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# Characterization of seediness attributes of blackberry genotypes

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Bethany Sebesta<sup>\*</sup>, John R. Clark<sup>†</sup>, Renee T. Threlfall<sup>§</sup>,  
and Luke R. Howard<sup>‡</sup>

## ABSTRACT

Fresh market blackberries can feel “seedy” when consumed. This “seediness” is associated with the presence of pyrenes which are comprised of a single seed enclosed in an endocarp. Small pyrene size (<3 mg) is preferred in both fresh-market and processed blackberry products. Yet, the proportion of pyrene weight to total berry weight can be more important than pyrene size. The objective of this study was to determine and compare descriptive sensory analysis and pyrene characteristics of blackberry genotypes from the University of Arkansas System Division of Agriculture blackberry breeding program. Panelists were trained according to Spectrum<sup>®</sup> methods and evaluated 20 genotypes for overall seediness. Pyrene characteristics, including weight and dimension, were measured for 22 genotypes. Seven of the 22 genotypes had an individual pyrene weight of 3 mg or less. Pyrene weight to berry weight ratio ranged from 2.7% (‘Tupy’) to 5.4% (‘Prime-Ark<sup>®</sup> 45’). ‘Tupy’ had low individual pyrene weights and a low ratio, which are most likely factors that contribute to its widespread acceptance by consumers. Pyrene weight to berry weight ratio was positively correlated to descriptive overall seediness ( $r = 0.70$ ) but not to number of pyrenes/berry. Therefore, finding a desirable pyrene weight to berry weight ratio is integral to decreasing perceived seediness in the development of new blackberry cultivars.

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## MEET THE STUDENT-AUTHOR



***Bethany Sebesta***

Having spent most of my childhood in Northwest Arkansas, attending University of Arkansas was a natural choice. An instilled love for things that grow and a passion for learning brought me to the Horticulture Department in spring 2012. Being someone who seeks a challenge and has an innate desire to set myself apart, I asked for a Special Topics project even though it was not required. It was the best decision of my academic career. This experience enhanced my research and writing skills along with project development and critical thinking. In addition, I had the chance to compete in the Southern Region American Society for Horticultural Sciences, Gamma Sigma Delta, and the Ozark Food Processors Association student competitions; I placed in the first two for my poster and oral presentation.

During my university career, I have come to be a part of Alpha Lambda Delta, Gamma Sigma Delta, and Pi Alpha Xi. I have had the opportunity to use my horticultural knowledge in the community by serving at The Farm and Feed Fayetteville and on campus through the GroGreen club. After graduating this December, I plan to attend graduate school for plant breeding and genetics starting fall 2014.

The Undergraduate Research Scholarship given by Mrs. Mitchener, who funded the Mitchener Undergraduate Research Grant Program, helped make much of this study possible. Thank you to Drs. John Clark and Renee Threlfall for guiding me in project development and research techniques among other things. Thank you to Dr. Luke Howard for lending input and resources. In addition, it was a privilege to work interdepartmentally and learn from a department outside of my own. I am thankful for all of you who helped make this research project such a wonderful experience.

## INTRODUCTION

Blackberries are grown throughout the United States and in other countries and used for both fresh market and processing. Blackberries are classified as a member of the *Rosaceae* family and *Rubus* genus (Finn and Clark, 2012). A blackberry fruit is an aggregate fruit comprised of many drupelets surrounding the receptacle or torus. The size of a blackberry fruit is determined by the combination of drupelet number and size (Clark et al., 2007). An individual drupelet includes a thin exocarp, a fleshy mesocarp and a hard, lignified endocarp, also known as a pyrene, which encloses a single seed (Tomlik-Wyremblewska et al., 2010).

