fruit firmness.

Location California (Lark Seeds) (Field)		**Location Uvalde (Field)**		**Location College Station-VFIC (Greenhouse)**	
Genotype	**Av. Pressure (lb)**	**Genotype**	**Av. Pressure (lb)**	**Genotype**	**Av. Pressure (lb)**
(J) J3	11.2 e [Π]	**(J)** Hyb.36	7.79 b	**(J)** 8	6.77 e
J4	26.7 a	Hyb.43	7.98 b	10-1	7.21 e
J5	13.8 de	Hyb.119	8.03 b	55	6.78 e
J6	18.7 bcd	Hyb.120	8.24 b	413p1-4	6.74 e
J7	22.9 abc	Dragon	8.53 b	413p1-8	7.04 e
J10	15.4 de	**(S)** Hyb.4	7.86 b	**(S)** SGH20H	10.31 a
J12	22.1 abc	Hyb.5	10.41 a	33-19	8.58 cd
J13	17.8 cd	Hyb.16	11.41 a	41	9.86 ab
J15	22.3 abc	Hyb.21	10.48 a	45a	9.77 abc
J16	21.5 abc	Hyb.24	11.23 a	45a-1	10.33 a
(S) S2	21.5 abc	Hyb.25	11.40 a	45a-1a	10.38 a
S4	23.5 ab	Hyb.27	10.71 a	45a-2	9.33 abc
S6	17.3 cd			45p2-4	9.19 abc
S7	25.9 a			107p5-1	8.68 bcd
S8	24.3 ab			108p1-2	10.29 a
S9	22.6 abc			111-2	8.71 bcd
S13	22.2 abc			112p6-1	8.59 cd
S14	26.5 a			117p7-2	7.54 de

[Π] Mean separations by Duncan at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

Table 2. Average monthly environmental conditions during May to August 2009 for maximum and minimum temperatures, relative humidity, solar radiation, and precipitation.

Location	Month	Max. Temp. (°C)	Min. Temp. (°C)	RHmin (%)	Solar (MJm^{-2})	Precip. (mm)
College Station-VFIC: Sandy Clay Loam						
	May	29	19	44	19.17	47.8
	June	34	23	33	21.82	0.0
	July	36	25	31	20.15	107.4
	Aug	35	24	31	19.59	5.1
Uvalde: Silty Clay Loam (fine-silty, mixed, hyperthermic Aridic Calciustoll)						
	May	33	20	32	20.78	29.0
	June	36	23	27	23.86	2.5
	July	37	24	26	23.72	30.5
	Aug	37	24	24	22.93	0.0
Weslaco: Hidalgo Fine Sandy Loam						
	May	32	22	48	22.57	45.7
	June	34	24	47	33.97	22.4
	July	36	26	37	38.94	8.9
	Aug	36	25	38	37.63	7.6

Max.: Maximum Min.: Minimum

Table 3. Average fruit number per plant, % dry matter, and correlation (r) analysis values between fruit yield (FY), fruit weight (FW), flavonoids (quercetin+luteolin), and capsaicinoids (capsaicin+DHC) for four select Habanero (*Capsicum chinense*) experimental hybrids grown in Uvalde, TX.

Genotype	**Fruit #**	**% Dry Matter**	**Correlation Analyses**				
H1-red	58 ab[Π]	16.10 b[Π]		**FY**	**FW**	**Flav.**	**Cap.**
H2-orange	41 b	20.01 a	**FY**	1.00 [α]	0.37	-0.56	0.24
			FW	0.37	1.00	-0.77	-0.11
H3-orange	93 ab	16.85 b	**Flav.**	-0.56	-0.77	1.00	-0.55
			Cap.	0.24	-0.11	-0.55	1.00
H5-dark orange	102 a	16.94 b					

FY: Fruit Yield; FW: Fruit Weight; Flav.: Flavonoids (Quercetin+Luteolin); Cap.: Capsaicinoids (Capsaicin+DHC)

[Π] Mean separations by Duncan at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[α] Signifies no significant associations were detected between any of these components at 5% level.

Table 4. F-values and their significances when data from five fruit (*C. chinense*) characteristics were analyzed by the main effects (location, genotype) and their interactions.

Source	df	Fruit Weight	Capsaicin	Dihydrocapsaicin	Quercetin	Luteolin
Location (L)	2	4.86 *	9.13 *	2.21 ^{NS}	8.83 *	0.54 ^{NS}
Genotype (G)	5	1.85 ^{NS}	11.37 *	14.68 *	5.13 *	1.62 ^{NS}
L x G	9	19.25 **	4.68 *	3.97 *	2.30 *	4.13 *

^{NS}, *, and **: Not Significant, significant, and highly significant values at 5% level, respectively.

Table 5. Fruit colors and fruit weights of mature Habanero peppers (*C. chinense*) grown in three Texas locations.

		Fruit Weight (g)		
Genotype	**Fruit Color**	**College Station**	**Uvalde**	**Weslaco**
Kuk	Orange	6.11 cd[Z] B[Y]	NA	8.87 c A
H1	Red	9.51 a B	7.80 ab C	14.43 a A
H2	Orange	8.61 ab A	6.11 d B	7.71 d A
H3	Orange	7.51 bc B	6.99 c B	9.95 b A
H5	Dark Orange	5.49 d B	7.22 bc A	6.70 e A
H6	Yellow	4.79 d C	7.97 a B	10.24 b A

NA: Entry not available in that location.

[Z] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Y] Mean separations across locations by LSD at $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 6. Capsaicinoid (capsaicin and dihydrocapsaicin) concentrations in mature Habanero pepper fruits (*C. chinense*) grown in three Texas locations.

Genotype	**Capsaicin Concn (μg g^{-1} FW)**			**Dihydrocapsaicin Concn (μg g^{-1} FW)**		
	CS	**UV**	**WE**	**CS**	**UV**	**WE**
Kuk-orange	372.25 a[Z] A[Y]	NA	491.72 a A	385.44 a A	NA	238.29 a B
H1-red	128.34 bc A	32.93 c B	154.58 c A	71.41 c A	30.78 c B	83.23 d A
H2-orange	121.81 bc B	60.26 b B	315.85 b A	80.98 c B	62.27 b B	135.95 c A
H3-orange	71.56 c A	9.18 d B	103.46 c A	41.63 c A	7.20 d B	44.01 d A
H5-dark orange	247.79 ab B	129.09 a B	435.14 a A	209.10 b A	99.96 a B	196.58 b AB
H6-yellow	0.00 c B	0.09 d B	0.65 d A	0.00 c B	0.04 d B	0.39 e A

CS: College Station-VFIC; UV: Uvalde; WE: Weslaco

NA: Entry not available in that location. FW: fresh weight.

[Z] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Y] Mean separations across locations by LSD at $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 7. Flavonoid (quercetin and luteolin) concentrations in mature Habanero pepper fruits (*C. chinense*) grown in three Texas locations.

Genotype	Quercetin Concn (µg g^{-1} FW)			Luteolin Concn (µg g^{-1} FW)		
	CS	UV	WE	CS	UV	WE
Kuk-orange	19.13 a[Z] A[Y]	NA	8.21 a B	2.88 b A	NA	9.35 a A
H1-red	3.87 b AB	5.67 a A	2.74 c B	0.00 b A	0.04 d A	0.06 b A
H2-orange	8.49 b A	4.53 a A	5.19 b A	5.36 ab AB	7.36 a A	3.03 b B
H3-orange	11.44 ab A	4.50 a B	6.51 ab B	9.61 a A	5.37 b B	3.82 b B
H5-dark orange	6.74 b A	1.63 b B	1.70 c B	10.20 a A	1.62 c B	0.75 b B
H6-yellow	11.61 ab A	2.40 b B	1.49 c B	1.73 b A	2.30 c A	0.00 b B

CS: College Station-VFIC; UV: Uvalde; WE: Weslaco

NA: Entry not available in that location. FW: fresh weight.

[Z] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Y] Mean separations across locations by LSD at $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 8. Genotype degree of freedom values, F-values, and their significances when six *C. chinense* genotypes were analyzed within three Texas locations (College Station-VFIC, Uvalde, and Weslaco) in 2009 for fruit weight and four different phytochemicals.

Location	df	Fruit Weight	Capsaicin	DHC	Quercetin	Luteolin
C.S.	5	6.75*	5.24*	9.44*	2.23^{NS}	2.04^{NS}
U.V.	4	11.64*	48.35**	51.75**	11.15**	84.91**
W.E.	5	47.74**	40.51**	36.10**	14.81**	4.45*

C.S.: College Station-VFIC; U.V.: Uvalde; W.E.: Weslaco

^{NS}, *, and **: Not significant, significant, and highly significant values at 5% level, respectively.

Table 9. F-values and their significances when 2009 data from five fruit (*C. annuum*) characteristics were analyzed by the main effects (location, genotype) and their interactions.

SOV	df	Ascorbic Acid	Capsaicin	DHC	Quercetin	Luteolin
Location (L)	2	10.96*	0.23^{NS}	8.50*	4.20*	16.07*
Genotype (G)	9	13.87**	3.92*	1.65^{NS}	3.65*	10.08*
L x G	15	3.01*	8.75*	3.29*	1.36^{NS}	2.51*

^{NS}, *, and **: Not significant, significant, and highly significant values at 5% level, respectively.

