2014 SRSFC Final Report

Title:
Determination and Inheritance of Firmness and Texture of the "Crispy" Trait in the Arkansas Blackberry Breeding Program

Final Report

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Objectives:

The main objective of this project was to evaluate and characterize the fruit morphology of firm and crispy genotypes within the Arkansas blackberry selections and possibly seedlings from these firm parents, and to evaluate its genetic potential as a source of exceptionally firm cultivars with high postharvest and shipping potential. If this unique trait can be incorporated into commercial cultivars then this would allow growers and marketers to have fruits of better quality after more extended storage periods.

Specific objectives:

1. To determine the fruit components that give rise to the firm and "crispy" trait found in genotypes within the Arkansas blackberry breeding program.

2. To quantify the flesh firmness of the firm and "crispy" trait.

3. To determine the postharvest potential of these new genotypes.

Justification and description:

Fruit firmness is critical for growers and shippers who are looking to produce and ship high-quality fruits to different markets around the world. For that reason this is an important trait that fruit breeders consider and want to improve; and blackberry is one of the crops that firmness is crucial for, especially during postharvest handling. Fruit firmness is suggested as an intractable trait, meaning that it is a difficult character to improve, and depends on cultivar, ripeness stage, and storage duration (Clark, 2005; Perkins–Veazie et al., 1996).

The blackberry breeding program of University of Arkansas is continuously releasing new cultivars for the fresh market industry and fruit firmness is a top priority within the program to introduce cultivars suitable for this purpose.

Crispy genotypes were first observed in the program a number of years ago in a floricane-fruiting, thorny selection. Since that time, this fruit trait has been advanced to improved selections, and currently, thornless selections that express the crispy trait consistently and have been used in crosses with the aim to transfer the crispiness into improved seedlings and resulting selections with increased yield, fruit size, fruit flavor, and primocane-fruiting. Two of these, A-2453T and A-2454T are believed to hold the most promise for use in breeding for this trait.

These crispy genotypes show an improved postharvest performance compared to released cultivars from the breeding program. They maintain the high firmness observed during harvest and after storage and also show a reduced color reversion (drupelets developing red color) after 7 d of cold storage. Color reversion causes are not well known yet, but this problem appears to have a genetic component, so selection against this problem and appropriate harvest and postharvest management appears to be the most likely solutions to reduce or eliminate this issue (Clark, 2005).

Methodology:

All evaluations and phenotyping during the 2013 and 2014 seasons were done at the University of Arkansas Fruit Research Station in Clarksville with exception of confocal image analysis, which was done at University of Arkansas, Fayetteville campus. Blackberry plants were grown with cultural components including annual routine plant management practices such us fertilization, weed control, and irrigation.

Genotypes used in this study were cultivars Prime-Ark® 45, Natchez, Osage, and Ouachita, and the Arkansas selections A-1790, A-1960T, A-2218, A-2252T, A-2297T, A-2416T, A-2417T, A-2418T, A-2428T, A-2453T, and A-2454T (Table 1). These selections represent a range of firmness values, although none are considered “soft” as older cultivars were often classified, and also have been used in hybridizations where increased firmness in seedling populations has been intended within the Arkansas program.

Table 1. Crispy and non-crispy genotypes used in the study.

Genotype	Crispy (Yes/no)
A-1790	Yes
A-1960T	No

A-2218	Yes
A-2252T	No
A-2297T	No
A-2416T	No
A-2417T	No
A-2418T	No
A-2428T	No
A-2453T	Yes
A-2454T	Yes
Natchez	No
Prime Ark® 45	No
Osage	No
Ouachita	No