Not until the late 1990s were fresh blackberries readily available in retail stores in the United States (Clark, 2005; Strik et al., 2007). Since then, blackberries have established a more prominent place in the market due to enhanced shipping capability, prolonged shelf life, and off-season availability (Clark, 2005; Strik et al., 2007). In 2005, worldwide blackberry area was 20,036 ha and was projected to increase by 2015 to over 27,000 ha (Strik et al., 2007).

The increase in acreage can be attributed in part to blackberry breeding programs. Blackberry breeding initiatives can be found on every continent with the exception of Antarctica (Strik et al., 2007). Blackberry breeding efforts have been in existence for over 100 years in the United States and continually work to enhance favored qualities and reduce undesirable ones. The first blackberry breeding program was initiated in 1909 at the Texas Agricultural Experiment Station (Clark and Finn, 2008). The oldest currently active program is at the U.S. Department of Agriculture–Agricultural Research Service at Corvallis, Ore. which began in 1928 (Clark and Finn, 2008; Finn and Clark 2012). In 1964, the University of Arkansas blackberry breeding program was initiated by Dr. James N. Moore (Clark, 1999). The University of Arkansas blackberry breeding program, based at the Fruit Research Station in Clarksville, Ark., has prioritized research efforts on attributes including thornlessness, erect growth habit, disease resistance, productivity, and adaptation (Clark, 1999; Clark and Finn, 2008). The fruit improvement objectives also included large fruit size, good flavor, firmness, and high fertility (Clark, 1999). The University of Arkansas breeding program also focuses

on primocane-fruiting genotypes which produce fruit on first-year canes in addition to floricanes-fruiting plants that produce fruit on second-year canes. Primocane-fruiting cultivars Prime-Jim<sup>®</sup>, Prime-Jan<sup>®</sup>, and Prime-Ark<sup>®</sup> 45 have been released (Clark and Finn, 2008). Even though breeding priorities may vary, most programs focus on promoting consumption through improved fruit quality (Finn and Clark, 2012).

Pyrene characteristics are distinctive to a blackberry genotype and can be classified into three groups: straight, concave, or convex (Wada et al., 2010; Wada and Reed, 2010). Variation in pyrene shape can occur in the same genotype and the outer layer of the endocarp typically has characteristic patterns of hollows (Tomlik-Wyremblewska et al., 2010). The structure, size, and number of pyrenes in the blackberry may influence mouth feel of the blackberries when consumed (Clark et al., 2007). Small seed size (<3 mg) is preferred in both fresh-market and processed blackberry products, and large seeds are objectionable (Moore et al., 1975). Fruit qualities such as seediness are important to consumers whether the berries are processed or consumed fresh (Clark and Finn, 2008). Large pyrene size, based on weight, length, or volume and seediness are also undesirable in processed blackberry products (Takeda, 1993). Yet, the proportion of pyrene weight to total berry weight is more important than pyrene size (Darrow and Sherwood, 1931). Pyrene weight and pyrene number were positively correlated with blackberry fruit weight (Moore et al., 1973).

Even though studies on blackberry pyrene characteristics and morphology on a limited number of cultivars have been published, there is little information on descriptive sensory analysis of fresh blackberries and the composition attributes that affect sensory scoring. The objective of this study was to investigate the descriptive sensory analysis and composition of blackberry genotypes from the University of Arkansas blackberry breeding program.

## **MATERIALS AND METHODS**

*Fruit.* Blackberry fruits were hand-harvested from the Fruit Research Station, Clarksville, Ark. in 2012. 'Ouachita', 'Natchez', 'PrimeArk<sup>®</sup> 45', APF-190, A-2434, A-2312 ('Stella'), and APF-227 were harvested on 29 May; 'Ouachita', A-2108, 'Osage', A-2215, APF-156, APF-185, APF-205, and A-2473 were harvested on 7 June; 'Ouachita', A-2252, A-2316, A-2418, A-2416, A-2419, 'Navaho', and 'Apache' were harvested on 14 June. After morning harvest, the berries were transported in coolers to the Food Science Department, University of Arkansas, Fayetteville. In addition, blackberries were purchased commercially including 'Tupy' (Naturipe, Salinas,

Calif.; fresh-market blackberries imported from central Mexico) and commercial frozen blackberries (Great Value, Wal-Mart Stores, Inc. Bentonville, Ark., cultivar unknown).

