

## ABSTRACT

HAYNES, BRIANNA NICOLE. Characterization of Fruit Composition of North Carolina Strawberry Germplasm. (Under the direction of Dr. Gina Fernandez and Dr. Penelope Perkins-Veazie)

Strawberry (*Fragaria xananassa*) fruits are highly desired for their attractive color, sweet flavor, and nutritional benefits. North Carolina ranks third nationally in fresh market strawberry production. The North Carolina strawberry breeding program was established with the primary goal of developing new strawberry cultivars that are adapted to the regions climate and exhibit improved fruit quality characteristics. Strawberry color and flavor are key components of fruit quality to consumers, with fruit composition profiles consisting of physicochemical variables including, soluble solids content (SSC), titratable acidity (Tacid), and pH and phytochemical variables including, anthocyanin content, pigment profiles, and phenolic content. Although the North Carolina strawberry breeding program encompasses advanced selections, commercial genotypes, and first-year seedlings, the fruit composition profile of much of the germplasm is largely unknown. The objective of this research was to characterize strawberry fruit composition profiles of strawberry genotypes from the North Carolina breeding program.

The relative effects of location and germplasm on fruit composition, defined as SSC, Tacid, pH, anthocyanin content and pigment profiles was determined using fruit from 17 strawberry genotypes. Fruit were collected from replicated trials at three locations in North Carolina (Castle Hayne, Clayton, and Salisbury) at three weekly harvest dates. The results of this study indicate that field-grown strawberries in this South Atlantic climate are more influenced by genetics and harvest date than by growing location. During the fruiting season, physicochemical variables generally increased and phytochemical variables decreased. Pelargonidin 3-glucoside was the

dominant pigment in all genotypes, with some material high in pelargonidin 3-(6''-malonylglucoside), which is often thought of as a minor pigment.

Strawberry fruit were harvested from 268 genotypes that consist of North Carolina breeding program's core germplasm collection to evaluate fruit composition profiles. Four multivariate statistical methods (Cluster, Correlation, MANOVA, and PCA) were used to determine genotype diversity, characterize relationships among the genotypes and the fruit composition parameters, and to visualize trends within the collection. Genotypes were split into four clusters based on strawberry type, breeding program of origin, and overall fruit composition profile. The first two principal components captured a significant portion of the variance, explaining 64.88% collectively, with pH and Tacid being the primary driving forces for germplasm separation and differentiation.

A final study was done to determine if rapid and nondestructive colorimetric methods, including subjective ratings of 1 to 9, or a Konica-Minolta colorimeter CR400, would be useful as good predictors of strawberry fruit anthocyanin and phenolic content for North Carolina germplasm. Strawberry fruit from 31 genotypes from the Florida and North Carolina breeding programs was evaluated for 21 fruit composition parameters. Neither method was useful in predicting total phenolic content. Using a Konica-Minolta colorimeter to measure color in a 0.8 cm diameter spot on the fruit, the colorimetric value of lightness ( $L^*$ ) was found to be the strongest predictor of anthocyanin content, with a correlation of  $R^2 = 0.9132$ . Additionally, 74.04% of the variability seen within anthocyanin content could be explained by using subjective color ratings. Critically, both colorimetry methods (CIE  $L^*a^*b^*$  and subjective color variables) were reliable predictors

of anthocyanin content only when a full spectrum of strawberry color (white-dark red fruit) was used in the regression models.

This work provided valuable preliminary information on fruit composition diversity and profiles in North Carolina strawberry genotypes, the breeding program's structure, and both genetic and environmental influences on physicochemical and phytochemical compositional parameters.

These insights will help guide future breeding decisions and strategies regarding the development of new commercial cultivars adapted to the North Carolina climate with improved fruit quality.

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Characterization of fruit composition of North Carolina Strawberry (*Fragaria xananassa*)  
Germplasm.

by  
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## **DEDICATION**

I dedicate this thesis and all of my graduate work to my beloved sister Lauren Haynes. Lauren, your strength, grace, humor, and beautiful spirit have always been a constant source of comfort and inspiration in my life. Thank you for being my rock during the tough times, my celebration partner during the highs, my biggest support, and my best friend all in one.

## BIOGRAPHY

Brianna Nicole Haynes, born in 2000, was lovingly raised in China Grove, North Carolina, by her parents, Ashley and Nicky Haynes. Her childhood was shared with younger sister Lauren and family dogs Bonsai, Patch, and PJ. Although she was not from an agricultural-based background, her love of science, nature, and teaching could be seen at a young age.

Brianna's journey in agriculture began in high school when she joined the school's National FFA program. She participated in several contests and conducted three research projects on maximizing strawberry production. She would go on to compete at State and National levels earning 35 awards, including the prestigious American FFA degree. To date, Brianna remains North Carolina's highest-ranking student in plant science research.

Her newfound love of horticulture and dream of becoming a professor led her to continue her education at North Carolina State University. In 2019, Brianna met Dr. Gina Fernandez after choosing to interview the small fruit breeder for a class project. Having previously worked with strawberries, she anticipated bonding over the shared love of the fruit. What began as Dr. Fernandez's interview quickly changed and resulted in Brianna being offered a position in the lab, making it the best "job interview" she had ever experienced. She continued working with the small fruits program throughout her undergraduate degree, delving into the art of fieldwork, plant crossing, tissue culture, and DNA extraction. Then, summer 2021 brought back a familiar face. In 2017, Brianna had the privilege of meeting and collaborating with Dr. Penelope Perkins-Veazie, who mentored her on a blueberry project required for Brianna's high school graduation. Little did Brianna know that four years later in 2021, she would return to work with Dr. Perkins-Veazie and eventually become one of her last graduate students.

In May 2022, Brianna officially became a first-generation college graduate by completing a B.S. in Horticultural Science and a B.S. in Plant and Soil Sciences with concentrations in Plant Breeding and Crop Biotechnology, while also becoming one of the first students to receive certification from the school's inaugural agriculture regulatory science program. However, the celebration was short-lived as she began harvesting strawberries for her soon-to-be graduate work just two days after graduation, after previously receiving acceptance into NC State's Department of Horticultural Science to pursue a M.S. earlier in the semester.

Here, Brianna was presented with the unique opportunity to continue working with both Dr. Fernandez and Dr. Perkins-Veazie due to her convenient hometown location, located just 12 minutes South of the Piedmont Research Station in Salisbury, NC housing the North Carolina strawberry germplasm collection and field trial and 17 minutes North of the Plants for Human Health Institute in Kannapolis, NC, home to the postharvest lab. As such, Brianna frequently traveled between main campus in Raleigh, NC, PRS, and PHHI, becoming one of the first graduate students to fully experience both campus and off-campus student life. Her current research focuses on characterizing the physicochemical and phytochemical diversity of strawberry fruit in North Carolina strawberry germplasm.

## ACKNOWLEDGMENTS

I would like to acknowledge and express my gratitude to all of those who have supported me throughout my studies and in life. Your unwavering support, encouragement, guidance, and presence have played pivotal roles in my academic, professional, and personal journeys.

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been possible without your help in plant, field, and greenhouse maintenance. I am thankful for the knowledge, patience, training, and many memories shared.

Additional thanks to my high school agriculture teachers and advisors, David Overcash and Laura Allen, for introducing me to the world of agriculture, research, and, of course, strawberries. Their encouragement and guidance led me to discover my love of horticultural science, both significantly contributing to my success as a student at NC State University.

Lastly, to my family and friends, I extend my deepest gratitude. I am incredibly and forever thankful for my personal “wolfpack” who have stood by my side throughout this journey and who I know will continue to give unconditional love and support. Thank you for keeping me grounded while watching me chase my dreams, lifting me up during the lows, and celebrating with me during the highs.

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## CHAPTER 1

### Introduction

#### Strawberry

The cultivated modern strawberry (*Fragaria xananassa* Duch.) is a hybrid developed from two wild American octoploid species, *Fragaria virginiana* Duch. and *Fragaria chiloensis* Duch. (Heide et al., 2013; Hernández-Martínez et al., 2023). In 2000, 4.6 million tons of strawberries were produced globally, and this has almost doubled to 8.9 million tons produced in 2019 (López & Olmo, 2021). China has been the largest strawberry producer in the world since 1994, producing over 3.3 million berries annually and accounting for up to 37% of the global strawberry production. Although China also has the greatest global strawberry acreage, at 125,637 hectares, yields are low at 25 t/ha, compared to the United States and Mexico at 56.3 t/ha and 52.4 t/ha, respectively (López & Olmo, 2021). The United States is the second largest producer of strawberries at 1.5 million tons of strawberries in 2023, accounting for 12% of the world total (*Strawberry Production by Country 2024*).

Within the United States, strawberries represent 13% of the total fruit production value in the United States and are the leading berry crop as well as the third most important fruit (after apple and grape) in volume (Yeh et al., 2023). California and Florida provide most of the winter and summer strawberry supply, totaling to 90% of the total strawberry production, while the South Atlantic region consisting of Alabama, Georgia, North Carolina, South Carolina, and Virginia supplies spring-grown fruit (Samtani et al., 2019). Within this region, North Carolina is the top producing state and, relative to California and Florida, ranks a distant third nationwide in fresh market strawberry production.

### North Carolina Strawberry Production

In 2017, roughly 485.6 hectares of strawberries were grown in North Carolina with fruit valued at over \$26 million to the industry (**Samtani et al., 2019**). North Carolina's strawberry production landscape is unique in that the state is highly decentralized with a few large farms ( $\geq 4.05$  hectares) producing 50% of the crop and the remaining half grown on smaller, generally family-owned, farms (0.41–1.21) hectares (**Samtani et al., 2019**). The state utilizes both commercial and direct markets with all 100 counties selling strawberries at U-pick operations, roadside stands, farmers markets, and other local sales (**Hoffmann, 2020**).

Open-field annual hill plasticulture is the most common production system in the state with soilless substrate greenhouse-grown production on the rise. Field-grown strawberry plug or bare-root plants are set in the fall (August–October) with fruit harvested the following spring (April–June) in a relatively short 6–8 window, dependent on weather (**Poling et al., 2005**). Genotypes can be classified as seasonal or June-bearing (short-day), ever-bearing (long-day), or a combination of both (day-neutral) based on their response to photoperiod or daylength cycles (**Heide et al., 2013**). Although all types of strawberries can be grown in North Carolina, growers often plant June-bearing genotypes for field production, while ever-bearing and day-neutral genotypes are more preferred for greenhouse production due to greater potential of longer seasons.

### National Strawberry Breeding Programs

Numerous strawberry breeding programs have been established globally with the goal of developing new commercial cultivars adapted to regional climates with enhanced agronomic and

fruit composition traits to help meet the needs of growers, markets, and consumers (**Zurn et al., 2022**). In 1902, the first strawberry breeding program in the United States was established in Sitka, Alaska with strawberry breeding and research continuing up into the 1990s (**Holloway, 1998**). The ARS strawberry breeding program at the Genetic Improvement of Fruits and Vegetables Laboratory in Beltsville, Maryland which was established in 1910, is currently the longest active breeding program (***Strawberry Fields, USDA***).

Since then, other notable public, federal, and private strawberry programs have been established throughout the United States, with the largest currently active programs residing in California, Florida, New York, North Carolina, Oregon, and Washington (**Table 1.1**). The newest breeding program was established at University of Wisconsin-River Falls in 1988 (***Fruit Breeding Extension and Research***). Private strawberry breeding programs Driscoll's and Lassen Canyon Nursery in California lead the market (***Strawberry***). Other states do not have dedicated strawberry breeding programs but are valuable strawberry research partners with some states pursuing strawberry work in controlled environment and jam industries (Ohio), genetics (New Hampshire), hydroponics (Alabama, Arizona), production systems (Minnesota), sustainability (Virginia), and vertical farming (Arizona) (**Table 1.1**).

**Table 1.1.** Historical and active public and private strawberry (*Fragaria xananassa*) breeding programs in the United States. States with universities considered strawberry research partners that greatly contribute to strawberry extension and research also included.

States	University, Location	Program Type	Activity
Alabama	Auburn University, Auburn	Research Partner	Active
Alaska	Alaska School of Agriculture and College of Mines, Sitka	Public	Historical
Arizona	Arizona State University, Tempe	Research Partner	Active
Arkansas	University of Arkansas, Fayetteville	Research Partner	Historical
California	UC-Davis, Davis	Public	Active
	Driscolls, Watsonville	Private	Active
	Lassen Canyon Nursery, Redding	Private	Active
	Cal Poly University, San Luis Obispo	Research Partner	Active
Florida	UF/IFAS, Wimauma	Public	Active
Maryland	USDA-ARS GIFVL, Beltsville	Public	Active
Michigan	Michigan State University, East Lansing	Public	Historical
Minnesota	UMN-Twin Cities, Twin Cities	Public	Historical
Missouri	Missouri State University, Springfield	Research Partner	Historical
New Hampshire	University of NH, Manchester	Research Partner	Active
New Jersey	Rutgers University, New Brunswick	Public	Active
New York	Cornell University, Geneva	Public	Active
North Carolina	NC State University, Raleigh	Public	Active
Ohio	Ohio State University, Columbus	Research Partner	Active
Oregon	USDA-ARS NCGR, Corvallis	Repository	Active
	HCPGIRU, Corvallis	Public	Active
Texas	Texas Tech, Lubbock	Research Partner	Active
	Texas A&M, College Station	Research Partner	Active
Virginia	Virginia Tech University, Blacksburg	Research Partner	Active
	Old Dominion University, Norfolk	Research Partner	Active
Washington	Washington State University, Puyallup	Public	Active
Wisconsin	UW-River Falls, River Falls	Public	Historical

The North Carolina strawberry breeding program was first established in the early 1900s with the primary goal of developing strawberry germplasm adapted to the South Atlantic climate with superior horticultural traits including increased disease resistance, adequate chilling hour requirements, steady yield, improved flavor, good appearance, and increased post-harvest shelf life (**Fernandez, personal communication**). Although DNA markers such as fruit and crown anthracnose resistance markers are being developed and utilized in breeding efforts, the program largely remains a traditional plant breeding program. The program currently houses over 300 genetically unique strawberry selections that the program has developed, along with commercial genotypes that serve as standards for the program, maintained in a greenhouse (Salisbury, NC) and in a micropropagation and respiratory unit (Raleigh, NC) part of the National Clean Plant Network (NCPN) which provides certified pathogen-tested true-to-type material (***NC State Micropropagation and Repository Unit***). To date, 12 commercial strawberry cultivars have been developed through traditional breeding methods and released by the program, with ‘Rocco’ and ‘Liz’ being the latest releases in 2020.

### Strawberry Fruit Composition

Strawberry fruit are highly desired for their attractive appearance, sweet flavor, and nutritional benefits. Strawberries provide minerals (manganese, potassium, magnesium copper, iron, and phosphorus), vitamins (C, B6, K, A, and E), folate, fiber, and several phytochemicals such as phenolic and anthocyanin compounds (**Giampieri et al., 2012; Tulipani et al., 2009; Voća et al., 2014**). With the development of high-yielding strawberry genotypes, year-round availability, and increased consumer awareness of the health aspects offered by strawberries, consumption has soared over the years from 0.91 kg per person in 1980, to 1.5 kg in 2000, and to 3.6 kg per

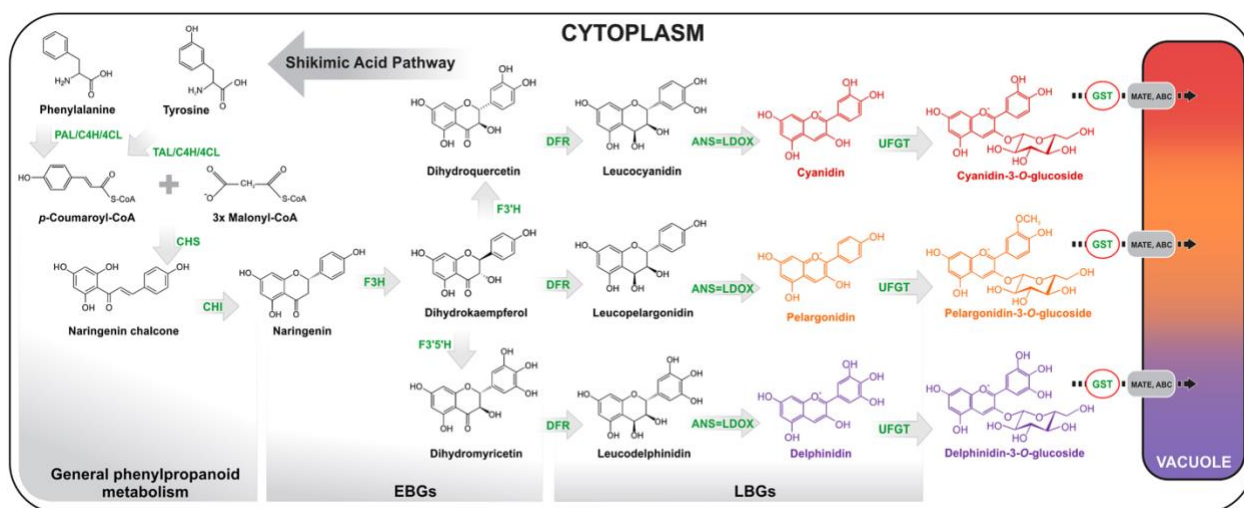
person in 2013 (**Samtani et al., 2019**). Strawberry production experienced a slight decline in 2013, dropping to 3.0 kg per person by 2017, before increasing again to reach the highest per capita consumption per person in 2020 at 3.9 kg (*Per capita consumption of fresh strawberries; Yet et al., 2020*).

Over the years, the plant breeding community has been quite successful in advancing genetic improvements within berries while still maintaining industry standards and demand (**Folta & Klee, 2016**). Despite increased fruit quality attributes of size, firmness and disease resistance, most berries on the market today still do not satisfy consumer expectations in terms of general sensory attributes (**Fan et al., 2021; Folta & Klee, 2016**). A consumer's initial impression of fruit quality is dependent on appearance (fruit color, shape, and size), with repeat purchases influenced by sweetness and overall flavor (**Voća et al., 2014**). However, consumers differ in their fruit sensory preferences, primarily pertaining to opinions on color and flavor.

### Strawberry Color and Phenolic Compounds

The external visual color of strawberry is diverse and can range from white to dark purple-red. Color is considered one of the most critical quality measurements for strawberry fruit as it is often associated with the ripeness of the fruit. Strawberry color is strongly related to the presence and amount of phenolic compounds, particularly anthocyanin, in the flesh of the fruit (**Timberlake & Bridle, 1982**). Phenolic compounds are naturally-occurring secondary metabolites present in many plants with important functions in sensory and nutritional quality in fruits and vegetables (**Guiné et al., 2020**). The largest and most diverse group of plant pigments are the anthocyanins. Anthocyanins are water-soluble pigments that provide most of the visual

color for many fruits, vegetables, flowers, and plants. In addition to color, anthocyanins provide numerous health benefits due to anti-inflammatory and antioxidant properties. Anthocyanin pigments are synthesized through the complex phenylpropanoid pathway, with regulation occurring at genetic and physiological levels (**Figure 1.1**). These compounds comprise an anthocyanidin base aglycon bound to a sugar group (**Jaakola, 2013**). The six main classes of anthocyanidin aglycons commonly found in plants contribute a different colored pigment: cyanidin (red), delphinidin (blue-purple), malvidin (red-blue-purple), pelargonidin (orange-red), peonidin (magenta), and petunidin (dark red-purple) (**Khoo et al., 2017; Bhatia, 2019; Mattioli et al., 2020**). As anthocyanidins rarely occur in nature due to their instability (**He & Giusti, 2010**), sugar attachments often occur at the 3' position to increase structural stability and provide water solubility, transforming the anthocyanidins into anthocyanins (**Belwal et al., 2020; De Rosas et al., 2018**).



**Figure 1.1.** Anthocyanin biosynthesis pathway schematic of cyanidin, pelargonidin, and delphinidin anthocyanidins. Image source: Chaves-Silva et al. 2018.

Most of the total anthocyanin in strawberry (50–80%) is represented by the dominant orange-red pigment pelargonidin 3-glucoside (P3G) (**Figure 1.1**). Other notable anthocyanins in the profiles

of strawberry fruit are pelargonidin 3-rutinoside (P3R), pelargonidin 3-(6''-malonylglucoside) (P3MG), and cyanidin 3-glucoside (C3G) (**Dzhanfezova et al., 2020; Lopes da Silva et al., 2007**). Cyanidin provides a darker red color in strawberry fruit (**Figure 1.1**). The total anthocyanin content reported among California and Florida commercial genotypes grown in multiple sites across the U.S. ranges from 8.5 to 59.8 mg/100g (**Anttonen et al., 2006; Chaves et al., 2017; Fredericks et al., 2012; Kelly et al., 2016; Nunes et al., 2005; Pelayo-Zaldivar et al., 2005; Van De Velde et al., 2013; Vinson et al., 2022**).

### Strawberry Flavor

Like anthocyanin, strawberry flavor is a complex system, affected by the amount and type of sugars, amino acids, organic acids, and aroma volatiles (**Fan et al., 2021; Jouquand et al., 2008; Schwieterman et al., 2014**). Sugars are the key components of overall strawberry flavor and in general, fruit with high sugar and relatively high titratable acidity (Tacid) are required for optimal strawberry flavor. Strawberry fruit with high sugars and lower titratable acidity have been described as having an artificially sweet taste, fruit with low sugars and high Tacid have a tart flavor, and strawberries containing low sugars and Tacid have little taste and are bland (**Kader, 1991; Mitcham et al., 1996**). To estimate sugar in strawberry fruit, soluble solids content (SSC) measured by refractometry is a quick method that provides an approximation of sucrose, glucose, and fructose as well as other components like organic acids, amino acids, pectins, phenols, and minerals that make up soluble sugars (**Beckles, 2012; Fan et al., 2021**). Citric acid is the dominant organic acid in strawberry fruits accounting for up to 49–75% of the total organic acid content (**Fait et al., 2008**).

The SSC reported among California and Florida genotypes grown in multiple sites across the U.S. ranges from 5.3 to 14.5% (**Agüero et al., 2015; Kader 1991; Menzel 2022; Perkins-Veazie et al., 2016; Whitaker et al., 2011; Whitaker et al., 2015**). The Tacid content of the same genotypes ranged from 0.49% to 1.06% (**Agüero et al., 2015; Kader 1991; Menzel 2022; Perkins-Veazie et al., 2016; Whitaker et al., 2011; Whitaker et al., 2015**). These differences in sugar and acid content can be influenced by environmental factors such as air temperature and rainfall during ripening and agronomic characteristics such as fertility. Additionally, both flavor and anthocyanin components are heavily influenced by genetics, the relative stage of ripeness, and environmental conditions (**Câmara et al., 2022**), indicating that different genotypes of the same species will likely have unique fruit composition profiles.

#### Previous Research on North Carolina Strawberry Germplasm

Two commercial genotypes developed and released by the North Carolina strawberry program were previously evaluated for fruit physicochemical content and phytochemical profiles. Strawberry cultivars, ‘Liz’ (previously NCS 10-038) and ‘Rocco’ (previously NCS 10-156), grown in Salisbury, NC, were evaluated for soluble solids content, titratable acidity, anthocyanin content and pigment profiles compared to commonly grown commercial genotypes, ‘Camarosa’ and ‘Chandler’ (**Perkins-Veazie et al. 2016**). In this study, fruit from ‘Liz’ and ‘Rocco’ had greater titratable acidity (0.72–0.75%) and relative pelargonidin 3-(6”-malonylglucoside) (13.8–14.1%) than fruit from ‘Camarosa’ (0.69%, 1.7%) and ‘Chandler’ (0.69%, 3.8%). Additionally, ‘Rocco’ berries were higher in SSC (7.8%) than those from ‘Camarosa’, ‘Chandler’, and ‘Liz’ (7.1%, 6.8%, and 7.1%, respectively), and ‘Liz’ berries were highest in relative cyanidin 3-glucoside (11.6%) than the other genotypes (2.8–7.4%) (**Perkins-Veazie et al. 2016**).

### Research Hypotheses and Objectives

The overall objective of these experiments was to characterize the fruit composition of strawberry germplasm in the North Carolina breeding program. Over the years, only a few genotypes, such as ‘Liz’ and ‘Rocco’ have been evaluated for both agronomic and fruit composition profiles. The NC breeding program encompasses advanced selections, parental germplasm, and seedling trials. Here, field trials that included advanced selections and established commercial cultivars were utilized to determine the relative importance of the environment during ripening compared to genetic differences. The parental germplasm, maintained in a plastic house, was utilized to determine relative genetic differences. A final study was done to explore the potential of using quantitative reflective color values to estimate the relative pigment (anthocyanin) in strawberry fruit.

In Chapter two, the complex interactions of genetics and environmental factors on strawberry fruit composition were explored. Each year, the North Carolina strawberry breeding program conducts replicated yield trials of several commercial genotypes, and both advanced and first-year selections developed by the program, at three locations in North Carolina. This approach offered the unique opportunity to investigate the effects of genetics, harvest date, and location factors on the soluble solids content, pH, titratable acidity, anthocyanin content, and pigment profiles of 17 genotypes from the North Carolina program. The hypothesis of this study was that North Carolina-derived germplasm would show genetic differences in the four composition parameters, and that harvest date will have the largest influence on composition responses.

Chapter three is more exploratory in nature and expands on the investigation of fruit composition and genetic diversity of North Carolina strawberry genotypes by profiling 268 genotypes maintained in the North Carolina strawberry core germplasm collection. Soluble solids content, pH, titratable acidity, and anthocyanin content from fruit grown in a more controlled greenhouse environment were used. Four multivariate statistical methods were used to visualize, determine, and characterize genotype diversity, relationships, program structure, and overall trends between the fruit composition parameters and genotypes. This work represented the first instance where the entire germplasm collection was assessed together in a single comprehensive evaluation.

Building off the results from the first two chapters, Chapter four transitions towards optimizing the plant material screening process breeders undergo by evaluating the effectiveness of using colorimetry methods to predict strawberry anthocyanin and phenolic content. As typical phytochemical analyses are time-consuming, expensive, resource- and labor-intensive, and impractical for hundreds to thousands of samples, the aim of this chapter was to potentially provide a simplified method for accurately quantifying strawberry phytochemical content using external non-destructive CIEL\*a\*b\* measurements and subjective RosBREED color ratings. The hypothesis of this study was that colorimeter measurements, specifically lightness ( $L^*$ ), would be most helpful in accurately predicting the range of anthocyanin and phenolic content in strawberry fruit.

Together, these chapters follow a progression of investigating the complex influences that environmental factors have on North Carolina strawberry fruit composition to broader focuses on the diversity in the parental strawberry population, and finally to evaluating a practical method to

assist and streamline breeding efficiency within the North Carolina strawberry program. This thesis expands the knowledge of North Carolina strawberry fruit composition profiles, genetics, diversity, and the influences of environmental factors that can be used to develop improved breeding strategies.

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## CHAPTER 2

### **Influence of Germplasm, Location, and Harvest Date on Strawberry Fruit Physicochemical Composition**

#### **Abstract**

Strawberry fruit are highly valued for their sweet and flavorful taste. Fruit composition, including soluble solids content (SSC) and titratable acidity (Tacid), affect flavor, while the brightness and saturation of the red coloration of strawberries are mainly attributed to anthocyanins. Together these fruit composition parameters impact consumer decisions. The North Carolina strawberry breeding program frequently trials advanced selections and commercial genotypes across multiple North Carolina locations to evaluate genotype performance and quality. In this study, strawberry fruit from 17 commercial cultivars and advanced selections were collected from replicated trials at three locations in North Carolina and at three weekly harvest dates to determine the relative effects of location and germplasm on fruit composition including, SSC, Tacid, pH, and anthocyanin. Among the strawberry genotypes, eight had more than 8% SSC while 11 had less than 0.8% Tacid, minimum levels for recent cultivar releases. Total anthocyanin content (TAC) ranged from 24.0 to 45.7 mg pelargonidin 3-glucoside (P3G) equivalents/100 g FWT, with five genotypes >40 mg P3G/100 g FWT. Pelargonidin 3-glucoside was the dominant pigment in all genotypes, followed by pelargonidin 3-rutinoside (P3R). Often thought of as a minor pigment, the percentage of pelargonidin 3-(6"-malonylglucoside) (P3MG) was higher in 'Liz', NC19-022, NC20-055, NC20-099, 'Rocco', and 'Ruby June' fruit (12.92–17.33%) compared to other genotypes (0.07–1.60%). During the fruiting season, TAC, pH, P3G, P3R, and P3MG generally decreased while SSC, Tacid, and

C3G increased. Our results indicate that composition of field-grown strawberries in this mid-Atlantic location were more influenced by genotype and harvest date than growing location.

## **Introduction**

### Strawberry Production

The modern strawberry (*Fragaria ×ananassa*) provides high amounts of dietary vitamin C (60–70 mg/100 g) and is valued for its sweet flavor. Globally, 8.2 million metric tons of fruit are produced annually, with more than 50% from China, the United States, and Mexico (**AtlasBig, 2022**). Florida and California provide most of the winter and summer strawberry supply in the U.S., while the South Atlantic Region (Alabama, Georgia, North Carolina, South Carolina, and Virginia) supplies spring-grown fruit for the eastern United States (**Samtani et al., 2019**). North Carolina had 485.6 hectares of production valued at over \$26 million in 2017 (**Samtani et al., 2019**). Challenges for strawberry production in this humid area include disease-resistant germplasm from the nursery through fruiting, moderate chilling hours, consistent fruit size, marketable fruit shape, full color, excellent flavor, and good postharvest life.

### Strawberry Physicochemical and Phytochemical Properties

The phytochemical and physiochemical properties of fruit, along with agronomic traits, can aid the success of a commercial strawberry genotype. A consumer's initial impression of fruit quality is appearance (fruit color, shape, and size), with repeat purchases dependent on sweetness and overall flavor (**Voća et al., 2014**). The concentration of anthocyanins in the flesh of the strawberry fruit has been correlated to the color of the fruit (**Timberlake & Bridle, 1982**). These water-soluble anthocyanin pigments provide most of the visual color of strawberries and range

from 10 to >40 mg P3G equiv/100 g fresh weight (FWT) in strawberry. Most of the total anthocyanin (TAC) in strawberry (50–80%) is from the orange-red pelargonidin 3-glucoside (P3G), followed by pelargonidin 3-rutinoside (P3R), cyanidin 3-glucoside (C3G), and pelargonidin 3-(6''-malonylglucoside) (P3MG) (**Dzhanfezova et al., 2020; Lopes da Silva et al., 2007**). Anthocyanin content can be affected by germplasm, the relative stage of ripeness, and the growing environment (**Câmara et al., 2022**).

Like anthocyanin, strawberry flavor is a complex system, affected by the amount and type of sugars, amino acids, organic acids, and aroma volatiles (**Fan et al., 2021b; Jouquand et al., 2008; Schwieterman et al., 2014**). The estimation of soluble sugars and non-volatile organic acids is often used as a rapid means of screening strawberry genotypes for flavor. Determination of soluble solids content (SSC) is simple and is often used to provide an approximate value of soluble sugars. The soluble sugars sucrose, glucose, and fructose make up 70–80% of the SSC values, with organic acids, amino acids, pectins, phenols, and minerals contributing to the remaining value (**Beckles, 2012; Fan et al., 2021b**). Perceived strawberry flavor and sweetness depend largely on titratable acidity (Tacid) and SSC. Titratable acidity is an indicator of the total amount of non-volatile organic acids within a sample. In earlier studies, target goals for strawberry SSC and Tacid were set at a minimum 7.0% SSC and a maximum of 0.8% Tacid (**Kader, 1991; Mitcham et al., 1996; Fan et al., 2021b**). However, recent findings suggest that most strawberries still do not meet consumer expectations for sweetness, giving rise to recommendations suggesting that the %SSC minimum for fresh strawberries be raised to 8.0–9.0%, while maximum Tacid remains unchanged at 0.8% (**Fan et al., 2021a; Fan et al., 2021b; Kubota Lab, 2015**).

### Environmental Influences on Strawberry Fruit Composition

Anthocyanin, sugar, and organic acids in strawberry fruit are all highly affected by preharvest environmental conditions, such as air temperature, rainfall, and the amount and type of light. The optimal strawberry fruit development temperature is 15 to 25°C, and elevated anthocyanin content is associated with day/night temperatures of 20–25°C/15°C–22°C (**Mao et al., 2022; Wang & Zheng, 2001**). Strawberry fruits are sensitive to low ( $\leq 10^{\circ}\text{C}$ ) and high temperatures ( $\geq 30^{\circ}$ ) and both extremes have the potential to severely delay strawberry fruit development and ripening, leading to lower anthocyanin content and uneven pigmentation (**Mao et al., 2022; Matsushita et al., 2016**). The effects of temperature on strawberry color and fruit composition have been previously evaluated (**Kalt et al., 1993**).

### Influence of Harvest Date on Strawberry Fruit Composition

In addition to temperature, the effects of location, harvest date, and genetics on strawberry fruit composition have also been previously studied. However, less literature exists that has collectively examined all three factors. Harvest date effects on SSC, Tacid, TAC, and volatile compound concentrations were examined in two studies conducted in Florida. The first study, taking place in February–March 2006 and January–March 2007, assessed fruit from ‘Strawberry Festival’, ‘Rubygem’, ‘Sugarbaby’, and five advanced genotypes from the University of Florida strawberry breeding program (**Jouquand et al. 2008**). Fruit was collected monthly, and purees from harvested fruit of each genotype were analyzed. Harvest date was strongly correlated with SSC, with higher values observed when air temperatures were  $< 20^{\circ}\text{C}$  a week prior to harvest. Fruit SSC decreased from 9.2 to 6.% throughout the fruiting season as temperatures increased from 14.5°C to 20.6°C. Titratable acidity among genotypes also varied considerably throughout

harvest dates and years. Fruit from the February 2006 harvest date ripened in a monthly average temperature of 16.1°C and had a Tacid content ranging from 0.75 to 1.02%, while March fruit ripened in a monthly average temperature of 17.2°C and was 0.58–0.70%. In 2007, January harvested fruit ranged from (0.76–0.95%) and declined to (0.58–0.79%) in March (**Jouquand et al., 2008**). In the second Florida study, conducted over a 6-week period from January to March in 2012 and 2013, fruit from ‘Strawberry Festival’, ‘Florida Radiance’, ‘Winterstar’, and four advanced genotypes were harvested twice weekly and fruit %SSC measured (**Cayo et al., 2016**). Soluble solids content was significantly influenced by harvest dates, with harvested fruit in January that ripened at average temperatures of 15°C having a higher SSC in both years than those in February at 16–16.5°C temperatures. In general, TAC content increased during January and decreased in February (**Cayo et al., 2016**). Lastly, a study taking place during the California summer production season used juice from the fruit of ‘Aromas’, ‘Diamante’, and ‘Selva’ genotypes to assess SSC and TAC over two harvest dates (**Pelayo-Zaldívar et al. 2005**). Although SSC was highest in May (8.4%) vs. August (7.2%) across all genotypes, genotypes were widely different when averaged over harvest dates (6.0–10.1%). Anthocyanin content was most affected by harvest date and was 14.1% higher for ‘Aromas’ and 48.9% higher for ‘Selva’ fruit collected in August than that of fruit collected in May. Fruit from ‘Diamante’ was similar in TAC in both months (**Pelayo-Zaldívar et al. 2005**).

#### Influence of Location on Strawberry Fruit Composition

Although location has an important environmental influence on strawberry fruit composition, few evaluation studies with multiple growing sites exist, likely due to limited resources and space. However, one study in 2011–2012 evaluated strawberry fruit from five short-day

genotypes from an Italian breeding program in three locations across Italy: Verona, Cesena, and Scanzano Jonico (**Cocco et al., 2015**). Although harvest began in three separate months (March–May) for each site, the average temperature 30 days before harvest began ranged from 12.2 to 13.1°C for each. In general, the highest %SSC was found in fruit from Scanzano J. (7.4–8.3) and the lowest in Cesena (5.0–5.9), while Tacid (mEq/100 g FW) was highest in Verona (6.1–10.4) and lowest in Cesena fruit (6.9–8.7). Total anthocyanin content ranged from 11.08 to 43.29 mg P3G/100 g, also showing significant location differences. Three genotypes recorded the highest TAC in Verona (15.93–29.82 mg/100 g) and two from Scanzano J. (26.81 and 43.29 mg/100 g). Anthocyanin was often lowest in fruit harvested from Cesena. The concentration of P3G was highest in Verona fruit, while the concentration of C3G was highest in Scanzano J. fruit (**Cocco et al., 2015**). **Kim & Shin, 2015** also found that location significantly affected strawberry fruit SSC, Tacid, pH, and TAC, amongst other fruit composition parameters. Fruit from three commercial Korean genotypes was harvested from plots at three locations in Korea, Gyeongsan, Nonsan, and Daegu, in April of 2013. Nonsan fruit was most often greater in %SSC (10.0–13.8%), while Gyeongsan fruit was most often greater in %Tacid (0.60–0.65) and higher in pH (3.92–4.01) in all genotypes. Furthermore, anthocyanin content in fruit harvested from Daegu was significantly greater (116.8–166.4 mg C3G/ kg) than that of Nonsan (85.6–125.2 mg C3G/ kg) and Gyeongsan (50.3–90.9 mg C3G/ kg) (**Kim & Shin., 2015**).

#### Influence of Genetic Variability on Strawberry Fruit Composition

Genetics is also responsible for the significant variation observed in fruit composition values between genotypes and among different years. Although not a large contributor to variation in %SSC and %Tacid, **Jouquand et al., 2008** determined that genetics strongly influenced both

volatile composition and perceived flavor in strawberry fruit. Similarly, **Pelayo-Zaldívar et al. 2005** found that genetic variation outweighed harvest date effects for all variables tested: color, firmness, SSC, pH, Tacid, TAC, organic acids, and aroma compounds. **Cayo et al., 2016** also found that genotype had the overall greatest effect on strawberry fruit composition and that ‘Winterstar’ fruit had the lowest Tacid values in both years (9.6 and 8.7% dry weight), while FL 10-47 had the highest values (14.7 and 14.1% DWT). Additionally, fruit from 2012 was significantly higher in Tacid (12.4% dwt) and TAC (22 mg P3G/100 g) compared to those in 2013 (11.4%; 13 mg P3G/100 g DWT). Regarding anthocyanin profiles, **Cocco et al., 2015** determined that TAC and the amount of P3G and C3G were more affected by genotype than by location. Another Florida study evaluated multiple fruit composition parameters from Florida127 (later to become ‘Florida Sensation’), ‘Strawberry Festival’, and ‘Florida Radiance’ genotypes over two years (2013–2014) (**Kelly et al., 2015**). Fruit from ‘Florida Radiance’ had the highest average TAC in both years (0.78 and 0.92 g/kg DWT), and Florida127 had the lowest average values (0.49 and 0.57 g/kg DWT), as well as lighter external and internal color. However, Florida127 also had the highest SSC values, with an average value of 70.9%, which was significantly higher than that of the other two genotypes (**Kelly et al., 2015**). Additionally, it has been noted that regardless of harvest date, high SSC levels have been consistently found in Florida127 compared to other genotypes in the Florida strawberry breeding program, further emphasizing the impact genetic background has on fruit composition (**Kelly et al., 2015; Whitaker et al., 2015**).

### Previous Research on North Carolina Strawberry Germplasm

Two commercial genotypes from the North Carolina breeding program have been previously evaluated for fruit composition profiles. Strawberry puree from ‘Liz’ (previously NCS 10-038) and ‘Rocco’ (previously NCS 10-156) fruit grown in Salisbury, NC, was used to measure fruit physiochemical and phytochemical contents and subjective quality ratings (**Perkins-Veazie et al. 2016**). Fruit from both genotypes had higher titratable acidity readings (0.72% and 0.75%) compared to the North Carolina industry standards ‘Camarosa’ (0.69%) and ‘Chandler’ (0.69%). Additionally, ‘Rocco’ strawberries were noticeably higher in soluble solids content (7.8%) than those from ‘Camarosa’, ‘Chandler’, and ‘Liz’ (7.1%, 6.8%, and 7.1%, respectively) (**Perkins-Veazie et al. 2016**). ‘Liz’ strawberries had less total anthocyanin (28.9 mg/100 g FWT), with a lower percent of pelargonidin 3-glucoside (69.4%) and higher cyanidin 3-glucoside (11.6%) than ‘Chandler’ (48.6 mg/100 g FWT; 83.6% P3G) or ‘Camarosa’ (41.3 mg/100 g FWT; 80% P3G). Although ‘Rocco’ was higher in total anthocyanin (34.0 mg/100 g), it had the lowest percentage of C3G (2.8%) of all materials tested. The percentages of pelargonidin 3-rutinoside were similar between ‘Chandler’ (7.6%) and ‘Rocco’ (7.8%), but slightly lower than in ‘Liz’ (9.9%). The percentage of pelargonidin 3-(6”-malonylglucoside) was significantly higher in ‘Rocco’ (14.1) and ‘Liz’ (13.8) than in fruit from ‘Camarosa’ and ‘Chandler’ (1.7 and 3.8%) (**Perkins-Veazie et al. 2016**). These results indicate that the anthocyanin profiles of North Carolina germplasm may differ slightly from California germplasm.