Each cultivar/selection was harvested into 0.24 L clamshells at two harvest each year. In 2013 two clamshells and an additional 10 berries were harvested at each harvest date for each genotype. The fruits of one clamshell (randomly selected) were used to measure fruit compression (15-20 fruits) and the other clamshell was used to measure drupelet penetration (10 fruits) and receptacle penetration (10 fruits). These same fruit were used for seed extraction and seed weight measurement. The additional 10 berries were used for reversion measurements after storage. In 2014, four clamshells were harvested at each harvest date. Two clamshells were randomly selected and used to measure firmness at harvest day (day 0) and the other two were used to measure firmness and reversion after one week (day 7) of cold storage at ~5 °C. At each day of measurement (day 0 and 7), the fruits of one clamshell were used to measure compression (15-20 fruits) and the other clamshell was used to measure drupelet penetration (10 fruits) and receptacle penetration (10 fruits). The measurements taken on fruits in this study are described:

<u>Fruit morphology:</u>

- Seeds: the weight of seeds of each genotype was measured in 2013. Three replicates per genotype were measured and analyzed. Each replicate consisted of 50 air-dried seeds.
- Torus and drupelet (Fig. 1) cell structure: In 2014 fresh tissue of individual torus and drupelet were cut in thin layers and placed on a microscope slide. Slides were examined with 20X magnification with a confocal microscope (Nikon Eclipse 90i, Nikon instrument Inc. Melville, NY) to examine cellular consistency. Pictures of two crispy genotypes (A-2453T and A-2454T) and three non-crispy genotypes (Natchez, Ouachita, and Shawnee) were taken immediately after placement to describe cellular differences among the genotypes.

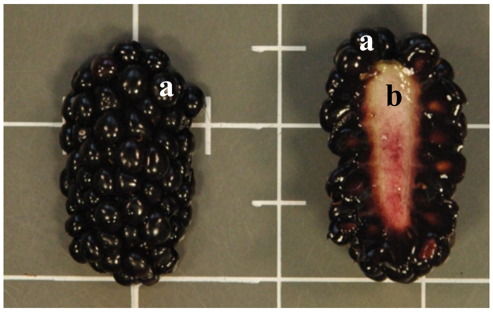

Fig. 1. Longitudinal section of cultivar Natchez showing drupelets (a) and torus or receptacle (b).

Fruit firmness:

- Compression: fruit compression was performed by placing individual fruits horizontally on a flat surface using a cylindrical and plane probe of 7.6 cm diameter (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).
- Penetration: each fruit was cut in half longitudinally. One half was used for drupelet penetration and the other half to measure the receptacle firmness (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).
    a. Drupelet penetration: drupelet skin firmness was assessed using a probe of 1 mm diameter. For this, three drupelets of similar shape and size were used per berry.
    b. Receptacle penetration: measured using a probe of 1 mm diameter in the middle of the receptacle.

Color reversion:

Color reversion after one week at cold storage at ~5°C was evaluated. For this evaluation each fruit was categorized in the following reddening scale:
RD_0: fruits with no red drupelets after cold storage.
RD_1: fruit only having one red drupelet after cold storage.
RD_2-3: fruits having two or three red drupelets scattered on the fruit after cold storage.
RD_4-5: fruits having four or five red drupelets scattered on the fruit after cold storage.
RD>6: fruits having six or more red drupelets scattered on the fruit after cold storage.

Statistical analysis:

Data were analyzed with SAS software (SAS Institute Inc. Cary, NC). When the analysis of variance (ANOVA) was significant at an α level of 0.05 the honest significant difference (HSD) was used to calculate the difference among means.

Results and discussion:

Fruit morphology examinations for cellular structure using the microscope for crispy and non-crispy genotypes showed differences among the berries. Shown are images of A-2454T (crispy) and 'Natchez' (non-crispy) (Fig. 2) drupelet skin (Fig. 2 a and b, respectively) and fruit receptacle (Fig. 2 c and d, respectively). The cells and cell walls can be clearly differentiated with A-2454T while 'Natchez' cells are not clearly differentiated. Similar results were found with the other crispy and non-crispy genotypes analyzed (images not shown). We believe that the crispy texture allowed the cells to maintain integrity of the tissue, thus have structural components that contribute to the crispy texture. Conversely, the cells of 'Natchez' did not maintain integrity and appeared to break apart, corresponding to its non-crispy texture. Therefore, the genotypes can likely be differentiated and identified by their maintenance of tissue and cell integrity and this is a morphological reason for the firmness differences.