Table 10. Means of ascorbic acid concentrations (µg g^{-1} FW) in various pepper (*C. annuum*) fruits grown in three Texas locations in 2009.

Entry	**Pepper Type**	**Ascorbic Acid Values**		
		Amarillo	**College Station**	**Uvalde**
J1	Jalapeño	777.98 bcd$^{\Pi}$ A$^{\Delta}$	886.07 cd A	562.75 ef B
J2	Jalapeño	516.23 d A	NA	468.43 g A
J3	Jalapeño	NA	591.00 d A	632.47 d A
J4	Jalapeño	723.31 bcd A	798.21 cd A	512.95 fg A
Dragon	Jalapeño	904.46 bc A	NA	514.20 fg B
Ixtapa	Jalapeño	608.11 cd B	758.84 cd A	369.36 h C
J1845	Jalapeño	947.25 bc A	1022.30 c A	807.54 c A
C1	cayenne	1439.01 a A	1623.63 b A	1014.71 b A
C2	cayenne	1583.57 a B	2300.17 a A	1355.48 a B
Mesilla	cayenne	1073.93 b A	899.31 cd A	568.50 e B

NA: Entry not available in that location

$^{\Pi}$ Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

$^{\Delta}$ Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 11. Means of capsaicinoid (capsaicin and dihydrocapsaicin) concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in three Texas locations in 2009.

Entry	**Pepper Type**	**Capsaicin Values**			**Dihydrocapsaicin Values**		
		A.M.	**C.S.**	**U.V.**	**A.M.**	**C.S.**	**U.V.**
J1	Jalapeño	96.07 cd[Π] A[Δ]	19.27 e B	17.40 e B	92.45 ab A	23.55 cd B	9.34 e B
J2	Jalapeño	60.05 de A	NA	17.42 e A	43.81 bc A	NA	7.59 ef B
J3	Jalapeño	NA	0.00 e A	0.00 e A	NA	0.00 d A	0.00 f A
J4	Jalapeño	139.07 bc A	101.13 c A	96.99 c A	118.57 a A	120.27 ab A	37.62 ab B
Dragon	Jalapeño	195.51 ab A	NA	59.51 d B	129.33 a A	NA	20.55 d B
Ixtapa	Jalapeño	120.25 cd B	235.12 a A	110.79 bc B	77.82 ab AB	185.11 a A	29.49 c B
J1845	Jalapeño	0.67 e B	132.76 bc A	112.61 bc A	0.60 c B	107.47 abc A	30.09 bc B
C1	cayenne	76.69 cd A	79.95 cd A	128.99 b A	90.15 ab AB	177.86 a A	41.54 a B
C2	cayenne	70.95 cde A	33.09 de AB	2.36 e B	84.41 ab A	65.76 bcd A	4.90 ef A
Mesilla	cayenne	241.56 a A	157.85 b B	291.52 a A	83.57 ab A	78.40 bcd AB	34.59 abc B

NA: Entry not available in that location

A.M.: Amarillo; C.S.: College Station-VFIC; U.V.: Uvalde

[Π] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Δ] Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 12. Means of flavonoid (quercetin and luteolin) concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in three Texas locations in 2009.

Entry	Pepper Type	Quercetin Values			Luteolin Values		
		A.M.	C.S.	U.V.	A.M.	C.S.	U.V.
J1	Jalapeño	9.75 bc[Π] AB[Δ]	18.74 b A	3.89 cd B	3.07 b B	8.54 b A	2.48 c B
J2	Jalapeño	2.73 c A	NA	1.04 e A	1.80 b A	NA	0.36 d B
J3	Jalapeño	NA	12.56 b A	5.05 c B	NA	3.29 c A	0.78 d B
J4	Jalapeño	0.58 c AB	1.95 b A	0.00 e B	1.71 b B	3.31 c A	1.07 d B
Dragon	Jalapeño	6.28 bc A	NA	1.08 e B	2.36 b A	NA	1.19 d B
Ixtapa	Jalapeño	5.53 bc A	5.45 b A	0.35 e B	1.88 b B	3.09 c A	0.92 d C
J1845	Jalapeño	6.87 bc A	5.27 b AB	2.83 d B	1.77 b A	1.90 c A	0.24 d B
C1	cayenne	19.34 ab A	12.68 b A	4.08 cd A	6.16 a AB	9.69 b A	4.77 b B
C2	cayenne	10.88 bc A	58.45 a A	20.01 a A	6.08 a B	15.91 a A	6.47 a B
Mesilla	cayenne	35.16 a A	40.61 ab A	13.69 b A	7.24 a A	13.09 ab A	7.14 a A

NA: Entry not available in that location

A.M.: Amarillo; C.S.: College Station-VFIC; U.V.: Uvalde

[Π] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Δ] Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 13. Genotype degree of freedom values, F-values, and their significances when ten *C. annuum* genotypes were analyzed within each Texas location (Amarillo, College Station-VFIC, and Uvalde) in 2009 for five different fruit characteristics.

Location	**df**	**Ascorbic Acid**	**Capsaicin**	**DHC**	**Quercetin**	**Luteolin**
A.M.	8	9.06*	8.48*	2.42^{NS}	3.69*	9.55*
C.S.	7	19.65**	17.91**	5.24*	2.26^{NS}	9.33*
U.V.	9	270.20**	68.98**	31.81**	185.93**	44.33**

A.M.: Amarillo ; C.S.: College Station-VFIC; U.V.: Uvalde

^{NS}, *, and **: Not significant, significant, and highly significant values at 5% level, respectively.

Table 14. Location degree of freedom values, F-values, and their significances when ten *C. annuum* genotypes were analyzed across three Texas locations (Amarillo, College Station-VFIC, and Uvalde) in 2009 for five different fruit characteristics.

Genotype	**df**	**Ascorbic Acid**	**Capsaicin**	**DHC**	**Quercetin**	**Luteolin**
J1	2	9.31*	5.58*	5.51*	6.58*	26.21**
J2	1	1.45^{NS}	4.77^{NS}	7.91*	6.92^{NS}	12.71*
J3	1	1.19^{NS}	0.00^{NS}	0.00^{NS}	282.90**	44.21**
J4	2	2.44^{NS}	1.02^{NS}	5.55*	3.90^{NS}	8.83*
Dragon	1	9.74*	98.94**	99.49**	44.55**	26.18*
Ixtapa	2	21.72*	6.76*	6.60*	9.47*	20.28*
J1845	2	2.98^{NS}	27.13**	18.51*	7.68*	19.14*
C1	2	2.43^{NS}	1.60^{NS}	4.03^{NS}	1.20^{NS}	4.82^{NS}
C2	2	11.24*	3.62^{NS}	2.20^{NS}	2.03^{NS}	6.77*
Mesilla	2	23.69*	12.43*	4.35^{NS}	1.09^{NS}	3.50^{NS}

^{NS}, *, and **: Not significant, significant, and highly significant values at 5% level, respectively.

Table 15. F-values and their significances for 2010 *C. annuum* study when data for fruit diameter, fruit length, capsaicin, dihydrocapsaicin (DHC), quercetin, and luteolin were analyzed by the main effects (location, genotype) with their interactions.

SOV	Fruit							
	Diameter	**Length**	**Wall Thickness**	**Ascorbic Acid**	**Capsaicin**	**DHC**	**Quercetin**	**Luteolin**
Location (L)	49.21**	29.00**	284.70**	50.90**	6.38*	20.30*	9.67*	8.37*
Genotype (G)	25.78**	79.89**	26.12**	11.96*	2.79*	6.13*	9.55*	16.61*
L x G	2.74*	7.09*	1.22^{NS}	35.51**	8.00*	4.61*	11.13**	1.75*

^{NS}, *, and **: Not significant, significant, and highly significant values at 5% level, respectively.

Table 16. Genotype F-values, and their significances when 21 *C. annuum* genotypes were analyzed within two Texas locations (Uvalde and Weslaco) in 2010 for fruit weight, fruit diameter, fruit length, fruit wall thickness, and five different phytochemicals.

	Fruit							
Location	**Diameter**	**Length**	**Wall Thickness**	**Ascorbic Acid**	**Capsaicin**	**DHC**	**Quercetin**	**Luteolin**
U.V.	49.27**	168.08**	15.13**	300.53**	19.30**	24.25**	70.49**	13.01**
W.E.	29.26**	521.58**	31.38**	199.04**	13.92**	14.68**	39.06**	19.68**

U.V.: Uvalde; W.E.: Weslaco

**: Highly significant values at 5% level, respectively.

Table 17. Mean fruit measurements of pepper (*C. annuum*) samples grown in two different Texas locations in spring 2010.