*Sensory Descriptive Analysis.* Descriptive sensory analysis of the fresh berries was performed at the Sensory and Consumer Research Center, Food Science Department, University of Arkansas, Fayetteville. Descriptive panelists ( $n = 8$ ) participated in a 3 h orientation session where the descriptive ballot was developed through consensus. The commercial frozen blackberries were used as the reference sample. The fruit was evaluated on the same day that it was harvested. 'Apache' and A-2252 were not evaluated because of limited quantity. Due to scheduling conflicts with the panelists, only four descriptive panelists ( $n = 4$ ) evaluated all the genotypes in the study.

Panelists evaluated the berries in duplicate using Spectrum<sup>®</sup> methods. Serving order was randomized across replication to prevent presentation order bias. The blackberry genotypes were served sequentially in 60-mL (2 oz) cups and were assigned random three-digit blinding codes. The blackberries were served at room temperature. Panelists were instructed to rinse their palettes with unsalted crackers and water between samples. Expecto-rant cups were provided. One paper ballot was completed per genotype for each replication. The descriptive panel scaled overall seediness of the berries on a 10-point scale (0 = no seeds to 9 = extremely seedy). The commercial frozen blackberries were thawed and used as a reference during each session and were scored a 7 for overall seediness.

*Composition Analysis.* All evaluations for composition of blackberries were done at the Grape and Wine Research Laboratory, Department of Food Science, University of Arkansas, Fayetteville. For each harvest date, samples were taken for descriptive sensory analysis. Three samples of approximately 100 g of berries were collected for each genotype, placed in plastic storage bags, and stored at -20 °C.

*Berry and Pyrene Attributes.* From the frozen berries, three berries per genotype and replication were used to determine attributes (individual berry weight, berry length, berry width, and drupelet number) and pyrene attributes (number/berry, dry weight/berry, and individual pyrene length, width, and height). The three-berry samples were weighed on a digital scale (Explorer, Ohaus Corporation, Switzerland) and the width and height of each blackberry were measured with a certified calibrated digital caliper.

To determine pyrene attributes, 0.1 mL of Pec5L enzyme (Scott Laboratories, Petaluma, Calif.) was added to each bag containing the three-berry frozen sample to break down the skin and pulp. Once the berries thawed,

they were hand-mashed in the bags. After 1.5 h at 21 °C, 100 mL of distilled water was added to each bag. The samples were then poured into a strainer. Under running water, the pulp was mashed against the strainer until only pyrenes remained. The pyrenes were placed onto paper towels and dried at ambient temperature (21 °C) for 1.5 h. The pyrenes for each three-berry sample were counted and weighed. The pyrenes were further dried in a laboratory oven (Fischer Scientific, Pittsburg, Pa., Isotemp<sup>®</sup>, Model 655F) at 55 °C (131 °F) for approximately 24 h. The pyrenes were removed from the oven and weighed, and then the length, width, and height of six randomly selected individual pyrenes per genotype and replication were measured with a digital caliper. Pyrene volume was calculated as length × width × thickness. The pyrenes for each genotype and replication were placed in plastic storage bags and stored in a freezer at -20 °C for further evaluations. Images of the individual pyrenes were taken, after freezing, using a camera with a macro lens attachment [Nikon D90, Tokyo, Japan; Nikon AF micro Nikkor 105 mm (1:2.8 D)].

*Experimental Design.* The experiment utilized a randomized complete block design with the 22 blackberry genotypes including the commercial sample, Tupy.