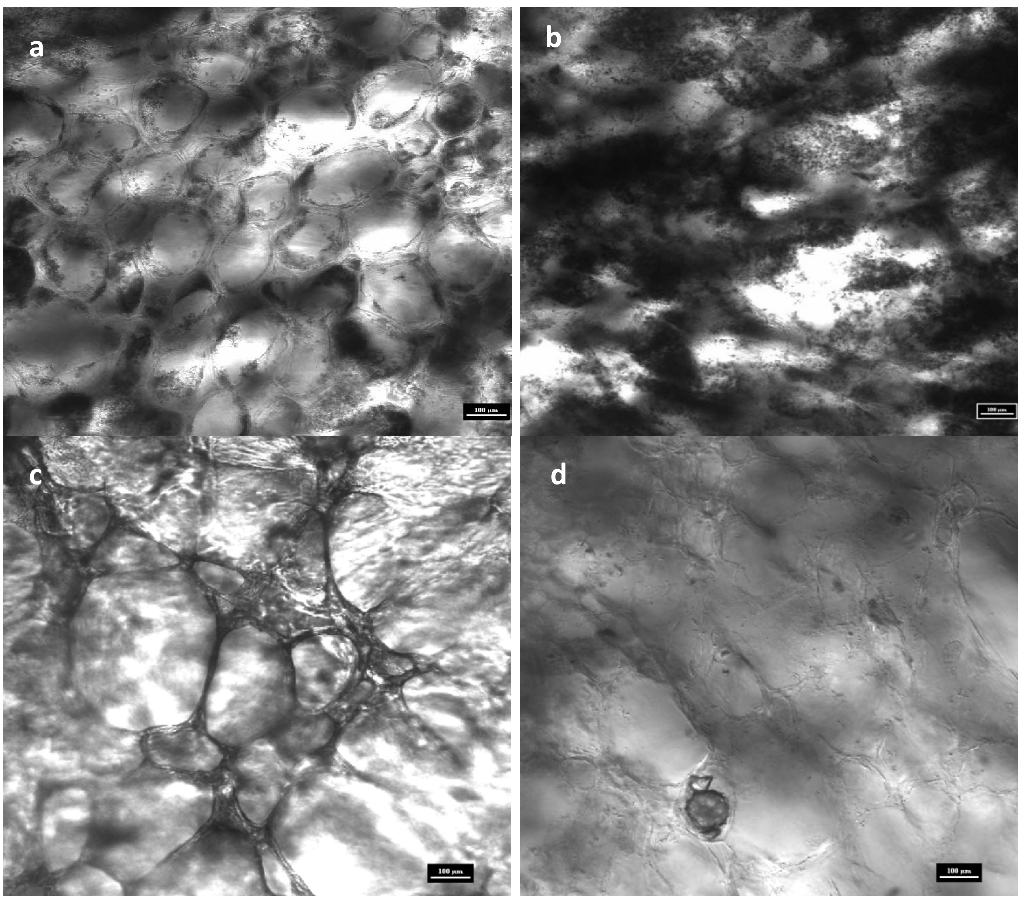

Fig 2. Ripe drupelet image taken by confocal microscope of selection A-2454T (a) and cultivar Natchez (b). Ripe receptacle image taken by confocal microscope of selection A-2454T (c) and cultivar Natchez (d). Bars = 100 µm.

