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Characterization and inheritance assessment of fruit and leaf shape in unique *Vitis* seedlings

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ABSTRACT

From August to October of 2009, two separate studies were conducted to assess fruit shape of *Vitis* section *Euvitis* hybrid bunch grapes using digital photography (Study 1) and evaluate inheritance of leaf shape in unique populations of *V. rotundifolia* hybrids (*Vitis* section *Muscadinia*) (Study 2). All vines studied were located at the University of Arkansas Fruit Research Station, Clarksville. Study 1 used SigmaScan® digital photography analysis software to calculate shape factor, major:minor axis ratio, and compactness of highly variable, unique-shaped fruit from a population of 182 *Euvitis* seedlings. SigmaScan® accurately characterized fruit shape elongation as had been recorded in previous studies. Although elongated shapes were measured accurately, the calculations used were unable to conclusively analyze ovoid or pear-shaped fruits. Study 2 evaluated the inheritance of leaf shape (lobing) in several populations of *V. rotundifolia* crosses within the University of Arkansas fruit breeding program. Based on previous studies, it was hypothesized that leaf lobing was a dominant trait. The two populations expected to segregate into a 3:1, lobed:standard, phenotypic ratio were successfully observed, while only two of the six expected to demonstrate a 1:1, lobed:standard, phenotypic ratio were observed. Previous studies suggest the unexpected ratios observed may be attributed to a lethal allele combination, where homozygous dominant progeny are not viable, or modifier genes impacted leaf shape of the seedlings. Further evaluation of these and other populations in addition to molecular analysis would be helpful in characterizing inheritance of leaf lobing in muscadine hybrids.

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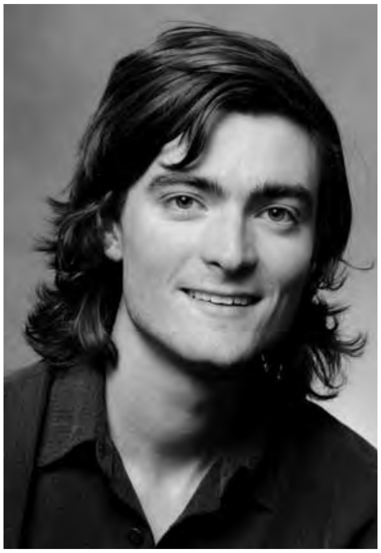

Paul J. Sandefur

After graduating from Fayetteville High School in 2006, I began my undergraduate studies at the University of Arkansas. Throughout my time as an undergraduate, I received the Bob and Marilyn Bogle scholarship for horticulture students. While completing my degree, I had the chance to experience the horticultural sciences hands-on by working at the Fayetteville Farmers Market, hiking in the Ozarks, raising a vegetable garden, and participating in a three-month internship at the Scottish Plant Explorer's Garden in Pitlochry, Scotland. Thanks to funding from the Bumpers College undergraduate research program, I began work on an undergraduate special project course under Dr. John R. Clark at the Fruit Research Station, Clarksville, during my senior year. After completing my research and graduating with a B.S. degree in horticulture and a minor in global agriculture, food, and life sciences in December of 2009, I started my master's studies in the Department of Horticulture advised by Dr. Clark. I would like to thank Drs. John R. Clark, Douglas Karcher, and the staff at the Fruit Research Station, Clarksville for all of their help, as well as Drs. Curt Rom and Jon Lindstrom for their guidance during my undergraduate studies.

INTRODUCTION

With over 7.2 million ha in production and 67.2 t being produced in 2007 worldwide, the grape is the world's most important fruit in terms of value and the second most important fruit crop in terms of overall production quantity (FAO, 2007). The history of grape production dates back to between 7000 BCE and 4000 BCE. Viticulture originated in the Middle East and quickly spread throughout the Mediterranean, reaching the North American coast by the late eighteenth century. Although the majority of grape production is processed for the wine industry, table grapes, raisins, and other processed products have been and continue to be major areas of production and research.

The grape family, Vitaceae, is made up of 14 genera, of which *Vitis* is the only genus utilized for food production. The genus *Vitis* is split into two sections, *Muscadinia* ($2n = 40$) and *Euvitis*, the bunch grape, ($2n = 38$). Although *Euvitis* heavily outweighs *Muscadinia* in its importance to world commerce, significant research has been conducted on both subgenera (Reisch and Pratt, 1996).

The grape breeding program at the University of Arkansas was started in 1964 by Dr. James N. Moore. In 1997, Dr. J.R. Clark replaced Dr. Moore as program director, and has continued and expanded the breeding objectives. The work at the University of Arkansas has resulted in the release of several table grape cultivars (*Euvitis*) including 'Reliance', 'Saturn', 'Venus', 'Mars', 'Jupiter', and 'Neptune' (Clark, 1997; Clark and Moore, 1999a and b). Although muscadine grapes have been grown in Arkansas for many years, it was not until 2005 that a muscadine (*V. rotundifolia*) breeding effort was incorporated into the grape breeding program. The major focus of the muscadine program is to develop adapted cultivars with enhanced quality and winter hardiness for central and southern Arkansas (Clark, 2003, 2008).