*Statistical Analysis.* Analyses were conducted using JMP<sup>®</sup> (V. 8.0; SAS Institute Inc., Cary, N.C.). Tukey's HSD (Honestly Significant Difference) was used for mean separation. Pearson's correlation was used to describe the relationship within and between descriptive intensity scores and composition.

## **RESULTS AND DISCUSSION**

The descriptive sensory attributes and the composition of blackberry genotypes from the University of Arkansas Breeding Program indicated a range of measurement values.

*Descriptive Sensory Analysis.* Scores for overall seediness as rated by the panelists were not significantly different among the genotypes evaluated (data not shown). The average overall seediness ratings ranged from A-2418 with 7.25, 'Natchez' with 6.75, and 'Prime-Ark<sup>®</sup> 45' with 6.63 for the genotypes with the highest scores, and 'Tupy' with 4.38 and A-2416 with 4.38 the lowest. Selections APF-205, A-2312, and A-2215 all scored 4.75, the second-lowest value. Rating of overall seediness proved to be challenging for the panelists, as reflected in the lack of differences among the ratings even though there was a near 3-point range among the means. The lack of differences reflects substantial variation in the seediness ratings recorded by panelists.

*Berry Attributes.* The size and shape of the berries varied by genotype; some were long and narrow, and others

short and wide. Average berry weight among genotypes varied from 5.1 g (A-2252) to 9.6 g (A-2434) (Table 1).

*Pyrene Attributes.* Pyrene attributes of each genotype were evaluated. 'Natchez' contained the greatest number of pyrenes having about 131/berry and 'Navaho' and 'Tupy' the least averaging 53/berry (Table 1). Number of pyrenes/berry for 'Natchez', A-2434, APF-156, and APF-205 were significantly higher than the averages for A-2108, A-2215, A-2252, A-2416, APF-185, APF-190, 'Apache', 'Navaho', 'Osage', 'Ouachita', and 'Tupy'. Genotypes having individual pyrene weight of 3.0 mg or less included: 'Tupy' (3.0 mg), APF-156 (3.0 mg), A-2416 (2.5 mg), APF-205 (2.6 mg), A-2252 (3.0 mg), A-2316 (2.9 mg) and A-2419 (2.7 mg); overall mean standard error for individual pyrene weight was 0.05. Genotypes A-2434, A-2108, and 'Apache' had individual pyrene weights of 4.0 mg or higher (data not shown). Average dry weight of pyrenes/berry varied from 160 mg ('Tupy') to 491 mg ('Natchez') (Table 1).

The proportion of pyrene weight per berry to total weight of berry is more important than pyrene size (Darrow and Sherwood, 1931). Of all genotypes, 'Prime-Ark<sup>®</sup> 45', 'Natchez', A-2473, A-2418, and A-2434 had high values for proportion of pyrene weight per berry to total berry weight; 'Tupy', A-2416, A-2215, and APF-190 had significantly lower values. The ratio for 'Prime-Ark<sup>®</sup> 45' and 'Natchez' was significantly higher than those for A-2215, A-2252, A-2416, A-2419, APF-185, APF-190, 'Navaho', and 'Tupy'. Even though some genotypes had a high proportion of pyrene weight to berry weight, these did not necessarily have the most pyrenes per berry. For example, the proportion of pyrene weight per berry to total berry weight for 'Apache' and APF-205 was 4.1%. However, the average number of pyrenes/berry for APF-205 was 122 pyrenes/berry and was significantly higher than that for 'Apache', which had 74 pyrenes/berry. This was especially notable for 'Prime-Ark<sup>®</sup> 45' which had 85 pyrenes per berry and a proportion of 5.4%. Conversely, A-2252, 'Navaho', and 'Tupy' had very low values for berry weight and average weight of pyrenes per berry. Pyrene volume ranged from 6.2 mm<sup>3</sup> (A-2416) to 10.7 mm<sup>3</sup> (A-2434) (Table 1).