Results for compression measurements indicated the crispy genotypes usually had higher values indicating greater firmness compared to non-crispy genotypes, particularly in 2013 at harvest (day 0) (Table 2). In 2014 at day 0 and after storage, the crispy genotypes usually had values in the highest mean separation grouping. However, the crispy genotypes were not always significantly higher in compression values compared to non-crispy. This same trend was usually maintained 7 d after harvest (Table 2). It was interesting to note that some compression values were higher after storage, and this was not an expected result; this could be due to weight loss and epidermal dehydration during storage resulting in an increased compression value (Perkins-Veazie et al., 1996). Compression values of the crispy genotypes were, on average, two times higher than non-crisp selections A-2297T and A-1960T. The industry standards 'Natchez' and 'Prime Ark® 45' averaged high values of firmness also without being in the crispy category. Unfortunately, no very soft genotypes were included in this comparison, such as older cultivars Choctaw or Shawnee, so differences were not as great as they could have been. Overall, the compression measurement appears to differentiate the firmness classifications quite well.

Table 2. Mean of compression force (N) of blackberry genotypes for years 2013 (0 day at harvest) and 2014 (0 and 7 d storage).

		Force (N)		
Genotype/type	Crispy	2013	2014	
		Day 0	Day 0	Day 7
A-1790	Yes	13.0 a[z]	9.6 bc	10.3 ab
A-1960T	No	6.5 fg	6.5 d	9.8 abc
A-2218	Yes	13.2 a	8.0 bcd	6.4 de
A-2252T	No	8.2 def	7.5 cd	11.4 a
A-2297T	No	6.2 f	6.0 d	6.6 de
A-2416T	No	10.0 bcd	9.8 b	8.1 bcde
A-2417T	No	8.6 cde	7.5 cd	6.6 de
A-2418T	No	11.5 ab	9.0 bc	8.2 bcde
A-2428T	No	7.5 efg	6.4 d	9.1 abcd
A-2453T	Yes	13.1 a	12.6 a	9.1 abcd
A-2454T	Yes	12.3 a	9.6 bc	9.8 abc
Natchez	No	10.1 bc	9.5 bc	7.1 cde
Osage	No	7.2 efg	8.7 bc	6.1 e
Ouachita	No	7.1 efg	6.5 d	6.2 de
Prime Ark® 45	No	9.8 bcd	9.0 bc	6.8 cde

[z] Different letters in columns indicate significant differences among genotypes at an α level of 0.05. Means were compared using HSD.

Drupelet skin and receptacle penetration force (Tables 3 and 4, respectively) were quite variable for the genotypes, with the crispy selections being higher in some measurements and not others. For instance, the selection A-1790 in 2013 was the highest for skin force but the crisp selections A-2453T and A-2454T were not in the higher mean value grouping. In 2014 at day 0, A-2453T had the higher mean value but overall means for crispy selections were not always higher than non-crispy. There were similar findings for receptacle firmness also, with some selections of the crispy type being highest (such as A-2218) but after storage the highest value was for A-2252T, a non-crispy selection.

Drupelet skin force usually showed a decrease after 7 d storage in most of the measured genotypes. However, a few genotypes presented a higher firmness after storage, and as with compression, could be due to weight loss and epidermal dehydration of fruits (Perkins-Veazie et al., 1996).

Table 3. Mean of drupelet skin penetration force (N) of blackberry genotypes, years 2013 (day 0 harvest) and 2014 (day 0 and after 7 d storage).

Genotype	Crispy	Force (N)		
		2013	2014	
		Day 0	Day 0	Day 7
A-1790	Yes	0.383 a[z]	0.149 bcd	0.165 ab
A-1960T	No	0.139 f	0.126 de	0.147 abcd
A-2218	Yes	0.251 bc	0.161 ab	0.152 abc
A-2252T	No	0.173 def	0.141 bcd	0.173 a
A-2297T	No	0.287 b	0.108 e	0.135 bcde
A-2416T	No	0.154 ef	0.129 cde	0.128 cde
A-2417T	No	0.180 def	0.153 abc	0.133 cde
A-2418T	No	0.159 ef	0.137 bcd	0.115 ef
A-2428T	No	0.189 def	0.126 de	0.126 cdef
A-2453T	Yes	0.159 ef	0.178 a	0.137 bcde
A-2454T	Yes	0.221 cd	0.124 de	0.136 bcde
Natchez	No	0.173 def	0.110 e	0.097 f
Osage	No	0.178 def	0.125 de	0.110 ef
Ouachita	No	0.181 def	0.156 ab	0.113 ef
Prime Ark® 45	No	0.197 cde	0.126 de	0.119 def

[z] Different letters in the columns indicate significant differences among genotypes at an α level of 0.05. Means were compared using HSD.