### Hypothesis and Objective

The North Carolina breeding program routinely screens material at three locations across the state, offering a unique opportunity to investigate the relative effects of location, genotype, and

harvest date on strawberry fruit quality and composition. This study incorporated a multi-location assessment and multiple harvest dates for advanced selections and commercial cultivars to follow the effects on fruit composition. This is the first instance where several North Carolina breeding program genotypes were assessed together in a field-study evaluation spanning multiple harvest dates and locations. Our hypothesis is that NC-derived strawberry germplasm will show genetic differences in SSC, Tacid, TAC, and anthocyanin profiles relative to genotypes developed in California and Florida, and that harvest date will significantly affect these responses. In the April-June harvest season in North Carolina, air temperatures can range from 12 to 27°C, and rainfall can range from 20 to 200 mm. As strawberry fruit composition appears to be sensitive to rain and air temperature, screening of germplasm across multiple locations and harvest dates is critical to separate genetic and environmental influences.

## **Materials and Methods**

### Experimental Design and Plant Material

Seventeen strawberry genotypes (encompassing commercial cultivars and advanced selections) were used in this study. All material was grown at three diverse locations in North Carolina over one growing season. These locations include the Central Crops Research Station (CCRS), the Piedmont Research Station (PRS), and the North Carolina State University Horticultural Crops Research Station (HCRS) (**Table 2.1**). Plant plugs were obtained from the NCSU strawberry breeding program, nurseries, and U.S. breeding programs. A completely randomized block design was used at each location, with three plots per genotype and 20 plants per plot established in an annual hill plasticulture system. Plugs were planted into raised beds covered with black plastic in August–September of 2021. Preplant, fertigation, and chemical protocols followed

commercially recommended practices, as outlined in the Southeast Regional Strawberry Plasticulture Production Guide (**Poling et al., 2005**). Information on plant maintenance practices regarding plant pest and disease control was collected (**Appendix Table A.1**). Additionally, average air temperature (°C), total precipitation (mm), and average relative humidity (%) during the harvest season were recorded at each research station (**Appendix Figure A.1**).

**Table 2.1.** Locations of replicated trials where fruit samples of each genotype were harvested for the 2021-2022 season in North Carolina.

Station	Longitude	Latitude	City	Soil Type	Planting Date	Harvest Dates
Central Crops Research Station (CCRS) <sup>z</sup>	35.66839°	-78.50631°	Clayton	Wagram loamy sand (0-6% slope)	10/11/21	4/25/22
						5/5/22
						5/12/22
Horticultural Crops Research Station (HCRS) <sup>z</sup>	34.32051°	-77.91533°	Castle Hayne	Torhunta loamy fine sand (0-2% slope)	10/26/21	4/28/22
				Sallings fine sand (2% slope)		5/5/22
						5/9/22
Piedmont Research Station (PRS) <sup>z</sup>	35.69501°	-80.62939°	Salisbury	Llyod clay loam (2-8% slope)	9/1/22	4/25/22
						5/2/22
						5/12/22

<sup>z</sup>Central Crops Research Station (CCRS), Horticultural Crops Research Station (HCRS), Piedmont Research Station (PRS).

### Sample Collection

Marketable fruit was collected from the field trials from April to June of 2022. Three harvests of five fruit per plot and harvest were collected from each genotype at all locations when available. The dates ranged from 4/25/2022 to 5/12/2022. Marketable fruit included those that were at least 10g in weight, free from visible defects such as disease, sunscald, water damage, and not misshapen. Fruit were placed into plastic loc bags, frozen at -15°C at each respective location, transported to the Plants for Human Health Institute in Kannapolis, NC, and held at -20°C until time for analysis.

### Fruit Compositional Analysis

Bags of fruit were thawed to room temperature and juice collected for fruit composition of soluble solids content (SSC), pH, titratable acidity (Tacid), and anthocyanin (TAC). A 0.5 mL aliquot of strawberry juice was placed on a handheld digital refractometer (Atago PAL-1, Bellevue, WA, USA) to determine %SSC. The pH of strawberry juice was determined using a pH meter (Thermo Scientific™ Orion Star™ A211, Waltham, MA, USA), and electrode (Thermo Scientific™ Orion™ Ross, Walham, MA, USA). For titratable acidity, 0.5 mL of juice was diluted with 24.5 mL of distilled deionized water, thoroughly mixed, and an aliquot placed on a digital acidity meter (Atago PAL-BX/ACID F5, Bellevue, WA, USA), with measurements expressed as % citric acid equivalents.

### Anthocyanin Analysis

Samples for anthocyanin profiles were prepared using 0.4 mL of juice and 1 mL of UPLC-grade methanol (Fisher Scientific, Fair Lawn, NJ, USA) acidified with formic acid (Sigma-Aldrich, Burlington, MA) at a ratio of 337:1, following the method in **Perkins-Veazie et al. (2016)**.

Supernatants were filtered through 0.2  $\mu\text{m}$  PTFE filters (VWR International, Radnor, PA, USA) into amber vials (Fisher Scientific, Rockwood, TN, USA) packed with  $\text{N}_2$ , sealed with screw caps (Fisher Scientific, Rockwood, TN, USA) and held at  $-80^\circ\text{C}$  until analyzed. Anthocyanin separation was performed using a Waters<sup>TM</sup> ACQUITY UPLC System (Waters, Milford, MA, USA), equipped with a photodiode array PDA detector, sample manager ( $10^\circ\text{C}$ ), column manager ( $45^\circ\text{C}$ ), and binary solvent manager. Empower 3 chromatography software (Waters, Milford, MA, USA) was used as the system run controller and for data processing. A 2  $\mu\text{L}$  injection volume was analyzed using a reversed-phase C18 column (ACQUITY UPLC BEH C18  $\mu\text{m}$ ,  $2.1 \times 100 \mu\text{m}$ , Waters, Milford, MA). The mobile phase consisted of 100% methanol (A1) and 5% formic acid in water (B1), and with a flow rate of 0.3 mL/min using a gradient of 0 min, 100% B1; 7 min, 88% B1; 10 min, 84% B1; 15 min, 75% B1; 18 min, 60% B1; and 20 min, 100% B1.

Anthocyanin concentrations were estimated from standard curves generated by injecting 1  $\mu\text{L}$  of 0.00625–0.1 mg/mL preparations of pelargonidin 3-glucoside, pelargonidin 3-rutinoside, and cyanidin-3-glucoside (Chromadex, Irvine, CA, USA; Sigma, St. Louis, MO, USA) as external standards. Pelargonidin 3-(6''-malonylglucoside) was identified using previously published reports (**Cerezo et al., 2010; Fredericks et al., 2012; Lopes da Silva et al., 2007**). Sample anthocyanin content was reported as mg pelargonidin 3-glucoside/100 g fresh weight (FWT), and total anthocyanins were the sum of identified anthocyanins.

### Statistical Analysis

The compositional analysis of strawberry samples was designed as a multi-factor, completely randomized block design with three replicates per genotype across locations and harvest dates. Independent variables consisted of genotype, location, and harvest date; replicates consisted of plots. Statistical analysis was conducted to explore relationships among the independent variables and the strawberry fruit composition parameters. Data was standardized, and all statistical analyses and visualizations were performed using R (version 4.2.2, Vienna, Austria) and RStudio (version 2022.12.0+353, Boston, Massachusetts).

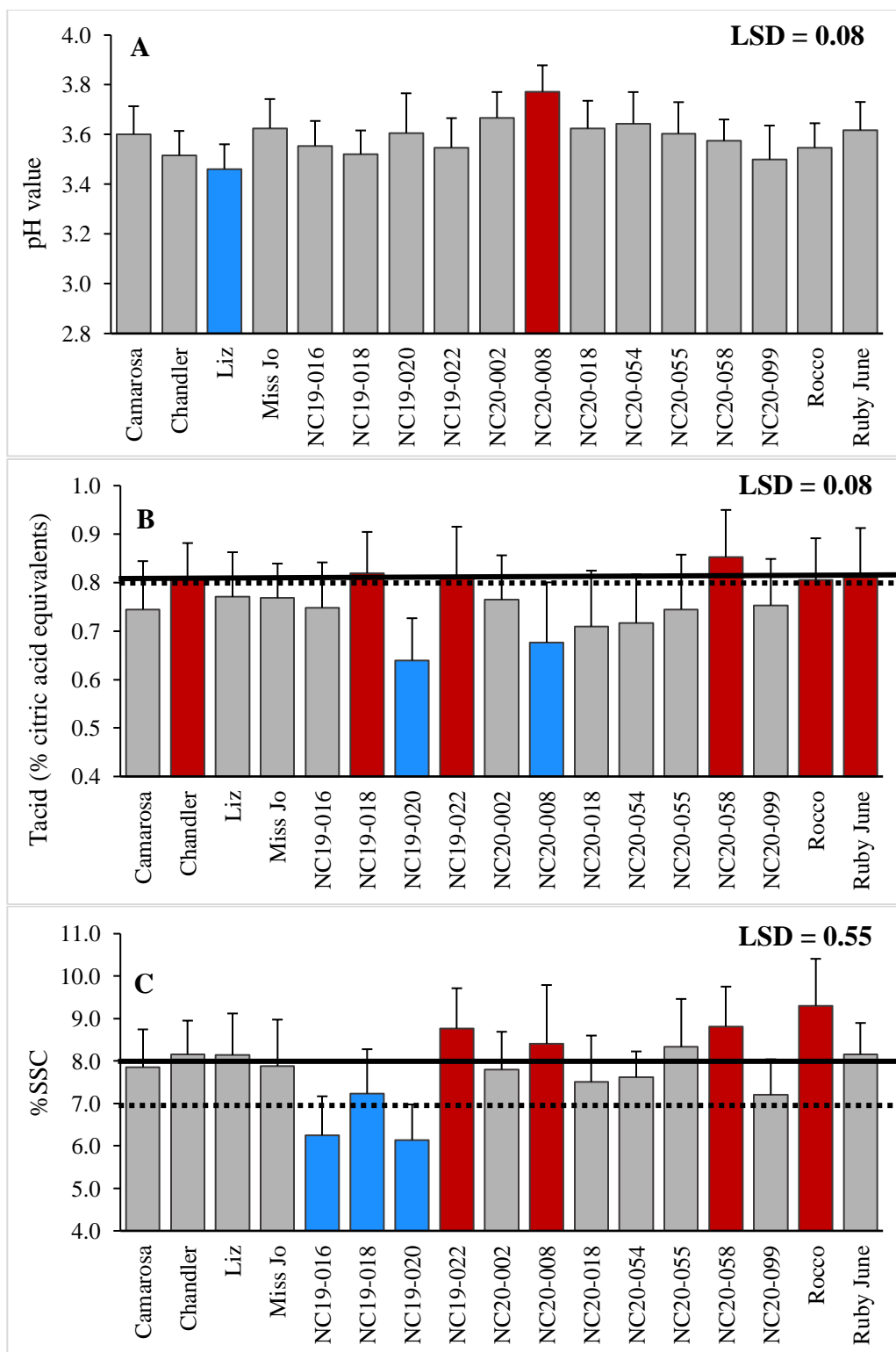
As field production of strawberry fruit was variable, not all genotypes yielded marketable berries at each date, leading to slightly unbalanced data. To ensure homogeneity and normality of sample population distributions, Levene's test for quality of variances and Shapiro-Wilk's test for normality were conducted. Effects demonstrating homogeneity and normality were analyzed by analysis of variance (ANOVA), and significant differences were detected using Tukey's post hoc analysis. Nonhomogeneous effects were analyzed using Welch's ANOVA and Games-Howell post hoc analysis, while non-normally distributed effects were analyzed using Kruskal-Wallis nonparametric ANOVA and Games-Howell post hoc analysis. Least significant difference (LSD) values were also computed.

Correlation and clustering analyses were also conducted to determine and explore relationships between the fruit composition parameters, the strawberry genotypes, and the interrelationships between the two. The "ggplot2" (**Wickham, 2016**) R package was used for correlation analysis to determine the strength and direction of relationships among the fruit composition variables,

with results presented in Pearson correlation coefficients. To visualize how genotypes differed in terms of fruit composition profile, a cluster heatmap was generated using the “factoextra” (Kassambara & Mundt, 2020) and “pheatmap” (Kolde, 2019) R packages. Euclidean distance and the Ward.D2 clustering method were used to determine distances between observations and the linkage method. Additionally, the “NbClust” (Charrad et al., 2014) R package was used to determine optimal cluster number.

## **Results**

Strawberry fruit composition components were affected nonuniformly by location, harvest, and genotype. Overall, genotype (collectively encompassing cultivars and selections) had the greatest effect on fruit composition, followed by harvest date. Location effects were significant for titratable acidity (Tacid), but not soluble solids content (SSC) or pH. Two-way interactions of location  $\times$  harvest date and location  $\times$  genotype were significant for all variables. The interaction between genotype  $\times$  harvest date was not significant, indicating that genotypes had similar results regardless of harvest date. A comprehensive analysis the impacts that location, harvest date, and genotype have on strawberry fruit composition was investigated (**Figures 2.1 and 2.2; Tables 2.2–2.4**).



**Figure 2.1.** (A) pH, (B) Titratable Acidity (% Tacid), and (C) Soluble solids content (% SSC). Dashed lines represent historical values before 2015 (7.0% SSC and maximum 0.8% Tacid) and solid lines represent current breeding aims (8.0% SSC and average 0.8% Tacid). The least significant different (LSD) values are listed at  $p < 0.05$ . Blue and red represent low and high genotypes.

### Strawberry Fruit Physicochemical Composition

Strawberry fruit pH varied the least among the components tested. Average juice pH values ranged from 3.64–3.77 for NC20-002, NC20-008, and NC20-054 to 3.46–3.52 for ‘Liz’, NC19-020, NC20-099, and ‘Chandler’, respectively (**Figure 2.1A**). ‘Camarosa’ juice pH averaged 3.60. The response of germplasm was similar and insignificant across the three locations, with juice pH decreasing from 3.64 to 3.49 with later harvest dates in all genotypes (**Table 2.2**). Juice pH was lowest for 11 genotypes in fruit harvested from CCRS and highest for eight genotypes in fruit harvested from PRS. The interaction of genotype  $\times$  location was significant, suggesting that the effect of genotype on fruit pH varies with location or that location differentially affects strawberry fruit pH across genotypes (**Table 2.2**). For all genotypes, juice pH was lowest in fruit harvested from the third (last) harvest than from previous harvests.

Strawberry Tacid was the most variable fruit composition component across all genotype, location, and harvest date factors, ranging from 0.47 to 1.17% (**Table 2.2**). The highest Tacid values were found in fruit from ‘Chandler’, NC19-018, NC19-022, NC20-058, ‘Rocco’, and ‘Ruby June’ (0.81-0.85%) (**Figure 2.1B**). Selections low in Tacid were NC19-020, NC20-008, NC20-018, and NC20-054 (0.64-0.71%) (**Figure 2.1B**). Titratable acidity for ‘Camarosa’ was 0.74%. Fruit harvested from HCRS was often greatest in Tacid (15 genotypes), and titratable acidity was usually least in fruit from PRS (14 genotypes). Fruit from the third harvest date had the highest Tacid values across genotypes (0.79%), with the exception of NC19-018, NC20-002, and ‘Ruby June’, which were highest during the first (**Table 2.2**). Sixteen genotypes had the lowest Tacid during the second harvest (0.73%) (**Table 2.2**). Interactions between location  $\times$  harvest date and location  $\times$  genotype were significant, suggesting the effect of location on Tacid

content was not consistent across harvest dates or strawberry genotypes. Overall, most of the selections and genotypes had titratable acidity values less than or equal to 0.8% citric acid equivalents.

**Table 2.2.** Effect of location, harvest date, and genotype on mean levels of fruit composition variables of 17 strawberry commercial genotypes and advanced selections grown at three locations in North Carolina (2022).

Source	Categories	pH	Tacid (% as citric acid) <sup>z</sup>	SSC (%) <sup>z</sup>
		mean ± SD		
Location <sup>y</sup>	CCRS <sup>z</sup>	3.58 ± 0.15 a	0.77 ± 0.13 b	7.9 ± 1.29 a
	HCRS <sup>z</sup>	3.57 ± 0.11 a	0.81 ± 0.09 a	7.9 ± 1.14 a
	PRS <sup>z</sup>	3.60 ± 0.14 a	0.71 ± 0.08 c	7.8 ± 1.34 a
HDate <sup>x</sup>	1st	3.64 ± 0.12 a	0.76 ± 0.11 b	7.3 ± 1.01 c
	2nd	3.63 ± 0.12 a	0.73 ± 0.12 c	7.7 ± 1.18 b
	3rd	3.49 ± 0.10 b	0.79 ± 0.10 a	8.6 ± 1.19 a
Genotype	Camarosa	3.60 ± 0.11 bcde	0.74 ± 0.10 bcde	7.9 ± 0.89 cdef
	Chandler	3.52 ± 0.10 def	0.81 ± 0.07 abc	8.2 ± 0.79 bcde
	Liz	3.46 ± 0.10 f	0.77 ± 0.09 abcd	8.1 ± 0.98 bcde
	Miss Jo	3.63 ± 0.12 bcd	0.77 ± 0.07 abcd	7.9 ± 1.09 cdef
	NC19-016	3.55 ± 0.10 cdef	0.75 ± 0.09 bcde	6.3 ± 0.92 g
	NC19-018	3.52 ± 0.10 def	0.82 ± 0.09 ab	7.2 ± 1.05 f
	NC19-020	3.61 ± 0.16 bcde	0.64 ± 0.09 f	6.1 ± 0.84 g
	NC19-022	3.55 ± 0.12 cdef	0.81 ± 0.10 abc	8.8 ± 0.95 abc
	NC20-002	3.67 ± 0.10 ab	0.76 ± 0.09 abcde	7.8 ± 0.89 def
	NC20-008	3.77 ± 0.11 a	0.68 ± 0.12 ef	8.4 ± 1.38 abcd
	NC20-018	3.62 ± 0.11 bcd	0.71 ± 0.12 def	7.5 ± 1.09 def
	NC20-054	3.64 ± 0.13 bc	0.72 ± 0.10 cdef	7.6 ± 0.61 def
	NC20-055	3.60 ± 0.13 bcde	0.74 ± 0.11 bcde	8.3 ± 1.13 bcd
	NC20-058	3.57 ± 0.09 bcde	0.85 ± 0.10 a	8.8 ± 0.94 ab
	NC20-099	3.50 ± 0.14 ef	0.75 ± 0.10 bcde	7.2 ± 0.84 f
	Rocco	3.55 ± 0.10 cdef	0.81 ± 0.09 abc	9.3 ± 1.11 a
	Ruby June	3.62 ± 0.11 bcd	0.81 ± 0.10 abc	8.2 ± 0.74 bcd
Location		<b>ns</b>	<b>***</b>	<b>ns</b>
Harvest Date		<b>***</b>	<b>***</b>	<b>***</b>
Genotype		<b>***</b>	<b>***</b>	<b>***</b>
Location × Harvest Date		<b>***</b>	<b>***</b>	<b>***</b>
Location × Genotype		<b>**</b>	<b>***</b>	<b>***</b>
Harvest Date × Genotype		<b>ns</b>	<b>ns</b>	<b>ns</b>

<sup>z</sup>CCRS = Central Crops Research Station (Clayton, NC), HCRS = Horticultural Crops Research Station (Castle Hayne, NC), PRS = Piedmont Research Station (Salisbury, NC), Tacid = titratable acidity and SSC = soluble solids content.

<sup>y</sup>Values averaged within location. Different letters within treatment are significantly different at P < 0.05

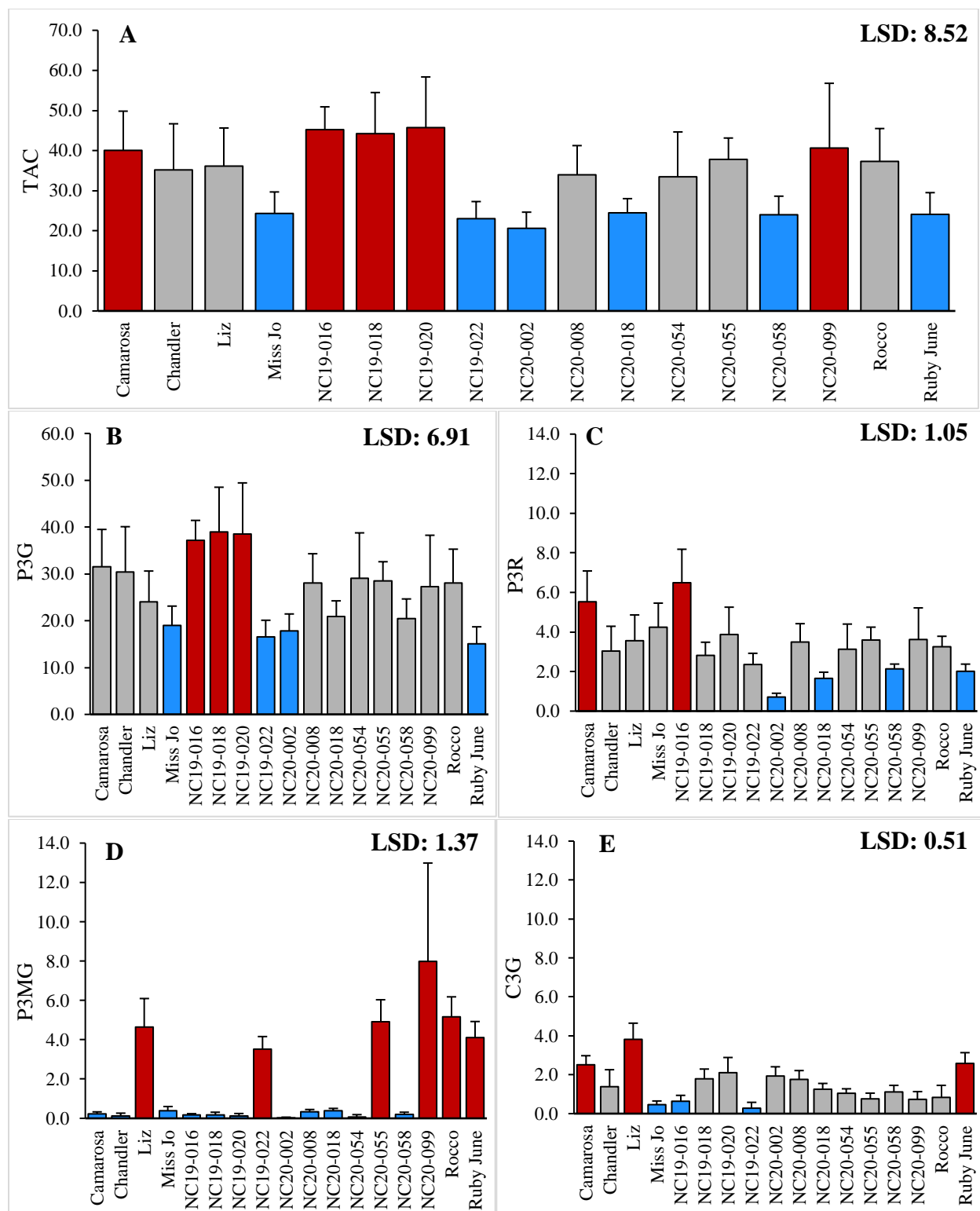
<sup>x</sup>Values averaged within harvest date. Different letters within treatment are significantly different at P < 0.05.

\*\*\*P < 0.0001, \*\*P < 0.01, \*P < 0.05

Strawberry SSC varied most across genotypes and ranged from 4.8 to 13.5. In general, SSC increased with later harvest dates (**Table 2.2**) across most genotypes. The highest SSC values were found in fruit from NC19-022, NC20-008, NC20-058, and ‘Rocco’ (8.4–9.3%) (**Figure 2.1C**). Genotypes low in SSC were NC19-016, NC19-018, NC19-020, and NC20-099 (6.1–7.2%) (**Figure 2.1C**). ‘Camarosa’ and ‘Chandler’, which are commonly grown in North Carolina, had 7.9 and 8.2% SSC, respectively (**Table 2.2**). Although location was not significant for SSC, values were lowest for 10 genotypes in fruit harvested from Piedmont Research Station (PRS) and highest in fruit of 11 genotypes from Central Crops Research Station (CCRS). Strawberry SSC increased from 7.3 to 8.6 between the first and third harvest dates for most of the germplasm (**Table 2.2**). The interaction of genotype  $\times$  location was significant, suggesting that the effect of location on SSC was not consistent across strawberry genotypes (**Table 2.2**). Strawberry SSC of selection NC19-018 was higher at HCRS and PRS (7.5–7.6) but lower at CCRS (6.7).

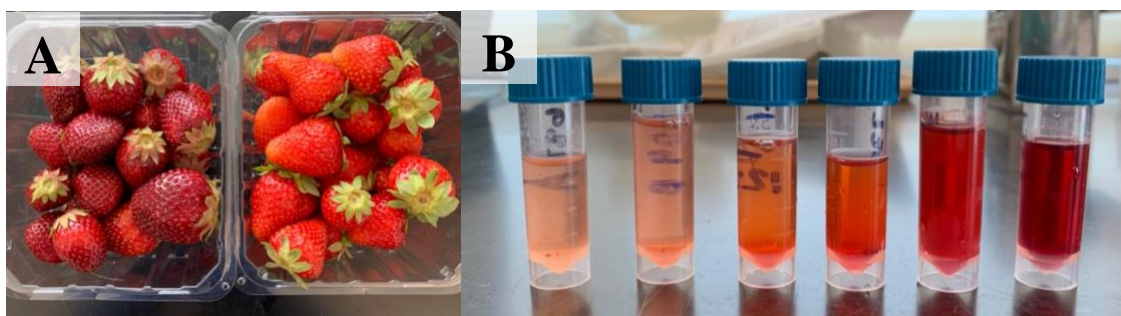
### Anthocyanin Pigment Profiles

Profiles of the individual anthocyanins were more affected by genotype rather than fruit harvest location or harvest date. Pelargonidin-3-glucoside was the dominant anthocyanin in all genotypes. Cyanidin-3-glucoside and pelargonidin 3-(6''-malonylglucoside) values differed mostly with genotype. Some interactions were found with location and harvest date, indicating differing genotype responses to these variables.



**Figure 2.2.** (A) Total Anthocyanin content TAC (mg pelargonidin 3-glucoside (P3G) equiv/100 g FWT), (B) pelargonidin 3-glucoside P3G (mg/100 g FWT), (C) pelargonidin 3-rutinoside P3R (mg/100 g FWT), (D) pelargonidin 3-(6''-malonylglucoside) P3MG (mg/100 g FWT), and (E) cyanidin 3-glucoside C3G (mg/100 g FWT). Least significant different (LSD) values listed at  $p < 0.05$ . Blue and red represent low and high genotypes.

A diverse range of total anthocyanin values and external strawberry color were found across genotypes, spanning from 14.77 to 74.17 mg pelargonidin 3-glucoside (P3G) equivalents/100 g FWT (**Figure 2.3**). Total anthocyanin content (TAC) is defined here as the sum of the individual anthocyanins identified in strawberry germplasm by UPLC and was most affected by genotype. Visually dark-colored genotypes ‘Camarosa’, NC19-016, NC19-018, and NC19-020 were highest in TAC (40.06, 45.23, 44.23, and 45.73) mg P3G/100 g FWT, respectively (**Figure 2.2A**). The light-colored genotypes NC19-022, ‘NC20-002, NC20-058, and ‘Ruby June’ were the lowest (23.02, 20.61, 24.00, and 24.09) mg P3G/100 g FWT, respectively (**Figure 2.2A**). Furthermore, TAC varied across harvest dates, with fruit from the third harvest exhibiting the lowest TAC across 13 genotypes, while fruit from the second harvest was highest across 10 genotypes. Location also heavily influenced TAC, with fruit from PRS having the highest levels in 15 genotypes. The lowest values were recorded in fruit from CCRS and HCRS. Two-way interactions of genotype  $\times$  location and of location  $\times$  harvest date were significant, highlighting the non-uniform response anthocyanin content has among genotypes, harvest dates, and across different locations, with the exception of the genotype  $\times$  harvest date interaction (**Table 2.3**).



**Figure 2.3.** (A) Selection NC19-020 (left) and selection NC19-022 (right), and (B) Strawberry fruit juice from different genotypes within the North Carolina Germplasm collection.

Pelargonidin 3-glucoside (P3G) was the dominant anthocyanin in all strawberry genotypes, with concentrations ranging from 11.31 to 57.44 mg P3G/100 g FWT (**Figure 2.2B**). The relative content of P3G (as %P3G of total anthocyanin) varied from 60.21 to 90.67%. Both P3G and %P3G levels were primarily influenced by genotype, followed by location (**Tables 2.3–2.4**). However, genotypes with fruit highest in P3G were not always highest in %P3G. Visually dark red selections ‘Camarosa’, NC19-016, and NC19-020 had high total P3G (31.53–38.52 mg/100 g FWT), while genotypes highest in %P3G (86.42–87.89%) were NC19-018, NC20-002, and NC20-054 (**Tables 2.3–2.4**). ‘Chandler’ and NC19-018 had high P3G and %P3G. A similar pattern was found for low P3G and %P3G, as genotypes NC20-002, NC20-018, and NC20-058 had low P3G (17.83–20.87 mg/100 g FWT) but high %P3G (85.26–86.68%). Genotypes ‘Liz’, NC20-099, and ‘Rocco’ were lowest in relative P3G (66.40–74.61%), while NC19-022 and ‘Ruby June’ low in both P3G and %P3G (**Tables 2.3–2.4**).

Eleven of the 17 genotypes harvested from PRS were highest in P3G and %P3G, while 10 genotypes were lowest in %P3G at CCRS. Additionally, eight genotypes harvested at both CCRS and HCRS were lowest in P3G. Although P3G decreased on average from 28.30 to 24.42 mg/100 g FWT across genotypes from the first to the third harvest and differed with location and harvest date, the amounts relative to total anthocyanin (%P3G) were not significantly different among harvest dates (**Table 2.4**).

Of the pigments identified in this study, pelargonidin 3-rutinoside (P3R) varied most across genotype, location, and harvest. P3R content ranged from 0.48 to 8.98 mg/100 g and relative amounts of P3R were 2.35 to 22.12% (**Figure 2.2C**). The highest content (4.24–6.49 mg/100 g

FWT) and relative amount (13.77–17.37%) of P3R were found in strawberries from ‘Camarosa’, ‘Miss Jo’, and NC19-016 (**Tables 2.3–2.4**). Genotypes high in P3R were generally high in relative amounts of P3R. However, fruit from NC19-020 was high in total P3R (3.87 mg/100 g FWT) but low in relative amount (8.84%). The lowest content (0.71–2.01 mg/100 g FWT) of P3R was found in fruit from NC20-002, NC20-018, and Ruby June, while the lowest relative P3R (3.45–6.83%) was found in fruit from NC19-018, NC20-002, and NC20-018 (**Tables 2.3–2.4**). Total P3R content decreased from 3.49 to 2.76 mg/100 g FWT between the first and third harvests. Fruit from the first harvest had high %P3R in 10 genotypes, and the second harvest had the highest %P3R in 13 genotypes. Fruit from the third harvest was consistently low in both total and relative content of P3R.

Additionally, fruit harvested from PRS was significantly lower in both P3R and %P3R than fruit from CCRS and HCRS. P3R and %P3R were lowest for 10 and 16 genotypes, respectively, in fruit harvested from PRS. Two-way interactions of location  $\times$  harvest date and location  $\times$  genotype were significant for total and relative P3R, indicating that genotypes have a non-uniform response to both location and harvest date (**Tables 2.3–2.4**).

**Table 2.3.** Effect of location, harvest date, and genotype on mean levels of total anthocyanin content and profiles of 17 strawberry commercial genotypes and advanced selections grown at three locations in North Carolina (2022).

Source	Categories	TAC <sup>z</sup>	C3G <sup>z</sup>	P3G <sup>z</sup>	P3R <sup>z</sup>	PM3G <sup>z</sup>
		mean ± SD				
Location <sup>y</sup>	CCRS <sup>z</sup>	33.4 ± 13.0 ab	1.48 ± 1.0 a	25.97 ± 10.2 b	3.56 ± 1.9 a	2.07 ± 3.3 a
	HCRS <sup>z</sup>	31.13 ± 8.6 b	1.38 ± 0.9 a	24.42 ± 7.3 b	3.31 ± 1.6 a	1.89 ± 2.5 a
	PRS <sup>z</sup>	36.13 ± 12.8 a	1.56 ± 1.1 a	29.14 ± 11.4 a	2.90 ± 1.5 b	1.90 ± 2.8 a
HDate <sup>x</sup>	1st	35.79 ± 12.2 a	1.49 ± 1.1 ab	28.30 ± 9.5 a	3.49 ± 1.7 a	2.15 ± 3.4 a
	2nd	33.92 ± 11.8 ab	1.38 ± 0.9 b	26.96 ± 10.5 ab	3.49 ± 1.9 a	1.77 ± 2.4 a
	3rd	31.03 ± 10.9 b	1.57 ± 1.1 a	24.42 ± 9.6 b	2.76 ± 1.3 b	1.92 ± 2.7 a
Genotype	Camarosa	40.06 ± 9.8 a	2.51 ± 0.5 b	31.53 ± 8.0 bc	5.53 ± 1.6 ab	0.21 ± 0.1 c
	Chandler	35.19 ± 11.5 abc	1.38 ± 0.9 cdef	30.46 ± 9.6 bc	3.04 ± 1.3 cdef	0.13 ± 0.1 c
	Liz	36.13 ± 9.5 abc	3.82 ± 0.8 a	24.05 ± 6.6 cdefg	3.56 ± 1.3 cde	4.64 ± 1.5 b
	Miss Jo	24.32 ± 5.4 bcd	0.46 ± 0.2 gh	19.00 ± 4.1 efg	4.24 ± 1.2 bc	0.38 ± 0.2 c
	NC19-016	45.23 ± 5.7 a	0.64 ± 0.3 fgh	37.18 ± 4.3 ab	6.49 ± 1.7 a	0.16 ± 0.1 c
	NC19-018	44.23 ± 10.3 a	1.79 ± 0.5 bcde	38.96 ± 9.6 a	2.82 ± 0.7 cdef	0.19 ± 0.1 c
	NC19-020	45.73 ± 12.6 a	2.10 ± 0.8 bc	38.52 ± 10.9 a	3.87 ± 1.4 bcd	0.13 ± 0.1 c
	NC19-022	23.02 ± 4.3 cd	0.28 ± 0.3 h	16.55 ± 3.6 fg	2.36 ± 0.6 defg	3.52 ± 0.6 b
	NC20-002	20.61 ± 4.0 d	1.93 ± 0.5 bcd	17.83 ± 3.6 efg	0.71 ± 0.2 g	0.02 ± 0.0 c
	NC20-008	33.96 ± 7.3 abc	1.76 ± 0.5 bcde	28.10 ± 6.2 cdef	3.49 ± 0.9 cde	0.32 ± 0.1 c
	NC20-018	24.48 ± 3.5 bcd	1.25 ± 0.3 defg	20.87 ± 3.4 efg	1.65 ± 0.3 fg	0.39 ± 0.1 c
	NC20-054	33.46 ± 11.2 abc	1.05 ± 0.2 efgh	29.02 ± 9.7 cd	3.13 ± 1.3 cdef	0.07 ± 0.1 c
	NC20-055	37.80 ± 5.3 ab	0.76 ± 0.3 fgh	28.49 ± 4.1 cd	3.59 ± 0.7 cde	4.91 ± 1.1 b
	NC20-058	24.00 ± 4.6 bcd	1.11 ± 0.3 defgh	20.48 ± 4.2 efg	2.13 ± 0.2 defg	0.21 ± 0.1 c
	NC20-099	40.64 ± 16.1 a	0.83 ± 0.4 fgh	27.25 ± 11.0 cdef	3.62 ± 1.6 cde	7.98 ± 5.0 a
	Rocco	37.31 ± 8.2 abc	0.83 ± 0.6 fgh	28.12 ± 7.3 cdef	3.25 ± 0.5 cdef	5.16 ± 1.0 b
	Ruby June	24.09 ± 5.4 bcd	2.58 ± 0.6 b	15.07 ± 3.6 g	2.01 ± 0.4 efg	4.11 ± 0.8 b
Location		**	ns	***	***	ns
HDate		*	*	*	***	ns
Genotype		***	***	***	***	***
Location × HDate		*	***	*	**	ns
Location × Genotype		*	ns	*	*	ns
HDate × Genotype		ns	ns	ns	ns	ns

<sup>z</sup>CCRS = Central Crops Research Station (Clayton, NC), HCRS = Horticultural Crops Research Station (Castle Hayne, NC), PRS = Piedmont Research Station (Salisbury, NC), TAC = total anthocyanin content, C3G = cyanidin 3-glucoside, P3G = pelargonidin 3-glucoside, P3R = pelargonidin 3-rutinoside, P3MG = pelargonidin 3-(6''-malonylglucoside). Units in mg/100 g FWT.

<sup>y</sup>Values averaged within location. Different letters within treatment are significantly different at  $P < 0.05$ .

<sup>x</sup>Values averaged within harvest date. Different letters within treatment are significantly different at  $P < 0.05$ .

\*\*\* $P < 0.0001$ , \*\* $P < 0.01$ , \* $P < 0.05$

**Table 2.4.** Effect of location, harvest date, and genotype on mean levels of anthocyanin content and profile percentages of 17 strawberry commercial genotypes and advanced selections grown at three locations in North Carolina (2022).