The images of individual pyrenes from the genotypes evaluated were visually classified into three groups, straight, slightly concave, and convex (Fig. 1). Classification was based on the shape of the raphe, the lower edge of the pyrene. The following genotypes were classified as straight: A-2316, A-2418, 'Apache', APF-205, 'Osage', 'Ouachita', and 'Prime-Ark<sup>®</sup> 45'. Selections A-2416 and APF-156 were slightly concave. Genotypes A-2215, A-2312 (Stella), A-2419, A-2473, APF-185, and APF-190 were straight to slightly convex. Genotypes A-2108, A-2252, A-2434, APF-227, 'Natchez', 'Navaho', and 'Tupy'

were convex. Blackberry pyrene shape and structure has been used previously to identify the genotype, based on the shape of the lower edge of the pyrene. Our results are in agreement with others. ‘Navaho’ and ‘Tupy’ were classified by others as convex (Wada et al., 2010). ‘Natchez’ was classified as straight to slightly convex and slightly convex to convex, ‘Ouachita’ as straight, and ‘PrimeArk® 45’ as straight to slightly convex (Bruce et al., 2012).

*Correlations Within and Between Descriptive Sensory Analysis and Composition Attributes.* Pearson’s correlation was used to understand the relationship within and between descriptive intensity scores and composition attributes. For all genotypes, positive and negative correlations with significance were  $r = 0.98-0.70$  ( $P < 0.001$ ),  $r = 0.69-0.56$  ( $P < 0.01$ ), and  $r = 0.55-45.0$  ( $P < 0.05$ ).

Berry weight was positively correlated to berry volume ( $r = 0.93$ ), number of pyrenes/berry ( $r = 0.70$ ) and pyrene weight/berry ( $r = 0.78$ ) (Table 2). Berry volume was positively correlated to pyrenes/berry ( $r = 0.58$ ) and pyrene weight/berry ( $r = 0.67$ ). Pyrenes/berry was positively correlated to pyrene weight/berry ( $r = 0.84$ ) and pyrene weight/berry weight ratio ( $r = 0.63$ ). Pyrene weight/berry was positively correlated to pyrene weight/berry weight ratio ( $r = 0.80$ ). Descriptively-evaluated overall seediness was positively correlated to pyrene weight/berry ( $r = 0.51$ ) and pyrene weight/berry weight ratio ( $r = 0.70$ ).

## **CONCLUSIONS**

Texture attributes, including seediness, are important to consumers and processors. Seven of the 22 genotypes had an individual pyrene weight of 3 mg or less and may be more accepted by consumers than those with higher pyrene weights. This may be a contributing factor to the widespread popularity of ‘Tupy’, which is grown in central Mexico but marketed in the United States from October until June. ‘Prime-Ark® 45’ had a high proportion of pyrene weight to berry weight and was scored among the highest for overall seediness. Conversely, ‘Tupy’ was among the lowest for both attributes. The positive correlation between pyrene weight to berry weight ratio and descriptive overall seediness supports Darrow and Sherwood’s (1931) findings that can be useful for evaluating new developments in the Arkansas blackberry breeding program.