Although the Arkansas muscadine breeding program focuses on environmental adaptation and fruit quality traits, one ornamental cultivar, 'Southern Home', has been used as a parent in the program. This hybrid release from the University of Florida was introduced for its unique ornamental value and potential disease resistance. 'Southern Home' is the only released hybrid between bunch grapes and muscadine grapes (Mortensen et al., 1994). The first population in Arkansas with 'Southern Home' as a parent was created in 2005, and this cultivar has since been crossed with other muscadine cultivars as well as a unique *Euvitis* hybrid, A-1665, developed in the University of Arkansas breeding program. The resulting populations included seedlings with unique-shaped leaves, exhibiting substantial lobing.

Fruit shape is also an important factor in the development of new grape cultivars, as high quality fruit with unique shapes can attract the attention of retail customers who are not enticed by standard oval grapes. Although fruit and leaf shape can be key traits in fruit breeding, little work has been done with shape as the specific breeding objective in the *Vitis* genus. Fruit with a unique shape and plants with unusual ornamental leaf characteristics have the potential to fill niche markets within fruit crop or ornamental plant production (Clark, 2003; Mortensen et al., 1994).

In 2005 Andrew Wycislo, M.S. student at the University of Arkansas, developed a simple method to characterize fruit shape using digital photography. The study compared digital findings to human raters and concluded that digital photography utilizing SigmaScan® software rapidly, simply, and accurately characterized fruit elongation with a strong correlation between the human subjective ratings and digital analysis. According to his report, SigmaScan® was able to differentiate elongation between berries in the population based on shape factor [$(4 \times \pi^2)/(\text{perimeter})^2$], major:minor axis ratio, and compactness value [$(\text{perimeter})^2/\text{area}$]. This method was found to be more reliable than subjective visual assessment as variation in individuals in rating shapes was substantial (Wycislo et al., 2008). To further test the ability of digital photography to characterize fruit elongation and other diverse fruit shapes, a new population of hybrid table grapes was created and our study used this method to analyze this more diverse-shaped population.

In breeding plants, the presence of genetic variation for traits, the ability to identify or classify this variation appropriately, and determining the type of inheritance are all very important components of an improvement program. Therefore, the two objectives of our study were to (1) characterize variation in fruit shape of a population of unique-shaped bunch (*Vitis* section *Euvitis*) grapes using digital image analysis, and (2) evaluate variation in leaf shape in a series of populations derived from a muscadine grape cultivar ('Southern Home', *Vitis* section *Muscadinia*) and estimate the inheritance of this trait. Quantifying the type of inheritance exhibited and characterizing the variation in leaf and fruit shape should facilitate future breeding efforts attempting to develop the ornamental and commercial value of interspecific muscadine and bunch grape hybrids.

MATERIALS AND METHODS

Study 1. The population studied was created from a 2005 cross of table grape selections A-2315 and V#1. The hybridization, seed collection, stratification, germination, and seedling production were all conducted at the University of Arkansas Fruit Research Station, Clarksville. During hybridization, emasculated female parent clusters were protected from contamination from foreign pollen by enclosure in a white, all-weather paper bag.

In August, 2009, 182 seedlings were examined and where possible, fruit was collected from each. Twenty five representative berries from each seedling bearing fruit and both parents were collected, placed in freezer bags, and stored in a freezer until data were collected. Within the population, 79 seedlings either produced no fruit or could not be collected due to Green June beetle (*Cotinis nitida*) damage.

From early September to late October, ten representative berries from each seedling sample were removed from the freezer, cleaned of any frost, and placed on a fluorescent-pink surface to be photographed. A Nikon D70S digital SLR camera (Nikon Corp., Tokyo, Japan) was suspended above the grape surface and set on the automatic 'A' mode with the flash turned off in order to prevent glare. One photograph of each seedling's 10 berries was taken, each using the same camera settings, downloaded to a computer, and labeled. The photographs were then uploaded onto a computer to be analyzed by SigmaScan® using the same macro and calculations used in Wycislo (2005), shape factor, major:minor axis ratio, and compactness. The data from each berry was placed in a Microsoft Excel® spreadsheet, and the minimum, maximum, and median of each variable were calculated. Once all data were collected, the results were compared to Wycislo's observations and visually assessed for accuracy of characterization of elongation (Wycislo, 2005).