Table 4. Mean receptacle penetration force (N) of blackberry genotypes, years 2013 (day 0 harvest) and 2014 (day 0 and after 7 d storage).

Genotype	Crispy	Force (N)		
		2013	2014	
		Day 0	Day 0	Day 7
A-1790	Yes	0.248 bcde[z]	0.132 bcde	0.123 b
A-1960T	No	0.156 fg	0.115 def	0.121 b
A-2218	Yes	0.302 abc	0.113 def	0.123 b
A-2252T	No	0.209 ef	0.138 bcde	0.180 a
A-2297T	No	0.243 cde	0.099 f	0.107 b
A-2416T	No	0.280 abcd	0.139 bcd	0.129 b
A-2417T	No	0.241 cde	0.135 bcde	0.102 b
A-2418T	No	0.333 a	0.170 a	0.121 b
A-2428T	No	0.228 de	0.155 ab	0.169 a
A-2453T	Yes	0.240 cde	0.147 abc	0.120 b
A-2454T	Yes	0.309 ab	0.112 ef	0.119 b
Natchez	No	0.217 def	0.111 ef	0.099 b

Osage	No	0.128 g	0.101 f	0.096 b
Ouachita	No	0.197 ef	0.117 def	0.105 b
Prime Ark® 45	No	0.235 de	0.122 cdef	0.106 b

[Z] Different letters in columns indicate significant differences among genotypes at an α level of 0.05. Means were compared using HSD.

Fruit firmness is believed to be a quantitative trait, meaning that several genes plus the environment contribute to the expression of this trait. It is not known how heritable fruit firmness is in blackberry, although increased firmness has been achieved in breeding and selection (Clark 2005; Clark and Finn, 2011). Our study investigated several populations that included crosses with crispy parents to see if the progeny reflected increased firmness compared to the non-crispy parent.

Fruit compression means (Table 5) of the analyzed populations were within the range of the values of their parents with the exception of population 1146 mean value, which was lower than both parents. Population means were closer to the less firm parent, suggesting that softer fruit in progeny may be partially dominant. Drupelet penetration force of populations (Table 6) were more variable and were often above or below the values of their parents. This was an unexpected finding, and possibly the drupelet skin penetration does not explain fruit firmness as well as compression. This was found also in the measurements on the genotypes including parent selections presented earlier. Receptacle penetration force of populations 1145 and 1148 were also in between the value of their parents; the other three populations were outside of the parent ranges (Table 7). Again, the receptacle firmness values may not parallel that of compression and thus not be as predictive of ultimate firmness.

Inheritance of the crispy trait was calculated with firmness measurements for 2014 using offspring-midparent regression (data not shown). Compression, drupelet skin penetration, and receptacle firmness heritability values were very low (10-27%), meaning that increased firmness of the crisp parents was not reflected consistently in the progeny. The environmental effects were likely high also, reducing heritability values. These values of inheritance need to be confirmed with additional data, but do provide some preliminary findings on trait inheritance. We had intended to conduct the study on the populations in 2013, but the plants were young and did not produce enough fruit for data collection.

Table 5. Mean of fruit compression year 2014, for five seedling populations and their parents where were intended to segregate for firmness in progeny.

Population	N° of seedlings	Parents		Force (N) Fruit compression		
		Female	Male	Seedling	Female	Male
1145	30	A-2297T	A-2454T	8.7 b[z]	6.0	9.6
1146	29	A-2416T	A-2453T	8.5 b	9.8	12.6
1147	35	A-2416T	A-2454T	9.6 a	9.8	9.6
1148	33	A-2417T	A-2454T	8.8 b	7.5	9.6
1151	34	A-2428T	A-2453T	9.1 ab	6.4	12.6

[Z] Different letters in columns indicate significant differences among populations at an α level of 0.05. Means were compared using HSD.