Entry	Pepper Type	Uvalde			Weslaco		
		F.L. (cm)	**F.D. (cm)**	**W.T. (cm)**	**F.L. (cm)**	**F.D. (cm)**	**W.T. (cm)**
J-1	Jalapeño	7.42 hi[Π] A[Δ]	3.43 bc A	0.47 ab A	6.82 efgh B	3.13 ab A	0.28 de B
J-2	Jalapeño	9.30 d A	3.77 a A	0.43 bc A	8.18 d B	3.33 a A	0.35 ab A
J-3	Jalapeño	9.00 de A	3.80 a A	0.50 ab A	6.96 ef B	2.97 bcde B	0.33 abc B
J-4	Jalapeño	8.26 efgh A	3.57 abc A	0.47 ab A	6.81 efgh B	2.77 cde B	0.32 bcd B
J-5	Jalapeño	8.30 efgh A	3.83 a A	0.47 ab A	6.69 efgh B	2.90 bcde B	0.32 bcd B
J-6	Jalapeño	7.98 fgh A	3.60 ab A	0.47 ab A	6.41 gh B	3.07 abc A	0.33 abc B
J-7	Jalapeño	7.70 ghi A	3.37 bc A	0.47 ab A	6.70 efgh B	2.70 de B	0.30 cd B
J-8	Jalapeño	7.36 hi A	3.37 bc A	0.53 a A	6.65 efgh B	3.17 ab A	0.36 a B
J-9	Jalapeño	8.54 defg A	3.27 c A	0.47 ab A	6.63 efgh B	2.67 e B	0.35 ab B
J-10	Jalapeño	8.16 efgh A	3.60 ab A	0.47 ab A	6.48 fgh B	3.07 abc B	0.33 abc B
Dragon	Jalapeño	8.00 fgh A	3.40 bc A	0.50 ab A	6.74 efgh B	3.03 abcd A	0.25 e B
Tormenta	Jalapeño	7.84 fgh A	3.60 ab A	0.47 ab A	6.37 h B	2.97 bcde B	0.34 abc B
S-1	serrano	8.74 def A	1.90 ef A	0.37 cd A	7.03 e B	1.57 f B	0.19 f B
S-2	serrano	8.49 defg A	1.90 ef A	0.33 de A	6.91 efg B	1.63 f B	0.15 fg B
S-3	serrano	8.04 fgh A	1.97 ef A	0.30 de A	7.10 e B	1.70 f A	0.16 fg A
S-4	serrano	7.49 hi A	1.90 ef A	0.30 de A	7.11 e B	1.80 f A	0.18 fg B
Halcon	serrano	5.98 j B	1.93 ef A	0.30 de A	6.45 fgh A	1.87 f A	0.17 fg B
Magnum45	serrano	6.75 ij A	1.80 f A	0.27 ef A	6.81 efgh A	1.73 f A	0.15 g B
C-1	cayenne	21.10 c A	2.83 d	0.27 ef	18.48 b B	NA	NA
C-2	cayenne	26.13 a A	2.13 e	0.10 g	25.55 a A	NA	NA
Mesilla	cayenne	22.24 b A	2.77 d	0.20 f	17.00 c B	NA	NA

U.V.: Uvalde location; W.E.: Weslaco location.

F.L.: Fruit length; F.D.: Fruit diameter; W.T.: Wall thickness

NA: Measurement was not available

[Π] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Δ] Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 18. Mean ascorbic acid concentrations (µg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in two different Texas locations in spring 2010.

Entry	Pepper Type	Ascorbic Acid Values	
		U.V.	W.E.
J-1	Jalapeño	465.07 e^{π} B^{Δ}	748.17 d A
J-2	Jalapeño	175.68 hij B	528.80 h A
J-3	Jalapeño	170.01 ij B	583.41 fgh A
J-4	Jalapeño	173.06 ij B	396.35 j A
J-5	Jalapeño	402.04 f B	542.05 gh A
J-6	Jalapeño	471.08 e B	676.40 de A
J-7	Jalapeño	230.13 gh B	430.80 ij A
J-8	Jalapeño	253.43 g B	575.15 fgh A
J-9	Jalapeño	500.81e B	681.46 de A
J-10	Jalapeño	405.53 f B	545.33 gh A
Dragon	Jalapeño	476.46 e A	441.46 ij A
Tormenta	Jalapeño	806.85 c A	927.83 c A
S-1	serrano	70.57 k B	722.55 d A
S-2	serrano	121.01 jk B	634.40 ef A
S-3	serrano	178.53 hi B	502.64 hi A
S-4	serrano	74.99 k B	666.35 de A
Halcon	serrano	265.85 g B	618.36 efg A
Magnum45	serrano	390.13 f B	683.43 de A
C-1	cayenne	1272.12 a B	2167.59 a A
C-2	cayenne	1214.74 b B	1557.65 b A
Mesilla	cayenne	610.93 d B	865.60 c A

U.V.: Uvalde location; W.E.: Weslaco location.

$^{\Pi}$ Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

$^{\Delta}$ Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 19. Mean capsaicin and dihydrocapsaicin concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in two different Texas locations in spring 2010.

Entry	**Pepper Type**	**Capsaicin Values**		**Dihydrocapsaicin Values**	
		U.V.	**W.E.**	**U.V.**	**W.E.**
J-1	Jalapeño	0.00 l[π] A[Δ]	0.00 i A	0.00 j A	0.00 h A
J-2	Jalapeño	39.49 jk A	70.41 fgh A	22.04 hi B	72.52 ef A
J-3	Jalapeño	0.00 l A	0.00 i A	0.00 j A	0.00 h A
J-4	Jalapeño	14.77 kl B	55.68 ghi A	12.77 ij B	58.60 efg A
J-5	Jalapeño	88.52 defg A	89.42 fgh A	71.60 c A	97.67 cde A
J-6	Jalapeño	90.65 def A	169.97 bcd A	62.92 cd B	132.53 bc A
J-7	Jalapeño	50.51 hij A	94.14 efgh A	21.56 hi B	76.03 ef A
J-8	Jalapeño	58.70 ghij A	79.07 fgh A	30.43 fghi B	77.13 def A
J-9	Jalapeño	44.74 ijk A	99.46 efg A	35.76 efgh A	98.56 cde A
J-10	Jalapeño	104.59 cde A	208.50 b A	54.82 cde B	157.46 b A
Dragon	Jalapeño	62.82 fghij B	281.41 a A	37.65 efgh B	121.53 bcd A
Tormenta	Jalapeño	139.35 ab A	125.85 def A	102.94 b A	142.32 bc A
S-1	serrano	79.22 efgh A	146.33 cde A	68.86 c B	134.04 bc A
S-2	serrano	124.56 abc A	160.85 bcd A	117.88 ab A	151.64 b A
S-3	serrano	61.08 fghij A	79.07 fgh A	46.95 def A	49.54 fg A
S-4	serrano	49.18 hij B	172.46 bcd A	27.51 fghi B	74.64 ef A
Halcon	serrano	111.67 bcd A	91.81 efgh A	63.50 cd A	34.10 fgh B
Magnum45	serrano	142.38 a A	189.27 bc A	127.60 a B	220.93 a A
C-1	cayenne	0.00 l A	0.00 i A	0.00 j A	0.00 h A
C-2	cayenne	74.14 fghi A	38.17 hi A	24.82 ghi A	18.73 gh A
Mesilla	cayenne	149.92 a A	65.70 gh B	44.17 defg A	25.30 gh A

U.V.: Uvalde location; W.E.: Weslaco location.

[Π] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Δ] Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 20. Mean quercetin and luteolin (flavonoid) concentrations (μg g^{-1} FW) in different pepper fruits (*C. annuum*) grown in two different Texas locations in spring 2010.

Entry	Pepper Type	Quercetin Values		Luteolin Values	
		U.V.	**W.E.**	**U.V.**	**W.E.**
J-1	Jalapeño	7.20 cde[Π] A[Δ]	3.13 def B	1.25 fg A	1.20 efg A
J-2	Jalapeño	5.42 cde A	1.35 ef B	3.53 cdef A	1.69 defg B
J-3	Jalapeño	5.95 cde A	2.15 def B	2.21 efg A	0.99 efg B
J-4	Jalapeño	2.32 de A	0.96 ef B	1.05 fg A	0.99 efg A
J-5	Jalapeño	9.45 cd A	3.49 def B	5.10 c A	3.16 cd B
J-6	Jalapeño	9.80 cd A	1.48 ef B	2.14 fg A	1.58 defg A
J-7	Jalapeño	4.18 de A	1.43 ef A	1.44 fg A	2.19 cdef A
J-8	Jalapeño	6.29 cde A	2.59 def B	4.75 cd A	3.93 c A
J-9	Jalapeño	12.46 bc A	1.76 ef B	4.66 cde A	2.52 cde A
J-10	Jalapeño	0.70 e A	0.41 f B	0.88 g A	0.97 efg A
Dragon	Jalapeño	8.48 cde A	0.91 ef B	2.47 defg A	0.91 efg B
Tormenta	Jalapeño	3.73 de A	1.34 ef B	2.48 defg A	2.57 cde A
S-1	serrano	18.64 b A	8.13 d A	2.69 cdefg A	2.33 cdef A
S-2	serrano	9.55 cd A	2.97 def B	2.65 cdefg A	1.97 defg A
S-3	serrano	8.14 cde A	2.27 def B	1.68 fg A	0.60 fg B
S-4	serrano	12.91 bc A	6.64 de B	2.61 cdefg A	1.51 defg B
Halcon	serrano	0.71 e A	0.13 f B	1.31 fg A	0.24 g B
Magnum45	serrano	3.18 de A	0.24 f B	2.08 fg A	0.18 g B
C-1	cayenne	88.14 a A	57.92 a A	8.69 b A	10.98 a A
C-2	cayenne	80.83 a A	29.11 b B	13.16 a A	7.77 b B
Mesilla	cayenne	18.39 b A	18.95 c A	9.51 b A	8.79 b A

U.V.: Uvalde location; W.E.: Weslaco location.