Study 2. From 2005 to 2008, nine populations with over 1,000 seedlings collectively were produced and established at the Fruit Research Station, Clarksville, and all segregated for leaf shape. All nine populations were progeny of 'Southern Home' crossed with a variety of other muscadine parents and one *Euvitis* parent (Table 1). During hybridization, emasculated female parent clusters were protected from contamination with foreign pollen by enclosure in a white, all-weather paper bag.

From August through October 2009, each population was characterized for leaf shape. Based on an initial evaluation of variation, there were two distinct classes of shape, the lobed types (usually deeply lobed), and the standard muscadine shape (Fig. 1). Thus, characterization was for one type or the other. Leaf shape observations were based on the leaf shape of each seedling between the sixth and ninth leaves from a major shoot tip, which resulted in uniform mature leaf assessment on each seedling. From the data collected, the appropriate genetic analysis, chi-square, was used to evaluate the qualitative inheritance pattern found for leaf shape variation with a 95% confidence level. We hypothesized that the lobed leaf trait was dominant and the standard leaf was recessive within the populations, and would be represented by either a 3:1 or 1:1 ratio (Table 1).

RESULTS AND DISCUSSION

Study 1. The *Euvitis* hybrid population studied in 2009 displayed a wide range of variation in fruit shape as indicated by the range in values for shape factor, major:minor axis ratio, and compactness (Table 2). Some of the grapes within the population were highly elongated, almost banana-shaped, while others were very small and perfectly round, although the majority were oblong (Fig. 2).

According to Wycislo (2005) and based on my results, shape factor decreased with elongation, and major:minor axis ratio and compactness both increased with elongation. Even with our population containing more extreme shape variation, all three measurements utilized in the previous study and repeated in our study accurately characterized fruit shape. Overall, digital photography analysis utilizing SigmaScan® software is a simple, cost effective, and accurate means of characterizing fruit shape in grape. Although the major:minor axis ratio was unable to determine the exact major axis of the slightly curved, highly elongated berries, and was therefore less accurate at determining elongation than shape factor and compactness, it still provided a useful overall measurement for the population.

One of the limiting aspects of all my calculations was the inability of any of the ratings to judge ovoid or pear-shaped fruit. If the SigmaScan® macro can be programmed to measure the maximum minor axis and major axis as well as the minimum minor axis and major axis, there is strong potential for the program to more accurately measure even more unique fruit shapes. Future studies assessing the usefulness of SigmaScan® with additional calculations would be beneficial to plant breeders for characterizing fruit shape in a variety of crops. In Wycislo's concluding remarks, he hypothesized that because a majority of his grapes tended toward a round shape, "this might indicate a partial dominance toward round shape" (2005). Our results support Wycislo's hypothesis, as the majority (~70%) of the grapes observed were round or oblong.

Study 2. Overall, the leaf-shape segregation in the populations was easily classified into lobed and standard muscadine shapes. The only challenges in the classifications were that some seedlings had intermediate leaf shapes (Fig. 1). Although this might be attributed to a quantitative inheritance of leaf shape, or possibly some modifying genes, only approximately 10% of each population showed an intermediate nature making quantitative inheritance of the lobed trait unlikely.

Based on the chi-square analysis, the null hypothesis was rejected. Therefore, the proposed ratio of 3:1 in the two predicted populations, 'Southern Home' open pollinated and 'Southern Home' x A-1665; with the lobed genotype being dominant, was supported (Table 1). These populations were projected to segregate in this manner due to both likely being heterozygous for the lobed leaf trait. While 'Southern Home' open pollinated had the cultivar as the female parent, but an unknown pollen parent, 'Southern Home' x A-1665 was a cross of two lobed parents. One might question why the ratio was not closer to the projected 3:1 rather than the observed 2.1:1. A possible explanation for this was the likely presence of a homozygous dominant lethal in one-fourth of the progeny. If this were the case, the lobed allele is dominant with respect to phenotype, but behaves as lethal when homozygous dominant. Therefore, all progeny that are homozygous recessive would display a standard leaf shape, all heterozygous offspring would display a lobed leaf shape, and any homozygous dominant would abort before any plant development occurred. A 2:1 (lobed to standard leaf) segregation ratio has been reported in muscadine grape hybrids (P. Conner, pers. comm.). In saucer-shaped peaches, it was found that a homozygous dominant allele was lethal and was the cause of the unique observed ratios (Guo and Jiang, 2002).

The 1:1 null hypothesis ratio, standard shape being homozygous recessive and lobed being heterozygous, was supported by two of the six populations ('Black Fry' x 'Southern Home' and 'Southern Home' x 'Granny Val') (Table 1). The most obvious observations from the data were the unexpected ratios that predominated in the other four populations of 'Southern Home' crossed with standard muscadine cultivars. Further, two of the most extreme variations in segregations from the expected (1 lobed: 1 standard) of Ga. 33-2-1 x 'Southern Home' (1.0 lobed: 2.2 standard) and 'Southern Home' x 'Doreen' (1.7 lobed:1.0 standard) segregated in opposite directions. One might consider a maternal or paternal effect, but this was not consistent among the populations.