Table 6. Mean of drupelet penetration force year 2014 for five seedling populations and their parents intended to segregate for firmness in progeny.

Population	N° of seedlings	Parents		Force (N)		
				Drupelet		
		Female	Male	Seedling	Female	Male
1145	11	A-2297T	A-2454T	0.130 b[z]	0.108	0.124
1146	16	A-2416T	A-2453T	0.114 c	0.129	0.178
1147	23	A-2416T	A-2454T	0.130 b	0.129	0.124
1148	20	A-2417T	A-2454T	0.138 a	0.153	0.124
1151	30	A-2428T	A-2453T	0.113 c	0.126	0.178

[z] Different letters in columns indicate significant differences among populations at an α level of 0.05. Means were compared using HSD.

Table 7. Mean of receptacle penetration force year 2014 for five seedling populations and their parents intended to segregate for firmness in progeny.

Population	N° of seedlings	Parents		Force (N)		
				Receptacle		
		Female	Male	Seedling	Female	Male
1145	11	A-2297T	A-2454T	0.109 c[z]	0.099	0.112
1146	20	A-2416T	A-2453T	0.128 b	0.139	0.147
1147	23	A-2416T	A-2454T	0.142 a	0.139	0.112
1148	20	A-2417T	A-2454T	0.125 b	0.135	0.112
1151	30	A-2428T	A-2453T	0.124 b	0.155	0.147

Results of color reversion are shown in Fig. 3. The crispy genotypes A-2453T and A-2454T showed the lowest levels of color reversion, in that higher percentages of these crispy fruits were scored in categories 0 and 1 of the reddening scale, and low percentages of fruits were in the two higher categories of reddening. Most other crispy genotypes had reduced reversion also. The industry standard cultivars also showed low levels of color reversion, with the exception of 'Natchez' with had greater reversion. However, none of the industry cultivars achieved the reduced levels of reversion as the two best crispy selections. Also, red drupe development values of crispy cultivars were more consistent and varied less between years when compared to non-crispy genotypes (data not shown).