Source	Categories	TAC <sup>z</sup>	%C3G <sup>z</sup>	%P3G <sup>z</sup>	%P3R <sup>z</sup>	%PM3G <sup>z</sup>
		mean ± SD				
Location <sup>y</sup>	CCRS <sup>z</sup>	33.4 ± 13.0 ab	4.72 ± 3.2 a	78.10 ± 7.5 b	10.49 ± 3.7 a	5.86 ± 7.4 a
	HCRS <sup>z</sup>	31.13 ± 8.6 b	4.79 ± 3.4 a	78.36 ± 8.3 b	10.35 ± 3.3 a	6.14 ± 7.6 a
	PRS <sup>z</sup>	36.13 ± 12.8 a	4.62 ± 3.6 a	79.96 ± 8.8 a	8.01 ± 3.5 b	5.27 ± 7.2 a
HDate <sup>x</sup>	1st	35.79 ± 12.2 a	4.45 ± 3.0 b	79.29 ± 7.8 a	9.65 ± 3.5 a	5.73 ± 7.5 a
	2nd	33.92 ± 11.8 ab	4.29 ± 3.2 b	78.86 ± 8.2 a	10.18 ± 3.9 a	5.54 ± 7.0 a
	3rd	31.03 ± 10.9 b	5.38 ± 3.9 a	78.34 ± 8.7 a	8.91 ± 3.5 b	5.96 ± 7.7 a
Genotype	Camarosa	40.06 ± 9.8 a 35.19 ± 11.5	6.44 ± 1.3 b	78.62 ± 1.5 de	13.77 ± 1.6 b	0.53 ± 0.3 c
	Chandler	abc	3.80 ± 1.5 cdef	86.71 ± 2.3 ab	8.70 ± 2.2 cde	0.39 ± 0.4 c
	Liz	36.13 ± 9.5 abc	11.02 ± 2.9 a	66.40 ± 2.2 h	9.62 ± 1.7 cd	12.78 ± 1.7 b
	Miss Jo	24.32 ± 5.4 bcd	1.93 ± 0.84 efg	78.19 ± 2.39 ef	17.37 ± 2.19 a	1.46 ± 0.76 c
	NC19-016	45.23 ± 5.7 a	1.44 ± 0.8 fg	82.33 ± 2.2 cd	14.19 ± 2.4 ab	0.36 ± 0.2 c
	NC19-018	44.23 ± 10.3 a	4.13 ± 1.1 cde	87.89 ± 2.1 a	6.45 ± 1.2 de	0.44 ± 0.4 c
	NC19-020	45.73 ± 12.6 a	4.54 ± 1.0 bcd	84.04 ± 2.8 bc	8.84 ± 3.5 cde	0.25 ± 0.2 c
	NC19-022	23.02 ± 4.3 cd	1.10 ± 1.0 g	71.64 ± 4.0 g	10.33 ± 2.5 c	15.33 ± 1.5 ab
	NC20-002	20.61 ± 4.0 d	9.46 ± 2.3 a	86.42 ± 2.2 ab	3.45 ± 0.6 f	0.07 ± 0.2 c
	NC20-008	33.96 ± 7.3 abc	5.20 ± 0.9 bc	82.62 ± 2.3 c	10.43 ± 2.4 c	0.98 ± 0.5 c
	NC20-018	24.48 ± 3.5 bcd 33.46 ± 11.2	5.19 ± 1.2 bc	85.09 ± 1.7 abc	6.83 ± 1.4 de	1.60 ± 0.3 c
	NC20-054	abc	3.36 ± 1.0 cdefg	86.68 ± 2.0 ab	9.39 ± 1.8 cde	0.15 ± 0.3 c
	NC20-055	37.80 ± 5.3 ab	2.03 ± 0.8 efg	75.34 ± 1.9 efg	9.58 ± 1.7 cde	12.92 ± 1.7 b
	NC20-058	24.00 ± 4.6 bcd	4.65 ± 1.3 bcd	85.26 ± 1.4 abc	9.05 ± 1.2 cde	0.83 ± 0.4 c
	NC20-099	40.64 ± 16.1 a	1.91 ± 0.9 efg	66.86 ± 1.6 h	8.80 ± 1.2 cde	17.33 ± 9.2 a
	Rocco	37.31 ± 8.2 abc	2.23 ± 1.6 defg	74.61 ± 3.2 fg	9.11 ± 2.5 cde	13.95 ± 1.5 ab
	Ruby June	24.09 ± 5.4 bcd	10.80 ± 1.6 a	62.47 ± 1.9 i	8.47 ± 1.2 cde	17.17 ± 1.3 a
Location		**	ns	***	***	ns
HDate		*	***	ns	***	ns
Genotype		***	***	***	***	***
Location × HDate		*	**	*	***	ns
Location × Genotype		*	ns	*	*	ns
HDate × Genotype		ns	ns	ns	ns	ns

<sup>z</sup>CCRS = Central Crops Research Station (Clayton, NC), HCRS = Horticultural Crops Research Station (Castle Hayne, NC), PRS = Piedmont Research Station (Salisbury, NC), TAC = total anthocyanin content, %C3G = cyanidin 3-glucoside, %P3G = pelargonidin 3-glucoside, %P3R = pelargonidin 3-rutinoside, %P3MG = pelargonidin 3-(6''-malonylglucoside).

<sup>y</sup>Values averaged within location. Different letters within treatment are significantly different at  $P < 0.05$ .

<sup>x</sup>Values averaged within harvest date. Different letters within treatment are significantly different at  $P < 0.05$ .

\*\*\* $P < 0.0001$ , \*\* $P < 0.01$ , \* $P < 0.05$

Pelargonidin 3-(6''-malonylglucoside) is a pelargonidin-3-glucoside with malonic acid (a dicarboxylic acid) attached to a glucoside. The content of P3MG was highly dependent on strawberry genotype and ranged from 0 to 16.79 mg/100 g FWT, and relative amounts were 0 to 23.47% (**Figure 2.2D**). The highest content (3.52 to 7.98 mg/100 g FWT) and relative amount of P3MG (12.78 to 17.33%) were found in strawberries from 'Liz', 'NC19-022', 'NC20-055', 'NC20-099', 'Rocco', and 'Ruby June'. The remaining genotypes were low in total (0.02–0.39 mg/100 g FWT) and relative P3MG (0.07–1.46%), with genotypes NC20-002 and NC20-054 both having the lowest P3MG and %P3MG values. Location and harvest date factors alone had no consistent effect on P3MG or %P3MG (**Tables 2.3–2.4**). Additionally, no significant two-way interactions were found between genotype, harvest date, or location.

Cyanidin-3-glucoside, also a minor pigment in strawberry fruit, varied most with genotype and harvest date. Total C3G in strawberry juice samples ranged from 0 to 4.9 mg/100 g FWT, and %C3G of total anthocyanin was 0 to 15.09% (**Figure 2.2E**). The highest total (2.10–3.82 mg/100 g FWT) and relative C3G (6.44–10.80%) were found in fruit from 'Camarosa', 'Liz', NC19-020, and 'Ruby June'. The lowest total (0.28–0.83 mg/100 g FWT) and relative C3G (1.10–2.23%) were found in strawberries from 'Miss Jo', NC19-016, NC19-022, NC20-055, NC20-099, and 'Rocco'. While total C3G amounts were consistent across harvest dates, relative amounts of C3G were higher in fruit collected from the last harvest date in 11 genotypes. No consistent patterns with location alone and the amount of C3G or %C3G are noted (**Tables 2.3–2.4**). However, the interaction of location  $\times$  harvest date was significant for C3G and %C3G, suggesting that location or harvest date effects on total and relative amounts of C3G are interrelated.

### Correlation and Hierarchical Clustering

Lastly, correlation analysis and hierarchical clustering were performed to determine and visualize relationships between the fruit composition parameters and determine interrelationships between the parameters and the genotypes. The strongest correlations included P3G and TAC ( $r = 0.96$ ), P3R and TAC ( $r = 0.69$ ), and P3G with P3R ( $r = 0.61$ ), with remaining correlations considered weak to negligible (**Appendix Table A.2**). Similarly, no significant groupings of genotypes from hierarchical clustering were seen (**Appendix Figure A.2**).

## **Discussion**

### Genotype Differences in Fruit Composition

In the current study, 15 of the 17 North Carolina genotypes had a %SSC >7.0, with ‘Chandler’, ‘Liz’, NC19-022, NC20-008, NC20-055, NC20-058, and ‘Ruby June’ having average SSC values of at least 8.0 across locations and harvest dates. ‘Rocco’ had the highest value at 9.3%. Values were comparable to those previously reported for strawberry commercial genotypes grown in California such as ‘Camarosa’ (7.1–8.5%), ‘Camino Real’ (6.8–7.9%), and ‘Chandler’ (6.0–8.9%), or in Florida for ‘Florida Beauty’ (8.2%), ‘Florida Brilliance’ (7.1%), ‘Florida Radiance’ (5.0–8.8%), ‘Strawberry Festival’ (5.6–8.6%), ‘Florida Sensation’ (6.7–9.6%), and ‘Sweet Charlie’ (7.1–9.8%) (**Agüero et al., 2015; Kader 1991; Menzel 2022; Perkins-Veazie et al., 2016; Whitaker et al., 2011; Whitaker et al., 2015**). Previous studies have shown that soluble solids content and perceived consumer sweetness of Florida germplasm indicated that above-average sweetness perception could be achieved with SSC values of 8.0–9.0%, with 0.64 to 0.86% confidence, respectively (**Fan et al., 2021b**).

Percent titratable acidity was moderately correlated with %SSC, with values ranging from high (0.81–0.85%), medium (0.74–0.77%), and low (0.64–0.72%) across the genotypes and locations. Six genotypes had Tacid levels of 0.81% and above, with ‘Camarosa’ and ‘Liz’ having moderate Tacid levels (0.74 and 0.77%), and ‘Chandler’ and ‘Rocco’ fruit having high Tacid levels (0.81%). These findings are similar to those reported by **Perkins-Veazie et al., 2016** for ‘Camarosa’ (0.69%), ‘Chandler’ (0.69%), ‘Liz’ (previously NCS10-038) (0.75%), and ‘Rocco’ (previously NCS10-156) (0.72%), grown at PRS in 2014 and 2015, and with those by **Vinson et al. 2022** who evaluated ‘Chandler’ and ‘Camarosa’ strawberries (0.68%) during a three-year study in Alabama. Of the North Carolina germplasm, five genotypes met the newly suggested flavor targets of minimum 8.0% SSC and 0.80% Tacid, as indicated by **Fan et al., 2021b and Kubota Lab, 2015**. Additionally, the pH of juice from the North Carolina genotypes, ranging from 3.46 to 3.77, was in the recommended range for ripe strawberry fruit (**Kader, 1991**).

Sugars are key components to strawberry flavor, whereas anthocyanins contribute largely to visual color. Strawberry sugar and anthocyanin content may be inversely correlated in strawberry. This relationship was seen in 14 of the 17 genotypes; notably, the three genotypes highest in TAC (NC19-016, NC19-018, NC19-020, 54.17–59.71 mg P3G/100 g FWT) were also the lowest in SSC (6.14–7.23%). Little information exists regarding the interrelationships of TAC and profiles with SSC. In a field study conducted in central Italy, seven genotypes (‘Albion’, ‘Cabrillo’, ‘Favette’, ‘Irma’, ‘Monterey’, ‘Portola’, and ‘San Andreas’) were evaluated across three harvest dates (**Amoriello et al., 2022**). As the harvest season progressed, %SSC increased and TAC decreased, presenting a negative correlation of  $r = -0.40$ . A similar pattern and negative correlation were also noted for North Carolina germplasm, suggesting a

potential trade-off between the two parameters. Further investigation of this relationship is needed to confirm and explore any implications.

### Genotype Differences in Anthocyanin Content and Profiles

In general, North Carolina strawberry germplasm was found to be higher in anthocyanin (mg P3G/100 g FWT) than in fruit in other studies. Fruit from 11 North Carolina genotypes had TAC values that exceeded 30.0 mg/100 g FWT, with five genotypes having at least 40.0 mg/100 g FWT. Strawberry fruit from genotypes originating from the California breeding program, such as ‘Albion’, ‘Aromas’, ‘Bounty’, ‘Camino Real’, ‘Portola’, ‘Ruby June’, ‘SanAndreas’, and ‘Selva’, had reported total anthocyanin values of 9.2 to 59.8 mg/100 g (**Anttonen et al., 2006; Chaves et al., 2017; Pelayo-Zaldivar et al., 2005; Van De Velde et al., 2013; Vinson et al., 2022**). Similarly, genotypes from the Florida breeding program, including ‘Florida Radiance’, ‘Strawberry Festival’, ‘Sweet Charlie’, ‘Florida Sensation’, and ‘Rubygem’ ranged from 8.5 to 54.0 mg/100 g (**Fredericks et al., 2012; Kelly et al., 2016; Nunes et al., 2005; Saridaş et al., 2022**). Possible causes for differences in anthocyanin content among studies include the relative fruit ripeness (fully colored vs.  $\frac{3}{4}$  red), extraction solvent (MeOH:water:formic acid, HCl:MeOH, acetone:HCL, KCl:HCl), equipment used (spectrophotometer, UPLC, HPLC), puree or juice, and choice of anthocyanin (cyanidin vs .pelargonidin) and respective extinction coefficient.

‘Camarosa’ and ‘Chandler’ are two of the most widely grown strawberry commercial genotypes in North Carolina and are often used as standards in breeding programs. The respective characteristics of these two cultivars help determine suitability for different market and industry

applications. ‘Camarosa’ fruit from our study and from fruit evaluated by **Perkins-Veazie et al., 2016**, had similar TAC values (40.1–41.3 mg P3G/100 g FWT) to strawberries grown in Argentina, Australia, Portugal, and Spain (38.4–48.2 mg/100 g) (**Castro et al., 2002; Fredericks et al., 2012; Van De Velde et al., 2013**). However, values up to 84.0 mg P3G/100 g have been reported in ‘Camarosa’ fruit (**Garcia-Viguera et al., 1998**). Similarly, anthocyanin content in ‘Chandler’ fruit in our study and in **Perkins-Veazie et al., 2016**, ranged from 35.2 to 48.6 mg P3G/100 g FWT, consistent with values reported by **Garcia-Viguera et al., 1998** (32.7 mg/100 g), but higher than **Nunes et al., 2005**, who reported values of 23.0 mg/100 g in ‘Chandler’ fruit. Furthermore, **Vinson et al. 2022**, observed slightly higher TAC in ‘Chandler’ (42.1–71.5 mg/100 g) and ‘Camarosa’ (49.3–51.3 mg/100 g) fruit grown in Clanton, Alabama, than what is typically expected in North Carolina fruit, with no statistical significance in TAC in both genotypes. Although TAC values are generally similar, ‘Chandler’ fruit tends to be visually lighter than ‘Camarosa’ fruit. Therefore, strawberries with dark external and internal color in addition to high TAC, more characteristic of ‘Camarosa’ fruit, are more desirable for the food and beverage industries, particularly preservative manufacturers (**Garcia-Viguera et al., 1998**). ‘Camarosa’ fruit is also known to have a slightly greater shelf life and perform better in pre-pick shipping operations, while ‘Chandler’ fruit has greater yields and is often preferred for U-pick and local markets (**Perkins-Veazie et al., 2016**). This suggests that the diverse range of fruit composition parameters, including TMAC, observed within the genotypes in this study may potentially be used for different market and industry applications.

Variation in anthocyanin content may also be caused by degree of ripeness. Strawberries used for processing are typically harvested at a riper stage than those for fresh market. Minimum color for

strawberries harvested for commercial markets is  $\frac{3}{4}$  full red (**Cayo et al., 2016; Jouquand et al., 2008; Pelayo-Zaldivar et al., 2005; Zhang et al., 2022**). California and Florida strawberries in particular are commonly harvested at no more than  $\frac{3}{4}$  red in order to maintain firmness during storage and transportation. In our study, fully red strawberries were used, in common with the direct market and local sales used in North Carolina (**Samtani et al., 2019**). The amount of TAC relative to ripeness stage has been explored in numerous studies (**Aaby et al., 2012; Amiri et al., 2021; Hwang et al., 2019; Nunes et al., 2005; Ornelas-Paz et al., 2013**). For instance, **Nunes et al. 2005** found that TAC of field-ripened Florida genotypes ('Chandler', 'Oso Grande', 'Sweet Charlie') increased by 31% as fruit ripened from  $\frac{3}{4}$  to full color (**Nunes et al., 2005**). A similar trend was observed in three Norwegian genotypes ('Blink', 'Polka', and 'Senga'), where TAC increased from 19.6–21.1 mg/100 g to 37.1–67.1 mg/100 g with increased ripeness stages (**Aaby et al., 2012**).

As noted in previous studies for red-fruited strawberries (**Cerezo et al., 2016; Lopes da Silva et al., 2007; Perkins-Veazie et al., 2016**), pelargonidin 3-glucoside (P3G) was the dominant anthocyanin pigment in North Carolina genotypes, contributing to the majority of the total anthocyanin. In the North Carolina germplasm, pelargonidin 3-glucoside and pelargonidin 3-rutinoside had the highest correlation of all pigments, and genotypes high in TAC were often high in P3G and P3R. In the present study, some genotypes were higher than others in the minor pigments pelargonidin 3-(6''-malyonylglucoside) (P3MG) and cyanidin 3-glucoside (C3G). P3MG has only been recently identified and recognized in strawberry compared to P3G and C3G (**Lukton et al., 1955; Tamura et al., 1995**); the amount of P3MG does not appear to significantly affect TAC in strawberry fruit (**Yoshida et al., 2002**).

In our study, P3MG was either very low (0.07–1.60%) or high (12.78–17.33%) with no correlation with total anthocyanin content, suggesting that the presence or absence of this anthocyanin may be dependent on a single gene. In previous research, a major quantitative trait locus (QTL) associated with P3MG biosynthesis was found within the *Fvb6-2* interval on the *Fragaria vesca* genome (**Davik et al., 2020**). As this locus has not been directly identified in cultivated *Fragaria xananassa* strawberry, **Davik et al. (2020)** performed linkage mapping using three cultivated strawberry mapping populations. A locus on linkage group LG6b was identified and hypothesized to contain a related gene involving P3MG biosynthesis in cultivated strawberry fruit, potentially explaining the absence of P3MG in certain genotypes. Additionally, mutations in this single major gene do not appear to induce any changes in P3G concentration (**Davik et al., 2020**).

With the exception of ‘Ruby June’, all high %P3MG genotypes were directly from the North Carolina strawberry breeding program. Conversely, cyanidin 3-glucoside (C3G) was the anthocyanin in the least amount among genotypes (1.1–11.0% of total anthocyanin) but was similar to P3MG in that no strong relationships between TAC and C3G amounts in strawberry genotypes could be found.

#### Harvest Date and Location Effects on Fruit Composition

Variation in fruit composition was mainly attributed to genotypic differences. Fruit soluble solids content, total sugar content, and organic acid content are quantitatively inherited (**Lerceteau-Kohler et al., 2006**). Several QTLs associated with strawberry fruit total and individual sugars, soluble solids content, organic acids, titratable acidity, anthocyanin, pH, and other fruit quality

measurements have been previously identified (Alarfaj et al., 2021; Castro et al., 2016; Fan et al., 2024; Lerceteau-Kohler et al., 2006; Vallarino et al., 2019; Zorrilla-Fontanesi et al., 2011). These QTL were detected using multiple environments and across different strawberry populations in order to find repeatable marker-trait associations for breeding programs.

In addition to genetics, harvest date also highly influenced North Carolina strawberry germplasm. Soluble solids content, Tacid, C3G, and %C3G generally increased throughout the season, while pH, TAC, and both the amount and relative P3G, P3R, and P3MG decreased. For each of the three harvest dates, there were notable differences in both the SSC (7.3, 7.7, and 8.6%) and Tacid values (0.76, 0.73, and 0.79%) of the harvested fruit. For all parameters, average fruit composition values were most often similar between the first and second harvest dates, while the third date statistically differed from one or both previous dates.

Location had the least influence on strawberry fruit composition. Although SSC, pH, and amount and percent of C3G varied across locations, differences could not be statistically attributed to location alone. Among the three locations, fruit from PRS was most often significantly different than CCRS and HCRS in each fruit composition variable. Fruit from PRS was higher in total and percent P3G (29.1 mg/100 g, 80.0%) and lower in total and percent P3R (2.9 mg/100 g, 8.0%) than P3G and P3R levels in fruit from CCRS (P3G: 26.0 mg/100 g, 78.1%; P3R: 3.6 mg/100 g, 10.5%) and HCRS (P3G: 24.4 mg/100 g, 78.4%; P3R: 3.3 mg/100 g, 10.4%) (**Table 2.2**). Total anthocyanin content was similar between CCRS and PRS and between CCRS and HCRS. Fruit Tacid was the only parameter that differed statistically at all three research stations (**Table 2.2**).

Lastly, significant location  $\times$  harvest date and location  $\times$  genotype interactions for most of the compositional variables illustrate the need to test advanced selections in multiple locations and over multiple dates to develop a comprehensive profile. Only C3G and %C3G were not significant for location  $\times$  genotype. Interestingly, neither the effects of location or harvest date alone nor their interaction together (location  $\times$  harvest date) had a notable impact on total and percent P3MG, indicating that variation may be influenced mostly by genotype. As the harvest date  $\times$  genotype interaction was primarily NS for variables tested, this suggests that the impact of harvest date was consistent regardless of the genotype. This result is supported by the fact that fruit was collected from all genotypes on the same date at each location, and the three harvests occurred within an 11- to 17-day timeframe due to the small fruiting window in North Carolina.

## **Conclusion**

Fruit composition variability was observed in strawberry fruit from 12 advanced selections from the North Carolina strawberry breeding program and five commercial genotypes. Genetics (genotype) explained the majority of variation in all traits measured in this study, with harvest date having a large secondary role. Location alone had the least influence on fruit composition but became more significant when considering interactions between location  $\times$  harvest date and location  $\times$  genotype. Soluble solids ranged from 6.1 to 9.3% and Tacid from 0.64 to 0.85%, with five genotypes meeting both the desired %SSC and %Tacid target values. Genotypes could also be grouped based on low, moderate, or high levels of total anthocyanin. Pelargonidin 3-glucoside was the dominant anthocyanin pigment (60.2 to 90.7%), followed by pelargonidin 3-rutinoside (3.5 to 17.4%). Notably, certain genotypes were unusually high in the minor pigment pelargonidin 3-(6''-malonylglucoside) (0.1 to 17.3%). Lastly, soluble solids content and total

anthocyanin were moderately and negatively correlated, implying a potential energy or carbohydrate tradeoff within strawberry fruits. This study provides insight into strawberry fruit composition profiles, particularly within the North Carolina germplasm collection and additional commercial genotypes, and the relative effects genetics, location, and harvest date have on the germplasm. Our results support the hypothesis that North Carolina-derived strawberry germplasm shows distinct genetic differences in fruit composition traits compared to California and Florida material, with harvest date heavily influencing variation in fruit composition.

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## CHAPTER 3

### Multivariate Analysis of Fruit Composition Diversity among the North Carolina Strawberry Germplasm Collection

#### Abstract

Strawberries are a highly sought-after fruit for their flavor, color, and nutritional benefits. The balance of soluble solids content (SSC) and titratable acidity (Tacid) is a large component of overall strawberry taste and perceived sweetness, while total monomeric anthocyanin (TMAC) is important for fruit color. Strawberry cultivation is particularly important in the United States, with North Carolina ranking 3<sup>rd</sup> nationally in fresh market strawberry production. However, as California and Florida commercial genotypes often underperform in North Carolina's climate and production season, there exists a strong need for the development of more genotypes adapted to North Carolina and nearby states. In this study, strawberry fruit from 268 commercial and advanced genotypic lines from the North Carolina breeding program were collected in a greenhouse germplasm collection to determine and characterize genotype diversity, relationships, and overall trends in fruit composition. Using multivariate statistical methods and hierarchical clustering, strawberry genotypes were separated into four distinct clusters based on fruit composition profiles. Florida commercial genotypes and 38 advanced genotypic lines were grouped together (Cluster 1) and exhibited the lowest SSC (7.0%), Tacid (0.72%), and TMAC (31.22 mg/100 g FWT). Cluster 2 had cultivars developed in North Carolina with the second lowest %SSC and %Tacid and high TMAC (54.57 mg/100 g FWT). California day-neutrals were found in Cluster 1, while short-day genotypes were split amongst the first two clusters. Cluster 3 was characterized by high SSC (10.3%) and pH (3.66), and fruit from Cluster 4 had the highest average Tacid (1.21%) and lowest pH (3.43). The first two principal components in PCA

analysis accounted for 64.88% of the total variance within total fruit composition, with both pH and Tacid contributing to PC1 (91.1%), SSC contributing to PC2 (71.1%), and TMAC associated with PC3 (77.4%). These differences in fruit composition among genotypes in the North Carolina core germplasm collection will be useful in determining crosses in the breeding program.

## **Introduction**

### Strawberry Production

In recent years, berries have emerged as some of the most highly valued fruit crops produced in the United States, with strawberries (*Fragaria ×ananassa*) leading the berry market in both value (\$2 billion) and volume (1.4 million tons) (Yeh et al., 2023). Strawberries represent 13% of the total fruit production value in the United States, making them the third largest fruit produced after grapes and apples. Strawberries contain vitamin C, anthocyanins, and other flavonoids that serve important functions in both plant and human health (Giampieri et al., 2014; Khoo et al., 2017). California and Florida combine to over 90% of the total strawberry production in the United States and are primarily responsible for supplies of winter and summer fruit (Samtani et al., 2019).

North Carolina consistently ranks 3<sup>rd</sup> nationwide in fresh market strawberry production. In 2017, 485.6 hectares of strawberries were grown in North Carolina with an estimated value near \$27 million (Samtani et al., 2019). In contrast to other states, the strawberry market in North Carolina is focused on direct markets. The production landscape is highly decentralized, with half the fruit produced for the state originating from a few large farms ( $\geq 4.05$  hectares) and the

remaining portion grown on smaller farms of 0.41 to 1.21 hectares (**Samtani et al., 2019**).

Additionally, all 100 counties in NC sell strawberries at U-pick operations, roadside stands, farmers markets, and other local sales (**Hoffmann, 2020**).

#### North Carolina Strawberry Production Practices and Breeding Program

The major production system in North Carolina uses open-field annual hill plasticulture, where plug or bare-root plants are set in the fall. Fruit is harvested the following spring (April–June) in a relatively short 6–8-week window, depending on weather. To date, field production methods follow those detailed in the 2005 Southeast Regional Strawberry Plasticulture Production Guide released by North Carolina, Georgia, and Clemson Universities (**Poling et al., 2005**). The commercial genotypes ‘Chandler’ and ‘Camarosa’ developed in the early 1980s and 1990s have been the dominant genotypes grown in North Carolina for over 25 years (**Samtani et al., 2019**). Other genotypes that have gained traction among growers in the state include ‘Albion’, ‘Camino Real’, ‘Sweet Charlie’, and the newest genotype, ‘Ruby June’. The development of new cultivars with disease resistance and firm well-colored fruit is needed to sustain or expand the North Carolina strawberry industry (**Samtani et al., 2019**).

The North Carolina strawberry breeding program was first established in the early 1900s and has been led by a number of breeders, including E. B. Morrow (1930–1955), G. J. Galleta (1959–1977), J. R. Ballington (1977–1984), R. Goldy (1984–1990), J. R. Ballington (1990–2010), J. Pattison (2010–2014), and G. Fernandez (2014–present). The program’s overall focus is to develop strawberry germplasm adapted to the South Atlantic climate with increased disease resistance, adequate chilling hour requirements, steady yield, improved flavor, good appearance, and increased post-harvest shelf life (**Fernandez, personal communication**). To date, 12

commercial strawberry genotypes have been developed through traditional breeding methods and released by the program, with ‘Rocco’ and ‘Liz’ being the latest releases in 2020.

### Strawberry Flavor and Color

Two of the most important sensory qualities in strawberries, and two that the NC breeding program highly prioritizes, are fruit flavor and color. The steady progression in strawberry breeding and better selection techniques over the last few decades have caused fruit composition values in most genotypes to easily surpass previously established goals for strawberry soluble solids content (minimum 7.0%) and titratable (maximum 0.80%) (**Fan et al., 2021b; Kader 1991; Mitcham et al., 1996**). Newer values for SSC have been raised to 8.0–9.0% but remain the same for Tacid (0.80%) (**Fan et al., 2021b; Kubota Lab, 2015**). Many studies choose to report on strawberry SSC/Tacid ratios as they are known to be highly associated with increased sweetness perception (**Jouquand et al., 2008**). However, basing strawberry sweetness on SSC/Tacid ratios alone is often misleading as it is difficult to accurately determine whether %SSC and %Tacid levels are in low, medium, or high categories.

This problem is illustrated in a 1994 study where researchers found average SSC/Tacid ratios of 17.05 and 17.89 in ‘Allstar’ and MDUS 5036 genotypes (**Wang et al., 1997**). In fact, ‘Allstar’ fruit had medium SSC (7.5%) and low Tacid (0.44%), while MDUS 5036 fruit contained very high SSC (10.2%) and medium Tacid (0.57%) and was more preferred in taste. In general, both high sugar and high titratable acidity are desirable for optimal strawberry flavor (**Kader et al., 1991**). In this study, strawberry fruit with high sugars and lower acids had an artificially sweet and almost bland taste, fruit with low sugars and high acids had a tart flavor, and strawberries

containing low sugars and acids have little taste at all (**Kader et al., 1991**). As such, strawberry fruits containing Tacid levels greater than 0.80% can still be perceived as sweet, if combined with higher %SSC. However, the demographics of taste preferences have changed in the US, with an increasing preference among consumers for fruit with higher SSC and lower acids (**Fan et al., 2021a; Porter et al., 2023**).

The visual color of strawberry is largely dependent on the concentration of water-soluble anthocyanin pigments, consisting mostly of pelargonidin and cyanidin anthocyanidins. Strawberry color can range from a white-blush to a deep purple-red, and as a result, considerable variation within consumer preferences on fruit color exists (**Denoyes et al., 2023**). As berry skin color tends to darken over time during storage, berries with already dark red skin are considered a liability and poor for commercial marketing purposes (**Hokanson & Finn, 2000; Nunes et al., 2005; Perkins-Veazie et al., 2016**). Therefore, California, Florida, and other markets with heavy shipping influences harvest berries no more than  $\frac{3}{4}$  commercial ripeness to maintain fruit firmness and slow development of deeper red shades; this leads to a lower anthocyanin content in fruit (**Cayo et al., 2016; Jouquand et al., 2008; Pelayo-Zaldivar et al., 2005; Zhang et al., 2022**). North Carolina growers utilize both commercial and direct marketing models. The commercial industry prefers a berry that is capable of shipping, while direct market growers prefer fully ripe and flavorful red fruit.

#### Strawberry Breeding Challenges

Target goals for sugar, acid, color, and other traits make breeding strawberries for improved sensory and quality traits challenging, with the additional challenge of an octoploid genome

**(Folta & Klee, 2016)**. For plant breeding programs to be successful, diversity in parental material is an important factor in determining the effectiveness of crosses. High genetic variability among genotypes in germplasm collections is needed to achieve greater genetic gains and obtain improved characteristics in new genotypes superior to commercial genotypes **(Rutz et al., 2023)**. Additionally, the preservation of unique genetics in elite genotypes requires vegetative maintenance. As screening germplasm populations is resource- and labor-intensive and time-consuming **(Rahaman et al., 2015)**, breeders are continuously seeking accurate and rapid methods for field evaluation, laboratory analyses, and data analysis tools for screening plant material.

#### Introduction to Multivariate Analysis

Multivariate analysis methods are statistical tools that allow for the simultaneous analysis of relationships between multiple variables and are very useful in breaking down complex datasets. Common analyses include multivariate analysis of variance (MANOVA), principal component analysis (PCA), multiple regression, factor analysis, discriminant analysis, and several types of cluster analyses **(Saed-Moucheshi et al., 2013)**. Trends, patterns, and correlations can be visualized and interpreted through different tests. Multivariate methods have been known since the 1960s and are often used in applied and physical chemistry, economics, and social science disciplines; use in the food and horticultural sciences has become more common only over the last two decades **(Cozzolino et al., 2009; Granato et al., 2018)**. Examples of multivariate analysis application includes prediction of the geographic area where honey samples and wine originated **(Capron et al., 2007; Scholz et al., 2020)**, determination of the optimal night temperature for lettuce growth **(Jeong et al., 2015)**, selection of apple genotypes to replace with

commercial apples as alternative options for juicing (**Tian et al., 2018**), and determining the most important sensory quality variables of blackberry, peach, and coconut fruits (**Li et al., 2018; Monicka et al., 2020; Nowicka et al., 2019**).

#### Multivariate Statistical Analysis for Strawberry Germplasm Evaluation

Multivariate methods have also been used to evaluate diversity and determine relationships in strawberry germplasm collections and populations. One diversity assessment of 13 production (bearing type, vigor, yield, fruit size, flowering, ripening time) and sensory (fruit appearance, firmness, internal and external color, glossiness, aroma, sweetness) traits in 91 strawberry genotypes from the Poland strawberry core collection was conducted during 2008–2010 (**Siezko et al., 2015**). Fruit was collected from 10 plants per genotype, spaced at  $0.25 \times 1.1$ m.

Hierarchical clustering analysis using Ward's method, Euclidean distance, and set cutoff values grouped the genotypes into six clusters based on similarity in traits. The researchers were able to characterize the clusters based on the dominant characteristics of the genotypes (**Siezko et al., 2015**). Cluster 1 contained genotypes with high yields, large fruit, and vigorous plant growth, while Cluster 3 fruit were the earliest flowering genotypes with increased firmness. Fruit from genotypes in Cluster 6 had the darkest internal and external color, greatest sweetness, and was the most aromatic. The greatest correlations were found between fruit aroma and sweetness ( $r = 0.84$ ) and between fruit size and glossiness ( $r = 0.61$ ). However, the remaining correlations had a coefficient of  $r = 0.59$  or lower, with the majority of relationships being negligible.

Another three studies done in Brazil, also used multivariate analysis methods to analyze South American strawberry germplasm. **Chiomento et al., 2021** followed strawberry performance and

quality of nine greenhouse-grown genotypes in Rio Grande do Sul during May–December, **Barth et al., 2022** used multivariate methods to screen for and improve the selection of heat-tolerant strawberries in 196 genotypes in a germplasm collection in Guarapuava Paraná, and **Rutz et al., 2023** evaluated a large number of genotypes arising from five main crosses to select for more short-day genotypes that do well in field settings in Ivaiporã Paraná.

Twenty fruits from strawberry genotypes ‘Albion’, ‘Aromas’, ‘Camarosa’, ‘Camino Real’, ‘Fronteras’, ‘Merced’, ‘Monterey’, ‘Portola’, and ‘San Andreas’, each with six replications, were harvested during peak production (November) to be used for fruit quality analysis (**Chiomento et al., 2021**). No significant differences were found in SSC, Tacid, or SSC/Tacid content.

However, total antioxidant, total flavonoid, and total phenolic content were 24–60% higher in ‘Aromas’ and ‘Camarosa’ fruit than ‘Camino Real’, ‘Portola’, and ‘San Andreas’ fruit.

Hierarchical clustering grouped the genotypes into four clusters, and the first two PC components explained 53.2% of the variance in the fruit. Based on the multivariate results, the researchers suggested using ‘Camarosa’ and ‘Merced’ genotypes for yield and fruit size, and to use ‘Aromas’ and ‘Camarosa’ for the best physiochemical and phytochemical properties (**Chiomento et al., 2021**).

In a second study, ‘Camarosa’ and ‘Camino Real’ genotypes were used as controls to screen 194 seedling lines for greater heat tolerance utilizing K-means clustering (**Barth et al., 2022**). Plants were transplanted in a humid mesothermal subtropical climate low-tunnel system organized in a randomized block design containing 10 plants per genotype with three replications each. Fruits were harvested when  $\frac{3}{4}$  red, frozen at  $-2^{\circ}\text{C}$ , and homogenized into puree when ready for analysis.

K-means clustering separated genotypes into two clusters, with ‘Camarosa’ and 154 seedling lines in one and ‘Camino Real’ and the remaining 40 lines in the second. Fruit size and yield were highly correlated ( $r = 0.96$ ), SSC/Tacid with fruit size and yield were moderately correlated ( $r = 0.52$  and  $0.53$ ), and weak correlations were found between combinations of fruit size, yield, and SSC/Tacid with ascorbic acid and anthocyanin ( $r = 0.18$ – $0.34$ ). Based on multivariate results, an advanced population of 53 genotypes was selected from the 194 lines, with the majority of lines coming from Cluster 1 (**Barth et al., 2022**).

To select for more short-day strawberry genotypes, **Rutz et al., 2023** utilized multivariate methods to increase their selection efficiency within 1500 seedling lines arising from population crosses between ‘Camino Real’ and five advanced genotypes (RVFS06, RVFS07, RVCA16, RVCS44, and RVDA11). Seedlings were grown in an augmented field block design and compared to eight commercial cultivars: ‘Albion’, ‘Aromas’, ‘Camarosa’, ‘Camino Real’, ‘Dover’, ‘Monterey’, and ‘San Andreas’. Fruit was harvested weekly, and both production and postharvest traits were assessed. Based on the results, researchers were able to select 44 strawberry lines that had superior traits greater than those of the commercial controls used. The ‘Camino Real’ x RVCS44 population had the highest number of lines selected (36%), compared to the other populations (4–12%). The principal component analysis explained 55% of the variation, and correlations between the traits indicated a strong positive correlation between fruit size and yield ( $r = 0.77$ ) and moderate negative correlations between lightness ( $L^*$ ) and chroma ( $C^*$ ) and between hue ( $h^*$ ) and fruit size ( $r = -0.59$  and  $0.51$ ). Remaining correlations involving SSC and firmness were weak or negligible.

**Li et al. (2016)** used principal component, correlation, and hierarchical clustering analyses to characterize strawberry size, firmness, pH, color, soluble solids, acidity, vitamin C, anthocyanin, and phenolic content in five commercial genotypes in Liaoning Province, China. Field grown and red mature fruit from strawberry cultivars ‘Akihime’, ‘All-star’, ‘99’, ‘R7’, and ‘Ever bearer’ were harvested between May and June and stored at 4°C before analysis. Twenty to twenty-five fruits per genotype were randomly selected, and puree was used for each chemical test in triplicate. Three PC components explained 85.0% of the total variation in the data set. PC1 mainly accounted for sensory qualities (fruit size, firmness, pH, SSC, and Tacid), color was the main attribute for PC2, and PC3 held nutritional content (vitamin C, anthocyanin, and phenolic content) (**Li et al., 2016**). Of two hierarchical clusters, Cluster 1 was composed of three genotypes characterized by firm berries with lower pH, SSC, and Tacid, and Cluster 2 (two genotypes) had softer berries with higher SSC, Tacid, and darker color (**Li et al., 2016**). Results provided insight on fruit composition variation within the five genotypes that will aid in the future selection of strawberry genotypes with specific desirable traits.

All five studies demonstrate the usefulness of multivariate analysis methods in characterizing strawberry germplasm populations with the goal of separating genotypes to select for desirable characteristics and discovering which sensory qualities contribute most to variation. Although statistical methods are thoroughly described, limitations in several key procedural steps and details exist in some studies, such as time of year (**Siezko et al., 2015**) number of fruits harvested or utilized (**Rutz et al., 2023; Siezko et al., 2015**), use of fresh or frozen berries (**Chiomento et al., 2021; Rutz et al., 2023; Siezko et al., 2015**), or use of juice or puree for fruit composition assays (**Chiomento et al., 2021; Siezko et al., 2015**).

## Objectives

The goal of this study was to evaluate the fruit composition profiles of the 268 genotypes currently maintained by the North Carolina strawberry breeding program and utilize multivariate statistical analysis methods to determine relationships. This work represents the first instance where the entire germplasm collection was assessed together in a single evaluation. Determining genotype diversity, characterizing relationships among the genotypes and fruit composition parameters, and visualizing trends within the collection will help guide future breeding decisions regarding developing new commercial cultivars with improved quality.

## **Materials and Methods**

### Experimental Location and Plant Material

Two hundred and sixty-eight strawberry genotypes were used in this study (**Appendix Table B.1.**). All material was grown in a 35x130-foot freestanding single-hoop greenhouse at Piedmont Research Station in Salisbury, North Carolina (35.69501°, -80.62939°) (**Figure 3.1**). The greenhouse was constructed with an aluminum and galvanized steel frame with a double layer of polyethylene-covered film and both a gravel and concrete base at the bottom. The greenhouse is equipped with four multidirectional circulating horizontal air flow fans, two large exhaust fans, two heaters, a cool cell with a curtain, and two thermostats (Hog Slat®). The irrigation system consists of two controllers that cover eight programmable zones (Hunter Pro-C® and Galcon), a container drip irrigation system, and an overhead sprinkler system (Netafilm Hanging Base and VibroNet Blue Sprinkler - 9.2 GPH).



**Figure 3.1.** North Carolina strawberry breeding program greenhouse germplasm collection at Piedmont Research Station in Salisbury in (A) production and (B) undergoing rejuvenation.

Strawberry plant plugs were obtained from the NCSU strawberry breeding program, nurseries, and U.S. breeding programs. Plants were grown in one-gallon plastic pots and placed in alphabetical and/or numerical order on 18 steel benches spanning across the different zones. The pots contained a Sungro Professional Growing Mix (Agawam, MA, USA). An automated drip fertigation system consisting of a Dosatron fertilizer injector and ½ gph pressure-compensated emitters delivered a balanced nutrient system and water to the plants. The master fertigation mixture consisted of 117.5 oz of 10-4-5 fertilizer (Triden Bionutrients) and 20 oz of 22-0-0 soluble fertilizer (Southern Agricultural Insecticides, Inc.) in a 5-gallon tank. Irrigation settings differed depending on the greenhouse zone of the plant location (**Appendix Table B.2**).

Fungicide sprays were applied every other week, and pesticides were applied as needed. Greenhouse climate control temperature settings differed depending on the time of year and weather conditions (**Appendix Table B.3**). Plant tissue, soil, and solution samples were taken periodically to check nutrition, disease, soil pH, and EC. All plants undergo a rejuvenation process every two years to maintain maximum plant growth and health in the germplasm collection. For this process, strawberry runner tips are collected from all mother plants, transplanted into trays to develop root balls, and transferred into gallon pots to eventually replace the “old” mother plants. Genotypes were last rejuvenated in 2021.

### Sample Collection

Marketable, fully red, firm fruit was collected from 16 commercial cultivars and 252 advanced selections from April to June of 2022. Fifteen marketable fruits were collected from each genotype and included those that were at least 10g in weight, not misshapen, and free from

visible defects such as disease, poor pollination, insect damage. Harvested fruit were placed into plastic ziploc bags and frozen at  $-15^{\circ}\text{C}$ . Samples were then transported to the Plants for Human Health Institute in Kannapolis, NC, at the end of the collection period and held at  $-20^{\circ}\text{C}$  until time for analysis.