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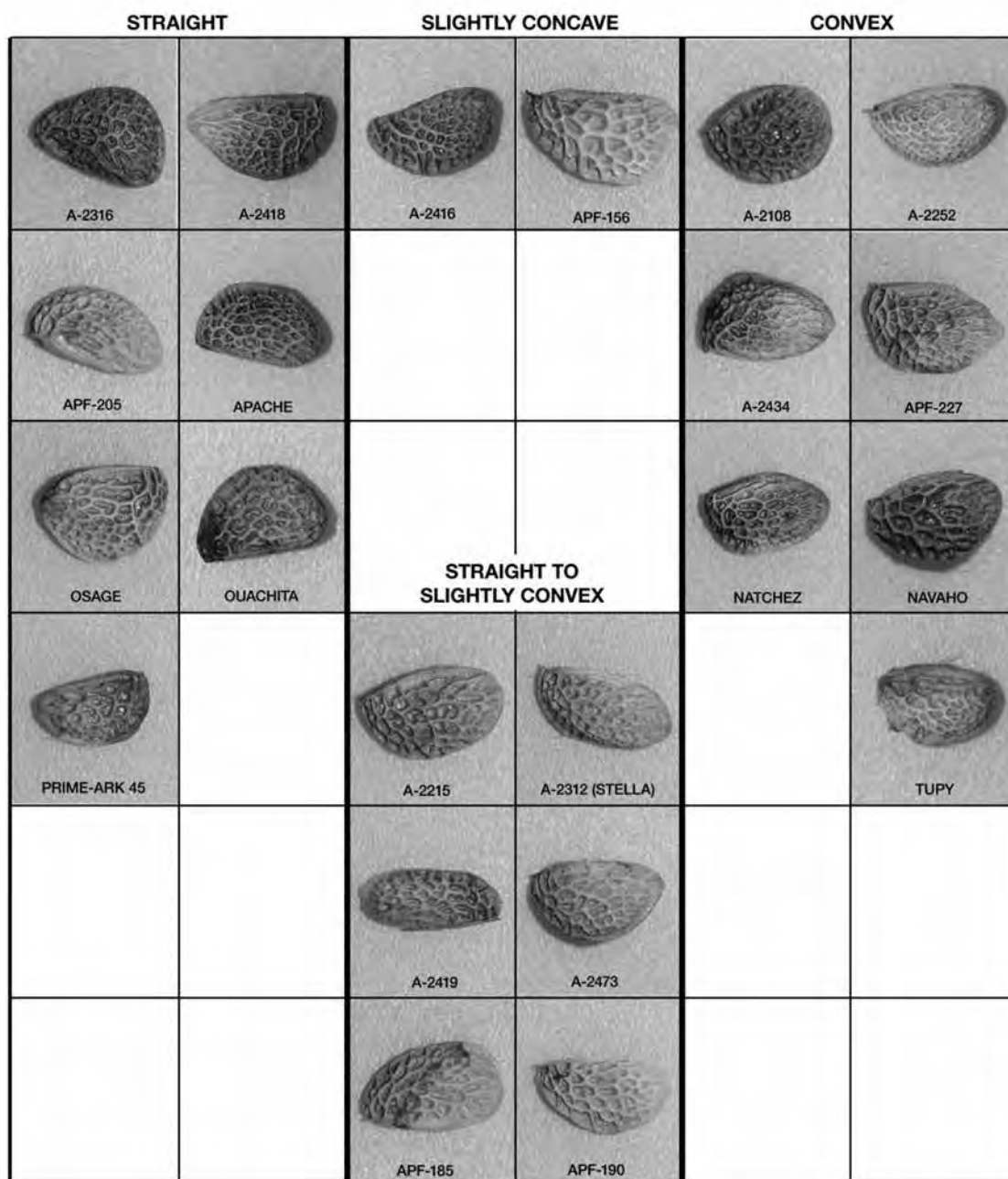
**Table 1. Berry and pyrene attributes of blackberry genotypes.**