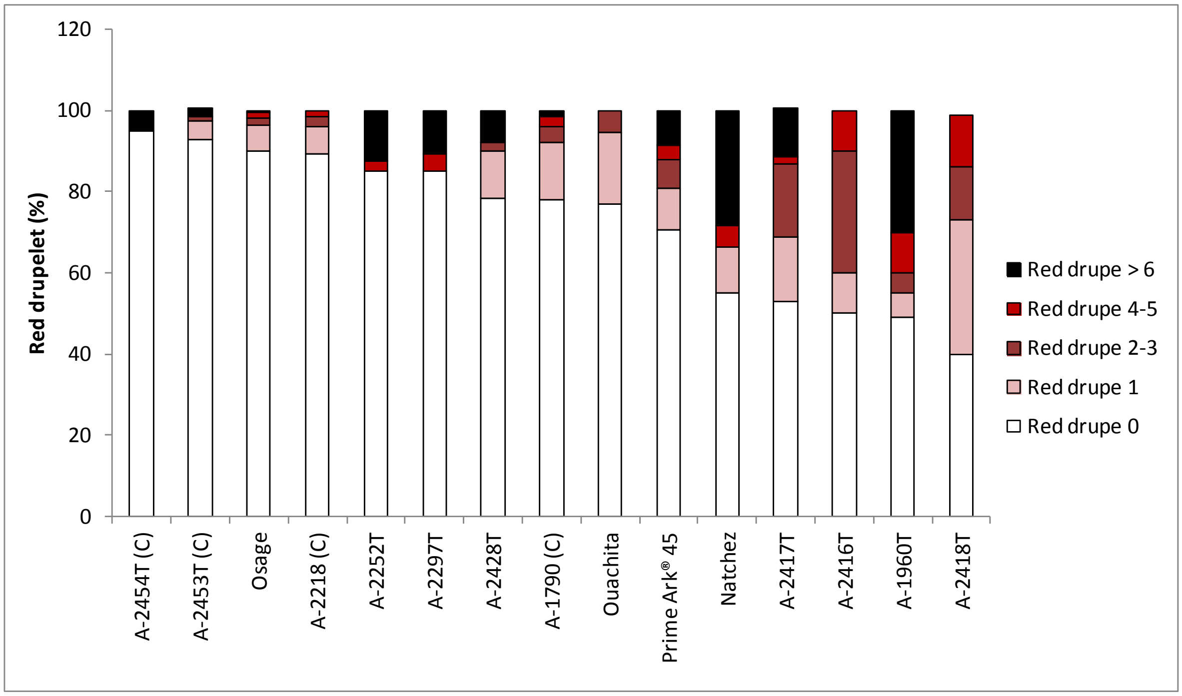

Fig 3. Red drupelet development (%) after one week of cold storage at 5 °C for blackberry genotypes. Mean of years 2013 and 2014. C indicates crispy genotype.

Seed size is a quantitative trait with partial dominance of small seed size (Clark and Finn, 2011). A low perception of seed content is preferred by consumers. For this reason, seed size (by measuring weight) of selections and cultivars was analyzed to determine if there is a relation between seed size and firmness. Seed weight was measured for the genotypes in 2013 only, and ranged from 0.24 g per 50 seeds for A-2418T to 0.14 g for A-2417T (Table 8). We further calculated if there was a relationship between compression and receptacle penetration and seed weight. Positive and significant correlations between higher fruit compression and higher seed weight (R^2 =0.33) and between higher receptacle penetration and higher seed weight (0.44) was found. This indicates that seed weight (and therefore size) likely are involved in these fruit firmness measurements.

Table 8. Mean of 50 seeds weight of blackberry genotypes, year 2013.

Genotype	Weight (g)
A-2418T	0.24 a^z
A-2218	0.22 ab
A-1790	0.20 bc
A-2454T	0.19 bc
Natchez	0.18 bc
Prime45	0.17 cd
A-1960T	0.17 cd
A-2428	0.17 cd
A-2453T	0.17 cd
A-2252T	0.16 cd

A-2297	0.16 cd
A-2417T	0.14 d

[Z] Different letters in columns indicate significant differences among genotypes at an α level of 0.05. Means were compared using HSD.

Conclusions:

Overall, many of the crispy selections/genotypes had superior firmness compared to non-crispy genotypes in 2013 and 2014. Compression values averaged 11.4 N in both years for crispy genotypes and 8.1 N for non-crispy genotypes and a similar but less consistent tendency was observed for drupelet and receptacle penetration.

Confocal image analysis of analyzed crispy and non-crispy selections/cultivars showed clear differences between both types of genotypes in cell characteristics. Drupelet and receptacle cells of crispy genotypes maintained their consistency and cell walls did not break apart, compared to non-crispy genotypes where cells did not remain intact.

Lastly, these superior firmness genotypes expressed lower levels of red drupelet after one week of cold storage, which is a positive quality for fresh the market blackberry industry.

Impact statement:

This study allowed us to dissect the components that influence the expression of the crispiness trait of blackberry fruits, using compression and penetration firmness and cellular structure. With these findings, the blackberry breeding program of the University of Arkansas can further develop a more refined firmness determination protocol involving the use of a texturometer to measure fruit firmness in a more accurate way. Also, the finding that the crispy genotypes expressed lower levels of color reversion is very significant, a relationship first reported in this research. The use of crispy genotypes as parents in crosses should continue to increase the firmness of future generations, resulting in enhanced postharvest performance cultivars for the blackberry shipping industry.

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Citations

Salgado, A.A., J.R. Clark, and T. Frett. 2014. Characterization of crispy selections of the Arkansas blackberry breeding program. ASHS Annual Conference, Orlando. HortScience 49(9) Supplement: S266-267. (abstract)