#### Fruit Compositional Analysis

Fruit were thawed to room temperature and juice collected for analysis of soluble solids content (SSC), pH, titratable acidity (Tacid), and anthocyanin. A 0.5 mL aliquot of strawberry juice was placed on a handheld digital refractometer (Atago PAL-1, Bellevue, WA, USA) to determine %SSC. The pH of strawberry juice was determined using a pH meter (Thermo Scientific™ Orion Star™ A211, Waltham, MA, USA), and electrode (Thermo Scientific™ Orion™ Ross, Waltham, MA, USA). For titratable acidity, 0.5 mL juice was diluted with 24.5 mL of distilled deionized water, thoroughly mixed, and an aliquot placed on a digital acidity meter (Atago PAL-BX/ACID F5, Bellevue, WA, USA), with results recorded as % citric acid equivalents.

#### Anthocyanin analysis

Total monomeric anthocyanin content of juice was determined for each sample using two aliquots of 0.1 mL juice, one combined with 1.5 mL of 0.025 M Potassium chloride KCl (pH 1.0) buffer and the other with 0.4 M Sodium acetate (pH 4.5) buffer. Samples were vortexed for 1 minute (Grant Instruments V-32 Multi-Vortex Mixer, Beaver Falls, PA, USA), sonicated for 10 minutes (Branson 3510R-MT Ultrasonic Cleaner, Danbury, CT, USA), and microfuged (Eppendorf 5417R, Framingham, MA, USA) at  $13,000 \times g$  for 10 minutes at  $4^{\circ}\text{C}$ . Supernatants of 150  $\mu\text{L}$  were placed on a 96-well plate with four replicates (wells) per sample and absorbance

red at 510 and 700 nm after 5 minutes using a microplate spectrophotometer (Biotek PowerWave35, Winooski, VT, USA). Total monomeric anthocyanin content (TMAC) was determined by adapting the methods of **Lee et al., 2005** and **Heredia et al., 2006** for a microplate reader where:

$$\text{TMAC (mg pelargonidin-3-glucoside (PG) equivalents/100 g fresh weight) =} \\ [((\text{Abs}_{510} - \text{Abs}_{700 \text{ pH} 1.0}) - (\text{Abs}_{510} - \text{Abs}_{700 \text{ pH} 4.5})) \times \text{A Slope (2.61)} \times \text{DF1} \times \text{DF2} \times \text{molecular} \\ \text{weight (PG)} \times 100] / \text{molecular coefficient (PG)}$$

where:

Abs<sub>510</sub> and Abs<sub>700</sub> represent absorbance values at 510 and 700 nm;

A slope value of 2.61 represents the difference between spectrophotometer and microplate;

Dilution Factor (DF1) is the dilution of the pigment in pH 1.0 buffer (, which was 3 in this study,

Dilution Factor 2 (DF2) represents (solvent volume + sample volume)/sample volume or

$$((0.4+1.2)/0.4)$$

Molecular weight of P3G pigment is 443;

Molecular coefficient of P3G is 156000

### Statistical Analysis

Multivariate assays including correlation, hierarchical cluster, and principal component analysis were conducted to determine the structure of the dataset and explore relationships between the fruit composition parameters, the strawberry genotypes, and the interrelationships between the two. Data was standardized, and all statistical analyses and visualizations were performed using

R (version 4.2.2, Vienna, Austria), Rstudio (version 2022.12.0+353, Boston, Massachusetts), and XLSTAT® (version 2023.1.1, Addinsoft, New York) software.

Correlation analysis using the “ggplot2” R package (**Wickham, 2016**) was computed to determine relationships among the four fruit composition parameters with correlations presenting in Pearson correlation coefficients ( $r$ ). For visualizing relationships among the germplasm, a phylogenetic agglomerative hierarchical cluster dendrogram was generated using the “cluster” (**Maechler et al., 2023**), “dendextend” (**Galili, 2015**), and “factoextra” (**Kassambara & Mundt, 2020**) R packages. Euclidean distance and the Ward.D2 clustering method were used to determine distances between observations and the linkage method. Additionally, the “NbClust” (**Charrad et al., 2014**) R package was used to calculate multiple clustering indices to determine the optimal cluster number.

The distribution and variability of the overall ranges in fruit composition and ranges in each cluster were visualized using box and whisker plots and bell curves generated by “ggplot2”. Multivariate analysis of variance (MANOVA) and Tukey’s post hoc analysis were run to determine significant differences among clusters. Levene’s test for the quality of variances was conducted prior to analysis to ensure the homogeneity of sample population distributions. Lastly, principal component analysis was used to determine and visualize any patterns among fruit composition and the strawberry genotypes, and to determine which composition parameters contributed the most variance within the dataset. XLSTAT software was used to generate eigenvalues, eigenvectors, factor loadings, and all visualizations.

## Results

### Correlation and Hierarchical Clustering

Correlation analysis on pH, soluble solids content, titratable acidity, and total monomeric anthocyanin indicated no significant relationships among the composition parameters (**Table 3.1**). Titratable acidity and pH had the strongest correlation ( $r = -0.36$ ) out of the relationships. Correlations of TMAC to %SSC, Tacid, and pH were  $r = 0.06$ ,  $0.06$ , and  $-0.06$  respectively. Correlations of %SSC with pH and %Tacid had positive correlations of  $r = 0.26$  and  $0.17$ , respectively (**Table 3.1**).

**Table 3.1.** Correlation matrix on pH, soluble solids content (SSC), titratable acidity (Tacid), and total monomeric anthocyanin content (TMAC) of 268 strawberry genotypes from the North Carolina germplasm collection.

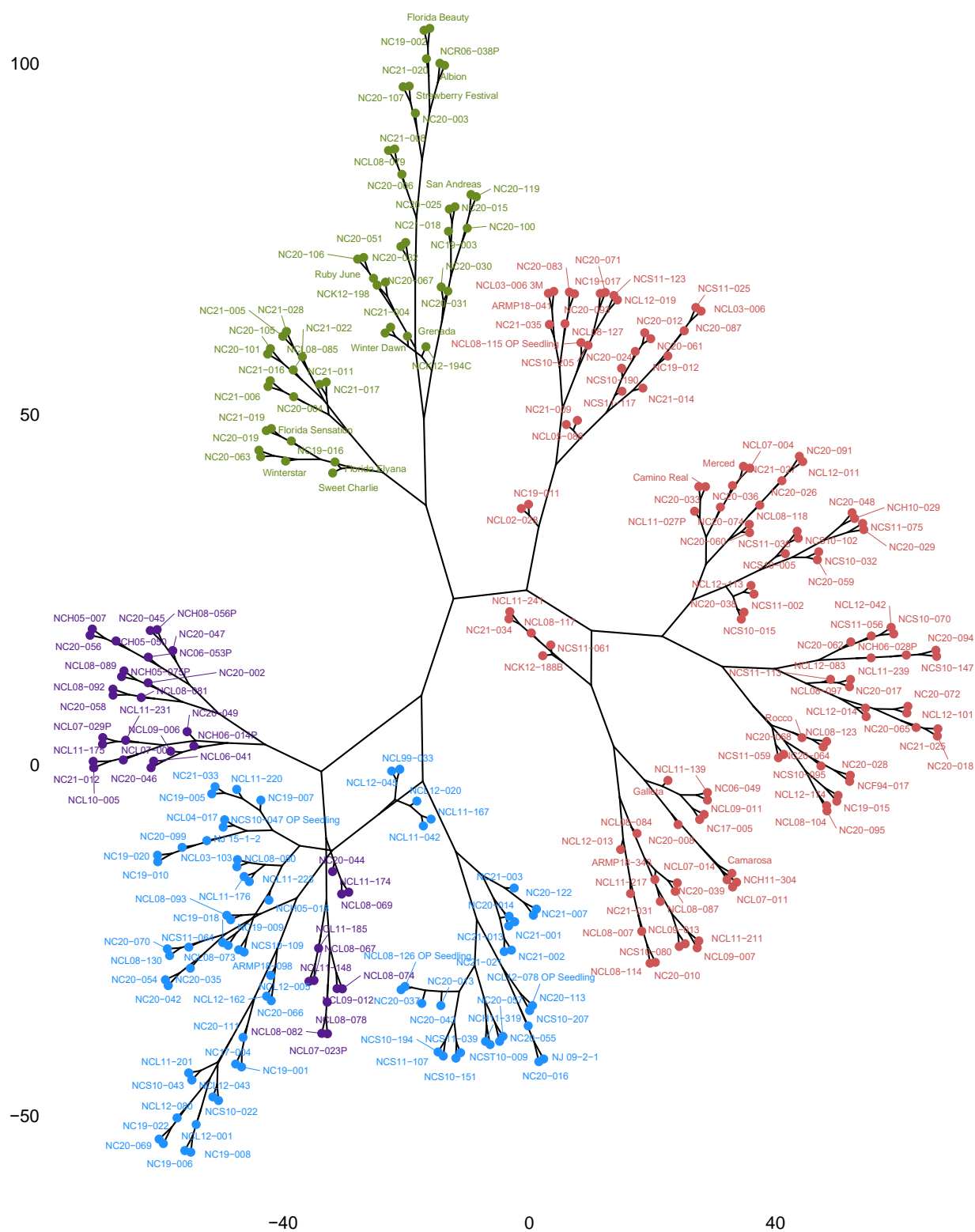
Attribute	pH	%SSC	%Tacid	TMAC
pH	1	-	-	-
SSC	0.26	1	-	-
Tacid	-0.36	0.17	1	-
TMAC	-0.06	0.06	0.06	1

Although correlations were weak, hierarchical clustering analysis showed significant differences in pH, SSC, Tacid, and TMAC ( $<0.0001$ ) with multivariate analysis of variance (MANOVA). Germplasm was separated into four distinct clusters based on overall fruit composition profiles (**Figure 3.2**). Four clusters were chosen as the optimal number based on 30 different clustering indices calculated prior to analysis. Ten of the 30 indices are presented in **Table 3.2**.

**Table 3.2.** Hierarchical clustering indices computed by the ‘NbClust’ package in R statistical programming language. Bolded values indicate the optimal cluster number for each index. Ten out of the 30 indices are represented.

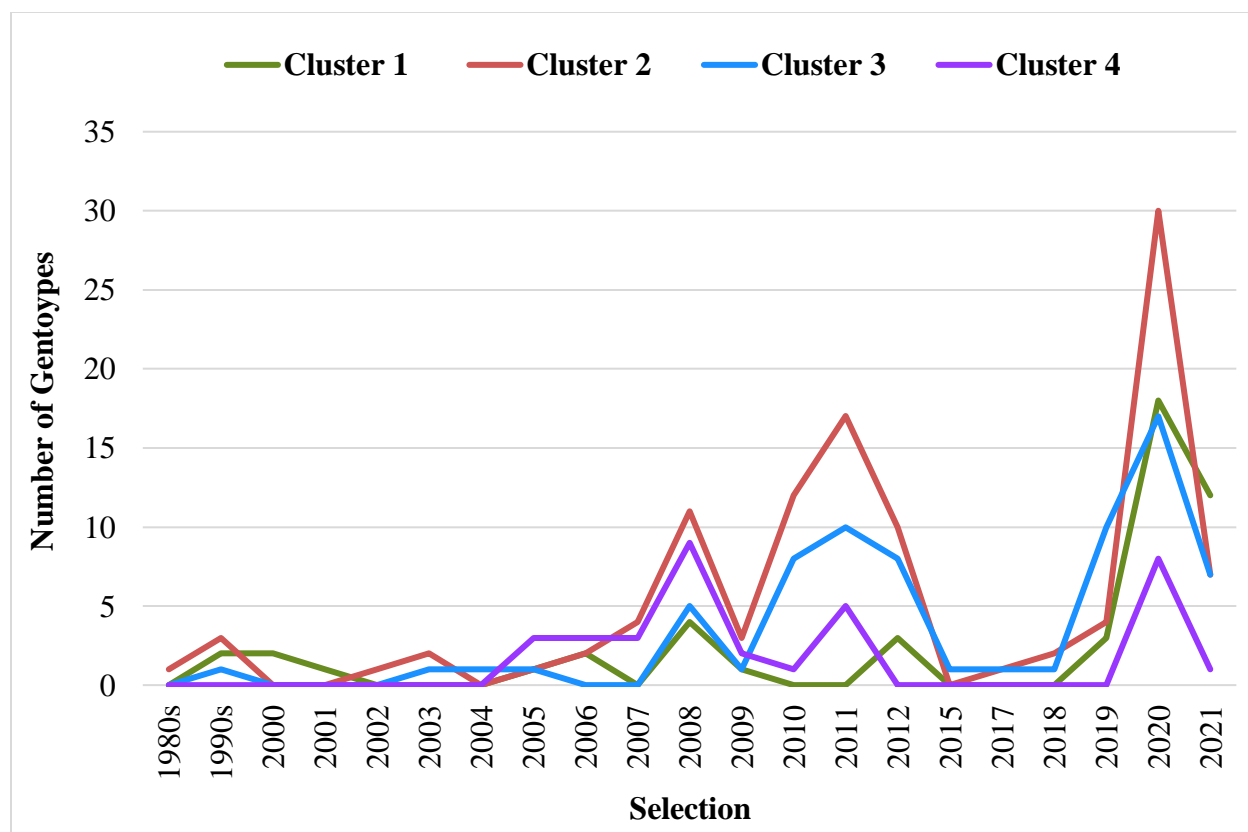
Number of clusters	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Calinski & Harabasz Index	56.295	64.579	<b>66.059</b>	63.245
Cindex Index	0.289	0.268	<b>0.297</b>	0.271
Duda Index	0.683	0.737	<b>0.767</b>	0.721
Elbow Method	3.065	10.519	25.949	<b>49.136</b>
Gap Statistics	<b>0.548</b>	0.351	0.485	0.453
Hartigan Index	-4.017	13.581	<b>14.997</b>	5.446
Marriot Index	6295	6674	<b>7295</b>	7000
Ratkowsky	0.286	0.303	<b>0.327</b>	0.31
Scott	224.846	426.512	556.877	<b>687.527</b>
Silhouette Scores	<b>0.256</b>	0.207	0.182	0.189

Cluster 1 (in green) contained a total of 49 genotypes and had the highest number of commercial genotypes of all the clusters (**Figure 3.2**). Commercial genotypes in this cluster included material from California (‘Albion’, ‘Grenada’, ‘Ruby June’, ‘San Andreas’) and Florida (‘Florida Beauty’, ‘Florida Elyana’, ‘Florida Sensation’, ‘Strawberry Festival’, ‘Sweet Charlie’, ‘Winter Dawn’, ‘Winterstar’) breeding programs. The largest cluster was Cluster 2 (red), which contained 111 genotypes and the rest of the California genotypes, ‘Camarosa’, ‘Camino Real’, and ‘Merced’, as well as the North Carolina genotypes, ‘Galleta’ and ‘Rocco’. Cluster 3 (blue) contained 73 genotypes, including two New Jersey-derived selections, NJ 15-1-2 and NJ 09-2-1. Lastly, the smallest cluster containing the least number of genotypes was Cluster 4 (purple) at 35 genotypes (**Figure 3.2**).



**Figure 3.2.** Agglomerative phylogenetic hierarchical clustering of 268 North Carolina strawberry genotypes. Cluster 1 in green, Cluster 2 in red, Cluster 3 in blue, and Cluster 4 in purple.

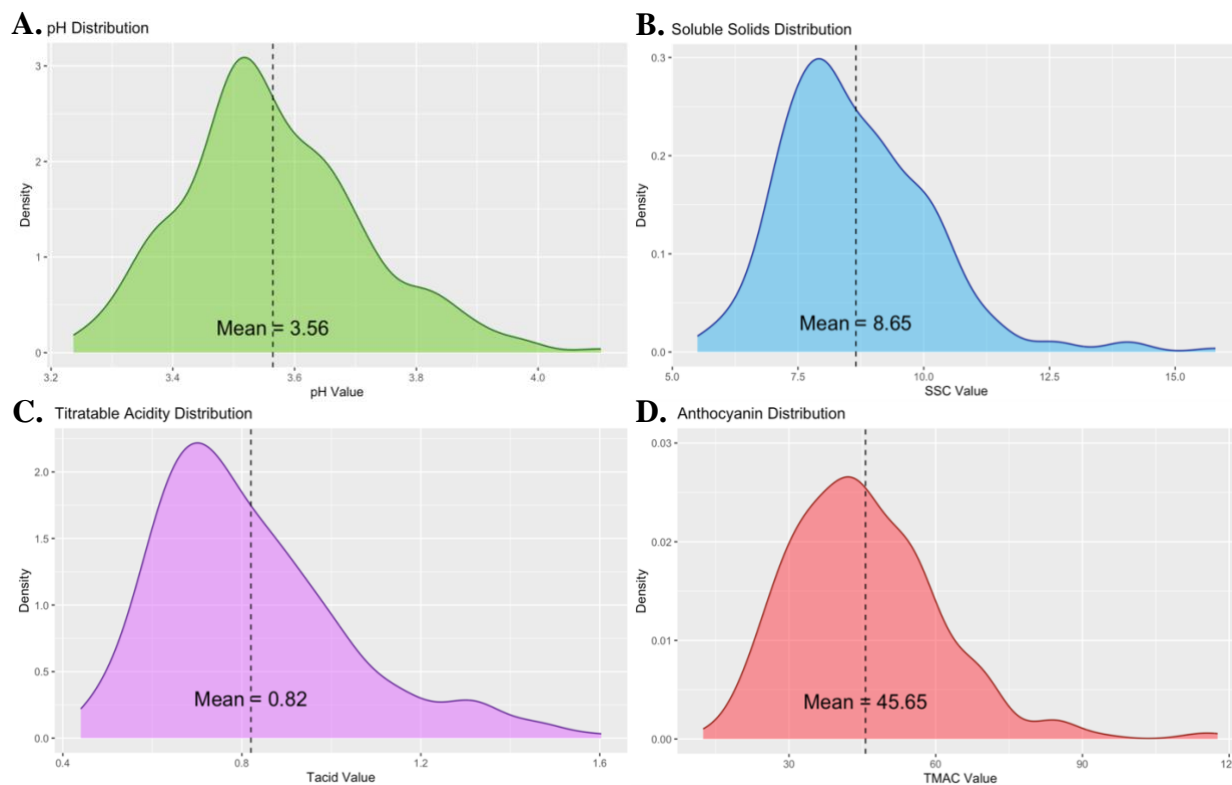
Additionally, the genotypic makeup of the four clusters was examined to determine if any trends were visible based on the year each of the North Carolina genotypes was crossed and selected (**Figure 3.3**). The majority of genotypes (84.7%) in the germplasm collection came from two plant crossing intervals, 2008–2012 and 2019–2021; the most genotypes (73) were selected in 2020. Genotypes selected in the 1980s to 2003 were the fewest in the collection and mostly found in Clusters 1 and 2. No selections before 2005 were included in Cluster 4; this group was largely comprised (74.3%) of genotypes from 2005 to 2008 and in 2020. Genotypes crossed from 2010–2012 were most often found in Clusters 2 and 3. Clusters 1–3 had the most selections in 2020 (18, 30, and 17, respectively), and Cluster 4 had the most in 2008 (9) (**Figure 3.3**).



**Figure 3.3.** Frequency of North Carolina germplasm genotypes in each of the four clusters based on selection year. The years 2013 and 2014 are not included because there were no selections during that period.

### Fruit Composition Analysis within Clusters

The diversity of fruit composition within the germplasm as a whole and in each cluster was analyzed. Strawberry fruit pH ranged from 3.11 to 4.22, with a mean pH of 3.56 (**Figure 3.4A**). Significant pairwise interactions among the four clusters for fruit pH were found, with the exception of the Cluster 1 × Cluster 3 interaction that did not statistically differ in fruit pH (**Figure 3.5A**). Average pH levels in Clusters 1 and 2 were 3.63 and 3.51, respectively. The highest levels were seen in NC21-022 (3.87) and NCS10-205 (3.81). Commercial genotypes in Cluster 1 ranged from pH 3.46 ('Winter Dawn') to 3.82 ('Florida Elyana'), and in Cluster 2 from pH 3.34 ('Rocco') to 3.58 ('Camarosa'). In general, pH was higher in the Florida-derived genotypes: 'Florida Beauty' (3.64), 'Florida Sensation' (3.71), 'Strawberry Festival' (3.57), 'Sweet Charlie' (3.80), and 'Winterstar' (3.77). Short-day California genotypes 'Camino Real', 'Grenada', 'Merced', and 'Ruby June' ranged from 3.48 to 3.59 in juice pH, and California day-neutral 'Albion' and 'San Andreas' had juice pH of 3.51 and 3.53. Fruit from genotypes in Cluster 3 had the highest average pH (3.66) of all clusters, with fruit from genotype NC21-007 producing the highest maximum value (4.10). On the other hand, genotypes in Cluster 4 had the lowest average pH of 3.43, with strawberries from NC20-049 having the highest maximum pH (3.60) in this group. Interestingly, Clusters 1 and 3 had the same minimum pH value (3.38), and both Clusters 2 and 4 had the same minimum pH value (3.24) (**Figure 3.4A**).



**Figure 3.4.** Bell curves of ranges of (A) pH, (B) soluble solids content (SSC), (C) titratable acidity (Tacid), and (D) total monomeric anthocyanin content (TMAC) in North Carolina strawberry germplasm. The means for each parameter are displayed by the dashed lines.

Strawberry soluble solids content was the most variable fruit composition parameter and ranged from 5.1 to 16.4%, with a mean SSC of 8.7% (**Figure 3.4B**). Each of the four clusters statistically differed for %SSC (**Figure 3.5B**). Strawberries in Cluster 1 genotypes contained the lowest average %SSC at 7.0, and Cluster 2 genotypes were the second lowest at 8.1%. Genotypes NC21-022 (5.5%) and NCL12-013 (6.3%) made up the minimum values, and NC20-032 (9.2%) and NCS11-113 (10.0%) were the maximum. However, out of the 16 commercial genotypes, the lowest and highest average %SSC were from California short-day ‘Grenada’ (5.7) and ‘Ruby June’ (9.1) fruit, both of which are grouped in Cluster 1. Also in Cluster 1, Florida germplasm ranged from 6.7 to 8.3%, and the remaining California genotypes ‘Albion’ and ‘San Andreas’, both day-neutral, ranged from 7.3 to 8.3%. In Cluster 2, the North Carolina industry

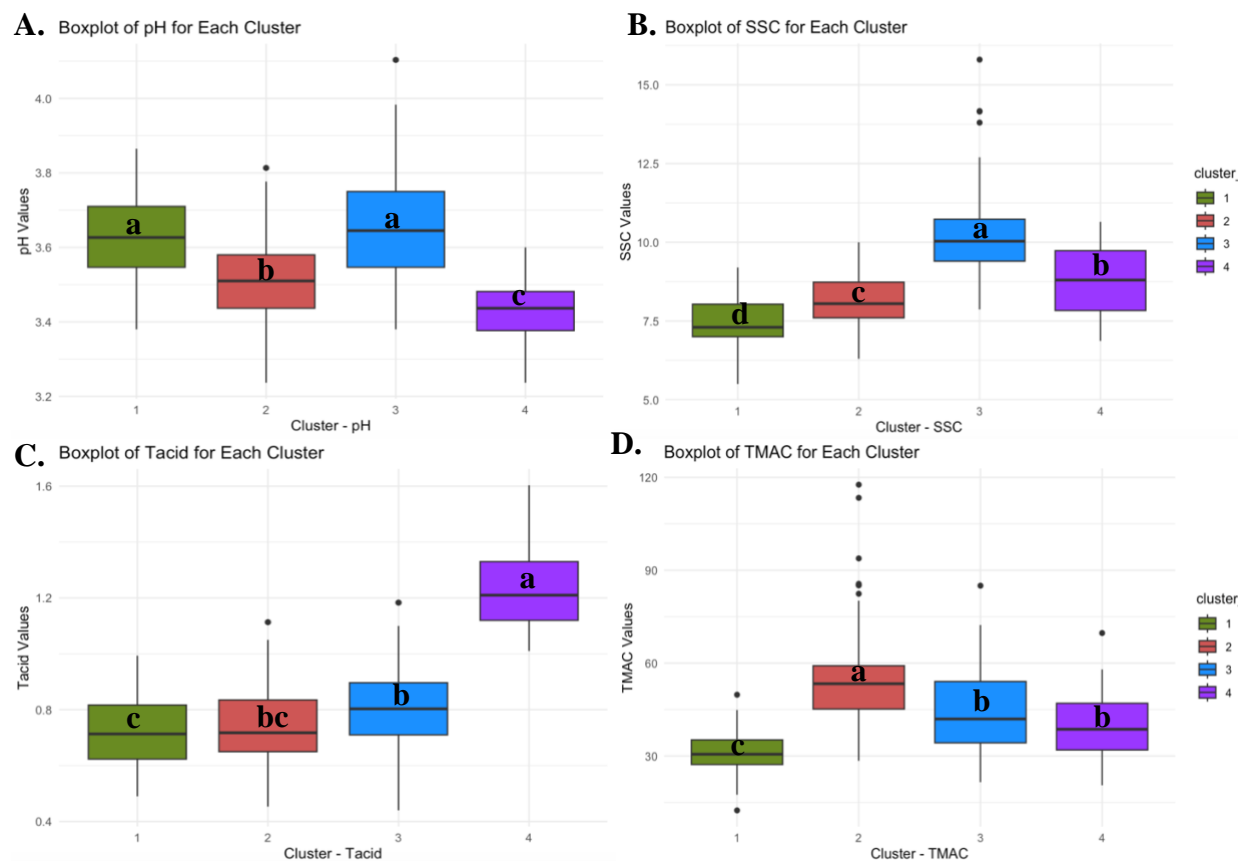
standard ‘Camarosa’ had an SSC of 8.1% compared to the NC-derived genotypes ‘Galleta’ and ‘Rocco’, which had 6.7 and 8.2 %SSC, respectively. Soluble solids content in California ‘Camino Real’ and ‘Merced’ were 7.0 and 7.7%. In addition to pH, fruit from genotypes in Cluster 3 also had the highest average SSC (10.3%) of all four clusters. Fruit from genotypes NCL12-020 and NC21-001 produced the highest maximum (15.8%) and minimum values (7.9%). Cluster 4 genotypes had the second-highest average SSC at 8.7%. NCH05-075P (6.9%) and NCL10-005 (10.7%) made up the minimum and maximum values in this cluster.

Titrate acidity was the least variable fruit composition parameter and ranged from 0.31 to 2.01% with a mean of 0.82% (**Figure 3.4C**). Of the pairwise interactions, Clusters 1, 3, and 4 significantly differed from one another for strawberry Tacid, while Cluster 2 only differed from Cluster 4 (**Figure 3.5C**). Similar to %SSC, strawberries in Cluster 1 genotypes contained the lowest average %Tacid at 0.72, and fruit in Cluster 2 genotypes had the second lowest (0.75). Cluster 1 Tacid ranged from 0.49% (NCL08-085) to 0.99% (NCL08-079), and Cluster 2 ranged from 0.45% (NCS10-005) to 1.60% (NCH06-028P). The commercial genotypes ranged from 0.55 (‘Winterstar’) to 1.05% (‘Galleta’) in Tacid. Titrate acidity values in California genotypes ranged from 0.69–0.94%, with an average of 0.77%. The remaining Florida genotypes, excluding ‘Winterstar’, ranged from 0.59%–0.84%, with an average of 0.72%. Titrate acidity in ‘Rocco’ was 0.79%. Cluster 4 fruit was lowest in pH but significantly greater in average %Tacid (1.21) than fruit in other clusters (0.72-0.81). All genotypes except genotype NCH06-014P (0.63%) had titrate acidity values >1.00%, with 18 genotypes  $\geq$ 1.20%. Minimum values in Clusters 1-3 were 0.44–0.49% while maximum values were 0.99–1.18% respectively. Strawberries in Cluster 3 had the second highest average Tacid.

Total monomeric anthocyanin content ranged from 10.66 to 158.67 mg P3G/100 g FWT, with a mean of 45.65 mg/100 g FWT (**Figure 3.4D**). All cluster interactions were significant for TMAC, except the interaction of Cluster 3  $\times$  Cluster 4 (**Figure 3.5D**). Total anthocyanin was greatest in fruit from Cluster 2 (54.57 mg/100 g FWT) and lowest in fruit from Cluster 1 (31.22 mg/100 g FWT). All but 10 genotypes out of 111 in Cluster 2 had TMAC values  $>40.0$  mg/100 g FWT, with fruit from genotype NC19-011 having the highest average (117.64 mg/100 g FWT). NCH06-028P had the lowest TMAC of 27.99 mg/100 g FWT in this cluster. Conversely, strawberries in Cluster 1 were lowest in TMAC (31.22 mg/100 g FWT) and ranged between 12.45 and 49.81 mg/100 g FWT. Selections NC 20-032 and NC21-028 were the maximum and minimum.

Among fruit breeding programs, Florida germplasm is represented in Cluster 1, and fruit was generally lower in TMAC than those from California and North Carolina. Florida TMAC values ranged from 22.65 ('Florida Elyana') to 37.47 ('Strawberry Festival'). California genotypes in Cluster 1 were 'Albion' (34.15 mg/100 g FWT), 'Grenada' (40.68 mg/100 g FWT), 'Ruby June' (23.36 mg/100 g FWT), and 'San Andreas' (30.00 mg/100 g FWT). In Cluster 2, 'Camarosa', 'Camino Real', and 'Merced' ranged from 42.44 to 54.43 mg/100 g FWT, and TMAC in NC commercial genotypes was 35.58–61.90 mg P3G/100 g FWT in 'Rocco' and 'Galleta', respectively. Fruit from genotypes in Clusters 3 and 4 had similar average TMAC (44.26 and 40.47 mg/100 g FWT) and minimum values (21.57 and 20.56 mg/100 g FWT). However, maximum TMAC was higher for Cluster 3 (85.03 mg/100 g FWT) than in Cluster 4 (69.73

mg/100 g FWT). Selections NCL11-042 and NC06-053P comprised the minimum values, and selections NC19-007 and NCL09-012 were the genotypes highest in TMAC for these clusters.



**Figure 3.5.** Box and whisker plots of each cluster for (A) pH, (B) soluble solids content (SSC), (C) titratable acidity (Tacid), and (D) total monomeric anthocyanin content (TMAC). Lowercase letters indicate significance between clusters ( $p < 0.05$ ) from MANOVA analysis, where clusters with the same letter do not statistically differ.

### Principal Component Analysis

Lastly, principal component analysis (PCA) was performed to assess overall diversity patterns in the 268 genotypes and to identify the fruit composition parameters that contribute to the most variation observed amongst the germplasm. The PCA eigenvalues, eigenvectors, factor loadings, and variable contributions are listed in the results summary table (**Table 3.3**).

Eigenvalues represent the amount of variance captured by each PC component for each of the four composition variables tested, and eigenvectors are the directions and weights of the composition variables in the variable space. The eigenvalues for each PC component are 1.486, 1.109, 0.944, and 0.460 (**Table 3.3**). Additionally, PC1–PC3 components explained 88.49% of the total variance in the data set, with PC1 attributing 37.16%, PC2 at 27.71%, and PC3 contributing 23.61%. For eigenvectors, pH was the only variable that was positive in each of the four principal components, and weights ranged from 0.163 (PC2) to 0.702 (PC1). Soluble solids content ranged from 0.039–0.843 in PCs 1-3 but was negative (-0.358) in PC4. Both titratable acidity and anthocyanin content were negative eigenvectors in PC1 (-0.647 and -0.296), with Tacid also negative in PC3 (-0.177) and TMAC negative in PC2 (-0.340). The remaining eigenvector weights were 0.384 and 0.634 for Tacid and 0.880 and 0.150 for TMAC (**Table 3.3**).

Factor loadings represent the Pearson correlation coefficients between the fruit composition variables and the principal components, with higher absolute values indicating stronger relationships. In the first principal component, pH and Tacid have the greatest correlations of  $r = 0.702$  and  $r = -0.647$  of the variables (**Table 3.3**). Soluble solids content was the weakest in PC1 ( $r = 0.039$ ), but the strongest in PC2 ( $r = 0.843$ ), while pH was the weakest ( $r = 0.163$ ). Principal component 3 had the strongest correlation with TMAC ( $r = 0.880$ ) and weakest relationships with pH and Tacid. Lastly, PC4 was characterized by stronger relationships with Tacid and pH ( $r = 0.635$  and  $0.668$ ), similar to PC1 (**Table 3.3**).

Lastly, the percentage of variance contributed by each of the four composition variables to each principal component is listed (**Table 3.3**). Following patterns from the eigenvectors and factor

loadings, the most important fruit composition variables for PC1 were pH and Tacid at 49.294 and 41.809%, while SSC was most important for PC2 (71.057%), and PC3 was largely comprised of TMAC (77.448%) (**Table 3.3**).

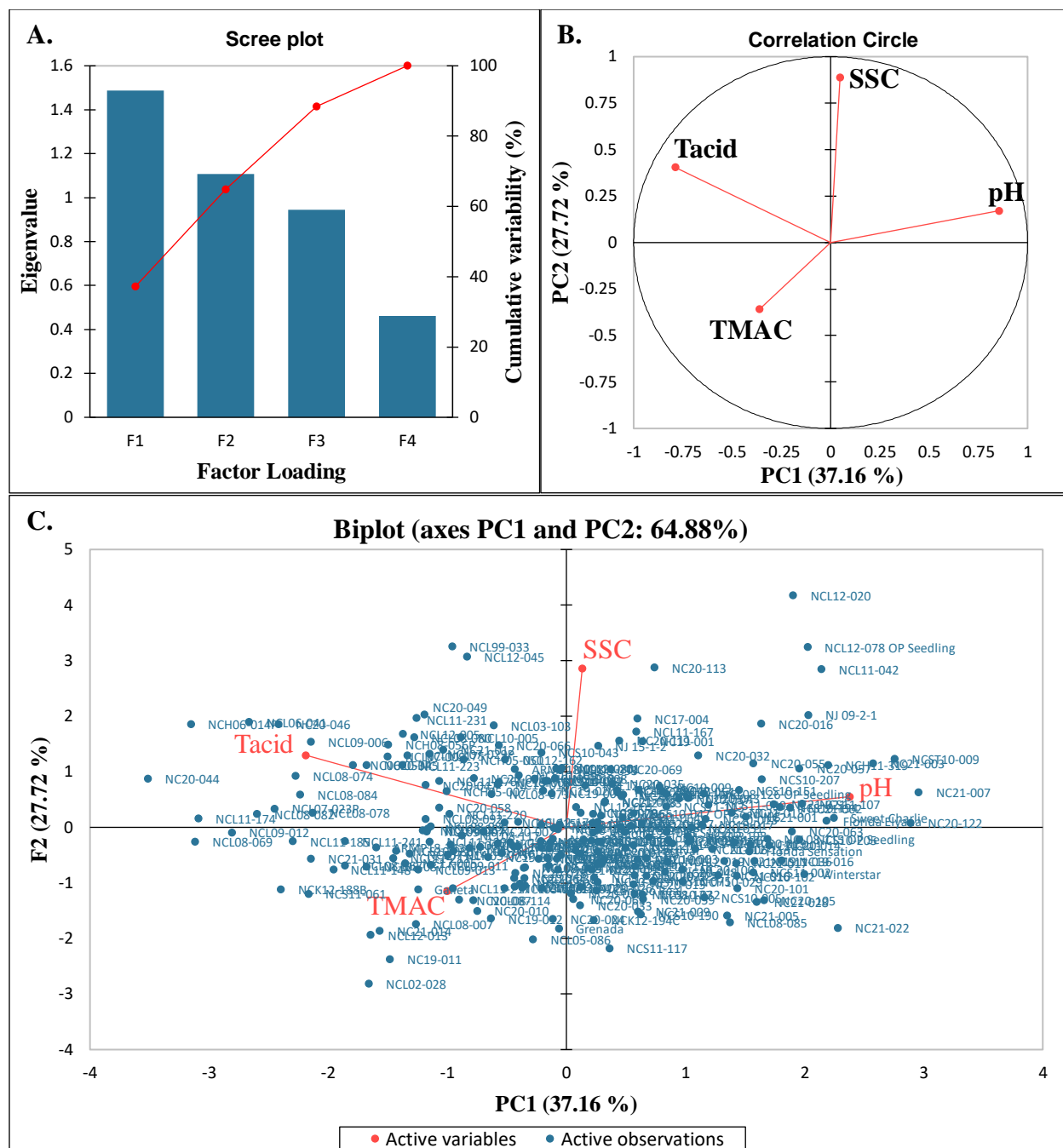
**Table 3.3.** Principal component analysis (PCA) results summary of 268 strawberry genotypes from the North Carolina strawberry breeding program germplasm collection.

<b>Eigenvalues</b>				
	PC1	PC2	PC3	PC4
Eigenvalue	1.486	1.109	0.944	0.460
Variability (%)	37.162	27.716	23.612	11.510
Cumulative %	37.162	64.877	88.490	100.000
<b>Eigenvectors</b>				
	PC1	PC2	PC3	PC4
pH	0.702	0.163	0.185	0.668
SSC	0.039	0.843	0.400	-0.358
Tacid	-0.647	0.384	-0.177	0.635
TMAC	-0.296	-0.340	0.880	0.150
<b>Factor Loadings</b>				
	PC1	PC2	PC3	PC4
pH	0.856	0.172	0.180	0.453
SSC	0.048	0.888	0.389	-0.243
Tacid	-0.788	0.404	-0.172	0.431
TMAC	-0.360	-0.358	0.855	0.102
<b>Contribution of the Variables (%)</b>				
	PC1	PC2	PC3	PC4
pH	49.294	2.655	3.428	44.623
SSC	0.154	71.057	15.989	12.799
Tacid	41.809	14.722	3.135	40.334
TMAC	8.742	11.566	77.448	2.244

To visualize the PCA results, a corresponding scree plot that displays eigenvalues, a variable correlation circle, and a biplot were generated that depict the relationships between the fruit composition variables and genotypes and the principal components (**Figure 3.6**). The scree plot indicates that all four principal components are required to capture 100% of the variation within the germplasm population but displays an “elbow” at the third PC (**Figure 3.6A**). This slope

change is traditionally a guideline for selecting the appropriate number of principal components.

Although three principal components best fit this data, a 3-dimensional space is required to visualize the components. Therefore, only PC1 and PC2 will be displayed.



**Figure 3.6.** Principal component analysis showing (A) PCA scree plot, (B) PCA fruit composition variable correlation circle, and (C) PCA biplot of fruit composition variables and genotypes.

The correlation circle projects the fruit composition variables (pH, SSC, Tacid, and TMAC) in the PCA space (**Figure 3.6B**). Variables close together dictate a strong positive correlation, while orthogonal relationships ( $90^\circ$ ) indicate no correlation. Variables opposite of each other on

the plot are negatively correlated. Titratable acidity and pH are opposite, indicating a negative relationship that can be confirmed by the correlation matrix value ( $r = -0.36$ ) (**Table 3.1**).

Additionally, the distance of a variable from the plot's center is an important factor in correlation circles. Closer variables require cautionary interpretation as some information could be carried on other axes. Total monomeric anthocyanin content is the closest variable to the center and does not quite follow the previously calculated coefficients, suggesting weak correlations ( $r = 0.06$ ,  $0.06$ , and  $-0.06$ ) (**Table 3.1**). Therefore, the interpretation of TMAC by the correlation circle alone is not entirely accurate and must be accompanied by the PCA results (**Table 3.3**), which show that TMAC is most associated with PC3 that is not pictured. The remaining variable relationships are nearly orthogonal, visually representing the weak correlations consistent with the calculated Pearson coefficients  $r = 0.06$ – $0.26$  (**Figure 3.6B**; **Table 3.1**).

The last figure, the PCA biplot, shows the simultaneous representation of the composition variables with all 268 genotypes (**Figure 3.6C**). Genotypes tend to aggregate towards the variable that most accurately characterizes their profile, resulting in genotypes with similar profiles being in close proximity. Examples include genotypes NC20-044, NCL11-174, NCL08-074, NCL09-006, and NCH-014P that are grouped near Tacid and are all in Cluster 4 (purple) that was highest in Tacid compared to the other clusters (**Figure 3.5C**; **Figure 3.6C**). However, most genotypes are concentrated around the plot's center, particularly heavy in the bottom right quadrant, suggesting that overall, the majority of the germplasm exhibited a relatively balanced fruit composition profile without extreme values in the variables (**Figure 3.6C**).

## Discussion

This one-year study revealed valuable preliminary insights into the fruit composition variability in the North Carolina strawberry breeding collection. Utilizing multivariate analysis methods including correlation analysis, multivariate analysis of variance (MANOVA), hierarchical clustering, and principal component analysis (PCA) allowed for the thorough exploration of trends, patterns, and correlations within the germplasm and among fruit composition variables.