[Π] Mean separations within each location by LSD at $P \leq 0.05$. Means followed by the same lower case letters are not significantly different.

[Δ] Mean separations across locations by LSD $P \leq 0.05$. Means followed by the same upper case letters are not significantly different.

Table 21. F-values and their significances when 21 *C. annuum* genotypes were analyzed across two Texas locations (Uvalde and Weslaco) in 2010 for eight different fruit characteristics.

Genotype	**FD**	**FL**	**WT**	**AA**	**Capsaicinoids**		**Flavonoids**	
					Capsaicin	**DHC**	**Quercetin**	**Luteolin**
J-1	7.36*	7.34*	12.70*	43.14*	0.00^{NS}	0.00^{NS}	17.40*	0.05^{NS}
J-2	5.28^{NS}	20.01*	5.70^{NS}	292.17**	2.47^{NS}	9.02*	31.45*	8.87*
J-3	12.02*	115.30**	89.29**	1009.07**	0.00^{NS}	0.00^{NS}	149.39**	87.38**
J-4	41.14*	42.16**	18.93*	184.38**	9.88*	9.12*	15.50*	0.05^{NS}
J-5	49.00*	27.87*	18.93*	51.73**	0.01^{NS}	4.65^{NS}	18.56*	14.74*
J-6	5.57^{NS}	77.57**	14.95*	91.62**	7.50*	12.34*	139.62**	0.76^{NS}
J-7	30.77*	11.46*	16.79*	148.28**	3.16^{NS}	10.14*	3.43^{NS}	3.08^{NS}
J-8	7.20*	18.25*	24.81*	826.46**	6.34*	40.54*	10.24*	0.26^{NS}
J-9	23.14*	66.06**	11.89*	155.52**	4.53^{NS}	5.88*	23.36*	1.33^{NS}
J-10	8.26*	84.46**	15.38*	29.66*	7.33*	15.63*	45.87*	0.74^{NS}
Dragon	1.73^{NS}	35.31*	186.32**	1.53^{NS}	47.99*	22.60*	179.34**	12.28*
Tormenta	22.56*	44.32**	12.12*	4.41^{NS}	0.16^{NS}	1.48^{NS}	8.57*	0.01^{NS}
S-1	10.00*	68.21**	26.77*	185.28**	7.06*	9.35*	5.35^{NS}	0.15^{NS}
S-2	64.00**	30.63*	28.87*	766.05**	2.04^{NS}	1.67^{NS}	19.78*	1.98^{NS}
S-3	4.00^{NS}	24.62*	5.88*	179.58**	0.36^{NS}	0.02^{NS}	41.14*	39.37*
S-4	0.75^{NS}	6.11*	195.57**	322.23**	37.40*	30.66*	11.93*	8.19*
Halcon	0.50^{NS}	17.05*	72.43**	218.04**	2.76^{NS}	40.16*	27.38*	23.00*
Magnum45	0.40^{NS}	0.11^{NS}	11.89*	110.86**	5.78^{NS}	19.23*	217.16**	1352.87**
C-1	--	7.42*	--	193.18**	0.00^{NS}	0.00^{NS}	6.40*	0.79^{NS}
C-2	--	0.28^{NS}	--	70.94**	4.70^{NS}	0.50^{NS}	45.82*	9.62*
Mesilla	--	19.22*	--	13.18*	10.70*	3.47^{NS}	0.01^{NS}	0.08^{NS}

FD: Fruit diameter; FL: Fruit length; WT: Wall thickness

AA: Ascorbic acid; DHC: Dihydrocapsaicin

^{NS}, *, and **: Not significant, significant, and highly significant values at 5% level, respectively.

Table 22. Means of four fruit characteristics (weight, length, diameter, and wall thickness) in F_1 fruits of different pepper (*C. annuum*) crosses grown in Weslaco, Texas, in Spring 2008.

Entry	Pedigree	Fruit			
		Weight (g)	Length (mm)	Diameter (mm)	Wall Thickness (mm)
Pap2	(PapP27xPapP30)	38.44 b	188.80 a	34.20 b	2.30 j-n
Pap4	(PapP27xPapP67)	62.95 a	169.60 b	42.00 a	3.20 c-g
Pap5	(PapP27xPapP26)	26.34 c	150.20 c	30.10 c	2.00 l-n
PapP26		38.37 b	139.30 c	35.80 b	2.60 g-l
PapP27		38.45 b	143.00 c	35.90 b	2.50 h-m
PapP30		36.55 b	188.33 a	35.67 b	2.50 h-m
PapP67		38.12 b	144.75 c	27.50 d	3.13 c-h
S11	(SP5xSP57)	13.34 e-j	67.10 o-r	19.50 f	3.20 c-g
S12	(SP5xSP71)	10.48 g-j	63.10 qr	19.00 f-i	3.20 c-g
S14	(SP5xSP73)	13.89 e-h	72.30 n-q	20.00 f	4.20 a
S27	(SP16xSP57)	11.66 f-j	91.00 f-k	15.40 l-q	2.40 i-n
S28	(SP16xSP73)	15.39 d-g	95.70 f-h	18.40 f-j	3.10 c-h
S30	(SP16xSP60)	13.96 e-h	77.80 k-p	18.70 f-i	3.20 c-g
S32	(SP16xSP15)	8.40 jk	83.10 h-n	14.00 q	2.40 i-n
S36	(SP15xSP57)	11.22 f-j	96.00 f-h	13.80 q	1.90 mn
S37	(SP15xSP73)	11.49 f-j	88.00 f-l	16.00 j-q	2.60 g-l
S38	(SP15xSP55)	15.04 d-g	89.10 f-k	18.10 f-k	3.30 b-f
S40	(SP15xSP60)	12.33 e-j	90.20 f-k	16.00 j-q	3.00 d-i
S41	(SP15xSP128)	13.86 e-i	98.60 ef	17.60 f-m	3.20 c-g
S43	(SP15xSP5)	17.09 de	96.50 fg	18.50 f-i	3.60 b-d
S46	(SP15xSP79)	9.58 h-k	91.30 f-j	14.10 pq	2.20 k-n
S47	(SP15xSP16)	10.66 g-j	77.90 k-p	17.10 g-n	2.20 k-n
S48	(SP15xSP2)	9.15 h-k	93.00 f-i	13.70 q	1.90 mn
S56	(SP47xSP60)	11.23 f-j	72.60 n-q	18.00 f-k	3.00 d-i
S60	(SP60xSP2)	10.54 g-j	79.00 j-o	16.90 h-o	2.90 e-j
S68	(SP79xSP128)	11.77 f-j	84.00 g-n	17.10 g-n	3.00 d-i
S70	(SP79xSP60)	9.71 h-k	84.10 g-n	14.90 n-q	2.20 k-n
S74	(SP79xSP2)	11.16 f-j	98.00 f	15.20 m-q	2.00 l-n
S90	(SP41xSP95)	8.95 h-k	66.50 o-r	17.00 g-n	2.90 e-j
S91	(SP41xSP15)	9.02 h-k	92.80 f-i	14.50 o-q	2.00 l-n
S95	(SP41xSP15)	10.40 g-j	97.70 f	14.90 n-q	2.50 h-m
S107	(SP50xSP15)	13.02 e-j	109.80 de	15.90 k-q	2.60 g-l
S108	(SP50xSP16)	13.59 e-j	99.50 ef	16.50 i-p	2.20 k-n
SP2		12.52 e-j	79.20 j-o	19.40 f-h	2.90 e-j
SP5		10.36 g-j	62.50 qr	18.40 f-j	3.60 b-d
SP15		9.01 h-k	79.20 j-o	15.50 l-q	2.40 i-n
SP16		11.31 f-j	74.40 m-q	17.60 f-m	2.40 i-n
SP41		12.24 e-j	89.80 f-k	16.90 h-o	2.90 e-j
SP47		4.92 k	56.80 r	13.60 q	1.80 n
SP50		16.20 d-f	116.20 d	17.70 f-l	2.90 e-j
SP55		13.78 e-i	80.50 i-n	19.00 f-i	3.40 b-e
SP57		13.56 e-j	75.10 l-q	19.30 f-h	3.50 b-e
SP60		13.48 e-j	79.30 j-o	18.70 f-i	3.70 a-c
SP71		11.56 f-j	65.90 p-r	18.80 f-i	3.30 b-f
SP73		15.17 d-g	86.60 f-m	19.10 f-h	3.50 b-e
SP79		8.67 i-k	74.40 m-q	14.70 n-q	2.20 k-n
SP95		10.78 g-j	65.60 p-r	18.30 f-k	2.70 f-k
SP128		19.18 d	78.50 j-p	24.90 e	3.90 ab

Means followed by the same lower case letters are not significantly different.