<b>Genotype</b>	<b>Berry weight (g)</b>	<b>Berry volume (mm<sup>3</sup>)<sup>†</sup></b>	<b>Pyrenes/ Berry</b>	<b>Pyrene weight (mg)/ berry</b>	<b>Pyrene weight/ berry weight (%)</b>	<b>Pyrene volume (mm<sup>3</sup>)<sup>‡</sup></b>
A-2108	8.6 abcd <sup>§</sup>	4532 a	74 defghi	324 cdef	3.8 bcdef	8.7 abcde
A-2215	7.6 abcdef	3614 abc	67 fghi	226 fgh	3.0 ef	8.1 bcdefg
A-2252	5.1 f	2395 c	61 ghi	179 gh	3.6 cdef	7.7 bcdefg
A-2312	8.0 abcde	3474 abc	92 cdef	330 cde	4.2 abcdef	8.9 abcd
A-2316	6.2 cdef	3066 abc	88 cdef	257 defgh	4.1 abcdef	7.0 defg
A-2416	6.5 cdef	3168 abc	73 efghi	183 gh	2.8 f	6.2 g
A-2418	7.5 abcdef	3453 abc	91 cdef	356 bcd	4.8 abc	9.4 abc
A-2419	7.8 abcdef	4027 abc	99 bcd	271 defg	3.5 cdef	6.5 fg
A-2434	9.6 a	4457 ab	110 abc	452 ab	4.7 abcd	10.7 a
A-2473	6.9 abcdef	3306 abc	94 cde	317 cdef	4.6 abcd	7.6 cdefg
APF-156	8.8 abc	4310 ab	125 a	380 bc	4.3 abcde	7.7 bcdefg
APF-185	6.8 bcdef	3532 abc	70 efghi	245 efgh	3.6 cdef	8.7 abcde
APF-190	8.5 abcd	3788 abc	84 defg	276 defg	3.3 def	8.4 bcdef
APF-205	7.7 abcdef	3635 abc	122 ab	313 cdef	4.1 abcdef	6.7 fg
APF-227	7.0 abcdef	3352 abc	91 cdef	301 cdef	4.3 abcde	8.5 bcdef
Apache	7.2 abcdef	3944 abc	74 defghi	298 cdef	4.1 abcdef	9.7 ab
Natchez	9.4 ab	4253 ab	131 a	491 a	5.2 ab	8.9 abcd
Navaho	5.3 ef	2804 bc	53 i	179 gh	3.4 cdef	8.0 bcdefg
Osage	6.9 abcdef	3725 abc	73 efghi	273 defg	4.1 abcdef	8.4 bcdef
Ouachita	6.3 cdef	3155 abc	78 defgh	259 defgh	4.1 abcdef	7.2 defg
Prime-Ark <sup>®</sup> 45	5.8 def	2768 bc	85 cdefg	318 cdef	5.4 a	8.1 bcdefg
Tupy	6.0 def	2846 abc	53 hi	160 h	2.7 f	8.1 bcdefg

<sup>†</sup>Volume calculated as a cone.

<sup>‡</sup> Volume calculated as length x width x height.

<sup>§</sup> Means with different letter(s) for each attribute are significantly different ( $P < 0.05$ ) using Tukey's honestly significant difference (HSD).



**Fig. 1.** Images of individual pyrenes from evaluated genotypes. Pyrene sizes in the images are not to scale relative to each other. Pyrenes were visually classified into the following four groups: straight, slightly concave, slightly convex, and convex, based on the shape of the raphe, the lower edge of the pyrene.



**Table 2. Correlations between sensory descriptive analysis and composition attributes.**

<b>Attributes</b>	<b>Berry weight (g)</b>	<b>Berry volume (mm<sup>3</sup>)</b>	<b>Pyrenes/ berry</b>	<b>Pyrene wt (mg)/ berry</b>	<b>Pyrene wt/ berry wt (%)</b>	<b>Overall seediness</b>
Berry weight (g)	1.00					
Berry volume (mm <sup>3</sup> )	0.93*** <sup>†</sup>	1.00				
Pyrenes/berry	0.70***	0.58**	1.00			
Pyrene wt (mg)/berry	0.78***	0.67**	0.84***	1.00		
Pyrene wt/berry wt (%)	0.27	0.18	0.63**	0.80***	1.00	
Overall seediness	0.08	0.12	0.25	0.51*	0.70***	1.00

<sup>†</sup>Correlations with significance were  $r = 0.98-0.70$  ( $P < 0.001$ ),  $r = 0.69-0.56$  ( $P < 0.01$ ), and  $r = 0.50-45$  ( $P < 0.05$ ), respectively, \*\*\*, \*\*, and \*.