Overall, the hypothesized dominance of the lobed-leaf trait was successfully observed in the field and supported by half of the populations. To fully study the inheritance of the lobed-leaf trait, a crossing design such as a diallel, where all parents in the study are crossed with each other, would be appropriate. Also, molecular characterization of leaf shape would provide more precise information on this trait. Although our inheritance study leaves some questions unanswered, the lobed-leaf trait found within the University of Arkansas breeding program will be useful for continued fruit breeding work.

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Table 1. Segregation of leaf lobing, expected and actual segregation ratios, and chi-square test results for crosses of 'Southern Home' lobed-leaf muscadine grape.

Population	Total seedlings	Lobed leaf %	Standard leaf %	Expected ratio	Actual ratio	Chi-Square Test
Southern Home (S.H.) x OP	84	67.8	32.1	3:1	2.1:1.0	Supports hypothesis
S.H x A-1665 (*Euvitis*)	153	66.0	34.0	3:1	2.1:1.0	Supports hypothesis
Supreme x S.H.	204	41.1	58.8	1:1	1.0:1.4	Rejects hypothesis
GA-33-2-1 x S.H.	107	30.8	69.1	1:1	1.0:2.2	Rejects hypothesis
S.H. x Doreen	181	63.5	36.4	1:1	1.7:1.0	Rejects hypothesis
Black Fry x S.H.	212	45.7	54.2	1:1	1.0:1.2	Supports hypothesis
Darlene x S.H.	156	34.6	65.3	1:1	1.0:1.9	Rejects hypothesis
S.H. x Granny Val	95	51.5	48.4	1:1	1.1:1.0	Supports hypothesis

Table 2. Overall distribution and median of fruit shape population for three SigmaScan® values.

SigmaScan® Calculations	Range	Median
Shape factor [$(4 \times \pi^2)/(\text{perimeter})^2$]	0.43 - 0.88	0.78
Compactness [$(\text{perimeter})^2/\text{area}$]	14.36 - 29.65	16.06
Axis ratio [major axis/minor axis]	1.17 – 4.10	1.72

Fig. 1. *Muscadinia* leaf types classified in Study 2. – Standard muscadine leaves lacked lobing on the leaf margins (a), while the lobed types (lobed and intermediate) had medium-deep lobing (b), which were found in a limited number of seedlings, to very deep lobing (c), the most common form of leaf lobing found.

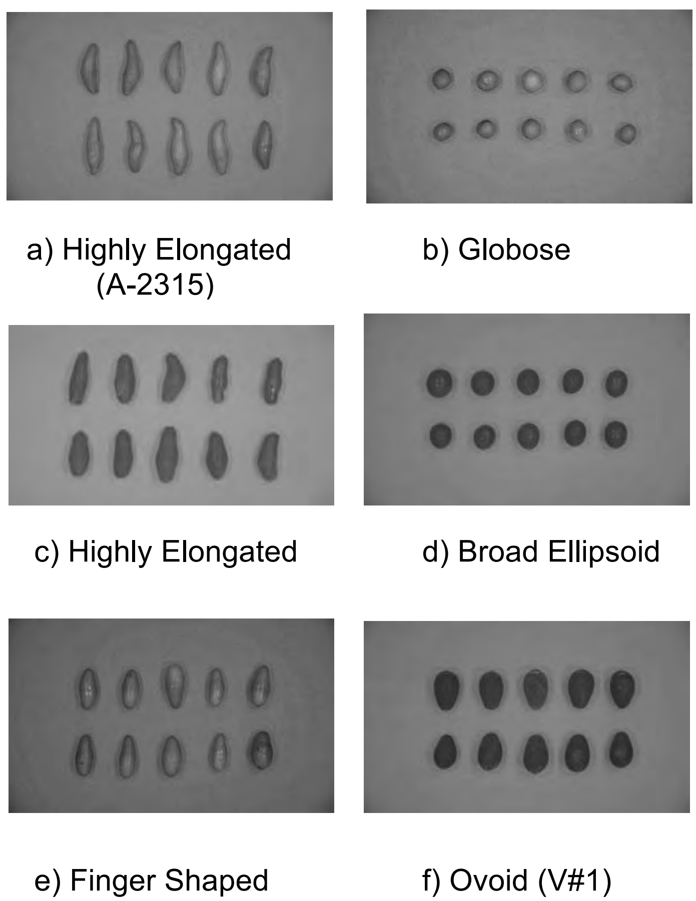

Fig. 2. Variation in characteristics of fruit shape in A-2315 (a) x V#1 (f) and some progeny.