PapP: Paprika parent; SP: Serrano parent.

Table 23. Degrees of freedom, sum of square, mean square, F-value, and broad sense heritability values (h^2) for four fruit characteristics and five phytochemical groups when data were analyzed by main effects (genotype).

Characteristic	**df**	**SS**	**MS**	**F-value**	**h^2 Value**
Fruit Weight	47	27109.65	576.80	54.57 *	0.91
Fruit Length	47	206555.78	4394.80	59.64 *	0.92
Fruit Diameter	47	10042.73	213.68	82.79 *	0.94
Wall Thickness	47	79.19	1.68	10.24 *	0.66
Ascorbic Acid	47	22138444.00	471030.72	23.44 *	0.88
Capsaicin	47	1007978.26	21446.35	15.00 *	0.82
Dihydrocapsaicin	47	474287.57	10091.22	18.73 *	0.86
Quercetin	47	164681.35	3503.86	83.47 *	0.96
Luteolin	47	6016.40	128.01	18.52 *	0.85

* Significant values at 5% level.

Table 24. Means of ascorbic acid, capsaicin, dihydrocapsaicin (DHC), quercetin, and luteolin concentrations (μg g^{-1} FW) in F_1 fruits of different pepper (*C. annuum*) crosses after grown in Weslaco, Texas, in Spring 2008.

Entry	Pedigree	Ascorbic Acid	Capsaicin	DHC	Quercetin	Luteolin
Pap2	(PapP27xPapP30)	2031.44 a	0.00 o	0.00 k	34.43 g-k	15.43 d
Pap4	(PapP27xPapP67)	1845.73 ab	0.00 o	0.00 k	80.28 c	11.98 d-h
Pap5	(PapP27xPapP26)	2078.93 a	0.00 o	0.00 k	64.93 d	25.27 b
PapP26		1781.36 bc	0.00 o	1.05 k	73.11 cd	21.82 bc
PapP27		1245.51 ef	0.00 o	1.07 k	49.69 ef	20.45 c
PapP30		1420.81 de	0.00 o	0.00 k	101.79 b	37.44 a
PapP67		1082.68 fg	0.00 o	0.00 k	211.70 a	21.11 bc
S11	(SP5xSP57)	763.83 j-r	34.44 j-o	41.26 f-k	10.54 p-t	9.30 e-j
S12	(SP5xSP71)	925.23 g-o	51.60 h-o	66.33 d-j	24.27 k-o	14.29 de
S14	(SP5xSP73)	938.25 g-n	56.83 h-o	58.96 e-j	16.04 n-s	8.74 f-j
S27	(SP16xSP57)	810.90 g-r	201.16 cd	80.26 d-g	38.91 f-j	7.34 g-l
S28	(SP16xSP73)	1050.73 f-i	89.63 f-k	34.99 g-k	33.37 h-k	8.49 f-j
S30	(SP16xSP60)	552.91 r-u	82.43 f-n	31.40 h-k	31.21 h-l	7.06 h-m
S32	(SP16xSP15)	1040.48 f-j	111.99 f-i	38.91 f-k	46.05 e-g	6.46 i-m
S36	(SP15xSP57)	1063.27 f-h	47.54 i-o	40.18 f-k	38.10 f-j	7.89 f-k
S37	(SP15xSP73)	691.66 l-s	69.08 g-o	38.83 f-k	28.83 i-m	6.46 i-m
S38	(SP15xSP55)	846.75 g-q	153.62 d-f	72.03 d-I	24.36 k-o	7.80 f-k
S40	(SP15xSP60)	993.56 f-k	64.18 g-o	37.36 g-k	29.53 i-m	7.73 f-k
S41	(SP15xSP128)	976.36 g-l	133.56 d-g	67.02 d-j	42.23 e-h	9.48 e-j
S43	(SP15xSP5)	849.19 g-q	13.43 k-o	11.65 k	5.56 r-t	2.16 mn
S46	(SP15xSP79)	933.45 g-o	283.36 ab	110.15 d	39.31 f-j	9.27 e-j
S47	(SP15xSP16)	717.71 k-s	17.76 k-o	7.58 k	43.01 e-h	6.92 h-m
S48	(SP15xSP2)	1492.87 de	69.95 g-o	33.79 h-k	51.38 e	12.60 d-g
S56	(SP47xSP60)	833.25 g-r	47.48 i-o	44.60 f-k	13.92 o-t	11.20 d-i
S60	(SP60xSP2)	578.37 q-u	83.43 f-m	83.97 d-f	10.10 p-t	8.66 f-j
S68	(SP79xSP128)	953.15 g-m	320.45 a	240.54 a	14.77 n-s	13.81 de
S70	(SP79xSP60)	914.73 g-p	235.93 bc	212.66 ab	13.93 o-t	8.67 f-j
S74	(SP79xSP2)	1369.60 de	224.37 bc	150.24 c	20.62 l-p	11.95 d-h
S90	(SP41xSP95)	807.20 g-r	73.44 g-o	91.97 de	19.37 l-q	7.86 f-k
S91	(SP41xSP15)	780.93 h-r	56.18 h-o	30.94 h-k	39.44 f-i	8.53 f-j
S95	(SP41xSP15)	959.01 g-l	23.01 k-o	12.12 k	37.10 g-j	7.54 f-l
S107	(SP50xSP15)	1090.38 fg	67.80 g-o	33.10 h-k	26.76 j-n	7.45 f-l
S108	(SP50xSP16)	655.37 n-t	66.91 g-o	23.05 jk	36.64 g-j	8.24 f-k
SP2		1599.78 cd	88.70 f-l	66.78 d-j	18.34 m-r	15.89 d
SP5		632.74 p-t	28.53 k-o	36.86 g-k	5.51 r-t	2.46 l-n
SP15		921.59 g-o	12.24 l-o	9.17 k	38.84 f-j	7.34 g-l
SP16		673.52 m-t	6.08 no	2.61 k	71.97 cd	6.42 i-m
SP41		328.94 u	22.67 k-o	26.29 i-k	9.79 p-t	3.08 k-n
SP47		406.69 tu	14.65 k-o	24.74 jk	3.32 st	0.96 n
SP50		823.46 g-r	10.71 m-o	5.97 k	1.55 t	1.00 n
SP55		882.99 g-p	198.80 cd	93.03 de	6.23 r-t	11.30 d-i
SP57		773.57 i-r	22.88 k-o	28.43 h-k	14.60 n-s	7.66 f-k
SP60		775.88 i-r	32.30 j-o	29.95 h-k	17.41 m-r	12.71 d-f
SP71		554.43 r-u	190.95 c-e	199.39 b	8.71 p-t	6.11 i-m
SP73		653.33n-t	33.10 j-o	29.28 h-k	8.13 p-t	5.32 j-n
SP79		650.44 o-t	285.71ab	181.00 bc	19.86 l-p	14.89 d
SP95		458.86 s-u	124.38 e-h	95.21 de	17.81 m-r	6.80 h-m
SP128		832.58 g-r	106.40 f-j	73.24 d-h	6.77 q-t	7.81 f-k

Means followed by the same letters are not significantly different. PapP: Paprika parent; SP: Serrano parent.

Table 25. Correlation (r) values for mid-parent and respective F_1 hybrids for all characteristics, as well as, r values between fruit characteristics and phytochemical groups.

Characteristic	Mid-parent / F_1	FW	FL	FD	WT	AA	Total Cap.	Total Flav.
Fruit Weight	0.871 *	1.000	0.854 *	0.952 *	0.096	0.659 *	-0.395	0.615 *
Fruit Length	0.956 *	0.854 *	1.000	0.799 *	-0.216	0.755 *	-0.374	0.643 *
Fruit Diameter	0.901 *	0.952 *	0.799 *	1.000	0.142	0.656 *	-0.406	0.536 *
Wall Thickness	0.712 *	0.096	-0.216	0.142	1.000	-0.229	-0.046	-0.206
Ascorbic Acid	0.752 *	0.659 *	0.755 *	0.656 *	-0.229	1.000	-0.217	0.475 *
Total Capsaicinoids	0.857 *	-0.395	-0.374	-0.406	-0.046	-0.217	1.000	-0.303
Capsaicin	0.825 *	-0.384	-0.335	-0.413	-0.083	-0.193	0.984 *	-0.266
Dihydrocapsaicin	0.858 *	-0.386	-0.408	-0.371	0.011	-0.240	0.965 *	-0.339
Total Flavonoids	0.755 *	0.615 *	0.643 *	0.536 *	-0.206	0.475	-0.303	1.000
Quercetin	0.790 *	0.580 *	0.595 *	0.476 *	-0.199	0.408 *	-0.323	0.990
Luteolin	0.632 *	0.577 *	0.666 *	0.662 *	-0.168	0.655 *	-0.092	0.693 *

FW: Fruit Weight; FL: Fruit Length; FD: Fruit Diameter; WT: Wall Thickness; AA: Ascorbic Acid.
Total Cap.: (Capsaicin + DHC); Total Flav.: (Quercetin + Luteolin)

* Signifies significant association at 5% level.