### Strawberry Fruit Composition Significance

Average juice pH spanned from 3.24 to 4.10, SSC ranged from 5.5% to 15.8%, Tacid ranged from 0.44% to 1.60%, and TMAC was 12.5–117.6 mg pelargonidin 3-glucoside (P3G)/100 g fresh weight (**Figure 3.4**). These values align with those typically observed in ripe strawberry fruit: pH 3.18–4.10, SSC 4.6–11.9%, Tacid 0.50–1.87%, and TMAC 55–145 mg P3G/100 g FWT (**Kader, 1991**). Although no significant correlations were observed in fruit composition parameters within the germplasm when considered collectively, significant differences ( $p < 0.0001$ ) in pH, soluble solids content (SSC), titratable acidity (Tacid), and monomeric anthocyanin (TMAC) content were evident between four hierarchical clusters (**Figure 3.5**). An important note is that hierarchical clustering algorithms automatically group observations based on similarity rather than statistical significance (**Sebastiani & Perls, 2016**). Therefore, dendrogram clusters may or may not be significant depending on the nature of the data set. However, MANOVA and Tukey's HSD did indicate significant differences within the fruit composition variables in this study.

The weak correlations, ranging from  $r = -0.06$  to  $r = -0.36$ , observed among the four fruit composition parameters were further investigated (**Table 3.1**). In the present study, strawberry fruit pH and Tacid presented a correlation of  $r = -0.36$ , which aligns with work by **Lewers et al. (2020)** that found a similar relationship ( $r = -0.30$ ) across 10 strawberry genotypes. The pH of a sample measures the concentration of free hydrogen ions, while titratable acidity measures the total acid concentration or the total amount of hydrogen ions. Despite the commonality in both assays in assessing acidity, no direct and predictable relationship between pH and Tacid has been reported in several commodities including grape juice and wine (*Acidity and pH*), apple (*Relationship between pH and Titratable acidity*), and tomato (**Paulston & Stevens, 1974**). Tacid provides a simple estimate of the total acid content within a sample, but many acids cannot be distinguished through titration methods; therefore, rendering Tacid as a weak predictor of pH (**Tyl & Sadler, 2017**). However, a general relationship commonly exists in that pH tends to decrease while Tacid increases with this relationship being heavily affected by genetic and environmental factors. Titratable acidity tends to be a better predictor of acid contribution to flavor than pH illustrating why soluble solids content and Tacid ratios are often reported when describing flavor of a commodity (**Tyl & Sadler, 2017**). Strawberry SSC and Tacid were also weakly positively correlated ( $r = 0.17$ ) within fruit from the 268 strawberry genotypes in this study, similar to correlation analysis results by **Chiomento et al. 2021** who also found a weak positive correlation ( $r = 0.06$ ).

### Clustering of Commercial Strawberry Genotypes

The 16 commercial genotypes in this study primarily split based on strawberry type (short-day vs. day-neutral) and breeding program from which they were developed. A prior study of 91 commercial strawberry genotypes within the Polish core germplasm collection found that ‘Camarosa’ and ‘Sweet Charlie’ separated into different clusters (Siezko et al., 2015). Here, ‘Camarosa’ and ‘Sweet Charlie’ were also in separate clusters (Figure 3.2). All seven Florida-derived genotypes included in this study grouped together (Cluster 1), along with the two California day-neutral genotypes and ‘Grenada’ and ‘Ruby June’. Furthermore, the two North Carolina-derived genotypes (‘Galleta’ and ‘Rocco’) were clustered together in Cluster 2, and the two New Jersey-derived genotypes (NJ 09-2-1 and NJ 15-1-2) were both present in Cluster 3. Interestingly, the short-day genotypes ‘Camarosa’ and ‘Camino Real’ bred in California and commonly grown in North Carolina as industry standards, were grouped alongside the NC genotypes in Cluster 2. ‘Merced’ which is not grown widely in NC, was also included in this group.

Our findings contradict those of Chiomento et al. (2021), who evaluated fruit quality and horticultural production of nine greenhouse-grown strawberry genotypes. In this study, ‘Camarosa’, ‘Camino Real’, and ‘Merced’ were each in different clusters. However, ‘Camarosa’ and ‘Merced’ fruit were characterized and grouped based on their dominant production traits (highest yield and largest berries for ‘Merced’ and greatest number of fruits per plant for ‘Camarosa’) rather than fruit composition traits, such as total sugar, acid, anthocyanin, antioxidant, flavonoid, and phenolic content did not statistically differ between the two (Chiomento et al. 2021). ‘Camino Real’ did not differ in production-based traits compared to

the ‘Camarosa’ and ‘Merced’ but was significantly lower in phenolic content (**Chiomento et al. 2021**). In our study, only fruit composition traits (pH, SSC, Tacid, and TMAC) were evaluated and could explain why the genotypes clustered together.

#### Strawberry Soluble Solids Content and Titratable Acidity

Of the 268 genotypes analyzed, 92.9% had soluble solids content  $\geq 7.0\%$ , while 65.3% (175 genotypes) were  $\geq 8.0\%$ , and 36.9% (99 genotypes) had an SSC value of at least 9.0%. Mean SSC of the germplasm (8.7%) fell within the new SSC minimum range of 8.0–9.0% (**Figure 3.4**). ‘Galleta’, ‘Grenada’, and ‘Winter Dawn’ were the only commercial genotypes lower than 7.0% in SSC, and genotypes ‘Albion’, ‘Camarosa’, ‘Florida Elyana’, ‘Rocco’, ‘Ruby June’, and ‘Sweet Charlie’ were  $\geq 8.0\%$ . Both New Jersey advanced genotype lines had high SSC (11.2 and 11.3%). For Tacid, 180 genotypes (67.2%) had a value of at least 0.70%, 123 genotypes (45.9%)  $\geq 0.80\%$ , and 49 genotypes (18.3%) were 1.0% or greater. ‘Galleta’ was the only commercial genotype greater than 1.0 (1.05%) and ‘Sweet Charlie’ and ‘Winterstar’ were 0.55 and 0.59%, respectively. In this study, ‘Florida Beauty’, ‘Florida Sensation’, and ‘Winterstar’ fruit had SSC contents of 7.1–7.7% and Tacid contents of 0.55–0.79%. In cultivar releases of these genotypes, SSC (5.3–10.3%) and Tacid (0.49–0.97%) varied depending on the year (**Whitaker et al., 2012; Whitaker et al., 2017**). This demonstrates the strong environmental influence on the variability of strawberry fruit composition. In our study, the fruit of greenhouse-grown ‘Florida Elyana’ and ‘Strawberry Festival’ had %SSC of 8.0 and 7.5 and %Tacid of 0.64 and 0.84. These values are similar to those reported by **Chandler et al. 2009** for these genotypes in a 2006-2007 variety trial (%SSC of 8.5 and 7.6 and %Tacid values of 0.72 and 0.78). **Brym et al (2022)** reported %SSC values of 7.2 to 8.0 for ‘Florida Beauty’, ‘Florida Sensation’, ‘Strawberry Festival’, and

‘Winterstar’ fruit and %Tacid was 0.65 and 0.70, for all except ‘Winterstar’, which lacked Tacid measurements. Although values are relatively similar across the five studies, it is important to note that fruit from the four Florida studies came from field plantings.

Although an ideal SSC/Tacid ratio in strawberry fruit has yet to be established, using desired %SSC and %Tacid values of 8.0 and 0.80 previously described by **Fan et al. 2021b and Kubota Lab, 2015**, a hypothetical ideal standard SSC/Tacid ratio of 10 is generated. Of the 268 total genotypes, 63.4% (170 genotypes) met this ratio, with 23 genotypes being greater than 15.0, and four with a ratio greater than 20.0. Of the entire germplasm collection, 40 genotypes met all three requirements of %SSC  $\geq$ 8.0, %Tacid  $\geq$ 0.80, and SSC/Tacid  $\geq$ 10.0, while 14 genotypes met none. Additional germplasm also had quality traits that may still be perceived as “good flavor”. As such, 86 genotypes achieved the desired %SSC, and the ratio requirements but the %Tacid was lower than 0.80; 44 genotypes met only the SSC/Tacid ratio standard of 10.0 but not %SSC and %Tacid values, and 49 genotypes had %SSC  $\geq$ 8.0 and %Tacid  $\geq$ 0.80 but had an SSC/Tacid ratio  $<$ 10.0.

The relative value of soluble solids, titratable acidity, and SSC/Tacid ratios to consumer preference and sensory panels has been re-evaluated recently. **Lewers et al. (2020)** utilized sensory taste panels and a consumer preference panel of 10 commercial strawberry cultivars. In this study, SSC/Tacid ratios ranged from 7.1 to 11.6; the four highest-ranked genotypes in flavor (‘Albion’, ‘Allstar’, ‘Earliglow’, and ‘Flavorfest’) had an average SSC/Tacid  $\geq$  10.0 and the lowest-ranked genotype (‘Seascape’) had a ratio of 7.1. Here, Albion had an SSC/Tacid of 10.2 and in the present study a ratio of 10.8. However, statistical correlations between the SSC/Tacid

ratio and consumer likeness were not done. Interestingly, ‘Earliglow’ and ‘Flavorfest’ recorded the same %SSC (7.6), %Tacid (0.72), and SSC/Tacid (10.6) values but ‘Flavorfest’ was rated significantly higher than Earliglow in overall sweetness and flavor (**Lewers et al., 2020**).

### Strawberry Anthocyanin Content

Much like SSC and Tacid, definitive standards and thresholds for anthocyanin (TMAC) have yet to be defined in strawberry fruit, primarily because of different opinions on what constitutes “low” vs “high” anthocyanin levels. These perspectives are driven by the needs of producers and consumers and include numerous considerations such as direct markets, shipping and distribution through wholesale channels, utilization in industrial processes (jam, beverage, pharmaceutical, cosmetic, etc.), or other uses. Previous work in (**Haynes, 2024**) has shown that fruit with  $\geq 40.0$  mg pelargonidin 3-glucoside (P3G)/100 g fresh weight is notably visually darker than fruit with less than 30 mg/100 g. Most of the germplasm collection exceeded 40 mg/100g of anthocyanin and was much visibly darker than what is likely desired for commercial markets. In total, TMAC levels in 10.8% (29 genotypes) of the germplasm were  $< 30.0$  mg/100 g FWT and 61.2% (164 genotypes) were  $\geq 40.0$  mg/100 g FWT. Of the 164 genotypes, 56.1% (92 genotypes) were greater than 50.0 mg/100 g FWT and 24.4% (40 genotypes) exceeded 60.0 mg/100 g FWT.

‘Florida Elyana’, ‘Florida Sensation’, ‘Ruby June’, and ‘Sweet Charlie’ fruit were considered low in TMAC (22.65–24.13 mg/100 g FWT), and fruit from ‘Albion’, ‘Florida Beauty’, ‘Rocco’, ‘San Andreas’, ‘Strawberry Festival’, ‘Winterstar’, and ‘Winter Dawn’ had medium TMAC content (30.0–37.47 mg/100 g FWT). ‘Grenada’, ‘Merced’, and ‘Camino Real’ fruit were high in TMAC (40.68–44.12 mg/100 g FWT) and fruit from ‘Camarosa’ and ‘Galleta’ were considered

very high ( $\geq 50.0$  mg/100 g FWT). Values were comparable to those previously reported for commercial strawberry genotypes ‘Albion’ (19.7–55.3), ‘Camarosa’ (27.6–53.6), ‘Camino Real’ (15.7–59.8), ‘Merced’ (18.7), ‘Rocco’ (22.2–34.0), ‘Ruby June’ (15.2–37.7), ‘San Andreas’ (18.2–21.2), ‘Strawberry Festival’ (18.0–54.1), ‘Sweet Charlie’ (23.0), ‘Winterstar’ (11.0–24.0), and ‘Winter Dawn’ (15.0–29.0) (Cayo et al., 2016; Chaves et al., 2017; Fredericks et al., 2012; Kannaujia et al., 2014; Nunes et al., 2005; Patel et al., 2023; Perkins-Veazie et al., 2016; Vinson et al., 2022).

### Genotype Separation and Differentiation through Principal Component Analysis

Principal component analysis also effectively summarized major trends within fruit composition diversity seen in the germplasm. The first three PC components accounted for a substantial 88.49% of the total variance (Table 3.3). While strawberry juice pH and Tacid had a narrower range of values in the germplasm compared to the other fruit composition parameters, they significantly contributed to PC1 (49.294 and 41.809%, respectively) that captures the most variability within a dataset (Table 3.3). From this, it can be inferred that large variation, especially extreme (low vs. high) values within these two parameters have a more pronounced effect on distinguishing genotype composition profiles and are the primary driving force for germplasm separation and differentiation. In the PCA biplot, the majority of genotypes are in close proximity to the pH vector, further emphasizing the strong correlation pH has with PC1 and the fact that the germplasm is separating primarily based on pH (Figure 2.3). Genotypes with similar pH are grouped together. Those not near pH are grouped near the fruit composition variable that has a bigger influence on and best characterizes their profile instead, potentially outweighing the influence of pH.

## Conclusion

Fruit composition variability was determined in strawberry fruit from 252 advanced selections from the North Carolina strawberry breeding program and in 16 commercial cultivars over a one-year period. Four multivariate statistical methods were used to classify and characterize genotype diversity, relationships, and overall trends in fruit composition. Four cluster groups were found among genotypes using cluster analysis based on overall fruit composition profiles. The majority of commercial genotypes were found in Cluster 1, which was distinguished by low %SSC, %Tacid, and %TMAC. In contrast, Cluster 2, containing 41.4% of germplasm, had fruit highest in %TMAC. Fruit from genotypes in Cluster 3 had high pH and %SSC, and the smallest group (Cluster 4) had 13.1% of the germplasm and had low pH and higher %Tacid. Furthermore, regional strawberry breeding programs were found to group together. The Florida germplasm were grouped in Cluster 1, North Carolina commercial genotypes in Cluster 2, and two advanced New Jersey genotypic lines were found in Cluster 3. California day-neutrals were found in Cluster 1, while short-day genotypes were split between the first two clusters. Additionally, the first two principal components (PC1 and PC2) collectively captured a significant portion of the variance, explaining 64.88%, with PC1 attributing to 37.16% and PC2 to 27.71%. Both pH and titratable acidity largely contributed to PC1, while soluble solids content was most important in PC2. Additionally, a third PC component (PC3) explained 23.61% of the total variance and was most influenced by total monomeric anthocyanin content (TMAC) (77.448%). This was the first comprehensive study to evaluate these chemical components, including TMAC, in wide set of germplasm. These insights regarding the relationships and interrelationships among the fruit composition variables and the genotypes provide valuable information on the structure of the

North Carolina germplasm collection and will be used to help guide future breeding decisions and strategies.

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## CHAPTER 4

### Evaluation of Colorimetry Methods as a Tool for the Estimation of Strawberry Fruit Anthocyanin and Total Phenolic Content

#### Abstract

The consumer interest in increasing phytochemical intake through dietary consumption of fresh fruits and vegetables has incentivized breeding programs towards developing cultivars with enhanced phytochemical content. Strawberries are among the most widely consumed fruits globally, highly regarded for their attractive appearance, sweet flavor, phytochemicals and nutrients. Many phytochemical assays require rapid analysis in order to make them feasible for annual screening of hundreds of genotypes selected in breeding programs. In this study, strawberry fruit representing 31 commercial and advanced genotypic lines from the Florida and North Carolina breeding programs were evaluated for 21 physicochemical, phytochemical, and color variables. Utilizing correlation and regression analyses, both objective CIE  $L^*a^*b^*$  measurements and subjective visual color ratings were evaluated to determine the predictability of the variables in accurately assessing strawberry anthocyanin content, pigment profiles, and total phenolic content. The colorimetric value lightness ( $L^*$ ) was the strongest predictor of anthocyanin content, with a correlation of  $R^2 = 0.9132$ . Hue ( $h^*$ ) and the red-green color axis ( $a^*$ ) were the next strongest variables, with  $R^2$  values of 0.7886 and 0.7067, respectively. Additionally, 74.04% of the variability seen within anthocyanin content could be explained by subjective color ratings. Critically, both the CIE  $L^*a^*b^*$  and subjective color variables were reliable predictors of anthocyanin content only when a full spectrum of strawberry color (white-dark red fruit) was used in the regression models. Additionally, color values were best correlated

with anthocyanin contents determined by high-performance liquid chromatography. Colorimetric values were poorly correlated with total phenolic content ( $r = -0.16$  to  $r = 0.32$ ). Pelargonidin 3-glucoside and pelargonidin 3-rutinoside, the primary anthocyanins in strawberry fruit, were most strongly correlated with  $L^*$  values ( $r = -0.80$  and  $-0.76$ , respectively). The minor cyanidin 3-glucoside and pelargonidin 3-(6''-malonylglucoside) anthocyanins were moderately correlated to hue ( $r = -0.48$  and  $0.31$ , respectively). These results offer a simplified and non-destructive means to estimate total anthocyanin content, and potentially the major anthocyanin pigments, in strawberry germplasm.

## **Introduction**

### Consumer Interest in Health-promoting Compounds

Results from research on phytochemicals and other health-promoting compounds in fresh produce have become more accessible and widespread to the public, leading to increased consumer awareness and interest in dietary health. Consumers have placed greater emphasis on fruit nutritional quality and wellness. This shift has encouraged berry fruit breeding programs to consider selecting and breeding for genotypes with enhanced nutritional profiles (**Dzhanfezova et al., 2020; Zhao et al., 2023**), such as increased polyphenolic compounds, vitamin C, and ellagic acid.

Strawberries (*Fragaria xananassa*) are economically important fruit crops and are highly desired by consumers. The fruit are light to dark red in color, sweet and with a distinctive fruity flavor, and are an excellent dietary source of vitamin C (60 mg/100 g). Strawberry fruit consumption in the United States has increased steadily over the last few decades, from 0.91 in 1980 to 3.63 kg

per person in 2013 (USDA), driven by year-round availability and greater consumer demand (Samtani et al., 2019). After a short decline in overall consumption, the highest per capita consumption per person was reached in 2020 at 3.9 kg (*Per capita consumption of fresh strawberries*; Yet et al., 2020), with 2.9 kg in fresh strawberries. In 2020, strawberries accounted for 13% of the total fruit production value in the United States, generating over \$2.2 billion in farm gate revenue (Yeh et al., 2023). In addition to vitamin C, fiber, minerals, and micronutrients, strawberry fruit are a rich source of phenolic and anthocyanin compounds that contribute to the fruit's bioactivity (Tulipani et al., 2008; Voca et al., 2014; Wang & Lewers, 2007).

#### Anthocyanin and Non-anthocyanin Phenolic Compounds

Phenolic compounds are secondary metabolites present in many plants that have multiple functions in preventing herbivory and pathogen infection, as well as providing sensory and nutritional quality in fruits and vegetables (Guiné et al., 2020). In strawberry, phenolic compounds include anthocyanins, flavonoids (catechin, quercetin and kaempferol), hydroxycinnamic acid, and ellagic acid derivatives (ellagitannins and ellagic acid glycosides) (Aaby et al., 2007; Aaby et al., 2012). Among these, anthocyanins represent the largest class of phenolic compounds, contributing up to 40% of the total phenolic content in some genotypes, and are primarily responsible for strawberry visual color (Aaby et al., 2012). Numerous anthocyanins have been identified in strawberry fruits; the primary pigments contributing 60-90% of the total anthocyanins are pelargonidin 3-glucoside (P3G), followed by pelargonidin 3-rutinoside (P3R). Cyanidin 3-glucoside (C3G), pelargonidin 3-(6''-malonylglucoside) and pelargonidin acetylglucoside have also been identified (Dzhanfezova et al., 2020; Lopes da

**Silva et al., 2007; van de Velde et al 2019).** Several preharvest factors influence anthocyanin and phenolic content in strawberry fruit, such as ripeness, temperature, genotype, harvest date, and location (**Aaby et al., 2012; Arend et al., 2017; Câmara et al., 2022; Zhang et al., 2022).**

And what about postharvest factors

#### Anthocyanin and Phenolic Compounds Relation to Color

The presence and amount of anthocyanin in strawberry is strongly related to fruit color (**Timberlake & Bridle, 1982**). Strawberry external visual color is diverse and can range from white to dark purple-red. In strawberry fruit, pelargonidin is known to provide an orange to bright red color, while cyanidin imparts a darker red to magenta color. Generally, greater fruit darkness is associated with higher levels of anthocyanin (**Ponder et al., 2021**). Therefore, breeding strawberry genotypes to contain higher amounts of anthocyanin will most often yield darker-colored fruits.

However, this presents a dilemma as consumers differ in their sensory preferences concerning optimal strawberry color. A consumer's initial impression of fruit quality is dependent on appearance (fruit color, shape, and size), with repeat purchases influenced by sweetness and overall flavor (**Voća et al., 2014**). Some consumers perceive light-red scarlet berries as fresher and potentially longer-lasting, while others favor darker red fruit, associating them with increased ripeness, sweetness, and juiciness (**Dzhanfezova et al., 2020**). Commercially available strawberries are often lighter in color than what is desired for direct markets.

### Anthocyanin and Phenolic Compounds in Wild and Modern *Fragaria* Species

Breeder selection for light red strawberry genotypes to meet commercial standards has inadvertently led to changes in the total anthocyanin content and anthocyanin profiles of strawberries over time. Fruit from wild strawberry species, including *Fragaria chiloensis*, *Fragaria virginiana*, and *Fragaria vesca*, are rich in cyanidin anthocyanins (Cheel et al., 2007; Peñarrieta et al., 2009; Simirgiotis et al., 2009; Wang & Lewers, 2007). In contrast, while modern-day cultivated octoploid strawberries (*Fragaria xananassa* Duch) are dominant in pelargonidin, comprising up to 70–95% of the total anthocyanin content (Sirijan et al., 2020; Zhao et al., 2021). In a study conducted in Pichihuillinco, Chile, anthocyanin and phenolic composition of ripe strawberry fruit from wild (*Fragaria vesca*), cultivated (*Fragaria xananassa* Duch cv. Chandler), wild white Chilean (*Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis*), and wild red Chilean (*Fragaria chiloensis* ssp. *chiloensis* f. *patagonica*) strawberry species were evaluated (Cheel et al., 2007). Whole fruits were frozen at -80°C, homogenized into strawberry puree, and filtered to determine total monomeric anthocyanin content (TMAC), total phenolic content (TPC), and anthocyanin profiles using UV/Vis spectrophotometry and high-performance liquid chromatography (HPLC) paired with diode array detection (DAD). Fruit from the wild *F. vesca* species contained the highest TPC at 268.1 mg gallic acid equivalents (GAE)/100g fwt, while the white Chilean fruit contained the least at 106.3 mg GAE/100g (Cheel et al., 2007). Similarly, the white strawberries also had the lowest TMAC (6.65 mg P3G/100g fwt), while ‘Chandler’ fruit was the highest (52.0 mg P3G/100g). However, no strong correlations between TMAC and TPC were found. Furthermore, the anthocyanin profile of ‘Chandler’ fruit consisted of 95% pelargonidin and 5% cyanidin, while the red Chilean fruit contained higher cyanidin at 37.4% (Cheel et al., 2007). Interestingly, fruit from both the wild and white Chilean species did

not contain any pelargonidin. Wild *F. vesca* fruit contained only cyanidin derivatives, while *F. chiloensis* ssp. *chiloensis* f. *chiloensis* fruit were comprised of 53.5% cyanidin, with the remaining amount possibly being petunidin or malvidin (Cheel et al., 2007).

A similar study by Simirgiotis et al. 2009 taking place in the same region also examined cultivated (*Fragaria xananassa* Duch cv. Chandler), wild white Chilean (*Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis*), and wild red Chilean (*Fragaria chiloensis* ssp. *chiloensis* f. *patagonica*) strawberry species and found cyanidin 3-glucoside (C3G) and pelargonidin 3-glucoside (P3G) in the anthocyanin profiles of all three. ‘Cyanidin 3-glucoside was dominant in white fruit ranging from 1.15 to 4.30 mg/100g, with P3G concentrations of 0.40, 16.80, and 20.40 mg/100g in white Chilean, red Chilean, and ‘Chandler’ fruit, respectively (Simirgiotis et al. 2009). Peñarrieta et al. 2009 also identified both C3G and P3G anthocyanins in the profiles of *F. vesca* strawberry puree samples from ripe fruit harvested in Patata and Quirambaya, Bolivia. Consistent with the two previous studies, C3G content was substantially higher than that of P3G, presenting a 20:1 ratio (Peñarrieta et al. 2009).

In a final study by Wang and Lewers, 2007 that evaluated phytochemical content of fruit puree from three strawberry species: *Fragaria chiloensis* (wild), *Fragaria virginiana* (wild), and *Fragaria xananassa* (cultivated) grown in Beltsville, Maryland, a similar pattern was found. *F. virginiana* strawberries had the greatest amount of anthocyanin (56.94 mg P3G/100g fwt) and phenolic content (212.28 mg GAE/100g fwt) compared to *F. chiloensis* fruit which had the lowest anthocyanin content (44.65 mg P3G/100g) and *F. xananassa* fruit which exhibited lower phenolic content (144.86 mg GAE/100g) (Wang & Lewers, 2007). Notably, the wild *F.*

*virginiana* and *F. chiloensis* fruit contained higher amounts of C3G (10.41 and 9.53 mg/100g, respectively) compared to *F. xananassa* fruit (3.80 mg/100g). However, fruit from *F. virginiana* and *F. xananassa* genotypes contained similar P3G (41.81 and 40.12 mg/100g), and *F. chiloensis* fruit contained the lowest amount (25.35 mg/100g) (**Wang & Lewers, 2007**).

In *Fragaria xananassa* Duch cultivars, pelargonidin 3-glucoside (P3G) was the most dominant anthocyanin, averaging 60 to 90% of the total anthocyanin content in strawberry fruit, and is often followed by pelargonidin 3-rutinoside (P3R) at 6 to 12% (**Aaby et al., 2012; Cerezo et al., 2010; Lopes da Silva et al., 2007**). Pelargonidin 3-(6''-malonylglucoside) (P3MG) and cyanidin 3-glucoside (C3G) are also present in cultivated strawberry fruit in much lower amounts. However, anthocyanin pigment profiles are highly dependent on genetics. Prior studies quantifying anthocyanins has shown P3MG to be the second most abundant anthocyanin in commonly-grown Italian (0–15%), Japanese (0–30%), and Norwegian (0–34%) cultivars (**Aaby et al., 2012; Tamura et al., 1995; Tulipani et al., 2008; Yoshida et al., 2002**) rather than P3R. Another study evaluating fully ripe red and deep burgundy purple-red fruit from genotypes at a breeding program in Queensland, Australia, found that although P3G concentrations were similar (78.3 to 76%) between the two colors, the burgundy fruit had greater averages of C3G (12–19%) compared to red fruit (0.8–7.3%) and was substantially greater in total anthocyanin (70–97 to 32.8 mg P3G/100g) (**O'Hare & Hong, 2021**). The concentration of P3MG was greater in red fruit (11.9%) compared to burgundy fruit (0.4%). However, the strawberry samples only included the outer flesh of the fruits rather than juice, with achenes removed before anthocyanin extraction (**O'Hare & Hong, 2021**). Apart from differences in genetics from the genotypes, differences in anthocyanin profiles may be caused by different extraction solvents, equipment,

the choice of anthocyanin standard and extinction coefficient, the relative ripeness of the fruit, and whether juice or puree was used.

#### Anthocyanin and Phenolic Compound Extraction, Quantification, and Identification

Anthocyanin content and profiles can be extracted and quantified by several methods. One of the quickest and most convenient ways to quantify total monomeric anthocyanin content (TMAC) is to extract pigments using the pH differential method and calculate absorbance using spectrophotometric methods (**Constantin et al., 2022; Lee et al., 2005; Perkins-Veazie et al., 2023**). As monomeric anthocyanins experience reversible structural and color changes at different pH levels, this method measures the change in absorbance that occurs from pH 1.0 to pH 4.5 to assess the total anthocyanin concentration at 520 nanometers (**Lee et al., 2005**). Total phenolic content (TPC) can also be quantified through spectrophotometric methods by using the Folin-Ciocalteu reagent method, which uses gallic acid as a standard and measures absorbance at (**Dzhanfezova et al., 2020; Singleton & Rossi, 1965; Singleton et al., 1999**).

However, microplate-based methods have limitations as only TMAC and TPC are measured, and the identification and quantification of the specific anthocyanins and phenolic compounds are unable to be determined. Other techniques, like chromatography, are required to characterize anthocyanin and phenolic profiles. Gas and liquid chromatography (HPLC and UPLC) combined with diode-array detection (DAD), mass spectroscopy (MS), UV/visible spectroscopy (UV-VIS), and coulometric detection are all common techniques of choice (**Aaby et al., 2007; Cheel et al., 2007; Dzhanfezova et al., 2020; Hernanz et al., 2008; Lee et al., 2022; Perkins-Veazie et al., 2016**).

Breeders are continuously in search of tools to assist in screening plant material that are rapid, inexpensive, simple, accurate and quantifiable. Along with the general labor- and resource-intensive nature of screening germplasm populations, the anthocyanin and phenolic assays are also time- and space-consuming, demand expensive specialized reagents and equipment, require trained personnel, and offer little margin for error (**Khanizadeh & Tremblay, 2011; Rahaman et al., 2015; Perkins-Veazie et al., 2023**). Additionally, breeders screen hundreds to thousands of samples throughout their breeding process, as it can take more than 10 years before the release of a new cultivar (**Khanizadeh and Tremblay, 2011**), rendering these assays largely impractical and unfeasible in such large quantities. The application of a rapid and non-destructive colorimetric method to predict anthocyanin and phenolic would provide a simpler method for quickly screening phytochemical content in germplasm populations.

#### Use of Colorimetry to Predict Anthocyanin Content and Profiles

One of the most developed methods for quantifying external color in fruits and vegetables is tristimulus colorimetry, where the reflected color of a given sample is measured and characterized by the three perceptual color attributes: lightness, chroma, and hue. The CIE  $L^*a^*b^*$  color system is a universally standardized 3-dimensional colorimetry system that provides numerical coordinates for lightness ( $L^*$ ) and chromaticity ( $a^*$  and  $b^*$ ) in samples (**“Defining and Communicating Color,” 2013; Phillips, 2023**). Changes in  $L^*$  measure differences in the relative lightness and darkness of a color, with a numerical value of 0 representing pure black and 100 representing pure white. The color axis  $a^*$  measures red to green (+a = redder, -a = greener), while the other axis  $b^*$  measures yellow to blue (+b = yellower, -b = bluer). Both the

$a^*$  and  $b^*$  measurements, along with  $L^*$ , combine to form  $h^*$  (hue angle), which measures the hue of a color, and  $C^*$  (chroma), which measures the relative intensity (brightness or dullness) of a color (“**Identifying Color Differences,**” 2022; “**Understanding the CIE  $L^*C^*H$  color space,**” 2022).

Colorimetry, utilizing the CIEALAB system, has been tested in several crops as a means to predict the content of anthocyanin and non-anthocyanin phenolic compounds. **Vieira et al. 2018** tested the feasibility of rapid color and anthocyanin evaluation on 13 fruits and vegetables from different regions of Brazil. Colorimeter values were compared to spectrophotometric assays of 70% acidified ethanol extracts. Different quantities of plant material and solvent were used depending on the sample, but all extracts were run using a SHIMADZU spectrophotometer at 535 nm to determine TMAC and color using a ColorQuest XE colorimeter.  $L^*$  values ranged from 44.53,  $a^*$  was 38.48,  $b^*$  was 26.42,  $C^*$  was 46.88, and  $h^*$  averaged 55.53 in the strawberry fruits (**Vieira et al. 2018**).

Another study explored the idea of estimating anthocyanin content in different strawberry genotypes using colorimeter measurements. In February 2011, fully red fruit were harvested from 19 strawberry genotypes in a greenhouse located in Suwon-si, South Korea. Whole fruit were blended and homogenized and analyzed for color, anthocyanin content, and anthocyanin profiles (**Kim et al., 2013**). Correlation analysis using Pearson coefficients and linear regression analysis was performed on the two anthocyanin assays and between the assays and colorimeter measurements ( $L^*$ ,  $a^*$ ,  $b^*$ ). A strong positive linear regression value of  $r = 0.972$  was found between TMAC and TAC (**Kim et al., 2013**). Similar to **Vieira et al, 2018** lightness ( $L^*$ ) was

the color measurement with the strongest correlation to TAC ( $r = -0.79$ ). When comparing the Pearson correlation ( $r = -0.79$ ) of TAC and  $L^*$  to the linear regression correlation ( $r = 0.63$ ) of the two variables, the linear regression relationship is lower (**Kim et al., 2013**). Despite this study's focus on several strawberry genotypes, there are also limitations due to the fruit composition parameters of each strawberry genotype being averaged for linear regression, which overlooks the variability of fruit within each genotype. Additionally, individual fruit samples were used in the correlation analysis and were not averaged for each genotype like in the regressions. This difference could explain the differences seen between TAC and  $L^*$  between the two statistical methods.

Subjective color ratings have been tried for assessing the visual color of strawberries. Different numbered scales are often used for these ratings, with added descriptions for the different scale numbers and visual images of fruit. A subjective color rating scale of 1 (darkest red) to 5 (lightest red) was used (**Nunes, 2017**) to follow changes in Radiance and Sensation strawberries. Subjective ratings ranged from 3.0 in 'Radiance' fruit to 3.3 in both 13.26-134 and 'Sensation' fruit. Although the ratings appear close in range, clear visual differences in light red versus dark red fruit could be seen.

### Hypothesis and Objectives

The overall objective of this study is to evaluate the usefulness of applying reflective colorimeter parameters and/or subjective visual color ratings as an estimate of total anthocyanin and phenolic content of strawberries from the North Carolina breeding program. The hypothesis is that colorimeter measurements, specifically lightness ( $L^*$ ), would be most useful in accurately

predicting the range of TAC and TPC. Additionally, color, physicochemical, and phytochemical differences of strawberries of the same genotype grown in different environmental locations (Florida and North Carolina) were followed to determine potential environmental influences on color, anthocyanin and total phenolic content.

## **Materials and Methods**

### Plant Material

For genotypes grown in North Carolina, fully-red marketable fruit was collected from a field trial at the NCDA&CS Piedmont Research Station in Salisbury, NC (35.69501°, -80.62939°) from April to June of 2023. Fruit was collected as available from three plot replications over eight harvest dates. Marketable fruit included those that were at least 10g in weight, free from visible defects such as disease, sunscald, and water damage, and not misshapen. Fruit were placed into plastic pint clamshells and transported to the Plants for Human Health Institute in Kannapolis, NC. Marketable fruit from five genotypes (20.12-99, 20.27-633, ‘Felicity’, ‘Festival’, ‘Medallion’, and ‘Pearl’) grown at the UF/IFAS Gulf Coast Research and Education Center in Balm, Florida (24.76121°, -82.22761°) were and shipped overnight to North Carolina in late-February 2023.

### Fruit Color Analysis

Five fruits per genotype and per harvest date from each of the North Carolina-grown genotypes were randomly selected and subjectively evaluated for visual color (Sbj Color) by trained lab personnel using color reference photos from the standardized RosBREED 1–9 phenotyping scale showing white/blush (1), light orange-red (3), scarlet (5), dark red “purple” (7), and very dark

red “black” (9) strawberry fruits (*RosBREED Strawberry Phenotyping Protocol*). Due to Florida-grown fruit being frozen, subjective color ratings were not conducted on those five genotypes. Colorimeter readings were then taken on both sides of each of the fruits evaluated from North Carolina and all fruits from Florida using a chroma meter (Konica Minolta CR-400, Ramsey, NJ, USA) calibrated with a white standard tile and set at color space D65 and CIEL\*a\*b\* coordinates with an 8mm aperture.

Strawberry fruit calyxes were removed, and berries were cut in half lengthwise. One set of fruit halves were frozen at -20°C in plastic locking bags for physicochemical assays and both anthocyanin and phenolic compound analyses by microplate. The other set was frozen at -80°C for anthocyanin profile analysis by high performance liquid chromatography (HPLC).

Anthocyanin and phenolic assays by microplate (TMAC and TPC) were not conducted on fruit from Florida.

#### Fruit Physicochemical Analysis

The first set of fruit was placed in large weigh boats and thawed to room temperature. Juice was collected to determine soluble solids content (SSC), pH, and titratable acidity (Tacid). A 0.5mL aliquot of strawberry juice was placed on a handheld digital refractometer (Atago PAL-1, Bellevue, WA, USA) to determine %SSC. The pH of strawberry juice was determined using a pH meter (Thermo Scientific™ Orion Star™ A211, Waltham, MA, USA), and electrode (Thermo Scientific™ Orion™ Ross, Waltham, MA, USA). For titratable acidity, 0.5mL of juice was diluted with 24.5mL of distilled deionized water, thoroughly mixed, and an aliquot placed

on a digital acidity meter (strawberry setting, Atago PAL-BX/ACID F5, Bellevue, WA, USA), with results as % citric acid equivalents recorded.

#### Total Monomeric Anthocyanin Content

Extracted juice was used to evaluate total monomeric anthocyanin content (TMAC), total phenolic content (TPC), and total anthocyanin content and profiles (TAC). Total monomeric anthocyanin content (TMAC) represents the quantified anthocyanin amount determined by spectrophotometric methods, while total anthocyanin content (TAC) is defined here as the sum of the individual anthocyanins identified in strawberry germplasm by HPLC.

Total monomeric anthocyanin content of juice was determined for each sample using two aliquots of 0.1 mL juice, one combined with 1.5 mL of 0.025 M Potassium chloride KCl (pH 1.0) buffer and the other with 0.4 M Sodium acetate (pH 4.5) buffer. Samples were vortexed for 1 minute (Grant Instruments V-32 Multi-Vortex Mixer, Beaver Falls, PA, USA), sonicated for 10 minutes (Bransonic 3510R-MT Ultrasonic Cleaner, Danbury, CT, USA), and microfuged (Eppendorf 5417R, Framingham, MA, USA) at  $13,000 \times g$  for 10 minutes at 4°C. Supernatants of 150  $\mu$ L were placed on a 96-well plate with four replicates (wells) per sample and absorbance read at 510 and 700 nm after 5 minutes using a microplate spectrophotometer (Biotek PowerWave35, Winooski, VT, USA). Total monomeric anthocyanin content (TMAC) was determined by adapting the methods of **Lee et al., 2005** and **Heredia et al., 2006** for a microplate reader where:

$$\text{TMAC (mg pelargonidin-3-glucoside (PG) equivalents/100 g fresh weight) =} \\ [((\text{Abs}_{510} - \text{Abs}_{700 \text{ pH} 1.0}) - (\text{Abs}_{510} - \text{Abs}_{700 \text{ pH} 4.5})) \times \text{A Slope (2.61)} \times \text{DF1} \times \text{DF2} \times \text{molecular} \\ \text{weight (PG)} \times 100] / \text{molecular coefficient (PG)}$$

where:

Abs<sub>510</sub> and Abs<sub>700</sub> represent absorbance values at 510 and 700 nm;

A slope value of 2.61 represents the difference between spectrophotometer and microplate;

Dilution Factor (DF1) is the dilution of the pigment in pH 1.0 buffer (, which was 3 in this study,

Dilution Factor 2 (DF2) represents (solvent volume + sample volume)/sample volume or

((0.4+1.2)/0.4)

Molecular weight of P3G pigment is 443;

Molecular coefficient of P3G is 156000

### Total Phenolic Content

Total phenolic content (TPC) sample preparation procedures followed those of TMAC. A separate 0.1 mL aliquot of juice was extracted with 1.5 mL of methanol (Fisher Scientific, Fair Lawn, NJ, USA) acidified with formic acid (67:30:1) (Sigma-Aldrich, Burlington, MA). Samples were vortexed for 1 minute (Grant Instruments V-32 Multi-Vortex Mixer, Beaver Falls, PA, USA), sonicated for 10 minutes (Branson 3510R-MT Ultrasonic Cleaner, Danbury, CT, USA), and microfuged (Eppendorf 5417R, Framingham, MA, USA) at 13,000 × g for 10 minutes at 4°C. Supernatants of 20 µL were placed on a 96 well plate with four replicates (wells) per sample, along with 20 uL of 0.25 N Folin-Ciocalteu Phenol reagent, 120 uL of dH<sub>2</sub>O, and 20 uL

of 1 M sodium carbonate. Methods followed those of **Singleton et al. 1999** and **Perkins-Veazie et al. 2016**, adapted for microplate. Absorbance was read at 765 nm after 5 minutes using a microplate spectrophotometer (Biotek PowerWave35, Winooski, VT, UDA). A 0.5% gallic acid stock solution (5 g/kg) was prepared by dissolving 0.5 g of dry gallic acid in 10 mL of ethanol and diluted to make standards of 25, 50, 75, 100, and 150 mg/L. Standards were also plated and run on the microplate to generate a gallic acid calibration curve where:

$$\text{TPC (mg gallic acid equivalents (GAE)/100g fresh weight)} = (\text{Abs}_{726\text{nm}} - \text{Abs}_{726\text{nm blank}}) \times \text{P-Slope} \times \text{DF1}$$

where:

Abs<sub>726nm</sub>=absorbance at 726 nm;

P-slope is the slope derived from the gallic acid standard curve and ranges from 218 to 225;

Dilution Factor (DF2) represented the [solvent volume + sample volume/sample volume] and was 16.