Table 26. Amount of variability (R^2 values) for mid-parent and respective F_1 hybrids for all characteristics, as well as, R^2 values between fruit characteristics and phytochemical groups.

Characteristic	Mid-parent / F_1	FW	FL	FD	WT	AA	Total Cap.	Total Flav.
Fruit Weight	0.759 *	1.000	0.729 *	0.906 *	0.009	0.434 *	0.156	0.378 *
Fruit Length	0.914 *	0.729 *	1.000	0.638 *	0.047	0.570 *	0.140	0.413 *
Fruit Diameter	0.812 *	0.906 *	0.638 *	1.000	0.020	0.430 *	0.165	0.287 *
Wall Thickness	0.507 *	0.009	0.047	0.020	1.000	0.052	0.002	0.042
Ascorbic Acid	0.566 *	0.434 *	0.570 *	0.430 *	0.052	1.000	0.047	0.226 *
Total Capsaicinoids	0.734 *	0.156	0.140	0.165	0.002	0.047	1.000	0.092
Capsaicin	0.681 *	0.147	0.112	0.171	0.007	0.037	0.968 *	0.071
Dihydrocapsaicin	0.736 *	0.149	0.166	0.138	0.0001	0.058	0.931 *	0.115
Total Flavonoids	0.570 *	0.378 *	0.413 *	0.287 *	0.042	0.226	0.092	1.000
Quercetin	0.624 *	0.336 *	0.351 *	0.227 *	0.040	0.166 *	0.104	0.980
Luteolin	0.399 *	0.333 *	0.444 *	0.438 *	0.028	0.429 *	0.008	0.480 *

FW: Fruit Weight; FL: Fruit Length; FD: Fruit Diameter; WT: Wall Thickness; AA: Ascorbic Acid.
Total Cap.: (Capsaicin + DHC); Total Flav.: (Quercetin + Luteolin)

* Signifies significant association at 5% level.

Table 27. Mid-parent (MP) heterosis (%) estimates for each fruit characteristic and phytochemical group.

Gen.	FW	FL	FD	WT	AA	Cap.	DHC	Total Cap.	Quercetin	Luteolin	Total Flav.
Pap2	2.51	13.96	-4.43	-8.00	52.38	0.00	-100.00	-100.00	-54.54	-46.69	-52.37
Pap4	64.42	17.88	32.49	13.68	58.55	0.00	-100.00	-100.00	-38.57	-42.35	-39.09
Pap5	-31.42	6.41	-16.04	-21.57	37.37	0.00	-100.00	-100.00	5.75	19.56	9.29
S11	11.54	-2.47	3.45	-9.86	8.63	33.98	26.39	29.73	4.82	83.79	31.26
S12	-4.38	-1.71	2.15	-7.25	55.87	-52.98	-43.85	-48.25	241.35	233.49	238.39
S14	8.81	-3.02	6.67	18.31	45.91	84.42	78.29	81.25	135.19	124.68	131.37
S27	-6.23	21.74	-16.53	-18.64	12.07	1289.23	417.14	838.07	-10.11	4.26	-8.10
S28	16.24	18.88	0.27	5.08	58.38	357.53	119.44	250.70	-16.68	44.63	-8.84
S30	12.63	1.24	3.03	4.92	-23.71	329.55	92.87	220.92	-30.16	-26.19	-29.46
S32	-17.32	8.20	-15.41	0.00	30.46	1122.60	560.61	902.66	-16.88	-6.10	-15.69
S36	-0.58	24.43	-20.69	-35.59	25.45	170.73	113.72	141.25	42.59	5.20	34.40
S37	-4.96	6.15	-7.51	-11.86	-12.17	204.72	101.98	157.57	22.76	2.05	18.36
S38	31.99	11.58	4.93	13.79	-6.16	45.58	40.96	44.07	8.10	-16.31	0.96
S40	9.65	13.82	-6.43	-1.64	17.06	188.19	91.00	142.74	5.00	-22.89	-2.33
S41	-1.67	25.05	-12.87	1.59	11.32	125.15	62.65	99.53	85.18	25.15	70.21
S43	76.46	36.20	9.14	20.00	9.27	-34.12	-49.38	-42.21	-74.93	-55.92	-71.49
S46	8.37	18.88	-6.62	-4.35	18.76	90.21	15.84	61.23	33.94	-16.60	20.05
S47	4.92	1.43	3.32	-8.33	-10.01	93.89	28.69	68.37	-22.37	0.58	-19.84
S48	-15.00	17.42	-21.49	-28.30	18.42	38.60	-11.02	17.29	79.71	8.48	59.13
S56	22.07	6.69	11.46	9.09	40.92	102.26	63.10	81.19	34.30	63.86	46.05
S60	-18.92	-0.32	-11.29	-12.12	-51.31	37.90	73.62	53.77	-43.50	-39.44	-41.69
S68	-15.48	9.88	-13.64	-1.64	28.54	63.45	89.22	73.59	10.93	21.67	15.87
S70	-12.33	9.43	-10.78	-25.42	28.26	48.38	101.62	69.61	-25.25	-37.17	-30.32
S74	5.33	27.60	-10.85	-21.57	21.73	19.85	21.27	20.42	7.96	-22.35	-5.57
S90	-22.24	-14.41	-3.41	3.57	104.93	-0.12	51.39	23.19	40.36	59.11	45.30
S91	-15.11	9.82	-10.49	-24.53	24.90	221.86	74.51	147.61	62.20	63.72	62.47
S95	-2.12	15.62	-8.02	-5.66	53.38	31.82	-31.64	-0.16	52.58	44.72	51.19
S107	3.29	12.38	-4.22	-1.89	24.97	490.85	337.25	429.80	32.51	78.66	40.41
S108	-1.20	4.41	-6.52	-16.98	-12.44	697.02	437.30	609.18	-0.33	122.10	10.90

FW: Fruit Weight; FL: Fruit Length; FD: Fruit Diameter; WT: Wall Thickness; AA: Ascorbic Acid; Cap.: Capsaicin; DHC: Dihydrocapsaicin; Total Cap.: Total Capsaicinoids; Total Flav.: Total Flavonoids

Highlighted values represent the highest MP heterosis estimate for each characteristic, respectively.

Table 28. High-parent (HP) heterosis (%) estimates for each fruit characteristic and phytochemical group.

Gen.	FW	FL	FD	WT	AA	Cap.	DHC	Total Cap.	Quercetin	Luteolin	Total Flav.
Pap2	-0.03	0.25	-4.74	-8.00	42.98	0.00	-100.00	-100.00	-66.18	-58.79	-64.19
Pap4	63.72	17.17	16.99	2.24	48.19	0.00	-100.00	-100.00	-62.08	-43.25	-60.37
Pap5	-31.50	5.04	-16.16	-23.08	16.70	0.00	-100.00	-100.00	-11.19	15.81	-4.98
S11	-1.62	-10.65	1.04	-11.11	-1.26	20.72	11.94	15.77	-27.81	21.41	-10.87
S12	-9.34	-4.25	1.06	-11.11	46.23	-72.98	-66.73	-69.79	178.65	133.88	160.19
S14	-8.44	-16.51	4.71	16.67	43.61	71.69	59.96	77.08	97.29	64.29	84.24
S27	-14.01	21.17	-20.21	-31.43	4.83	779.20	182.31	448.47	-45.94	-4.18	-41.00
S28	1.45	10.51	-3.66	-11.43	56.01	170.79	19.50	99.78	-53.63	32.24	-46.60
S30	3.56	-1.89	0.00	-13.51	-28.74	155.20	4.84	82.86	-56.63	-44.45	-51.18
S32	-25.73	4.92	-20.45	0.00	12.90	814.95	324.32	604.81	-36.02	-11.99	-33.01
S36	-17.26	21.21	-28.50	-45.71	15.37	107.78	41.33	70.96	-1.91	3.00	-0.41
S37	-24.26	1.62	-16.23	-25.71	-24.95	108.70	32.62	72.99	-25.77	-11.99	-23.58
S38	9.14	10.68	-4.74	-2.94	-8.12	-22.73	-22.57	-22.68	-37.28	-30.97	-30.36
S40	-8.53	13.75	-14.44	-18.92	7.81	98.70	24.74	63.12	-23.97	-39.18	-19.32
S41	-27.74	24.49	-29.32	-17.95	5.94	25.53	-8.49	11.66	8.73	21.38	11.97
S43	64.96	21.84	0.54	0.00	-7.86	-52.93	-68.39	-61.65	-85.68	-70.57	-83.28
S46	6.33	15.28	-9.03	-8.33	1.29	-0.82	-39.14	-15.68	1.21	-37.74	5.20
S47	-5.75	-1.64	-2.84	-8.33	-22.12	45.10	-17.34	18.36	-40.24	-5.72	-36.31
S48	-26.92	17.42	-29.38	-34.48	-6.68	-21.14	-49.40	-33.28	32.29	-20.70	38.54
S56	-16.69	-8.45	-3.74	-18.92	7.39	47.00	48.91	47.92	-20.05	-11.88	-16.60
S60	-21.81	-0.38	-12.89	-21.62	-63.85	-5.94	25.74	7.67	-44.93	-45.50	-45.19
S68	-38.63	7.01	-31.33	-23.08	14.48	12.16	32.90	20.20	-25.63	-7.25	-17.76
S70	-27.97	6.05	-20.32	-40.54	17.90	-17.42	17.49	-3.88	-29.86	-41.77	-34.96
S74	-10.86	23.74	-21.65	-31.03	-14.39	-21.47	-16.99	-19.73	3.83	-24.80	-6.27
S90	-26.88	-25.95	-7.10	0.00	75.91	-40.96	-3.40	-24.67	8.76	15.59	10.65
S91	-26.31	3.34	-14.20	-31.03	-15.26	147.82	17.69	77.94	1.54	16.21	3.88
S95	-15.03	8.80	-11.83	-13.79	4.06	1.50	-53.90	-28.25	-4.48	2.72	-3.33
S107	-19.63	-5.51	-10.17	-10.34	18.32	453.92	260.96	371.28	-31.10	1.50	-25.92
S108	-16.11	-14.37	-6.78	-24.14	-20.41	524.74	286.10	439.33	-49.09	28.35	-42.75