### Anthocyanin Pigment Profiles

The second set of fruit was thawed, and strawberry juice samples for total anthocyanin content (TAC) and anthocyanin profile analysis were prepared using 0.4mL of juice and 1mL of 60:37:3 MeOH:water:formic acid solvent, using UPLC-grade methanol (Fisher Scientific). All samples were vortexed for 1 minute (Grant Instruments V-32 Multi-Vortex Mixer, Beaver Falls, PA, USA), sonicated for 20 minutes (Branson 3510R-MT Ultrasonic Cleaner, Danbury, CT, USA), and microfuged (Eppendorf 5417R, Framingham, MA, USA) at 4,000 × g for 20 minutes at 4°C.

Supernatants were filtered into HPLC vials using 0.2µm PTFE filters (Fisher Scientific, Pittsburg, PA, USA), packed with N<sub>2</sub>, and sealed. Filtered samples (10 µL) were injected into an Elite LaChrom Hitachi HPLC System (Hitachi Ltd., Tokyo, Japan), equipped with a UV-VS diode array detector (DAD) controlled temperature auto sampler (4°C), column compartment (30°C). D-2000 chromatography software (Hitachi Ltd., Tokyo, Japan) was used as the system run controller and for data processing.

Anthocyanin separation was performed using a reversed phase C18 column (Synergi 4µ Hydro-RP 80Å, 6 × 250 µm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of 5% formic acid in water (A1) and 100% methanol (B1) with a flow rate of 1 ml/min using a step gradient of 0 min, 10% B1; 5 min, 15% B1; 15 min, 20% B1; 20 min, 25% B1; 25 min, 30% B1; 45 min, 60% B1; 47 min, 10% B1; 60 min, 10% B1. Compound concentrations were estimated using standard curves generated by injecting 1µL of 0.00625–0.1 mg/mL preparations of pelargonidin 3-glucoside as external standards. Anthocyanins cyanidin-3-glucoside and pelargonidin-3-glucoside were identified using retention time compared to standards (Chromadex, Irvine, CA, USA; Sigma, St. Louis, MO, USA) and previously published reports for pelargonidin 3-(6''-malonylglucoside) (**Cerezo et al., 2010; Fredericks et al., 2012; Lopes da Silva et al., 2007**). Sample anthocyanin content was reported as mg/100g fresh weight (fwt), and total anthocyanins were considered the sum of identified anthocyanins.

### Statistical Analysis

The experimental design comprised of 31 commercial and advanced strawberry genotypic lines, each with three sample replications across three harvest dates. Each replication consisted of five

berries. All statistical analyses and visualizations were performed using R (version 4.2.2, Vienna, Austria), Rstudio (version 2022.12.0+353, Boston, Massachusetts, USA), and Microsoft Excel (version 16.83, Redmond, Washington, USA). To ensure homogeneity and normality of sample population distributions, Levene's test for quality of variances and Shapiro-Wilk's test for normality were conducted. All genotypes demonstrated homogeneity and normality and were analyzed by analysis of variance (ANOVA), with significant differences detected using Tukey's post hoc analysis. Colorimeter CIEL\*a\*b measurements were translated into HEX codes, which served as visual color codes that allowed for the visualization of variations in strawberry fruit color (*NIX Color Sensor Converter*).

Correlation and regression analyses were also conducted to determine and explore relationships between the fruit composition parameters. The "ggplot2" (**Wickham, 2016**) R package was used for correlation analysis to determine the strength and direction of relationships among the fruit composition variables, with results presented in Pearson correlation coefficients. However, due to variability in strawberry production and limited fruit in some genotypes, not all physicochemical, phytochemical, and color variables could be measured for each individual fruit sample, preventing the simultaneous analysis of all fruit composition variables. To address this limitation, pairwise correlations between two variables at a time were calculated for each different pair of variables. While this approach meant that some samples were included in certain interactions and excluded from others, this method enabled the exploration of relationships across all variables. Following correlation analysis, only the correlations involving anthocyanin (TMAC and TAC) and phenolic (TPC) variables that were deemed moderate or strong ( $r \leq 0.50$ ) were considered for subsequent regression analysis. Regression analysis was performed in

Microsoft Excel to determine the potential predictability of phytochemical content in strawberry fruit by other variables.

## **Results**

### Strawberry Fruit Physicochemical Composition

A diverse range of fruit physicochemical, phytochemical, and color profiles were found across genotypes. Strawberry soluble solids content (SSC) was the most variable fruit physicochemical composition parameter, with fruit juice values ranging from 3.0 to 14.7 in all samples, with a mean SSC of 7.7%. Average SSC values per genotype are displayed in **Table 4.1**, where average values ranged from 5.9 (NC19-020) to 9.5 (NC21-033) among the 31 genotypes. Fruit from Florida-grown ‘Felicity’ and ‘Medallion’ commercial genotypes had the lowest SSC at 7.2%, while Florida ‘Festival’ had the highest SSC at 8.1%. North Carolina ‘Felicity’ fruit also had an average of 7.2% SSC, but ‘Medallion’ fruit was higher when grown in North Carolina (8.6% vs. 7.2%), while ‘Pearl’ was higher in Florida (7.3% vs. 6.5%). The soluble solids content of other commercial cultivars included Camarosa (7.5%), Chandler (7.9%), D’Light (8.3%), Liz (7.9%), Rocco (7.5%), Ruby June (8.5%), Scarlet (7.0%), and Sweet Charlie (8.8%) (**Table 4.1**).

Strawberry juice pH was the least variable parameter and ranged from 3.4 to 4.0 in all genotypes, with a pH mean of 3.5 (**Table 4.1**). Juice pH from Florida ‘Medallion’ fruit averaged 3.4, while North Carolina ‘Medallion’ fruit averaged 3.6. Florida ‘Felicity’ and ‘Pearl’ fruit had a higher pH at 3.8 and 3.6, respectively, than North Carolina ‘Felicity’ and ‘Pearl’ fruit at 3.5 each.

Lastly, titratable acidity (Tacid) in all samples ranged from 0.34 to 1.2, with a mean Tacid value of 0.72 (**Table 4.1**). Average Tacid values ranged from 0.5 to 0.8 in Florida genotypes and from

0.6 to 1.0 in North Carolina genotypes. 'Medallion' had the highest Tacid in both locations. North Carolina 'Felicity' and 'Pearl' fruit had higher Tacid (0.8 and 0.9%, respectively) compared to Florida-grown 'Felicity' and 'Pearl' (0.5 and 0.6%, respectively) (**Table 4.1**).

**Table 4.1.** Mean physicochemical and color properties of 31 genotypes from the University of Florida and North Carolina State University strawberry breeding programs grown in Florida and North Carolina (2023).

Loc <sup>y</sup>	CV	SSC <sup>z</sup>	pH	Tacid <sup>z</sup>	L*	a*	b*	h*	C*	Sbj Color <sup>z</sup>	Color <sup>z</sup>
		mean ± SD									
FL	20.12-99	7.8 ± 1.0 c-f	3.7 ± 0.1 ab	0.6 ± 0.1 c	55.6 ± 5.3 b	16.8 ± 6.7 h	19.0 ± 4.4 a-e	48.7 ± 0.0 a	25.5 ± 6.5 g-i	n.d.	
FL	20.27-633	7.7 ± 0.7 d-f	3.7 ± 0.1 ab	0.6 ± 0.1 c	32.0 ± 2.3 e	33.5 ± 2.7 b-f	16.5 ± 2.6 c-g	26.1 ± 0.0 c	37.3 ± 3.3 c-f	n.d.	
FL	<b>Felicity</b>	7.2 ± 0.6 ef	3.8 ± 0.0 a	0.5 ± 0.1 c	32.6 ± 2.5 de	37.8 ± 3.4 a-c	20.5 ± 3.7 a-f	28.3 ± 0.0 bc	43.1 ± 4.5 a-c	n.d.	
FL	Festival	8.1 ± 0.6 b-e	3.6 ± 0.1 b	0.6 ± 0.1 c	31.0 ± 4.3 e	32.1 ± 3.0 b-e	16.0 ± 6.4 c-d	26.3 ± 0.0 c	35.8 ± 5.7 d-f	n.d.	
FL	<b>Medallion</b>	7.2 ± 0.4 ef	3.4 ± 0.1 b	0.8 ± 0.1 bc	34.6 ± 3.3 c-e	35.3 ± 3.1 ab	31.1 ± 4.5 a	23.5 ± 0.0 d	34.1 ± 4.2 fh	n.d.	
FL	<b>Pearl</b>	7.3 ± 0.7 d-f	3.6 ± 0.1 b	0.6 ± 0.1 c	65.3 ± 2.1 a	11.7 ± 6.7 i	19.9 ± 4.6 a-e	59.9 ± 0.0 a	23.7 ± 3.6 i	n.d.	
NC	Ashley Jay	n.d.	n.d.	n.d.	37.5 ± 2.1 c	39.4 ± 2.2 a	23.9 ± 3.0 ab	29.3 ± 2.8 bc	46.1 ± 2.8 a	n.d.	
NC	Camarosa	7.5 ± 1.6 d-f	3.6 ± 0.1 b	0.9 ± 0.6 a	35.0 ± 3.7 c-e	32.2 ± 4.2 b-e	18.4 ± 6.5 c-g	27.1 ± 6.8 c	37.3 ± 6.2 c-e	5.4 ± 1.0 c	
NC	Chandler	7.9 ± 1.3 c-f	3.5 ± 0.1 b	0.8 ± 0.4 bc	33.3 ± 3.9 de	31.1 ± 3.4 ef	17.1 ± 5.1 c-g	26.6 ± 5.5 c	35.7 ± 4.9 ef	5.1 ± 0.5 cd	
NC	D'Light	8.3 ± 1.1 b-e	3.9 ± 0.2 ab	0.8 ± 0.2 bc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
NC	<b>Felicity</b>	7.2 ± 1.3 ef	3.5 ± 0.1 b	0.8 ± 0.5 bc	35.0 ± 3.9 de	34.6 ± 4.8 bc	18.1 ± 6.7 c-g	25.1 ± 6.7 cd	39.3 ± 6.8 b-e	5.1 ± 0.5 cd	
NC	Liz	7.9 ± 1.6 c-f	3.6 ± 0.1 b	0.9 ± 0.7 a	33.8 ± 4.7 c-e	31.6 ± 3.9 ef	16.4 ± 6.8 c-g	24.8 ± 7.8 cd	35.9 ± 6.0 d-f	5.0 ± 1.3 cd	
NC	<b>Medallion</b>	8.6 ± 2.0 a-c	3.6 ± 0.1 b	1.0 ± 0.7 a	34.1 ± 3.3 c-e	32.2 ± 4.7 b-e	16.4 ± 6.0 c-g	24.4 ± 6.6 cd	36.4 ± 6.4 d-f	5.8 ± 0.3 bc	
NC	NC19-016	6.4 ± 0.8 ef	3.7 ± 0.1 ab	0.6 ± 0.1 c	37.8 ± 4.9 c	34.8 ± 2.8 a-e	24.7 ± 5.4 a	33.0 ± 5.0 b	42.8 ± 4.6 a-c	n.d.	
NC	NC19-020	5.9 ± 1.3 f	3.7 ± 0.2 ab	0.8 ± 0.5 bc	32.2 ± 4.9 e	28.0 ± 6.2 g	13.7 ± 7.5 fg	22.6 ± 8.2 d	31.5 ± 8.6 fh	6.8 ± 0.4 a	
NC	NC19-022	7.6 ± 0.8 d-f	3.5 ± 0.1 b	0.8 ± 0.1 bc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
NC	NC20-008	7.6 ± 1.4 d-f	3.8 ± 0.2 a	0.7 ± 0.5 bc	34.1 ± 3.3 c-e	33.0 ± 3.4 c-f	17.2 ± 4.9 c-g	25.5 ± 5.0 cd	37.4 ± 4.9 c-e	5.6 ± 0.5 bc	
NC	NC20-012	7.6 ± 1.2 d-f	4.0 ± 2.3 a	0.7 ± 0.1 bc	31.8 ± 3.9 e	31.5 ± 4.7 ef	15.8 ± 6.2 d-g	24.2 ± 6.6 cd	35.5 ± 6.5 ef	6.2 ± 0.8 b	
NC	NC20-055	8.1 ± 1.9 b-e	3.7 ± 0.1 ab	0.6 ± 0.1 c	33.2 ± 1.7 de	32.4 ± 4.0 d-f	15.5 ± 2.0 d-g	24.2 ± 2.9 cd	35.9 ± 4.1 d-f	n.d.	
NC	NC20-058	7.8 ± 1.4 c-f	3.6 ± 0.1 b	0.7 ± 0.1 bc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
NC	NC20-099	6.5 ± 1.4 ef	3.5 ± 0.1 b	0.7 ± 0.1 bc	36.5 ± 4.4 cd	33.8 ± 3.1 b-e	20.9 ± 5.3 a-d	29.4 ± 4.9 bc	39.9 ± 4.8 b-e	6.0 ± 0.0 bc	
NC	NC21-006	6.3 ± 1.3 ef	3.8 ± 0.1 a	0.6 ± 0.1 c	38.5 ± 2.9 c	37.2 ± 4.0 a-d	23.3 ± 3.4 a-c	30.3 ± 5.4 bc	44.1 ± 3.1 a-c	n.d.	
NC	NC21-020	8.2 ± 1.9 b-e	3.6 ± 0.1 b	0.7 ± 0.1 bc	36.9 ± 5.1 c	31.9 ± 4.9 d-f	20.3 ± 7.4 a-e	29.5 ± 8.3 bc	38.2 ± 7.1 b-e	3.0 ± 0.0 d	
NC	NC21-031	8.0 ± 1.3 b-f	3.4 ± 0.1 b	0.9 ± 0.5 a	34.4 ± 4.5 c-e	29.8 ± 4.5 f	15.5 ± 6.5 e-g	24.8 ± 7.0 cd	33.9 ± 6.6 fg	5.9 ± 0.8 bc	
NC	NC21-033	9.5 ± 1.5 a	3.6 ± 0.1 b	0.9 ± 0.1 a	36.6 ± 4.5 c	34.1 ± 3.8 bc	20.3 ± 5.9 a-e	28.3 ± 5.8 bc	39.9 ± 5.7 b-e	5.0 ± 0.8 cd	
NC	NC21-035	8.6 ± 1.5 a-c	3.7 ± 0.1 ab	0.7 ± 0.1 bc	33.5 ± 3.5 de	28.2 ± 5.2 g	13.5 ± 6.3 g	22.7 ± 7.7 d	31.6 ± 7.1 fh	6.3 ± 0.4 ab	
NC	<b>Pearl</b>	6.5 ± 1.2 ef	3.5 ± 0.1 b	0.9 ± 1.2 a	63.1 ± 4.7 a	14.0 ± 7.0 i	22.0 ± 3.3 a-c	54.9 ± 13.2 a	26.9 ± 3.8 i	n.d.	
NC	Rocco	7.5 ± 1.4 d-f	3.6 ± 0.1 b	0.8 ± 0.5 bc	36.0 ± 3.3 cd	32.5 ± 4.5 b-e	18.8 ± 6.0 b-g	27.6 ± 6.1 bc	37.7 ± 6.3 c-e	5.2 ± 0.5 c	
NC	Ruby June	8.5 ± 1.5 b-d	3.6 ± 0.1 b	0.9 ± 0.7 a	33.9 ± 3.2 c-e	33.7 ± 4.2 b-e	19.9 ± 5.1 a-e	28.2 ± 5.4 bc	39.3 ± 5.6 b-e	4.4 ± 0.5 cd	
NC	Scarlet	7.0 ± 0.6 ef	3.5 ± 0.1 b	0.8 ± 0.1 bc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
NC	Sweet Charlie	8.8 ± 1.3 ab	3.8 ± 0.1 a	0.6 ± 0.1 c	37.4 ± 3.5 c	37.5 ± 4.3 ab	22.5 ± 6.3 a-c	28.5 ± 5.9 bc	44.0 ± 6.1 a-c	4.9 ± 0.5 cd	

<sup>z</sup>SSC = soluble solids content (%), Tacid = titratable acidity (% as citric acid), L\* = lightness, a\* = red-green color axis, b\* = yellow to blue color axis, h\* = hue, C\* = chroma, Sbj Color = subjective color (1–7 scale), Color = HEX code

<sup>y</sup>Location. FL = University of Florida breeding program, NC = North Carolina State University breeding program. Genotypes in bold were in both locations.

### Colorimeter and Subjective Color

To assess strawberry color, both tristimulus reflective colorimetry (CIE  $L^*a^*b$  coordinates) and subjective external visual color ratings were performed. Lightness ( $L^*$ ) measures the relative lightness of a color and ranged from 18.2 to 71.5 in all samples, with a mean  $L^*$  of 34.8. Fruit from Florida-grown genotypes averaged between 31.0 and 65.3  $L^*$  and North Carolina-grown genotypes from 31.8 to 63.1 (**Table 4.1**). White-fruited ‘Pearl’ and pink-fruited 20.12-99 had the highest  $L^*$  values, while ‘Festival’, NC19-020, NC20-012, and NC20-055 had the lowest. ‘Felicity’ (32.6–35.0), ‘Medallion’ (34.1–34.6), and ‘Pearl’ (63.1–65.3) fruit were similar between states. Twenty-four genotypes had  $L^*$  values in the 30s. ‘Camarosa’ and ‘Chandler’ fruit had lightness measurements of 35.0 and 33.3, respectively (**Table 4.1**).

Values of the red to green color axis  $a^*$  parameter ranged from 0.13 to 47.2, with a mean  $a^*$  value of 31.8. Average  $a^*$  values per genotype are displayed in **Table 4.1**, where average values ranged from 11.7 to 39.4, with a mean value of 32.1. ‘Pearl’ (11.7–14.0), 20.12-99 (16.8), NC19-020 (28.0), NC21-031 (29.8), and NC21-035 (28.2) had the lowest  $a^*$  values, while Ashley Jay (39.4), Florida-grown ‘Felicity’ (37.8), NC21-006 (37.2), and Sweet Charlie (37.5). Similar to  $L^*$ , most genotypes (20) had  $a^*$  values in the 30s. The other color axis ( $b^*$ ), which measures yellow to blue, ranged from -2.1 to 40.7 across individual samples and averaged 13.5 to 31.1 among genotypes (**Table 4.1**). ‘Festival’ and 20.27-633 had the lowest  $b^*$  values of the Florida genotypes at 16.0 and 16.5, respectively, while ‘Medallion’ was considerably greater at 31.3. In strawberry fruit grown in North Carolina, genotypes NC19-020, NC20-012, NC20-055, and NC21-035 averaged 13.5–15.8  $b^*$  values, and genotypes ‘Ashley Jay’, NC19-016, NC21-006, Pearl, and Sweet Charlie averaged 22.0–24.7. The majority of  $b^*$  values were <20.0. ‘Camarosa’

and ‘Chandler’ fruit had  $a^*$  and  $b^*$  measurements of 32.3–31.1 and 18.4–17.1, respectively (**Table 4.1**).

Hue ( $h^*$ ) values ranged from 22.6 (NC19-020) to 54.9–59.9 (Pearl) in genotypes, with most of the genotypes having values between 20 and 30 (**Table 4.1**). Individual  $h^*$  values were between 9.3 and 82.5, with a mean value of 25.6. The  $h^*$  was similar between Florida and North Carolina-grown ‘Felicity’ (25.1–28.3), ‘Medallion’ (23.5–24.4), and ‘Pearl’. Lastly, chroma ( $C^*$ ) measures the relative intensity or saturation of a color, and strawberry fruit values ranged from 11.9 to 57.7, with a mean of 36.6. Average  $C^*$  values per genotype are displayed in **Table 4.1** and range from 23.7 to 46.1. Similar to  $L^*$  and  $a^*$  values, ‘Pearl’ and 20.12-99 also had the lowest  $C^*$  values (23.7–26.9). ‘Ashley Jay’ (46.1), Florida-grown ‘Felicity’ (43.1), NC19-016 (42.8), NC21-006 (44.1), and ‘Sweet Charlie’ (44.0) were highest. Most of the 27 genotypes had  $C^*$  values between 30 and 40, with indicator cultivars ‘Camarosa’ and ‘Chandler’ fruit had  $C^*$  values of 37.3 and 35.7, respectively (**Table 4.1**).

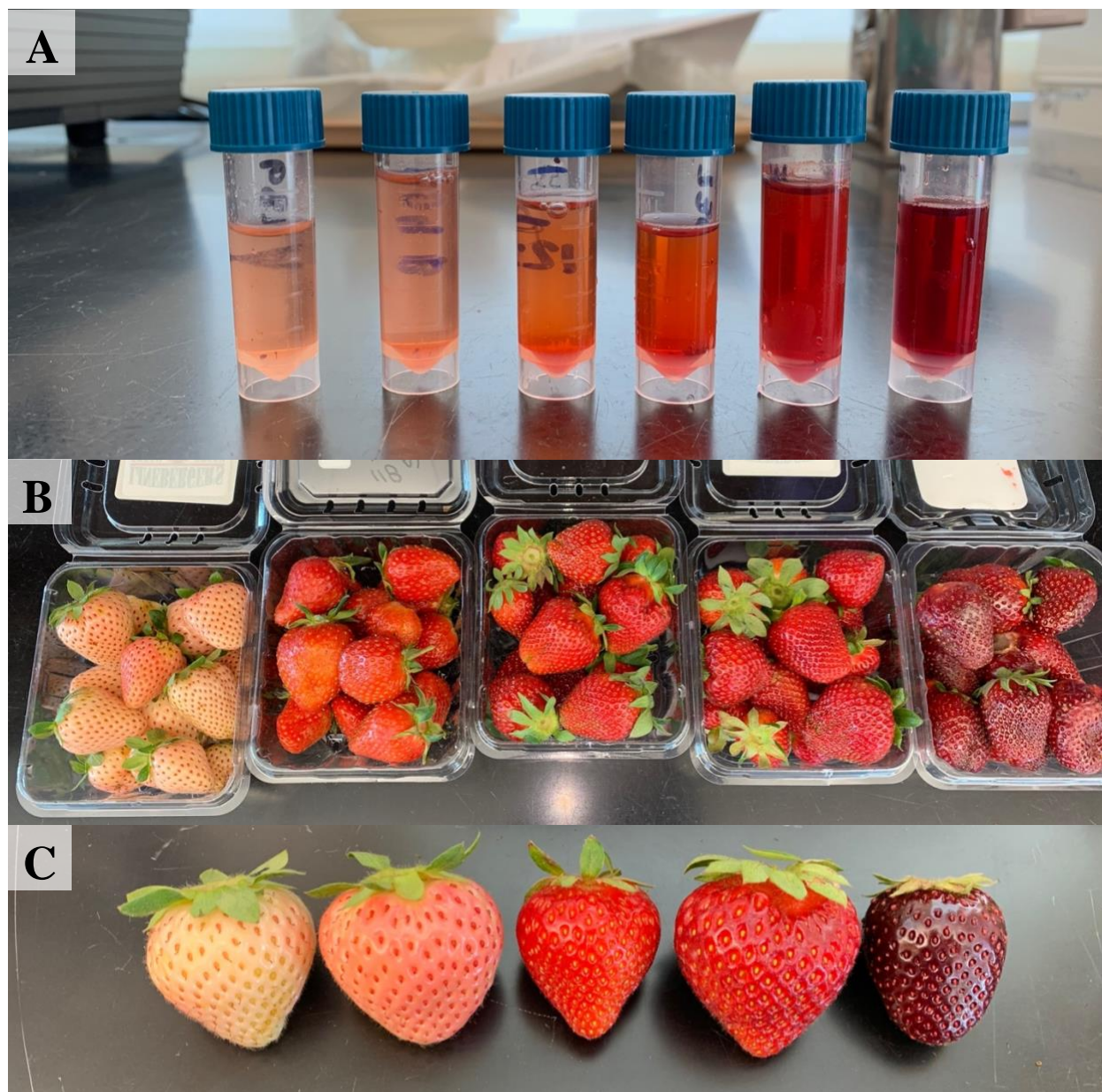
Delta E ( $\Delta E$ ) values represent the distance and difference between the coordinates of two colors in the three-dimensional CIEL<sup>\*</sup> $a^*b^*$  color space (**McGrath et al., 2017**). To assess color differences between the strawberry genotypes and provide a comprehensive understanding of the perceived color differences visualized when analyzing samples,  $\Delta E$  values and visual RGB hex code colors translated from the corresponding CIEL<sup>\*</sup> $a^*b^*$  color space measurements are presented in **Table 4.2** to offer both an additional quantitative color value and enhance the visual clarity of the fruit. Values ranged from  $\Delta E = 0$  to 36 in 21 North Carolina genotypes, with an average  $\Delta E$  value of 8 for all pairwise comparisons between genotypes. A visual color difference

between Medallion and ‘NC20-008’ fruit was undiscernible ( $\Delta E = 0$ ), while Pearl had the greatest color differences with all remaining genotypes with  $\Delta E$  values of 31 (NC21-020) to 36 (Ashley Jay and ‘NC20-012’). Of the 210 comparisons, 74 pairs had a  $\Delta E < 5$ , 136 pairs had a  $\Delta E \geq 5$ , and 56 pairs were  $> 10$  (**Table 4.2**). Though not pictured, the  $\Delta E$  values between Florida-grown and North Carolina-grown genotypes were ‘Felicity’ (4.7), ‘Medallion’ (15.21), and ‘Pearl’ (3.8).

Strawberry fruits were also subjectively rated for external strawberry color using the RosBREED 1–9 phenotyping scale developed from a USDA-NIFA Specialty Crop Research Initiative project devoted to standardizing phenotyping protocols for Rosaceae crops (*RosBREED Strawberry Phenotyping Protocol*). Fruit were given a value between 1 (white) and 9 (deep red “black”) based on external peel color. Subjective color ratings averaged 3.0 (NC21-020) to 6.8 (NC19-020), spanning colors from a light orange-red to a dark purple-red (**Figure 4.1**). Of the 16 genotypes measured, 10 had a color rating of between 5.0 and 6.0, indicative of scarlet to red fruit. Commonly grown genotypes ‘Camarosa’ and ‘Chandler’ had subjective color measurements of 5.4 and 5.1. Other commercial genotypes Felicity, Liz, Medallion, and Rocco had color ratings between 5.0 (‘Liz’) and 5.8 (‘Medallion’). Fruit from ‘Ruby June’ and ‘Sweet Charlie’ had rankings in the 4.0 range. Fruit from Florida-grown genotypes was not ranked subjectively.

**Table 4.2.** ΔE values and visible color of 21 North Carolina strawberry cultivars with CIEL\*a\*b\* color values.

CV	AJ	Cam	Chan	Feli	Liz	Mdln	19-16	19-20	20-08	20-12	20-55	20-99	21-06	21-20	21-31	21-33	21-35	Pearl	Rocco	RJ
AJ	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cam	9	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chan	11	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Feli	8	2	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Liz	11	2	1	3	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mdln	10	2	2	2	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19-16	5	7	9	7	9	9	0	-	-	-	-	-	-	-	-	-	-	-	-	-
19-20	16	8	5	9	6	6	14	0	-	-	-	-	-	-	-	-	-	-	-	-
20-08	10	2	2	2	1	0	8	7	0	-	-	-	-	-	-	-	-	-	-	-
20-12	13	5	2	5	3	3	11	4	3	0	-	-	-	-	-	-	-	-	-	-
20-55	11	3	2	3	2	1	10	6	2	2	0	-	-	-	-	-	-	-	-	-
20-99	6	3	6	3	5	5	4	10	4	7	6	0	-	-	-	-	-	-	-	-
21-06	2	8	11	7	10	10	3	16	9	12	11	5	0	-	-	-	-	-	-	-
21-20	8	3	5	5	5	5	5	10	5	8	6	2	7	0	-	-	-	-	-	-
21-31	14	5	3	6	3	4	12	3	4	3	4	8	13	7	0	-	-	-	-	-
21-33	6	3	6	3	6	5	4	11	5	8	6	1	5	3	13	0	-	-	-	-
21-35	17	9	6	10	6	7	15	2	7	6	7	11	16	10	3	12	0	-	-	-
Pearl	36	34	34	35	34	35	33	35	35	36	35	33	34	31	34	33	34	0	-	-
Rocco	9	1	3	3	3	3	6	8	3	5	4	2	8	2	5	3	9	33	0	-
RJ	8	2	4	2	3	3	6	9	2	5	4	3	8	4	6	3	10	35	3	0
SwtC	2	7	10	6	9	8	4	15	8	11	9	5	2	6	12	4	15	35	7	6



**Figure 4.1.** Panels displaying range of strawberry fruit juice and relative visual color in North Carolina germplasm. (A) Strawberry fruit juice, (B) From left to right, clamshells of ‘Florida Pearl’, ‘Sweet Charlie’, ‘Felicity’, NC21-031, and NC19-020 genotypes from North Carolina germplasm, and (C) Closer image of singular fruits from different genotypes.

#### Anthocyanin and Phenolic Content

A diverse range of total anthocyanin (TMAC and TAC) and non-anthocyanin phenolic values were detected across all genotypes, spanning from 0.2 to 69.0 mg pelargonidin 3-glucoside (P3G) equivalents/100g fwt and from 76.5 to 121.2 gallic acid equivalents (GAE)/100g fwt

(**Table 4.3**). Total monomeric anthocyanin content (TMAC) represents the quantified anthocyanin amount determined by spectrophotometric methods, while total anthocyanin content (TAC) is defined here as the sum of the individual anthocyanins identified in strawberry germplasm by HPLC. Spectrophotometric methods were also used to determine the total non-anthocyanin phenolic content (TPC) in the strawberry fruit. Only TAC and corresponding anthocyanin profiles by HPLC were measured for the six genotypes from Florida.

Total monomeric anthocyanin content by microplate spectrophotometry was the most variable phytochemical variable and ranged from 0.2 to 72.3 mg pelargonidin 3-glucoside (P3G)/100g fwt in all samples, with a mean of 32.0 mg/100g. Average TMAC values per genotype are displayed in **Table 4.3** where average values ranged from 1.1 mg P3G/100g ('Pearl') to 46.6 mg P3G/100g ('Scarlet'). Among the germplasm evaluated, fruit from 12.5% (three genotypes) exhibited TAC values of at least 40.0 mg pelargonidin 3-glucoside (P3G)/100g fwt, while an additional 50% (12 genotypes) had lower TAC values of <30.0 mg P3G/100g. With the exception of 'Pearl' and 'Scarlet' that exhibited the lowest and highest TMAC values, fruit from NC19-016, NC21-020, and 'Ruby June' was also low (15.9–17.6 mg P3G/100g), while genotypes 'Chandler', NC19-020, NC20-012, NC21-031, and NC21-033 were high (37.5–41.7 mg P3G/100g) (**Table 4.3**).

Total phenolic content was the least variable phytochemical variable and ranged from 47.7 to 218.98 mg gallic acid equivalents (GAE)/100g fwt in individual samples, with genotypes averaging between 75.5 and 121.2 mg GAE/100g fwt (**Table 4.3**). Average TPC in North Carolina strawberry fruit was 93.3 mg GAE/100g. High phenolic content ( $\geq 100.0$  mg

GAE/100g) was found in fruit from ‘Chandler’, ‘Liz’, NC19-020, NC20-058, NC21-031, and ‘Scarlet’, while lower phenolic content ( $\leq 85.0$  mg GAE/100g) was present in ‘Felicity’, NC19-016, NC20-099, NC21-006, NC21-020, ‘Pearl’, and ‘Ruby June’ (**Table 4.3**).

Lastly, total anthocyanin content measured by high performance liquid chromatography (TAC), in addition to anthocyanin profiles, was measured in the same strawberry fruit. Among the germplasm evaluated, fruit from 42% (13 genotypes, including Florida and NC) of the collection exhibited TAC values of at least 40.0 mg pelargonidin 3-glucoside (P3G)/100g fwt, while an additional 29% (nine genotypes) had lower TAC values of  $< 30.0$  mg P3G/100g. Anthocyanin ranged from 0.2 and 74.9 mg P3G/100g across all samples, with genotypes averaging between 0.3 to 69.0 mg P3G/100g (**Table 4.3**). Excluding Pearl (0.3–0.8) and 20.12-99 (1.1), fruit from genotypes ‘D’Light’ (12.6), NC19-016 (19.2), NC21-020 (27.1), ‘Ruby June’ (21.2), and ‘Sweet Charlie’ (27.4) had the lowest TAC values at mg P3G/100g. ‘Scarlet’ fruit had the highest TAC of all genotypes at 69.0 mg P3G/100g, and ‘Festival’ fruit was the highest out of the six Florida genotypes at 44.2 mg P3G/100g. Additional genotypes, ‘Camarosa’, ‘Chandler’, FL-‘Felicity’, NC-‘Medallion’, NC19-020, NC20-008, NC20-012, NC21-031, NC21-033, and ‘Rocco’ also had fruit high in TAC ranging from 40.2 to 51.2 mg P3G/100g (**Table 4.3**).

Total anthocyanin content measured by HPLC (TAC) was higher in all genotypes than total anthocyanin content measured by microplate (TMAC), with the exception of ‘D’Light’ fruit, where microplate values were higher. Average TMAC values were 34.7 and 37.4 mg P3G/100g for ‘Camarosa’ and ‘Chandler’, while average TAC values for the two genotypes were 46.5 and 43.0 mg P3G/100g, respectively (**Table 4.3**). Of the 24 genotypes assessed for both anthocyanin

assays, 10 genotypes surpassed 40.0 mg P3G/100g in TAC, while only three genotypes reached that same level for TMAC. Additionally, fruit from 11 genotypes had TAC values between 20.0–39.0 mg P3G/100g, predominantly in the 30s, while 17 genotypes had TMAC values of the same range and were distributed fairly evenly between the 20s and 30s (**Table 4.3**).

**Table 4.3.** Mean phytochemical content of 31 strawberry genotypes from the University of Florida and North Carolina State University strawberry breeding programs grown in Florida (FL) and North Carolina (NC) in 2023.

Loc <sup>y</sup>	CV	mean ± SD		
		TMAC <sup>z</sup>	TPC <sup>z</sup>	TAC <sup>z</sup>
FL	20.12-99	n.d.	n.d.	1.1 ± 0.3 ef
FL	20.27-633	n.d.	n.d.	35.6 ± 5.5 b-f
FL	<b>Felicity</b>	n.d.	n.d.	40.2 ± 9.8 b-e
FL	Festival	n.d.	n.d.	44.2 ± 10.5 b-d
FL	<b>Medallion</b>	n.d.	n.d.	26.8 ± 6.8 ef
<b>FL</b>	<b>Pearl</b>	n.d.	n.d.	0.3 ± 0.1 f
NC	Ashley Jay	n.d.	n.d.	n.d.
NC	Camarosa	34.7 ± 7.6 cd	90.8 ± 1.2 cd	46.5 ± 7.2 b-d
NC	Chandler	37.4 ± 5.5 a-c	104.8 ± 1.1 ab	43.0 ± 9.2 b-d
NC	D'Light	21.5 ± 4.9 hi	95.6 ± 1.8 b-d	12.6 ± 3.0 ef
NC	<b>Felicity</b>	33.4 ± 6.4 c-e	84.5 ± 1.3 d	35.1 ± 3.7 c-f
NC	Liz	29.0 ± 5.8 d-g	100.1 ± 1.5 bc	35.5 ± 7.9 c-f
NC	<b>Medallion</b>	28.1 ± 5.6 d-h	98.0 ± 1.6 bc	43.0 ± 13.3 b-d
NC	NC19-016	15.9 ± 4.8 ij	75.5 ± 0.8 d	19.2 ± 6.3 ef
NC	NC19-020	39.1 ± 10.0 a-c	101.6 ± 1.3 b	46.8 ± 11.3 b-d
NC	NC19-022	26.8 ± 8.2 e-h	91.5 ± 1.3 cd	36.9 ± 11.9 b-e
NC	NC20-008	34.9 ± 7.2 b-d	96.8 ± 1.5 bc	47.0 ± 8.1 a-d
NC	NC20-012	40.4 ± 8.3 ab	96.0 ± 1.2 bc	48.3 ± 11.7 a-c
NC	NC20-055	32.6 ± 10.8 c-f	90.8 ± 1.2 cd	37.7 ± 8.0 b-e
NC	NC20-058	24.5 ± 7.7 f-i	105 ± 1.9 ab	31.1 ± 7.6 d-f
NC	NC20-099	24.4 ± 5.8 f-i	82.7 ± 1.1 d	37.8 ± 8.0 b-e
NC	NC21-006	23.5 ± 5.5 g-i	83.3 ± 1.0 d	35.0 ± 8.0 c-f
NC	NC21-020	16.8 ± 6.7 ij	81.5 ± 1.2 d	27.1 ± 6.5 ef
NC	NC21-031	41.7 ± 9.4 a	101.7 ± 1.3 b	40.8 ± 12.2 b-e
NC	NC21-033	39.1 ± 8.5 a-c	92.8 ± 0.8 b-d	51.2 ± 11.5 ab
NC	NC21-035	34.4 ± 8.3 cd	99.0 ± 2.1 bc	38.0 ± 10.5 b-e
NC	<b>Pearl</b>	1.1 ± 0.8 j	80.1 ± 1.0 d	0.8 ± 0.4 f
NC	Rocco	31.0 ± 7.4 d-f	98.0 ± 1.2 bc	42.6 ± 8.5 b-e
NC	Ruby June	17.6 ± 5.8 ij	76.6 ± 1.2 d	21.2 ± 7.3 ef
NC	Scarlet	46.6 ± 6.4 a	121.2 ± 1.6 a	69.0 ± 3.0 a
NC	Sweet Charlie	22.7 ± 8.0 hi	92.2 ± 1.5 cd	27.4 ± 6.4 ef

<sup>z</sup>TMAC = total monomeric anthocyanin content (units in mg pelargonidin 3-glucoside (P3G)/100g fwt), TPC = total phenolic content (units in mg gallic acid equivalents (GAE)/100g), and TAC = total anthocyanin content (units in mg P3G/100g fwt).

<sup>y</sup>FL = University of Florida breeding program, NC = North Carolina State University breeding program. Genotypes in bold were in both locations.

**Table 4.4.** Mean pigment profiles of 31 strawberry genotypes from the University of Florida and North Carolina State University strawberry breeding programs grown in Florida (FL) and North Carolina (NC) in 2023.

Loc <sup>y</sup>	CV	C3G <sup>z</sup>	P3G <sup>z</sup>	P3R <sup>z</sup>	P3MG <sup>z</sup>	%C3G	%P3G	%P3R	%P3MG
		mean ± SD							
FL	20.12-99	0.1 ± 0.0g	0.9 ± 0.3 de	0.1 ± 0.0 c	0.0 ± 0.0 d	9.2 ± 3.0 c-e	84.4 ± 5.0 a-c	5.0 ± 2.9 bc	0.6 ± 1.1 d
FL	20.27-633	1.2 ± 0.3 fg	26.7 ± 3.8 c-e	3.1 ± 0.8 bc	4.5 ± 0.8 a-d	3.3 ± 0.5 h-k	75.0 ± 0.9 cd	8.7 ± 1.2 bc	12.8 ± 1.1 a-c
FL	<b>Felicity</b>	1.2 ± 0.3 fg	34.5 ± 8.8 b-d	4.3 ± 0.7 bc	0.2 ± 0.1 d	3.0 ± 0.7 i-k	85.5 ± 1.3 a-c	11.0 ± 1.8 a-c	0.4 ± 0.2 d
FL	Festival	1.4 ± 0.6 fg	38.0 ± 8.8 a-c	4.7 ± 1.2 a-c	0.1 ± 0.1 d	3.2 ± 0.9 h-k	85.9 ± 1.1 a-c	10.6 ± 0.7 a-c	0.3 ± 0.1 d
FL	<b>Medallion</b>	0.4 ± 0.1 g	21.6 ± 6.6 de	3.0 ± 0.7 bc	1.9 ± 1.2 d	1.5 ± 0.3 k	80.0 ± 5.2 b-d	11.1 ± 1.6 a-c	7.4 ± 4.9 c
<b>FL</b>	<b>Pearl</b>	0.1 ± 0.0 g	0.2 ± 0.1 e	0.0 ± 0.0 c	0.0 ± 0.0 d	28.2 ± 8.5 a	53.7 ± 9.0 d	8.5 ± 8.3 bc	0.0 ± 0.0 d
NC	Ashley Jay	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
NC	Camарosa	3.0 ± 0.8 cd	33.3 ± 11.6 b-d	9.0 ± 9.1 a	1.2 ± 2.2 d	6.3 ± 1.3 e-g	72.0 ± 21.6 cd	19.2 ± 17.8 a	2.5 ± 4.8 cd
NC	Chandler	1.5 ± 0.5 fg	37.3 ± 8.1 bc	4.1 ± 0.9 bc	0.1 ± 0.1 d	3.4 ± 1.0 h-k	86.6 ± 1.1 ab	9.7 ± 1.0 bc	0.3 ± 0.2 d
NC	D'Light	0.3 ± 0.0 g	9.2 ± 1.8 de	0.6 ± 0.1 bc	2.6 ± 1.0 cd	2.2 ± 0.2 i-k	73.2 ± 3.6 cd	4.4 ± 0.3 bc	20.2 ± 3.9 a
NC	<b>Felicity</b>	1.0 ± 0.2 fg	30.8 ± 3.3 b-d	3.0 ± 0.6 bc	0.3 ± 0.1 d	2.7 ± 0.4 i-k	87.9 ± 1.1 ab	8.5 ± 1.5 bc	0.9 ± 0.2 d
NC	Liz	4.7 ± 1.2 b	22.6 ± 6.1 de	4.6 ± 5.6 bc	3.6 ± 1.3 cd	13.3 ± 2.2 c	64.1 ± 9.8 cd	12.6 ± 13.9 a-c	10.1 ± 2.7 bc
NC	<b>Medallion</b>	1.6 ± 1.3 ef	36.9 ± 9.6 bc	4.2 ± 2.5 bc	0.3 ± 0.1 d	3.2 ± 1.9 h-k	87.0 ± 4.1 ab	9.1 ± 2.7 bc	0.7 ± 0.3 d
NC	NC19-016	0.5 ± 0.3 g	17.8 ± 5.7 de	0.8 ± 0.3 bc	0.1 ± 0.0 d	2.2 ± 1.1 i-k	93.3 ± 1.2 a	4.1 ± 0.3 c	0.5 ± 0.2 d
NC	NC19-020	2.6 ± 1.0 de	40.4 ± 9.6 ab	3.6 ± 1.2 bc	0.2 ± 0.1 d	5.5 ± 1.3 f-h	86.4 ± 2.7 ab	7.7 ± 2.1 bc	0.4 ± 0.3 d
NC	NC19-022	0.3 ± 0.2 g	30.1 ± 12.0 b-d	4.2 ± 1.7 bc	2.3 ± 2.4 d	0.8 ± 0.3 k	79.8 ± 7.1 b-d	11.2 ± 1.6 a-c	8.2 ± 8.7 c
NC	NC20-008	2.6 ± 0.7 de	38.8 ± 6.4 a-c	5.3 ± 1.2 a-c	0.3 ± 0.1 d	5.6 ± 1.0 e-h	82.7 ± 1.0 a-c	11.1 ± 1.4 a-c	0.6 ± 0.3 d
NC	NC20-012	3.8 ± 1.2 bc	38.5 ± 9.2 a-c	5.6 ± 1.6 ab	0.4 ± 0.2 d	7.7 ± 0.6 ef	79.8 ± 2.1 b-d	11.7 ± 1.6 a-c	0.8 ± 0.3 d
NC	NC20-055	0.6 ± 0.3 fg	29.1 ± 6.1 b-d	4.1 ± 0.9 bc	3.8 ± 0.9 b-d	1.7 ± 0.5 k	77.3 ± 0.9 b-d	11.0 ± 0.7 a-c	10.1 ± 0.9 bc
NC	NC20-058	1.1 ± 0.7 fg	26.2 ± 6.2 c-e	3.6 ± 1.9 bc	0.2 ± 0.1 d	3.6 ± 2.4 g-k	84.6 ± 2.3 a-c	11.1 ± 3.1 a-c	0.6 ± 0.3 d
NC	NC20-099	0.5 ± 0.2 g	26.8 ± 6.8 cd	4.0 ± 0.9 bc	6.5 ± 1.0 a	1.3 ± 0.7 k	70.6 ± 3.6 cd	10.4 ± 0.9 a-c	17.6 ± 3.1 a
NC	NC21-006	1.8 ± 0.8 ef	25.4 ± 5.9 c-e	2.8 ± 0.7 bc	5.1 ± 0.8 a-c	4.9 ± 1.1 g-j	72.5 ± 0.6 cd	7.9 ± 0.3 bc	14.8 ± 1.4 ab
NC	NC21-020	0.8 ± 0.2 fg	21.9 ± 5.5 de	4.4 ± 1.0 bc	0.1 ± 0.1 d	2.9 ± 1.2 h-k	80.4 ± 1.3 a-c	16.2 ± 0.9 ab	0.4 ± 0.2 d
NC	NC21-031	1.8 ± 0.6 ef	33.8 ± 11.1 b-d	4.7 ± 1.9 bc	0.5 ± 0.6 d	4.9 ± 2.5 g-i	81.8 ± 6.7 a-c	11.5 ± 3.0 a-c	1.8 ± 3.3 cd
NC	NC21-033	0.5 ± 0.2 g	40.9 ± 8.9 ab	4.9 ± 1.4 a-c	4.9 ± 1.2 a-c	0.9 ± 0.3 k	80.0 ± 1.4 bc	9.5 ± 1.0 bc	9.5 ± 0.4 bc
NC	NC21-035	0.6 ± 0.4 g	28.7 ± 8.1 cd	3.1 ± 1.0 bc	5.5 ± 2.6 ab	1.7 ± 1.5 k	75.3 ± 4.6 cd	8.4 ± 2.1 bc	14.6 ± 5.0 ab
NC	<b>Pearl</b>	0.1 ± 0.1 g	0.6 ± 0.3 e	0.1 ± 0.0 c	0.1 ± 0.0 d	17.0 ± 4.5 b	76.9 ± 3.0 b-d	5.7 ± 2.2 bc	0.4 ± 0.8 d
NC	Rocco	0.9 ± 0.2 fg	33.1 ± 6.5 b-d	3.5 ± 1.0 bc	5.1 ± 1.2 a-c	2.2 ± 0.7 jk	77.9 ± 1.6 b-d	8.1 ± 1.2 bc	11.9 ± 1.3 bc
NC	Ruby June	1.7 ± 0.8 ef	15.3 ± 6.3 de	2.2 ± 0.7 bc	1.9 ± 1.5 d	8.8 ± 3.2 de	71.2 ± 8.1 cd	10.3 ± 2.3 bc	9.7 ± 5.9 bc
NC	Scarlet	8.3 ± 1.0 a	56.7 ± 2.7 a	3.8 ± 0.2 bc	0.3 ± 0.1 d	12.0 ± 1.4 cd	82.2 ± 1.1 a-c	5.4 ± 0.2 bc	0.4 ± 0.1 d
NC	Sweet Charlie	0.5 ± 0.3 g	22.3 ± 5.3 de	2.1 ± 0.4 bc	2.5 ± 0.6 d	1.9 ± 0.8 k	81.2 ± 1.2 a-c	7.7 ± 0.6 bc	9.2 ± 1.2 c

<sup>z</sup>C3G = cyanidin 3-glucoside, P3G = pelargonidin 3-glucoside, P3R = pelargonidin 3-rutinoside, P3MG = pelargonidin 3-(6"-malonylglucoside), TMAC = total monomeric anthocyanin content. Units in mg/100g fwt.

<sup>y</sup>FL = University of Florida breeding program, NC = North Carolina State University breeding program. Genotypes in bold were in both locations.

### Anthocyanin Pigment Profiles

The individual anthocyanin concentrations (mg/100g) and relative percentages of total anthocyanin content (%) also varied across germplasm. Cyanidin-3-glucoside (C3G) is a minor pigment in modern octoploid strawberries (*Fragaria x ananassa* Duch). Total C3G in juice ranged from 0.1 to 8.3 mg/100g fwt across genotypes, and the relative content of C3G (as %C3G of total anthocyanin) was 0.8 to 28.2% (**Table 4.4**). The highest total C3G (3.8–8.3 mg/100g) and relative C3G (7.7–13.3%) levels were found in fruit from ‘Liz’, NC20-012, and ‘Scarlet’. Conversely, the lowest total (0.1–0.9 mg/100g) and relative C3G (0.8–2.2%) were found in strawberries from ‘D’Light’, Florida-grown ‘Medallion’, NC19-016, NC19-022, NC20-055, NC20-099, NC21-033, NC21-035, ‘Rocco’, and ‘Sweet Charlie’. White-fruited ‘Pearl’ strawberries contained the lowest content of C3G (0.1 mg/100g) but had the highest relative C3G (17.0–28.2%) of all genotypes (**Table 4.4**). Similarly, fruit from genotype 20.12-99 also exhibited the lowest C3G content (0.1 mg/100g) and the second highest relative C3G percentage at (9.2%). The commonly grown commercial genotypes ‘Camarosa’ and ‘Chandler’, had C3G contents of 3.0 and 1.5 mg/100g and relative C3G of 6.3 and 3.4% (**Table 4.4**).

Pelargonidin 3-glucoside (P3G) was the dominant anthocyanin in all strawberry genotypes, with concentrations ranging from 0.2 to 56.7 mg/100g fwt (**Table 4.4**). The relative content of P3G (as %P3G of total anthocyanin) varied from 53.7 to 93.3%. Fruit from ‘Festival’ had the greatest P3G and %P3G content of the six Florida genotypes (38.0 mg/100g and 85.9%). Within North Carolina strawberries, genotypes highest or lowest in P3G were not always the highest or lowest in %P3G. For instance, fruit from NC19-016 had the fourth lowest P3G value (17.8 mg/100g) but also the highest relative P3G (93.3%) (**Table 4.4**). Genotypes NC19-020 (40.4 mg/100g),

NC21-033 (40.9 mg/100g), and ‘Scarlet’ (56.7 mg/100g) were high in P3G but had average %P3G values of 80.0–86.4, while genotypes ‘Felicity’, ‘Medallion’, and NC19-016 had high %P3G values of 87.0–93.3 but were lower in P3G (17.8 to 36.9 mg/100g). Aside from NC19-016, commercial genotypes ‘D’Light’ (9.2 mg/100g), ‘Pearl’ (0.6 mg/100g), and ‘Ruby June’ (15.3 mg/100g) were lowest in P3G, while Liz (64.1%), NC20-099 (70.6%), and ‘Ruby June’ (71.2%) were lowest in relative P3G (**Table 4.4**). ‘Medallion’ (36.9 mg/100g and 87.0%) and ‘Pearl’ (0.6 mg/100g and 76.9%) fruit grown in North Carolina were higher than those from Florida with P3G of 21.6 and 0.2 mg/100g and relative P3G of 80.0 and 53.7%, respectively. Although Florida-grown ‘Felicity’ fruit had higher P3G levels (34.5 vs. 30.8 mg/100g), North Carolina ‘Felicity’ fruit was higher in relative P3G (87.9 vs. 85.5%). Lastly, fruit from ‘Camarosa’ and ‘Chandler’ had average P3G contents of 33.3 and 37.3 mg/100g and relative P3G of 72.0 and 86.6% (**Table 4.4**).

Pelargonidin 3-rutinoside (P3R) was the least variable anthocyanin pigment across genotypes, with concentrations ranging from 0.1 to 9.0 mg/100g fwt and relative percentages (%P3R) from 5.0 to 19.2% (**Table 4.4**). ‘Camarosa’ fruit had the highest P3R (9.0 mg/100g) and relative P3R (19.2%) of all genotypes. Other genotypes high in P3R mg/100g were ‘Festival’ (4.7), ‘Liz’ (4.6), NC20-008 (5.3), NC20-012 (5.6), NC21-031 (4.7), NC21-033 (4.9), and genotypes FL-‘Felicity’, FL-‘Medallion’, ‘Liz’, NC19-022, NC20-008, NC20-012, NC20-055, NC20-058, and NC21-031 were high in %P3R (11.0–16.2). Conversely, genotypes low in P3R (0.1–2.2 mg/100g) were 20.12-99, ‘D’Light’, NC19-016, ‘Pearl’, ‘Ruby June’, and ‘Sweet Charlie’, while all genotypes except ‘Ruby June’ were low in relative P3R (5.0–7.7%) (**Table 4.4**).

Pelargonidin 3-(6''-malonylglucoside) is a pelargonidin-3-glucoside with malonic acid (a dicarboxylic acid) attached to a glucoside and is often thought of as a minor pigment. Average genotype total P3MG in juice ranged from 0.0 to 6.5 mg/100g fwt, and the relative content of P3MG (as %P3MG of total anthocyanin) was 0.0 to 20.2% (**Table 4.4**). The highest P3MG (4.5–6.5 mg/100g) and relative P3MG (11.9–17.6%) concentrations were found in fruit from 20.27-633, NC20-099, NC21-006, NC21-035, and Rocco. Fruit from D'Light were lower in P3MG compared to other genotypes (2.6 mg/100g) but were highest in relative P3MG (20.2%). Lowest P3MG (0.0–0.2) and relative P3MG (0.0–0.6%) were found in fruit from 20.12-99, FL-‘Felicity’, ‘Festival’, ‘Chandler’, NC19-016, NC19-020, NC20-058, NC21-020, ‘Pearl’, and ‘Scarlet’. North Carolina-grown ‘Felicity’ fruit had lower P3MG (0.3 mg/100g) but higher relative P3MG (10.1%) (**Table 4.3**).

#### Correlation Analysis

Correlation analysis of physiochemical, colorimetric, anthocyanin, total phenolic content, and subjective color ratings revealed both weak and strong relationships among the fruit composition variables (**Table 4.5**). A total of 115 pairwise correlations were computed for each set of variables.

**Table 4.5.** Correlation matrix on soluble solids content (SSC), pH, titratable acidity (Tacid), lightness ( $L^*$ ), red-green axis ( $a^*$ ), yellow-blue color axis ( $b^*$ ), hue ( $h^*$ ), chroma ( $C^*$ ), subjective color (Sbj Color), total monomeric anthocyanin content (TMAC), total phenolic content (TPC), total anthocyanin content (TAC), cyanidin 3-glucoside (C3G), pelargonidin 3-glucoside (P3G), pelargonidin 3-rutinoside (P3R), and pelargonidin 3-(6''-malonylglucoside) (P3MG) of strawberry genotypes from the Florida and North Carolina germplasm collections. Correlations in bold underwent subsequent regression analysis.

	SSC	pH	Tacid	$L^*$	$a^*$	$b^*$	$h^*$	$C^*$	Sbj Color	TMAC	TPC	TAC	C3G	P3G	P3R
SSC	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	-0.02	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Tacid	0.09	-0.38	1	-	-	-	-	-	-	-	-	-	-	-	-
$L^*$	0.15	-0.03	-0.08	1	-	-	-	-	-	-	-	-	-	-	-
$a^*$	0.26	0.04	0.04	-0.81	1	-	-	-	-	-	-	-	-	-	-
$b^*$	0.23	0.00	0.00	0.31	0.22	1	-	-	-	-	-	-	-	-	-
$h^*$	0.18	-0.03	0.00	0.93	-0.78	0.40	1	-	-	-	-	-	-	-	-
$C^*$	0.27	0.03	0.02	-0.59	0.94	0.54	-0.52	1	-	-	-	-	-	-	-
Sbj Color	-0.44	-0.02	-0.14	-0.48	-0.45	-0.45	-0.41	-0.46	1	-	-	-	-	-	-
TMAC	-0.05	0.26	-0.22	-0.35	-0.22	-0.31	-0.31	-0.28	<b>0.82</b>	1	-	-	-	-	-
TPC	0.18	0.01	0.26	-0.27	-0.26	-0.24	-0.16	-0.27	0.32	0.44	1	-	-	-	-
TAC	-0.14	0.28	-0.04	<b>-0.83</b>	<b>0.59</b>	-0.33	<b>-0.75</b>	0.40	-	0.76	0.27	1	-	-	-
C3G	-0.13	0.09	0.12	-0.47	0.17	-0.41	-0.48	0.00	-	0.23	0.26	0.61	1	-	-
P3G	-0.25	0.25	-0.03	-0.80	0.54	-0.30	-0.72	0.35	-	0.39	0.29	0.99	0.55	1	-
P3R	-0.10	0.13	-0.07	-0.76	0.57	-0.24	-0.68	0.40	-	0.22	0.07	0.92	0.56	0.91	1
P3MG	0.00	0.13	-0.12	-0.26	0.23	-0.13	-0.31	0.15	-	-0.05	-0.13	0.16	0.05	0.05	0.01

Physicochemical variables (SSC, pH, Tacid) exhibited the weakest correlations ( $r = 0.0$  to  $r = -0.44$ ) of the pairwise interactions overall, while color and phytochemical variables had some of the strongest (**Table 4.4**). Lightness ( $L^*$ ) had strong correlations with  $a^*$  ( $r = -0.81$ ),  $h^*$  ( $r = 0.93$ ), TAC ( $r = -0.83$ ), P3G ( $r = 0.83$ ), and P3R ( $r = -0.76$ ). The weakest correlation was between  $L^*$  and P3MG ( $r = -0.26$ ). Interactions between  $a^*$  and  $h^*$  ( $r = -0.78$ ) and with  $C^*$  ( $r = 0.94$ ) were also strong, while remaining  $a^*$  interactions were between  $r = 0.04$  (pH and Tacid) and  $r = 0.59$  (TAC). Similar to the physicochemical variables, the correlations of  $b^*$  and  $C^*$  to all other variables in this study were fairly weak ( $r = 0.0$  to  $r = 0.59$ ), with the exception of  $a^*$  and  $C^*$  previously mentioned. The last color variable (Sbj Color) had a strong positive relationship with TMAC but weak relationships ( $r = -0.05$  to  $r = -0.48$ ) with other variables (**Table 4.4**).

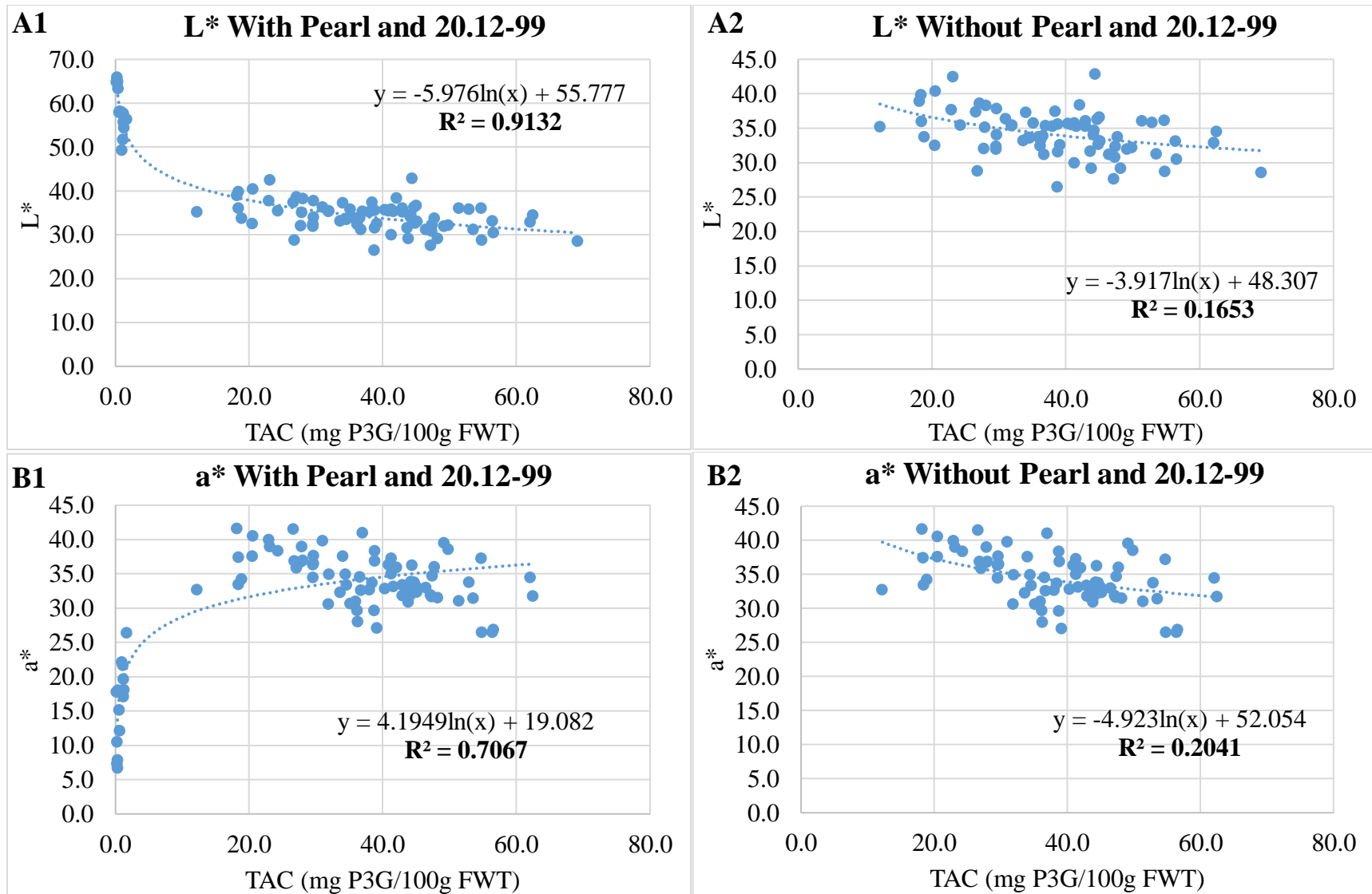
The two anthocyanin assays, TMAC and TAC, had a correlation of  $r = 0.76$  together and correlations of  $r = 0.44$  and  $r = 0.27$  with TPC, respectively (**Table 4.4**). For pigment profiles, P3G and TAC had the strongest correlation of all pairwise interactions in this study ( $r = 0.99$ ), with P3R also having a strong positive correlation with TAC ( $r = 0.92$ ). The two pigments also shared a strong correlation of  $r = 0.91$ . Pelargonidin 3-(6''-malonylglucoside) was the variable with the weakest correlations ( $r = 0.0$  to  $r = -0.31$ ) to all other variables in this study. Lastly, C3G had a strong positive relationship with TAC ( $r = 0.61$ ) with remaining correlations between  $r = 0.0$  ( $C^*$ ) and  $r = 0.56$  (P3R) (**Table 4.4**).

### Regression Analysis

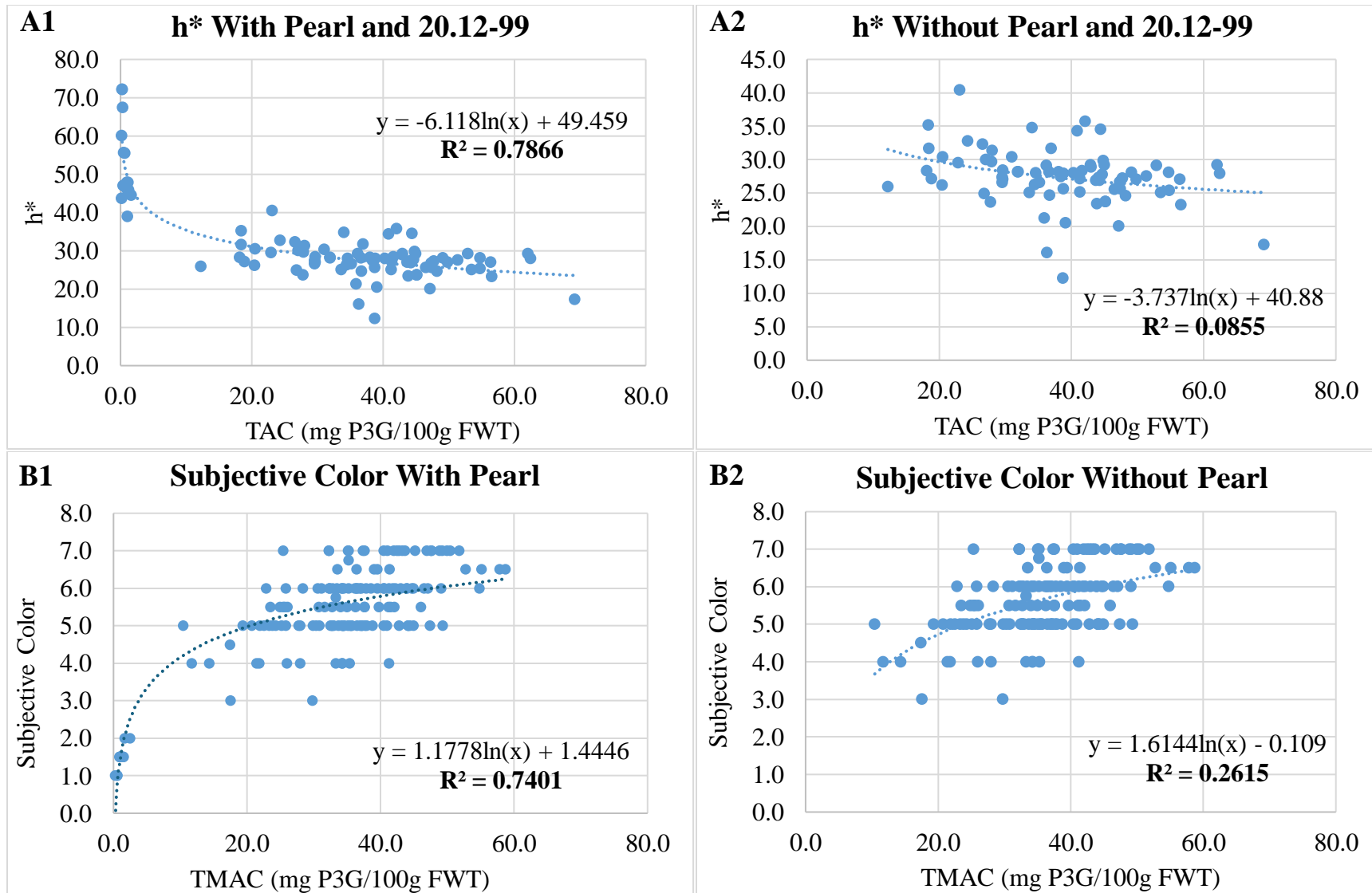
For regression analysis, only pairwise interactions between color variables (CIEL  $a^*b^*$  and Sbj Color) and phytochemical variables (TMAC, TAC, and TPC) that exhibited an  $r$  value of 0.50 or

greater were examined (**Figure 4.2**). These interactions included TAC with  $L^*$ ,  $a^*$ , and  $h^*$  and Sbj Color with TMAC. Additionally, two regressions were computed for each of the variable pairs, with the first regression containing fruit from white-fruited ‘Pearl’ and pink-fruited 20.12-99 and the other regression without the two genotypes.

In each regression analysis conducted between pairs of variables, a logarithmic fitted trend line consistently provided the highest coefficient of determination ( $R^2$ ) values, which were comparable to the Pearson coefficients obtained from correlation analysis. Regression analysis of  $L^*$  with TAC, which included fruit from ‘Pearl’ and 20.12-99 in the model, produced an  $R^2$  value of 0.9132 (**Figure 4.2 A1**). The corresponding  $L^*$  and TAC model without ‘Pearl’ and 20.12-99 produced a lower  $R^2$  value of 0.1653 (**Figure 4.2 A2**). A similar pattern was observed for the other color variables. The regressions of  $a^*$  with TAC produced values of  $R^2 = 0.7067$  and  $R^2 = 0.2041$  (**Figure 4.2 B1–B2**), and the regressions of  $h^*$  with TAC had values of  $R^2 = 0.7866$  and  $R^2 = 0.0855$  (**Figure 4.3 A1–A2**) with the first values corresponding to the model with the two genotypes. Lastly, the regression model between subjective color and TMAC with ‘Pearl’ fruit included produced an  $R^2$  value of 0.7401, while the model without ‘Pearl’ fruit was  $R^2 = 0.2615$  (**Figure 4.3 B1–B2**).



**Figure 4.2.** Logarithmic Regressions of color variables (A) lightness ( $L^*$ ) and (B) red-green axis ( $a^*$ ) with total anthocyanin content (TAC), both with white-fruited Pearl and pink-fruited 20.12-99 (A1–B1) and without the two genotypes (A2–B2).



**Figure 4.3.** Logarithmic Regressions of color variables (A) hue ( $h^*$ ) and total anthocyanin content (TAC), with white-fruited Pearl and pink-fruited 20.12-99 (A1) and without the two genotypes (A2), and (B) subjective color and total monomeric anthocyanin content (TMAC).

## Discussion

### Strawberry Fruit Composition Profiles

In general, fruit composition values were similar to previously published results for strawberry fruit (Kader, 1991). Average soluble solids content (SSC) spanned 5.9% to 9.5%, juice pH ranged from 3.4 to 4.0, and titratable acidity (Tacid) ranged from 0.5% to 1.0% (**Table 4.1**).

In red-fruited genotypes, total monomeric anthocyanin content (TMAC) was 15.9–46.6 mg pelargonidin 3-glucoside (P3G)/100g fwt, total anthocyanin content (TAC) was 12.6–69.0 mg P3G/100g, and total phenolic content (TPC) ranged 75.5–121.2 mg gallic acid equivalents (GAE)/100g fwt (**Table 4.3**). Anthocyanin concentrations were slightly lower than values reported in **Kader, 1991**, where anthocyanin ranged from 55 to 145 mg P3G/100g, while TPC values were similar (58–210 mg GAE/100g). Additionally, white-fruited ‘Pearl’ and pink-fruited 20.12-99 had lower TMAC and TAC values than the red-fruited genotypes (0.8 and 1.1 mg P3G/100g), and fruit from ‘Pearl’ exhibited similar TPC at 80.1 mg GAE/100g (**Table 4.3**). Total phenolic content in strawberry fruit ranged from 75.5 to 121.2 mg GAE/100g, which is similar to previously reported ranges for strawberry (80.2–99.3 mg GAE/100g) (**Khanizadeh & Tremblay, 2011**).

The concentration and percentage of the individual anthocyanin pigments appeared to be primarily driven by genetics, as profiles were independent of the amount (low vs. high) of total anthocyanin. Cyanidin 3-glucoside (C3G) ranged from 0.1 to 8.3 mg/100g (relative C3G: 0.8–24.2%), pelargonidin 3-glucoside (P3G) from 0.1 to 56.7 mg/100g (relative P3G: 46.0–93.3%), pelargonidin 3-rutinoside (P3R) from 0.0 to 9.0 mg/100g (relative P3R: 4.1–19.2%), and

pelargonidin 3-(6''-malonylglucoside) ranged from 0.0 to 6.5 mg/100g with relative P3MG: 0.0–20.2% (**Table 4.4**). The dominant pigment in all genotypes was P3G, but interestingly, although C3G was only 0.1 mg/100g in white-fruited ‘Pearl’, fruit was noticeably greater in relative C3G (17.0–24.2%) than in the majority of the red-fruited genotypes (0.8–8.8%) (**Table 4.4**). Pink-fruited 20.12-99 also had an average C3G concentration of 0.1 mg/100g but was higher in relative C3G (11.9%) than all genotypes except ‘Liz’ and ‘Scarlet’ red fruit, averaging 13.3% and 12.0%, respectively. These results align with previous work by **Cheel et al. 2007**, **Peñarrieta et al. 2009**, **Simirgiotis et al. 2009**, and **Wang & Lewers, 2007** that found white-fruited and wild populations of strawberry germplasm had increased C3G content compared to red-fruited and modern cultivated counterparts. Pelargonidin 3-glucoside content still ranged from 25.3 to 41.8 mg 100/g in wild strawberry populations; however, pelargonidin was undetectable in some white-fruited genotypes.

#### Differences between Florida and North Carolina Genotypes

Of the 17 fruit physicochemical, phytochemical, and color variables assessed between Florida and North Carolina genotypes, fruit from ‘Felicity’ and ‘Pearl’ grown in both locations had similar composition profiles overall, with the exception of pH, titratable acidity (Tacid), and relative cyanidin 3-glucoside (%C3G). Florida-grown ‘Felicity’ fruit had a higher average pH (3.8 vs. 3.5), and ‘Pearl’ fruit had higher relative C3G (28.2 vs. 17.0%), while North Carolina-grown ‘Pearl’ fruit was higher in average Tacid (0.9 vs. 0.6%). ‘Medallion’ was the genotype that statistically differed the most between both locations. Florida ‘Medallion’ fruit had higher average  $b^*$  (31.1 vs. 16.4) and relative pelargonidin 3-(6''-malonylglucoside) (7.4 vs. 0.7%), and North Carolina ‘Medallion’ fruit had higher soluble solids content (SSC) (8.6 vs. 7.2%), Tacid

(1.0 vs. 0.8), C3G (1.6 vs 0.4 mg/100g), pelargonidin 3-glucoside (P3G) (36.9 vs. 21.6 mg/100g), and total anthocyanin content (TAC) (43.0 vs. 26.8 mg P3G/100g).

Although uniformly colored red fruit was harvested from both locations, differences seen between ‘Medallion’ fruit may be attributed to the degree of ripeness. North Carolina growers often harvest fruit riper as the state utilizes both commercial and direct marketing models. The commercial industry prefers a berry that withstands shipping, while direct market growers prefer fully ripe and flavorful red fruit (**Pritts et al., 1998**). Therefore, California, Florida, and other commercially driven high-shipping strawberry markets harvest berries no more than  $\frac{3}{4}$  commercial ripeness to maintain fruit firmness and slow development of deeper red fruit color during storage, leading to lighter fruit color, lower anthocyanin content, and different fruit composition (**Cayo et al., 2016; Hokanson & Finn, 2000; Jouquand et al., 2008; Kalt et al., 1993; Nunes et al., 2005; Pelayo-Zaldivar et al., 2005; Zhang et al., 2022**). The  $\Delta E$  color difference between Florida-grown and North Carolina-grown ‘Medallion’ fruit was 15.21, with North-Carolina ‘Medallion’ fruit exhibiting darker hues due to lower  $L^*$ .

### Strawberry Color Profiles

Color measurements of red strawberry fruits in this study were  $L^*$  (lightness): 31.0–38.5,  $a^*$  (red-green axis): 28.0–37.8,  $b^*$  (yellow-blue axis): 13.5–31.3,  $h^*$  (hue): 22.6–33.0, and  $C^*$  (chroma): 31.5–46.1 (**Table 4.1**). These values are lower than those reported by **Fernández-Lara, 2015**, where  $L^*$ : 38.8–42.03,  $a^*$ : 49.3–52.4,  $b^*$ : 32.0–39.4,  $h^*$ : 32.0–36.6,  $C^*$ : 59.8–65.9, and by **Kim et al., 2013**:  $L^*$ : 32.9–50.5,  $a^*$ : 35.1–45.2,  $b^*$ : 20.1–29.0. Values were similar to those reported by **Nunes et al., 2005**, that evaluated physicochemical changes during strawberry

development in ‘Chandler’, ‘Oso Grande’, and Sweet Charlie’ fruit that were field-ripened versus fruit that been fully ripened during storage. Here, field-ripened fruit were higher in  $L^*$ ,  $h^*$ , and  $C^*$ , while storage-ripened fruit had greater average  $a^*$  measurements. Between the two berry systems,  $L^*$  ranged from 31.0 to 35.0,  $a^*$  from 33.0 to 40.0,  $h^*$  was from 22.0 to 27.0, and  $C^*$  ranged from 35.0 to 45.0 (Nunes et al., 2005).

White-fruited ‘Pearl’ fruit grown in Florida and North Carolina ranged from  $L^*$ : 63.1–65.3,  $a^*$ : 11.7–14.0,  $b^*$ : 19.9–22.0,  $h^*$ : 54.9–59.9, and  $C^*$ : 23.7–26.9 (Table 4.1). Values of both red and white/pink genotypes were also similar to those of Smith and Nunes, 2023, who compared the physicochemical, phytochemical, and color parameters of ‘Pearl’ and ‘Brilliance’ fruit grown in Florida. However, one thing to note is the skin color variability of ‘Pearl’ fruit, which ranges from white to blush pink and sometimes has yellowing of the skin. Although ‘Pearl’ fruit received from Florida was whiter in comparison to fruit harvested from North Carolina, Florida-grown Pearl berries did not have uniform color, with fruit exhibiting all three shades mentioned, while North Carolina-grown ‘Pearl’ fruit was more uniformly pink with slight yellowing. This non-uniform coloration of ‘Pearl’ fruit was also seen by Smith and Nunes, 2023, who found that differences in the color of ‘Pearl’ fruits depends on the time of harvest. Strawberries harvested in January appeared much whiter, while those harvested in February and March were noticeably pinker, to the extent that the fruits looked like a separate genotype. North Carolina ‘Pearl’ fruit was most visually and quantitatively similar to the pink ‘Pearl’ berries harvested in March in the Smith and Nunes, 2023 study. This indicates that fruit composition reference values for white and pink color variations may need to be established with each production season.

Florida-grown ‘Pearl’ fruit and North Carolina-grown ‘Pearl’ had a  $\Delta E$  color difference of 3.8. While two colors with an  $\Delta E$  value of at least 3.0 and even as low as 1.0 may be discernible by human eyes, this ability is likely influenced by factors such as age, greater visual acuity (20/20 vision), prior training in color-based industries, or even prior knowledge that a color difference is present. Additionally, the minimum  $\Delta E$  threshold for perceivable differences varies depends on the specific color and saturation being examined, as deciphering differences between two shades of one color may be easier than two shades of another. This variability in color thresholds is illustrated by **Martínez et al., 2001**, which found that only red wine shades with  $\Delta E$  differences  $\geq 3.0$  could be visibly detected as different. In our study,  $\Delta E$  values ranged between 1.0 and 36.0. With the red and beige shades of strawberry fruit presented in **Table 4.2**, color differences among the strawberry genotypes are most likely noticeable to untrained eyes at a minimum  $\Delta E$  value of 5.0 to 6.0, with values  $\geq 10.0$  being easily detectable from a distance.

#### Prediction of Strawberry Phytochemical Content from External Fruit Color

When comparing different regression models to determine whether colorimetry measurements could accurately predict phytochemical content, a logarithmic model best fit the variable interactions in this study, meaning that as one anthocyanin variable increased (TAC or TMAC), the rate of change in the predictor variables ( $L^*$ ,  $a^*$ ,  $h^*$ , Sbj Color) slowed.  $L^*$  was the most important value in predicting anthocyanin content, followed by  $h^*$ , subjective color, and  $a^*$ . All of the colorimetry variables were weakly correlated with phenolic content.

$L^*$  and TAC had the highest  $R^2$  value (0.9132), indicating that 91.32% of the variability in TAC could be explained by changes in  $L^*$  and that  $L^*$  could serve as the strongest predictor of

anthocyanin content (**Figure 4.2 A1**). These results align with previous research conducted by **Kim et al, 2013** and **Vieira et al, 2018** that found the highest correlation between  $L^*$  and anthocyanin content. Hue ( $h^*$ ) had the second highest  $R^2$  value (0.7866) that explained 78.66% of the variability in TAC, and  $a^*$  had the lowest relationship of the three CIEL\*a\*b\* color variables with TAC ( $R^2 = 0.7067$ ) (**Figure 4.2–4.3**). As the quickest and simplest color method, subjective color (Sbj Color) ratings explained 74.01% of the variability in TAC (**Figure 4.3 A1**). However, high  $R^2$  values were only seen when white and pink berries from ‘Pearl’ and 20.12-99 were included in the models. When fruit from these two genotypes was taken out, the predictability of the color parameters to anthocyanin content drastically decreased ( $R^2 = 0.0855$  to  $R^2 = 0.2615$ ). Therefore, for colorimetry methods to be useful in predicting anthocyanin content in hopes of replacing the need for anthocyanin assays, including light-colored berries in the model is essential. Although Sbj Color and  $a^*$  had the lowest predictability of the four variables for anthocyanin content when Pearl’ and 20.12-99 fruit were included, Sbj Color and  $a^*$  had the highest predictability rates of anthocyanin content when the two genotypes were removed.