FW: Fruit Weight; FL: Fruit Length; FD: Fruit Diameter; WT: Wall Thickness; AA: Ascorbic Acid; Cap.: Capsaicin; DHC: Dihydrocapsaicin; Total Cap.: Total Capsaicinoids; Total Flav.: Total Flavonoids

Highlighted values represent the highest HP heterosis estimate for each characteristic, respectively.

Table 29. ANOVA table showing df, SS, MS, F, and CV values for quercetin concentrations across this F_2 family.

SOV	df	SS	MS	F Value	CV Value
Entry	116	2291391.46	19753.38	28.44**	19.70
Error	317	220181.20	694.58		
Total	433	2511572.66			

** Highly Significant at 5% level.

Table 30. ANOVA table showing df, SS, MS, F, and CV values for luteolin concentrations across this F_2 family.

SOV	df	SS	MS	F Value	CV Value
Entry	116	335861.41	2895.36	14.97**	38.81
Error	317	61309.08	193.40		
Total	433	397170.49			

** Highly Significant at 5% level.

Table 31. ANOVA table showing df, SS, MS, F, and CV values for total flavonoid (quercetin+luteolin) concentrations across this F_2 Family.

SOV	df	SS	MS	F Value	CV Value
Entry	116	3787669.70	32652.33	31.77**	18.90
Error	317	325838.07	1027.88		
Total	433	4113507.77			

** Highly Significant at 5% level.

Table 32. ANOVA table showing df, SS, MS, F, and broad sense heritability values (h^2) for quercetin concentrations across this F_2 family with two commercial jalapeño checks (Ixtapa and TMJ).

SOV	df	SS	MS	F Value	h^2 Value
Entry	118	2494002.16	21135.61	31.39**	0.84
Error	327	220189.48	673.36		
Total	445	2714191.64			

** Highly Significant at 5% level.

Table 33. ANOVA table showing df, SS, MS, F, and broad sense heritability values (h^2) for luteolin concentrations across this F_2 family with two commercial jalapeño checks (Ixtapa and TMJ).

SOV	df	SS	MS	F Value	h^2 Value
Entry	118	348901.03	2956.79	15.77**	0.71
Error	327	61311.05	187.50		
Total	445	410212.07			

** Highly Significant at 5% level.

Table 34. Means of quercetin, luteolin, and quercetin+luteolin concentrations (μg g^{-1} FW) in mature F_2 pepper fruits (*C. annuum*) grown in Uvalde, TX, in increasing concentrations.

Entry	Quercetin	Luteolin	Quercetin + Luteolin
19	NA	NA	NA
27	NA	NA	NA
39	NA	NA	NA
61	NA	NA	NA
63	NA	NA	NA
71	NA	NA	NA
85	NA	NA	NA
90	NA	NA	NA
92	NA	NA	NA
118	NA	NA	NA
18[Π]	4.15 SS	5.92 II	10.07 WW
16	9.64 SS	9.60 EE - II	19.24 VV - WW
70	15.11 RR - SS	7.98 GG - II	23.10 UU - WW
59	22.34 QQ - SS	8.01 GG - II	30.35 TT - WW
1	26.80 QQ - SS	9.84 EE - II	36.64 SS - WW
‘B22’	17.70 QQ - SS	21.31 v - II	39.01 RR - WW
78	28.14 PP - SS	11.97 CC - II	40.11 RR - WW
23	31.26 OO - SS	9.27 FF - II	40.53 QQ - WW
79	29.13 PP - SS	11.58 CC - II	40.71 QQ - WW
120	26.98 QQ - SS	13.95 z - II	40.93 QQ - WW
49	36.85 NN - SS	11.01 DD - II	47.86 PP - WW
44	38.98 MM - SS	9.61 EE - II	48.59 PP - WW
77	44.97 KK - SS	6.80 HH - II	51.77 OO - WW
73	42.62 LL - SS	10.33 DD - II	52.96 OO - WW
11	49.62 JJ - SS	5.92 II	55.54 NN - WW
12	47.62 JJ - SS	9.44 EE - II	57.06 MM - WW
110	49.05 JJ - SS	8.94 FF - II	57.99 MM - WW
114	45.97 JJ - SS	14.65 y - II	60.62 LL - WW
17	52.32 II - SS	13.26 z - II	65.58 KK - WW
121	48.88 JJ - SS	17.61 w - II	66.49 KK - WW
113	46.02 JJ - SS	20.53 v - II	66.55 KK - WW
56	61.34 HH - RR	11.20 DD - II	72.54 JJ - VV
4	63.95 GG - RR	9.36 EE - II	73.32 JJ - VV
20	62.39 GG - RR	13.38 z - II	75.77 JJ - VV
68	65.18 FF - QQ	11.56 DD - II	76.74 II - VV
84	61.40 GG - RR	18.97 w - II	80.37 HH - UU
55	76.53 DD - PP	10.51 DD - II	87.05 GG - TT
‘Ca377’ x ‘B22’ F_1[Δ]	59.47	29.91	89.38
60	80.64 CC - NN	12.41 BB - II	93.04 FF - SS
52	79.83 CC - OO	14.08 z - II	93.92 EE - SS
24	76.65 DD - PP	17.32 x - II	93.98 EE - SS
88	65.88 EE - QQ	30.19 q - II	96.07 EE - SS
25	77.13 DD - PP	20.34 v - II	97.47 DD - RR

Table 34 Continued.

Entry	Quercetin	Luteolin	Quercetin + Luteolin
125	86.68 z - MM	12.54 AA - II	99.22 CC - RR
48	80.18 CC - OO	20.51 v - II	100.68 BB - QQ
32	80.68 CC - NN	22.07 v - II	102.74 BB - PP
100	82.31 BB - NN	26.43 r - II	108.74 AA - OO
57	94.09 w - KK	18.95 w - II	113.04 z - NN
69	91.45 x - LL	22.39 t - II	113.84 z - NN
26	84.70 AA - NN	32.22 n - II	116.92 y - MM
106	88.99 y - LL	29.62 q - II	118.61 y - LL
51	99.86 v - II	20.52 v - II	120.38 y - LL
47	89.28 y - LL	32.95 n - II	122.22 x - KK
116	95.49 v - JJ	31.10 o - II	126.59 x - JJ
117	101.44 v - II	27.99 r - II	129.43 w - JJ
67	103.56 v - HH	27.01 r - II	130.57 w - JJ
22	111.80 t - GG	20.49 v - II	132.29 v - JJ
41	105.86 u - HH	26.72 r - II	132.58 v - JJ
76	104.06 v - HH	32.33 n - II	136.38 u - II
96	105.36 v - HH	34.73 l - GG	140.09 t - HH
101	114.36 s - FF	29.21 q - II	143.57 s - GG
37	115.63 s - DD	28.72 q - II	144.35 s - GG
6	124.83 r - DD	22.87 t - II	147.70 r - FF
123	120.88 s - DD	27.19 r - II	148.08 r - FF
40	115.08 s - EE	33.52 m - HH	148.60 r - FF
82	121.16 s - DD	30.69 p - II	151.84 q - FF
9	130.78 q - BB	21.70 v - II	152.48 p - FF
58	123.13 r - DD	30.17 q - II	153.29 o - FF
5	131.82 p - BB	22.12 u - II	153.94 n - EE
46	137.10 n - y	19.44 w - II	156.54 n - DD
38	132.39 p - AA	24.30 s - II	156.69 n - DD
108	122.51 r - DD	35.75 l - FF	158.26 m - CC
29	135.40 o - z	23.36 s - II	158.76 m - CC
119	123.13 r - DD	35.82 l - FF	158.94 m - CC
3	138.09 n - y	21.70 v - II	159.80 l - CC
115	121.01 s - DD	39.14 j - BB	160.15 l - BB
2	142.78 m - w	22.62 t - II	165.40 k - AA
43	126.41 r - DD	39.96 j - z	166.37 k - AA
75	128.28 q - CC	39.51 j - AA	167.79 j - AA
80	138.34 n - y	29.90 q - II	168.23 j - AA
105	143.02 m - w	27.95 r - II	170.97 j - z
10	144.66 m - v	31.45 n - II	176.11 I - y
42	157.05 l - t	24.45 s - II	181.51 I - x
15	160.09 l - t	27.40 r - II	187.49 I - w
30	157.08 l - t	35.47 l - FF	192.55 I - v
122	115.93 s - DD	79.16 d - e	195.08 I - u
104	155.34 l - u	44.45 i - w	199.78 h - t
36	142.19 m - w	59.67 e - m	201.85 h - s
95	140.51 m - x	61.82 d - k	202.33 h - s
8	170.80 j - r	31.99 n - II	202.78 h - s
31	185.85 g - n	21.19 v - II	207.03 h - r
64	180.34 h - p	28.78 q - II	209.12 h - q
74	170.87 j - r	38.65 j - CC	209.51 h - q
45	177.33 h - q	35.31 l - FF	212.65 h - p
34	164.24 k - s	49.08 g - t	213.32 h - o
53	184.38 h - o	29.05 q - II	213.43 h - o
81	158.67 l - t	54.96 e - q	213.63 h - o

Table 34 Continued.

Entry	Quercetin	Luteolin	Quercetin + Luteolin
21	185.00 h - n	29.13 q - II	214.13 h - n
72	187.81 g - m	29.71 q - II	217.52 g - m
50	195.07 f - l	24.53 s - II	219.60 g - l
124	183.22 h - o	37.20 k - DD	220.43 g - k
35	196.64 f - l	24.65 s - II	221.29 g - k
111	172.08 i - r	49.88 g - s	221.96 g - k
103	193.76 f - l	33.67 m - HH	227.43 f - j
86	159.75 l - t	71.90 d - h	231.64 f - i
112	183.97 h - o	48.98 g - u	232.95 f - i
107	160.67 l - t	73.28 d - g	233.95 f - i
98	219.98 d - i	36.49 k - EE	256.47 e - h
109	197.31 f - l	74.40 d - g	271.71 d - g
62	209.23 e - k	68.74 d - i	277.97 c - f
14	245.58 a - e	41.45 j - y	287.03 b - e
97	217.57 d - j	76.46 d - f	294.03 b - e
83	249.73 a - e	44.51 i - w	294.25 b - e
54	247.85 a - e	46.88 h - v	294.73 b - e
94	238.96 b - f	56.77 e - p	295.72 b - e
33	232.55 b - g	70.82 d - h	303.36 b - e
‘Ca377’	273.06 a - b	32.49 n - II	305.55 b - e
99	245.57 a - e	60.03 e - l	305.60 b - e
91	257.15 a - d	63.99 d - j	321.13 b - d
66	269.45 a - c	52.00 f - r	321.45 b - d
87	274.40 a - b	57.68 e - n	332.08 b - c
7	275.53 a - b	57.22 e - o	332.75 b - c
13	290.06 a	44.32 i - x	334.38 b - c
93	289.88 a	50.14 g - s	340.01 b
65	257.65 a - d	85.36 d	343.01 b
89	291.51 a	116.95 c	408.45 a
28	262.44 a - d	148.25 b	410.69 a
102	223.45 c - h	187.58 a	411.02 a

NA: Fruit tissue not available for harvest on that plant.

Π Mean separations by Duncan at $P \leq 0.05$. Means followed by the same letters are not significantly different.

Δ Fruit tissue harvested from plant growing inside College Station-VFIC greenhouse to obtain idea of phytochemical concentration. Value not included in statistical analysis.

Table 35. ANOVA table showing df, SS, MS, F, and CV values for ascorbic acid concentrations across this F_2 family.

SOV	df	SS	MS	F Value	CV Value
Entry	116	30122653.40	259678.05	4.62*	20.98
Error	318	17875211.84	56211.36		
Total	434	47997865.25			

* Significant at 5% level.

Table 36. ANOVA table showing df, SS, MS, F, and broad sense heritability values (h^2) for ascorbic acid concentrations across this F_2 family with two commercial jalapeño checks (Ixtapa and TMJ).

SOV	df	SS	MS	F Value	h^2 Value
Entry	118	37297436.18	316079.97	5.80*	0.41
Error	328	17878461.09	54507.50		
Total	446	55175897.27			

* Significant at 5% level.

Table 37. Means of ascorbic acid concentrations (µg g^{-1} FW) in mature F_2 pepper fruits (*C. annuum*) grown in Uvalde, TX, in increasing concentrations.

Entry	**Ascorbic Acid**
19	NA
27	NA
39	NA
61	NA
63	NA
71	NA
85	NA
90	NA
92	NA
118	NA
112$^{\Pi}$	576.35 EE
123	645.59 DD - EE
78	649.55 CC - EE
82	677.36 BB - EE
46	725.62 AA - EE
47	743.84 z - EE
102	774.46 y - EE
79	776.64 y - EE
77	778.28 y - EE
122	793.63 x - EE
119	803.28 w - EE
86	822.95 v - EE
84	832.35 u - EE
73	833.89 u - EE
58	845.03 t - EE
121	846.45 t - EE
115	846.64 t - EE
38	853.22 s - EE
40	881.70 r - EE
87	886.32 r - EE
33	900.30 q - EE
109	922.77 p - EE
83	927.47 p - EE
107	929.70 p - EE
51	934.00 p - EE
111	937.97 p - EE
50	951.35 o - EE
76	954.87 o - EE
34	963.02 n - EE
103	965.55 m - EE
114	967.04 m - EE
5	980.08 l - EE
74	982.69 l - EE
48	999.23 l - EE
52	1007.70 l - EE
25	1012.23 l - EE
41	1015.26 k - EE
36	1038.16 j - DD
80	1050.09 i - DD
75	1053.70 i - DD
117	1054.90 i - DD
66	1057.85 i - DD

Table 37 Continued.

Entry	**Ascorbic Acid**
37	1059.63 i - DD
97	1082.36 h - DD
81	1086.29 h - DD
105	1086.92 h - DD
88	1089.79 h - DD
57	1091.67 h - DD
95	1096.44 h - DD
32	1096.45 h - DD
120	1099.40 h - DD
69	1104.65 h - DD
106	1110.76 h - CC
55	1113.27 h - BB
11	1123.72 h - BB
67	1128.56 h - BB
18	1132.26 h - BB
28	1136.90 h - BB
70	1143.08 g - AA
43	1145.68 g - AA
‘Ca377’x’B22’ F_1^{Δ}	1153.00
108	1154.84 g - AA
42	1155.06 g - AA
29	1165.11 g - AA
16	1169.45 f - AA
17	1178.24 f - AA
62	1179.87 f - AA
104	1180.00 f - AA
30	1188.49 f - AA
21	1196.35 f - z
2	1198.09 f - z
89	1201.19 f - z
49	1203.05 e - z
12	1211.41 e - y
124	1231.67 d - y
‘B22’	1235.51 d - y
53	1240.90 c - y
13	1241.18 c - y
15	1247.60 c - x
68	1249.69 c - x
98	1253.66 c - x
31	1269.71 c - w
65	1280.85 c - v
113	1294.93 c - u
99	1302.55 b - t
125	1305.49 b - t
10	1306.93 b - t
14	1319.42 b - s
8	1340.31 a - r
72	1344.42 a - r
22	1354.14 a - q
7	1356.29 a - q
35	1368.83 a - p
23	1373.28 a - p
26	1373.68 a - p

Table 37 Continued.

Entry	**Ascorbic Acid**
60	1377.65 a - p
1	1408.86 a - o
4	1413.25 a - o
110	1415.05 a - o
6	1422.40 a - n
91	1426.45 a - n
24	1430.18 a - n
44	1431.12 a - m
20	1435.52 a - l
59	1480.93 a - k
56	1490.77 a - j
101	1516.03 a - i
64	1530.87 a - h
54	1533.62 a - h
9	1605.56 a - g
94	1630.59 a - f
96	1660.85 a - e
'Ca377'	1675.66 a - d
116	1677.32 a - d
45	1688.62 a - d
93	1696.92 a - c
100	1756.22 a - b
3	1788.02 a

NA: Fruit tissue not available for harvest on that plant.

Π Mean separations by Duncan at $P \leq 0.05$. Means followed by the same letters are not significantly different.

Δ Fruit tissue harvested from plant growing inside College Station-VFIC greenhouse to obtain idea of phytochemical concentration. Value not included in statistical analysis.

VITA

Justin David Butcher was born in Hot Springs, Arkansas. He earned his B.S. degree in agricultural sciences from Southern Arkansas University in Magnolia, AR, in May 2003. He then had the opportunity to attend the University of Arkansas in Fayetteville, AR, where he studied under Dr. Teddy Morelock and earned his M.S. degree in horticulture in December 2006. He completed his Ph.D. in horticulture from Texas A&M University with an emphasis in plant breeding under the esteemed direction of Dr. Kevin Crosby in December 2011. His experience includes breeding cowpea, spinach, pepper, tomato, cantaloupe, and to a lesser degree, watermelon, onion, and carrot. His goal is to pursue a career in the private vegetable seed industry and become a renown traditional plant breeder. His current contact information is the following: 1500 Research Parkway Suite, 120A, College Station, Texas, 77845
horticulture13@yahoo.com.