CIEL\*a\*b\* color variables were also correlated with the individual anthocyanin pigments, pelargonidin 3-glucoside (P3G), pelargonidin 3-rutinoside (P3R), pelargonidin 3-(6’’-malonylglucoside), and cyanidin 3-glucoside (C3G). Similar to TAC,  $L^*$  was also the strongest predictor of P3G and P3R content ( $r = -0.80$  and  $-0.76$ , respectively). P3G was the dominant pigment in all genotypes evaluated in this study followed by P3R in red-fruited genotypes and C3G in white-fruited genotypes (**Table 4.5**). Although correlations were weak,  $h^*$  was the strongest predictor of C3G and P3MG content ( $r = -0.48$  and  $0.31$ , respectively). Additionally,

both the objective CIEL\*a\*b\* color measurements and subjective color ratings were more strongly associated to anthocyanin content measured by high performance liquid chromatography than with anthocyanin content measured by microplate spectrophotometry (**Table 4.5**).

#### Limitations in Colorimetry Methods

Interestingly, while the CIEL\*a\*b\* readings were fairly accurate in predicting strawberry anthocyanin content, these measurements were less reliable in assessing overall strawberry color and appearance. After converting the CIEL\*a\*b\* readings into HEX color codes that can be visually seen, all of the red-fruited genotypes had colors that leaned towards darker brick-red shades rather than bright and scarlet-red shades, as observed in **Figure 4.1**, as well as indicated by the subjective ratings (**Table 4.1**). Similarly, the HEX codes for white and pink-fruited genotypes appeared beige and light brown in color. These results suggest that evaluating strawberry appearance and quantifying fruit color using CIEL\*a\*b\* measurements alone can be misleading. Reasons as to why CIEL\*a\*b\* measurements did not accurately reflect the true strawberry color could be attributed to several factors, including non-uniform fruit color, uneven fruit contact with the colorimeter head, environmental background noise such as different lighting, and the impact of achene color on the overall color perception of the fruits (**Ngo et al., 2007**).

In the standardized RosBREED phenotyping protocol, reference pictures of different strawberry colors are provided, which helps prevent some bias and discrepancies. Reference photos and ratings for orange-colored strawberries would also benefit graders. However, more detailed

criteria on the environmental conditions under which evaluations should take place as well as the criteria of the evaluators themselves are needed for subjectively rating strawberry color. For instance, lighting significantly influences visual perception of color and includes factors such as evaluation setting (in field or in laboratory), if in a laboratory what type of light is used (LED, fluorescent, halogen, incandescent), and what color temperatures the lights emit (daylight or variations of cool, soft, bright, true, neutral, and warm white) (**Bustamante et al., 2021; Jost-Boissard et al., 2009; Kim et al., 2014; Shih et al., 2017**). Additionally, concerning the  $\Delta E$  values and perceived differences previously mentioned, subjective ratings will likely vary between individuals due to different visual acuities, ages, backgrounds, etc. Therefore, if subjective ratings are to be used more frequently to potentially replace the need for anthocyanin assays during quick germplasm screening, it is essential that the RosBREED protocols be updated to provide more detailed guidelines addressing some of these implications to optimize fruit ratings.

## **Conclusion**

The main objective of this study was to evaluate whether using external colorimetry methods are able to accurately estimate the total anthocyanin and phenolic content of strawberries.

Strawberry fruit from 31 genotypes from the Florida and North Carolina strawberry breeding programs were evaluated for 21 physicochemical, phytochemical, and color variables over a one-year period. Results showed that both objective (CIE  $L^*a^*b^*$ ) and subjective (visual ratings) colorimetry methods may be useful in assessing anthocyanin content, but only when a full spectrum of strawberry colors is used (white–dark red). Lightness ( $L^*$ ) was the most important color variable for the prediction of anthocyanin content ( $R^2 = 0.9132$ ), followed by hue ( $h^*$ ) ( $R^2$

= 0.7866) and subjective color (Sbj Color) ratings explained ( $R^2 = 0.7401$ ).  $L^*$  also exhibited strong associations with pelargonidin 3-glucoside and pelargonidin 3-rutinoside ( $r = -0.80$  and  $-0.76$ , respectively), while  $h^*$  was strongest predictor of cyanidin 3-glucoside and pelargonidin 3-(6''-malonylglucoside) with  $R^2$  values of  $-0.48$  and  $0.31$ , respectively. Colorimetry methods were not able to accurately predict the phenolic content in fruit. Notably, this study highlighted the importance of utilizing both analyses when assessing strawberry fruit color. While the CIEL\*a\*b\* provided similar quantitative values to those previously reported in strawberry, measurements were not entirely representative of the true variability in strawberry color as seen in person. Conversely, subjective ratings of fruit color were more representative of the true strawberry colors, but graders may have a tendency to exaggerate perceived differences in color unless provided with more detailed assessment guidelines. Combining both objective (CIEL\*a\*b\*) and subjective (visual ratings) colorimetry methods will provide a more comprehensive understanding of strawberry color and will help to discern differences between genotypes.

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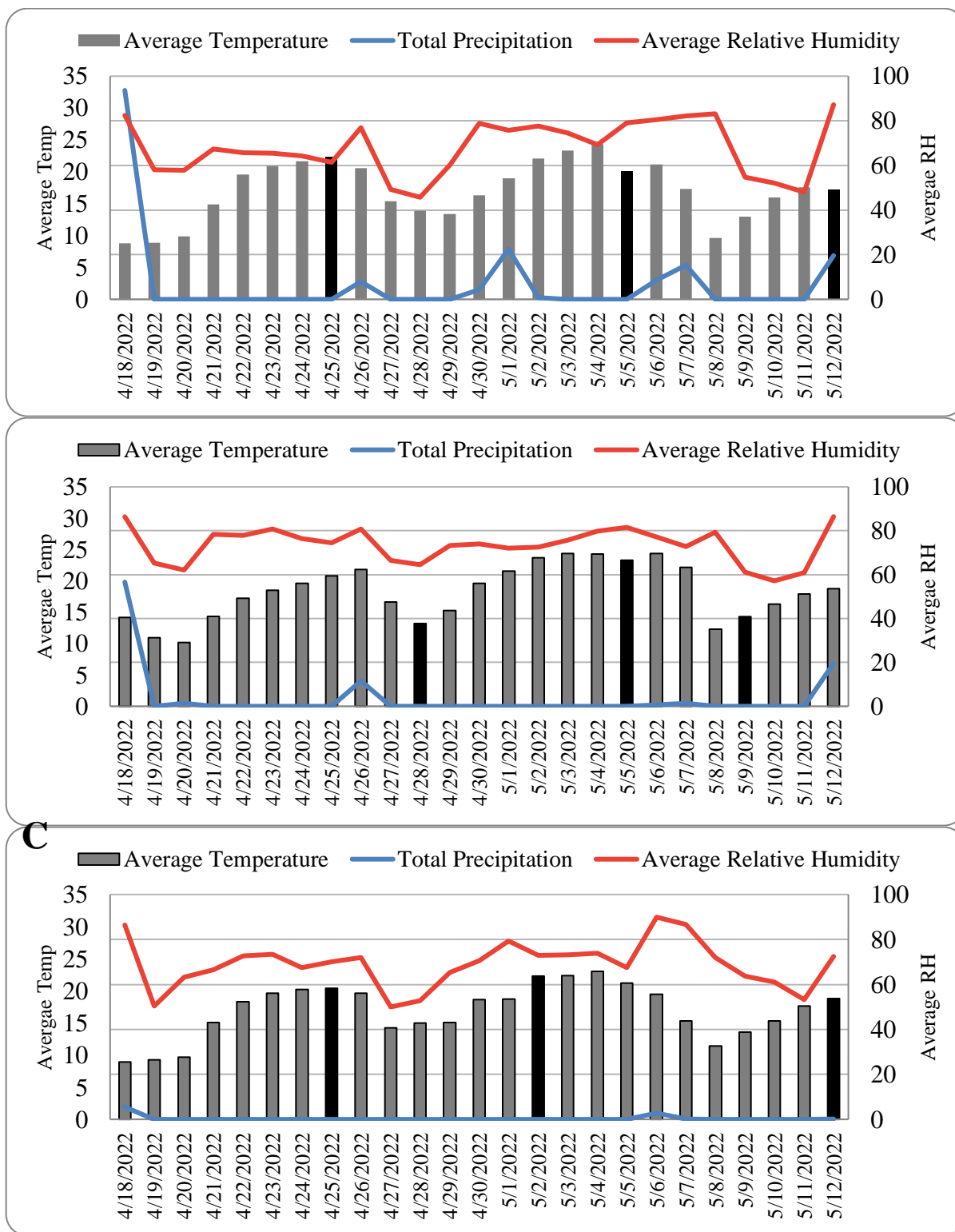
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**APPENDICES**

## Appendix A



**Figure A.1.** Average Temperature (C°), Total Precipitation (mm), and Average Relative Humidity (%) of (A) Central Crops Research Station, (B) Horticultural Crops Research Station, and (C) Piedmont Research Station. Harvest dates are colored black.

**Table A.1.** Commercial strawberry production practices for three NCDA&CS Research Stations in North Carolina for the 2021-2022 season.

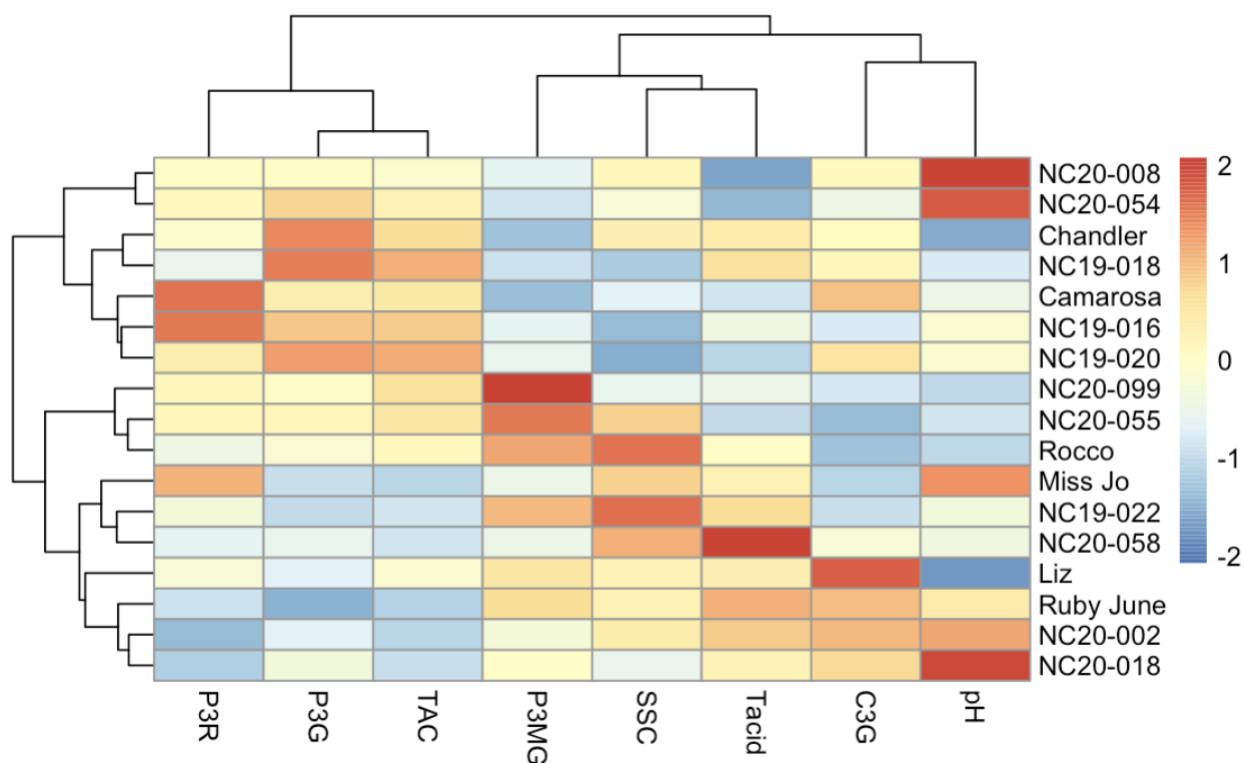
Station	Product Used	Category	Company	Rate	Frequency	
PRS <sup>y</sup>	15.5-0-0 Calcium Nitrate	Fertilizer	TCC <sup>z</sup>	13.5 lbs app	Three (4/22-5/22)	
	17-17-17 NPK	Fertilizer	TCC	81 lbs app	One (8/21)	
	34-0-0 Ammonium Nitrate	Fertilizer	TCC	6.56 lbs app	Two (3/22)	
	Borate (21% B)	Fertilizer	TCC	0.122 lbs app	One (3/22)	
	MgSO <sub>4</sub>	Fertilizer	TCC	5.14 lbs app	Five (3/22-5/22)	
	Cabrio EG	Fungicide	TCC	2.8 oz app	One (3/22)	
	Captan 4L	Fungicide	TCC	13-24 oz app	One 13 oz (10/21), two 19 oz (3/22), one 24 oz (5/22)	
	Capevate 68 WDG	Fungicide	TCC	1.3 lbs app	One (4/22)	
	Ridomil Gold SL	Fungicide	TCC	3.2-4.3 oz app	One 4.3 oz (10/21), two 3.2 oz (3/22, 4/22)	
	Switch 62.5 WG	Fungicide	TCC	2.5-3.8 oz app	One 3.8 oz (10/21), two 2.5 oz (3/22), one 3.5 oz (5/22)	
	Gramaxone SL 3.0	Herbicide	TCC	0.675 pt app	One (6/22)	
	Tide Clethodim 2EC	Herbicide	TCC	1 oz app	One (3/22)	
	HCRS	4-0-8 Strawberry FCI Liq Epsom Salt Magriculture Granular	Fertilizer	NAS/MA	4.93 gal	Five (3/22-5/22)
		10-10-10 NPK	Fertilizer	NAS/MA	7.06 lbs	Five (3/22-5/22)
		6-3-18 NPK	Fertilizer	TWG	150 lbs	One (4/21)
Solubor (9% B)		Fertilizer	NAS/MA	1000 lbs	One (9/21)	
Captan 4L		Fungicide	LL	120.7 mL	One (3/22)	
Dominus		Fungicide	NAS/MA	96 oz/acre	Eight (3/22-5/22)	
Luna Sensation		Fungicide	NAS/MA	250 lbs	One (9/21)	
Ridomil Gold SL		Fungicide	NAS/MA	7.5 oz/acre	Four (3/22-5/22)	
Switch 62.5 WG		Fungicide	NAS/MA	3.3 pt	Two (11/21, 3/22)	
Agri-Mek 0.15 EC		Insecticide	NAS/MA	11 oz/acre	Four (3/22-5/22)	
Chateau EZ		Herbicide	NAS/MA	16 oz/acre	Three (3/22-5/22)	
Gly Star Plus		Herbicide	NAS/MA	6 oz/acre	Two 6 oz/acre (3/22, 4/22), one 0.4 oz/acre (3/22)	
Gramaxone SL 2.0		Herbicide	NAS/MA	96 oz/acre	Four 96 oz/acre (6/21-3/22)	
Liberty 280 SL		Herbicide	NAS/MA	32 oz/acre	Three 32 oz/acre (4/21, 6/22, 8/55)	
CCRS		24 S Nitrogen	Fertilizer	TCC/HC	64 oz/acre	One (4/22)
	6-6-18 NPK	Fertilizer	TCC/HC	5.6 gal	Four (3/22-5/22)	
	Boron	Fertilizer	TCC/HC	1000 lbs/acre	One (9/21)	
	MgSO <sub>4</sub>	Fertilizer	TCC/HC	196 grams	Two (3/22 and 5/22)	
	Captan 4L	Fungicide	TCC/HC	17.5-35.6 lbs	One 17.5 lbs (3/22), one 18 lbs (3/22), three 35.6 lbs (3/22-5/22)	
	Elevate 50 WDG	Fungicide	TCC/HC	3 lbs/acre	Five 3 lbs/acre (4/22-5/22), one 3.25 lbs/acre (5/22)	
	Pristine	Fungicide	TCC/HC	1 lbs/acre	Two (4/22)	
	Ridomil Gold SL	Fungicide	TCC/HC	15 oz/acre	One (5/22)	
	Switch 62.5 WG	Fungicide	TCC/HC	15 oz/acre	One (3/22)	
	Agri Mite	Insecticide	TCC/HC	14 oz/acre	Two (4/22 and 5/22)	
	Gramaxone	Herbicide	TCC/HC	.75 lbs/acre	Two (5/22)	
			3 pt/acre	Four (3/22)		

<sup>z</sup>TCC = Triangle Chemical Company, NAG = Nutrient Ag Solutions, MA = Merherrin Ag, TWG = Tribute Weaver Grangular, LL = Loveland, HC = Helena Chemical

<sup>y</sup>PRS = Piedmont Research Station, HCRS = Horticultural Crops Research Station, CCRS = Central Crops Research Station

**Table A.2** Correlation matrix on pH, titratable acidity (Tacid), soluble solids content (SSC), total anthocyanin content (TAC), pelargonidin 3-glucoside (P3G), pelargonidin 3-rutinoside (P3R), pelargonidin 3-(6''-malonylglucoside), and cyanidin 3-glucoside (C3G) of 17 strawberry genotypes from the North Carolina germplasm collection.

Attribute	pH	Tacid	SSC	TAC	P3G	P3R	P3MG	C3G
pH	1	-	-	-	-	-	-	-
Tacid	-0.27	1	-	-	-	-	-	-
SSC	-0.29	0.39	1	-	-	-	-	-
TAC	0.03	-0.26	-0.30	1	-	-	-	-
P3G	0.08	-0.30	-0.36	0.96	1	-	-	-
P3R	0.10	-0.20	-0.21	0.69	0.61	1	-	-
P3MG	-0.17	0.09	0.20	0.25	0.01	0.10	1	-
C3G	-0.06	0.13	0.04	0.22	0.16	-0.01	-0.02	1



**Figure A.2.** Hierarchical cluster heatmap of fruit composition and anthocyanin profiles in 17 strawberry commercial genotypes and advanced selections grown at three locations in North Carolina (2022). Red hues indicate positive correlation, white/yellow represent negligible/minimal correlation, and blue hues indicate negative correlations. C3G = cyanidin 3-glucoside, P3G = pelargonidin 3-glucoside, P3R = pelargonidin 3-rutinoside, P3MG = pelargonidin 3-(6''-malonylglucoside), TAC = total anthocyanin content, SSC = soluble solids content, Tacid = titratable acidity.

## Appendix B

**Table B.1.** Greenhouse grown strawberry genotypes used for fruit composition data, dendrogram cluster, and cluster color.

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
1	Albion	8.3	3.53	0.77	34.15	1	green
2	ARMP18-041	8.8	3.63	0.79	51.47	2	red
3	ARMP18-098	10.6	3.55	0.91	53.36	3	blue
4	ARMP18-343	8.7	3.35	0.92	61.67	2	red
5	Camarosa	8.1	3.58	0.94	54.43	2	red
6	Camino Real	7.0	3.54	0.69	44.12	2	red
7	Florida Beauty	7.7	3.64	0.79	31.90	1	green
8	Florida Elyana	8.0	3.82	0.64	22.65	1	green
9	Florida Sensation	7.3	3.71	0.66	24.13	1	green
10	Galleta	6.7	3.52	1.05	61.90	2	red
11	Grenada	5.7	3.48	0.71	40.68	1	green
12	Merced	7.7	3.54	0.82	42.22	2	red
13	NC06-049	8.2	3.50	0.97	56.68	2	red
14	NC06-053P	8.9	3.33	1.21	20.56	4	purple
15	NC17-004	11.3	3.60	0.82	26.45	3	blue
16	NC17-005	7.7	3.45	1.00	58.58	2	red
17	NC19-001	10.7	3.62	0.81	30.67	3	blue
18	NC19-002	7.5	3.60	0.85	33.20	1	green
19	NC19-003	7.8	3.50	0.60	27.27	1	green
20	NC19-005	10.2	3.65	1.02	66.71	3	blue
21	NC19-006	9.4	3.51	0.85	36.41	3	blue
22	NC19-007	8.9	3.63	0.89	85.03	3	blue
23	NC19-008	9.6	3.54	0.89	34.27	3	blue
24	NC19-009	9.4	3.78	0.90	47.79	3	blue
25	NC19-010	9.8	3.62	0.71	64.63	3	blue
26	NC19-011	7.2	3.56	0.84	117.64	2	red
27	NC19-012	7.9	3.50	0.67	85.21	2	red
28	NC19-015	8.9	3.44	0.82	42.10	2	red
29	NC19-016	7.2	3.73	0.60	25.26	1	green
30	NC19-017	9.4	3.74	0.65	56.45	2	red
31	NC19-018	10.2	3.52	0.90	54.79	3	blue
32	NC19-020	10.3	3.59	0.84	61.07	3	blue

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
33	NC19-022	9.5	3.54	0.74	29.91	3	blue
34	NC20-002	7.3	3.46	1.01	25.35	4	purple
35	NC20-003	7.2	3.56	0.92	32.85	1	green
36	NC20-004	7.0	3.69	0.85	37.71	1	green
37	NC20-006	8.4	3.68	0.94	43.40	1	green
38	NC20-008	7.7	3.56	0.94	45.06	2	red
39	NC20-010	7.0	3.38	0.72	54.19	2	red
40	NC20-012	7.8	3.52	0.63	61.78	2	red
41	NC20-013	8.8	3.65	0.65	33.33	3	blue
42	NC20-014	8.2	3.82	0.64	47.91	3	blue
43	NC20-015	7.0	3.50	0.61	29.53	1	green
44	NC20-016	11.7	3.83	0.73	44.11	3	blue
45	NC20-017	8.7	3.49	0.62	46.49	2	red
46	NC20-018	8.4	3.52	0.73	39.25	2	red
47	NC20-019	8.0	3.70	0.64	32.55	1	green
48	NC20-024	6.9	3.53	0.68	62.14	2	red
49	NC20-025	7.2	3.55	0.64	32.97	1	green
50	NC20-026	7.5	3.55	0.69	55.02	2	red
51	NC20-028	7.7	3.44	0.75	46.37	2	red
52	NC20-029	7.8	3.54	0.60	42.93	2	red
53	NC20-030	7.1	3.48	0.72	23.66	1	green
54	NC20-031	7.4	3.43	0.75	24.43	1	green
55	NC20-032	9.2	3.70	0.88	12.45	1	green
56	NC20-033	6.8	3.53	0.69	49.81	2	red
57	NC20-035	9.7	3.64	0.85	42.22	3	blue
58	NC20-036	7.6	3.50	0.78	53.36	2	red
59	NC20-037	9.2	3.67	0.74	35.10	3	blue
60	NC20-038	8.5	3.65	0.67	45.30	2	red
61	NC20-039	8.3	3.56	0.83	54.19	2	red
62	NC20-042	10.0	3.64	0.74	47.08	3	blue
63	NC20-043	8.9	3.70	0.79	31.90	3	blue
64	NC20-044	8.8	3.24	1.47	46.13	4	purple
65	NC20-045	9.0	3.48	1.30	38.42	4	purple
66	NC20-046	10.0	3.36	1.40	32.28	4	purple
67	NC20-047	8.0	3.52	1.25	27.33	4	purple

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
68	NC20-048	7.5	3.61	0.64	39.61	2	red
69	NC20-049	10.5	3.60	1.31	42.34	4	purple
70	NC20-051	8.4	3.61	0.65	28.11	1	green
71	NC20-054	9.7	3.69	0.76	43.88	3	blue
72	NC20-055	10.2	3.72	0.67	27.87	3	blue
73	NC20-056	9.0	3.43	1.01	22.29	4	purple
74	NC20-057	10.0	3.75	0.61	23.61	3	blue
75	NC20-058	8.5	3.47	1.05	40.16	4	purple
76	NC20-059	7.1	3.52	0.55	40.79	2	red
77	NC20-060	8.0	3.58	0.72	46.96	2	red
78	NC20-061	7.6	3.54	0.65	62.73	2	red
79	NC20-062	9.6	3.53	0.66	51.47	2	red
80	NC20-063	8.2	3.76	0.60	28.53	1	green
81	NC20-064	7.5	3.37	0.70	36.88	2	red
82	NC20-065	9.1	3.50	0.74	47.32	2	red
83	NC20-066	11.0	3.41	0.86	33.20	3	blue
84	NC20-067	8.9	3.51	0.62	30.24	1	green
85	NC20-068	8.4	3.37	0.66	28.46	2	red
86	NC20-069	10.0	3.57	0.80	32.14	3	blue
87	NC20-070	9.5	3.59	0.76	38.42	3	blue
88	NC20-071	8.8	3.72	0.66	55.26	2	red
89	NC20-072	8.6	3.55	0.71	45.42	2	red
90	NC20-074	8.1	3.57	0.69	51.82	2	red
91	NC20-083	8.2	3.59	0.64	65.82	2	red
92	NC20-087	7.7	3.44	0.76	69.37	2	red
93	NC20-091	8.0	3.58	0.72	55.14	2	red
94	NC20-093	8.4	3.64	0.72	69.26	2	red
95	NC20-094	8.5	3.50	0.71	55.97	2	red
96	NC20-095	8.1	3.48	0.84	42.87	2	red
97	NC20-099	10.0	3.66	0.77	54.08	3	blue
98	NC20-100	7.0	3.61	0.67	27.67	1	green
99	NC20-101	6.5	3.69	0.62	29.53	1	green
100	NC20-105	6.1	3.71	0.58	28.58	1	green
101	NC20-106	7.9	3.63	0.67	28.34	1	green
102	NC20-107	7.4	3.53	0.83	38.07	1	green

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
103	NC20-111	10.4	3.65	0.92	29.71	3	blue
104	NC20-113	12.7	3.80	0.99	44.12	3	blue
105	NC20-119	7.6	3.56	0.74	30.02	1	green
106	NC20-122	8.2	3.98	0.59	33.62	3	blue
107	NC21-001	7.9	3.96	0.93	45.42	3	blue
108	NC21-002	8.9	3.84	0.68	39.13	3	blue
109	NC21-003	9.9	3.97	0.68	35.34	3	blue
110	NC21-004	6.2	3.45	0.86	29.53	1	green
111	NC21-005	6.0	3.72	0.62	41.27	1	green
112	NC21-006	7.7	3.73	0.74	41.98	1	green
113	NC21-007	8.7	4.10	0.73	37.83	3	blue
114	NC21-008	8.2	3.70	0.99	34.15	1	green
115	NC21-009	7.4	3.75	0.68	80.17	2	red
116	NC21-011	7.1	3.85	0.82	33.80	1	green
117	NC21-012	10.2	3.52	1.12	43.76	4	purple
118	NC21-013	8.1	3.89	0.89	41.98	3	blue
119	NC21-014	6.6	3.49	0.96	85.15	2	red
120	NC21-016	7.2	3.74	0.67	44.89	1	green
121	NC21-017	6.6	3.83	0.82	33.44	1	green
122	NC21-018	7.2	3.63	0.91	17.91	1	green
123	NC21-019	7.1	3.67	0.66	25.32	1	green
124	NC21-020	8.3	3.59	0.85	30.95	1	green
125	NC21-021	8.6	3.90	0.80	40.56	3	blue
126	NC21-022	5.5	3.87	0.55	39.67	1	green
127	NC21-025	8.7	3.55	0.68	38.07	2	red
128	NC21-027	7.6	3.52	0.76	46.72	2	red
129	NC21-028	6.6	3.81	0.63	49.81	1	green
130	NC21-031	7.9	3.28	1.03	53.36	2	red
131	NC21-033	10.4	3.68	0.93	67.95	3	blue
132	NC21-034	9.3	3.50	0.95	72.69	2	red
133	NC21-035	9.0	3.64	0.83	68.07	2	red
134	NCF94-017	8.2	3.48	0.74	48.62	2	red
135	NCH05-007	8.6	3.41	1.04	25.50	4	purple
136	NCH05-018	10.1	3.55	0.88	47.91	3	blue
137	NCH05-050	9.1	3.47	1.12	20.99	4	purple

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
138	NCH05-075P	6.9	3.46	1.04	34.86	4	purple
139	NCH06-028P	9.4	3.34	0.63	51.23	2	red
140	NCH06-014P	9.3	3.32	1.60	27.99	4	purple
141	NCH08-056P	9.2	3.49	1.31	27.87	4	purple
142	NCH10-029	7.3	3.64	0.62	43.76	2	red
143	NCH11-304	8.0	3.50	0.93	50.16	2	red
144	NCH11-319	10.5	3.79	0.55	31.31	3	blue
145	NCK12-188B	8.1	3.30	0.97	82.42	2	red
146	NCK12-194C	5.7	3.38	0.61	17.51	1	green
147	NCK12-198	8.4	3.56	0.58	27.28	1	green
148	NCL02-028	7.2	3.38	0.65	113.40	2	red
149	NCL03-006	7.7	3.53	0.80	67.12	2	red
150	NCL03-006 3M	8.0	3.67	0.86	58.79	2	red
151	NCL03-103	11.5	3.65	1.09	60.12	3	blue
152	NCL04-017	11.0	3.62	0.83	71.42	3	blue
153	NCL05-086	7.1	3.64	0.72	93.83	2	red
154	NCL06-041	9.9	3.38	1.49	36.76	4	purple
155	NCL07-004	7.4	3.50	0.84	44.47	2	red
156	NCL07-009	10.0	3.47	1.18	46.37	4	purple
157	NCL07-011	8.1	3.51	0.86	53.48	2	red
158	NCL07-014	8.2	3.39	0.92	55.14	2	red
159	NCL07-023P	8.0	3.38	1.34	46.66	4	purple
160	NCL07-029P	9.6	3.47	1.15	31.78	4	purple
161	NCL08-007	6.8	3.31	0.74	59.06	2	red
162	NCL08-067	7.3	3.37	1.08	53.60	4	purple
163	NCL08-069	7.0	3.33	1.42	53.01	4	purple
164	NCL08-073	10.0	3.51	0.89	55.97	3	blue
165	NCL08-074	9.5	3.43	1.30	56.69	4	purple
166	NCL08-078	8.0	3.48	1.34	54.43	4	purple
167	NCL08-079	7.8	3.64	0.99	30.60	1	green
168	NCL08-080	10.9	3.56	1.18	58.35	3	blue
169	NCL08-081	7.6	3.55	1.17	47.43	4	purple
170	NCL08-082	8.1	3.36	1.32	51.23	4	purple
171	NCL08-084	9.5	3.29	1.11	49.81	2	red
172	NCL08-085	6.2	3.62	0.49	35.22	1	green

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
173	NCL08-087	8.0	3.35	0.95	60.72	2	red
174	NCL08-089	7.3	3.49	1.16	32.85	4	purple
175	NCL08-092	8.2	3.44	1.05	38.66	4	purple
176	NCL08-093	9.7	3.66	1.00	46.13	3	blue
177	NCL08-097	9.4	3.48	0.62	44.23	2	red
178	NCL08-104	7.8	3.44	0.93	44.83	2	red
179	NCL08-114	7.6	3.38	0.70	58.82	2	red
180	NCL08-115 OP Seedling	9.3	3.78	0.52	57.99	2	red
181	NCL08-117	9.8	3.43	0.76	69.02	2	red
182	NCL08-118	8.0	3.64	0.69	54.25	2	red
183	NCL08-123	7.6	3.40	0.73	36.29	2	red
184	NCL08-126 OP Seedling	9.2	3.68	0.72	29.53	3	blue
185	NCL08-127	7.8	3.67	0.64	68.43	2	red
186	NCL08-130	9.8	3.59	0.77	40.56	3	blue
187	NCL09-006	9.9	3.38	1.30	38.19	4	purple
188	NCL09-007	8.8	3.36	0.88	47.32	2	red
189	NCL09-011	7.3	3.49	1.02	53.60	2	red
190	NCL09-012	8.1	3.40	1.34	69.73	4	purple
191	NCL09-013	7.6	3.40	0.92	51.76	2	red
192	NCL10-005	10.7	3.51	1.08	41.27	4	purple
193	NCL11-027P	7.0	3.49	0.77	45.06	2	red
194	NCL11-042	13.8	3.65	0.44	21.57	3	blue
195	NCL11-139	8.4	3.46	0.96	57.28	2	red
196	NCL11-148	7.1	3.40	1.13	57.99	4	purple
197	NCL11-167	12.3	3.47	0.57	36.88	3	blue
198	NCL11-174	7.2	3.30	1.46	38.90	4	purple
199	NCL11-175	9.9	3.45	1.14	37.83	4	purple
200	NCL11-176	10.7	3.51	0.96	69.61	3	blue
201	NCL11-185	7.7	3.35	1.20	51.82	4	purple
202	NCL11-201	10.0	3.58	0.91	39.96	3	blue
203	NCL11-211	8.1	3.33	0.90	50.52	2	red
204	NCL11-217	7.4	3.35	0.78	48.38	2	red
205	NCL11-220	9.1	3.64	1.10	71.33	3	blue
206	NCL11-223	11.0	3.48	1.03	67.12	3	blue
207	NCL11-231	10.3	3.51	1.26	32.17	4	purple

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
208	NCL11-239	8.0	3.41	0.63	55.62	2	red
209	NCL11-241	9.2	3.37	0.97	72.66	2	red
210	NCL12-001	9.2	3.52	0.78	37.36	3	blue
211	NCL12-005	11.5	3.38	1.00	47.67	3	blue
212	NCL12-011	7.7	3.59	0.71	56.09	2	red
213	NCL12-013	6.3	3.24	0.78	53.72	2	red
214	NCL12-014	8.8	3.56	0.61	46.96	2	red
215	NCL12-019	8.9	3.65	0.56	62.97	2	red
216	NCL12-020	15.8	3.83	0.69	41.98	3	blue
217	NCL12-042	9.4	3.51	0.76	54.08	2	red
218	NCL12-043	10.0	3.53	0.76	39.85	3	blue
219	NCL12-045	14.2	3.55	1.01	62.38	3	blue
220	NCL12-078 OP Seedling	12.7	3.90	0.86	21.70	3	blue
221	NCL12-080	10.0	3.48	0.84	28.35	3	blue
222	NCL12-083	9.9	3.51	0.66	41.51	2	red
223	NCL12-101	8.8	3.49	0.77	41.51	2	red
224	NCL12-113	8.3	3.70	0.62	46.25	2	red
225	NCL12-162	10.5	3.45	0.88	37.47	3	blue
226	NCL12-174	8.9	3.50	0.85	46.61	2	red
227	NCL99-033	14.2	3.47	1.01	49.10	3	blue
228	NCR06-038P	8.4	3.58	0.76	35.22	1	green
229	NCS10-005	7.6	3.59	0.45	48.50	2	red
230	NCS10-015	8.7	3.64	0.68	43.88	2	red
231	NCS10-022	10.2	3.50	0.80	42.96	3	blue
232	NCS10-032	7.3	3.50	0.54	39.25	2	red
233	NCS10-043	10.3	3.54	0.94	33.68	3	blue
234	NCS10-047 OP Seedling	10.4	3.68	0.66	72.34	3	blue
235	NCS10-070	9.2	3.51	0.72	59.18	2	red
236	NCS10-080	7.8	3.39	0.99	49.10	2	red
237	NCS10-095	8.1	3.45	0.71	41.03	2	red
238	NCS10-102	7.9	3.63	0.47	40.56	2	red
239	NCS10-109	10.6	3.53	0.87	54.19	3	blue
240	NCS10-147	8.5	3.46	0.65	54.08	2	red
241	NCS10-151	10.1	3.71	0.60	41.03	3	blue
242	NCS10-190	7.4	3.56	0.51	59.18	2	red

**Table B.1.** (continued).

ID	Genotype	%SSC	%Tacid	pH	TMAC	Dendrogram Cluster	Cluster Color
243	NCS10-194	9.2	3.75	0.59	34.58	3	blue
244	NCS10-205	9.1	3.81	0.51	54.91	2	red
245	NCS10-207	10.5	3.87	0.69	58.35	3	blue
246	NCS11-002	7.9	3.72	0.53	46.25	2	red
247	NCS11-025	8.1	3.52	0.73	68.43	2	red
248	NCS11-036	8.4	3.65	0.45	42.45	2	red
249	NCS11-039	9.5	3.71	0.67	43.76	3	blue
250	NCS11-056	9.1	3.57	0.69	57.04	2	red
251	NCS11-059	8.9	3.44	0.71	34.98	2	red
252	NCS11-061	8.7	3.27	0.82	85.62	2	red
253	NCS11-064	9.0	3.61	0.75	42.34	3	blue
254	NCS11-075	7.8	3.60	0.59	49.93	2	red
255	NCS11-107	9.2	3.81	0.61	36.53	3	blue
256	NCS11-113	10.0	3.57	0.57	43.64	2	red
257	NCS11-117	6.5	3.58	0.56	68.78	2	red
258	NCS11-123	9.3	3.67	0.59	62.73	2	red
259	NCST10-009	10.5	3.86	0.51	25.69	3	blue
260	NJ 15-1-2	11.2	3.67	0.87	54.19	3	blue
261	NJ 09-2-1	11.3	3.91	0.78	36.24	3	blue
262	Rocco	8.2	3.34	0.79	35.58	2	red
263	Ruby June	9.1	3.59	0.73	23.36	1	green
264	San Andreas	7.3	3.51	0.73	30	1	green
265	Strawberry Festival	7.5	3.57	0.84	37.47	1	green
266	Sweet Charlie	8.3	3.8	0.59	22.82	1	green
267	Winter Dawn	6.7	3.46	0.8	37.12	1	green
268	Winterstar	7.1	3.77	0.55	32.61	1	green

**Table B.2.** Greenhouse irrigation (overhead and drip) settings for 2022 strawberry germplasm collection at Piedmont Research Station, Salisbury, NC.

<b>Left side of Greenhouse (Propagation)</b>						
Greenhouse Zone	Location	Type	Irrigation System	Time of Day	Duration (mins)	Frequency
Zone 1	Front of Greenhouse	Overhead	Galcon	-	-	-
Zone 2	Upper Middle of Greenhouse	Overhead	Galcon	-	-	-
Zone 3	Back Middle of Greenhouse	Overhead	Galcon	1:56 PM	5	Daily
Zone 4	Back of Greenhouse	Overhead	Galcon	2:01 PM	5	Daily
<b>Right side of Greenhouse (Mother Plants)</b>						
Zone 1	Front of Greenhouse	Overhead	Hunter Pro	-	-	-
Zone 2	Upper Middle of Greenhouse	Overhead	Hunter Pro	-	-	-
Zone 3	Back Middle of Greenhouse	Overhead	Hunter Pro	-	-	-
Zone 4	Back of Greenhouse	Overhead	Hunter Pro	-	-	-
<b>Entire Greenhouse</b>						
Zone 5	Right Side of Greenhouse	Emitters	Hunter Pro	8:00am; 12:00pm	1.5	Daily
Zone 6	Left Side of Greenhouse	Emitters	Hunter Pro	8:20am; 12:11pm	1.5	Daily
<b>Additional Programmable Locations</b>						
Zone 5	Greenhouse #2	Overhead	Galcon	8:00pm; 5:00pm	10 secs/10 mins	Daily
Zone 7	Gravel Pad	Overhead	Hunter Pro	7:14am; 6:31pm	30	Daily
Zone 8	Propagation Tunnel	Emitters	Hunter Pro	-	-	-

**Table B.3.** Greenhouse climate control temperature settings for 2022 strawberry germplasm collection at Piedmont Research Station, Salisbury, NC.

<b>November-February</b>				
Heating/Cooling	Temp (°C)	Differential $\Delta$ (°C) <sup>z</sup>	Setting	Delay (min)
Cool Cell	OFF	-	-	-
Curtain	10.6	3	COOL	4
Exhaust Fans	15.6	5	COOL	0
Front Heater	4.4	-	-	-
Back Heater	4.4	-	-	-
<b>March-October</b>				
Heating/Cooling	Temp (°C)	Differential $\Delta$ (°C)	Setting	Delay (min)
Cool Cell	21.1	-	-	-
Curtain	10.6	3	COOL	4
Exhaust Fans	15.6	5	COOL	0
Front Heater	4.4	-	-	-
Back Heater	4.4	-	-	-
<b>Snow/Ice Conditions</b>				
Heating/Cooling	Temp (°C)	Differential $\Delta$ (°C)	Setting	Delay (min)
Cool Cell	OFF	-	-	-
Curtain	29.4	3	COOL	4
Exhaust Fans	35	5	COOL	0
Front Heater	37.8	-	-	-
Back Heater	37.8	-	-	-

<sup>z</sup> Temperature Differential  $\Delta$  is the difference between the minimum required temperature in the greenhouse and the lowest outside temperature.