Title of Project: Evaluation of Advanced Southern Highbush and Rabbiteye Blueberry Breeding Selections for Fruit Quality Traits

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Objective:

The main objective of this proposal is to examine the postharvest fruit quality attributes of southern highbush and rabbiteye advanced breeding selections. The goal of this project is to identify elite germplasm within the breeding program with superior fruit quality for release as named cultivars and for use as parents in crossing. This will provide the breeding program with information that will aid in the advancement of desired fruit quality traits.

Justification and Description:

In Georgia and in the southeastern US, two main types of blueberries are commercially grown: southern highbush (species complex between *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (*V. virgatum* Aiton) blueberries. Within the US in 2012, the state of Georgia accounted for over 13% of US fresh fruit production ranking number four (37 million pounds), and accounted for over 12% of US processed fruit production ranking number five (33 million pounds) (USDA-ERS, 2013). In 2014, Georgia ranked number one in total harvested blueberry acreage in the US, accounting for over 20% of US harvested acreage (16,600 acres) and over 17% of total US metric tons (283,400 metric tons) (USDA-NASS, 2014).

Often southern highbush fruit are perceived to have higher fruit quality characteristics as compared to rabbiteye fruit; however, evidence for this is inconclusive at best. In 2008, Saftner et al. examined instrumental fresh fruit quality measurements of ten highbush and two rabbiteye cultivars grown in New Jersey. They reported variations for firmness (compression test), soluble solid content, titratable acidity, sugar/acid ratio, pH, and the aromatic volatile concentration, associated with cultivar differences, rather than species differences. A sensory panel was also used to examine consumer acceptability of these cultivars. Panel results reported differences in highbush and rabbiteye cultivars for numerous acceptability traits. However, not all highbush ranked higher than the rabbiteye cultivars. In another study, three rabbiteye and two highbush cultivars were examined for fruit characteristics including skin puncture, berry firmness (compression test), collective fruit firmness with multiple berries (Kramer shear press), carbohydrates and fiber content (Silva et al., 2005). In this study, the rabbiteye cultivars were reported to be higher than the highbush cultivars for all traits examined, yet no significant cultivar differences existed for sensory panelist evaluations. A confounding factor in this study was that rabbiteye cultivars were harvested in a different location/environment than the highbush fruit, with fruit collected from Mississippi and Michigan, respectively. Different preharvest environmental conditions may influence postharvest quality. Swift (2010) studied fresh and frozen highbush and rabbiteye fruit from different cultivars. The study was conducted over two years: four rabbiteye, one highbush and one highbush rabbiteye hybrid evaluated in year one; and four rabbiteye and one highbush genotype evaluated in year two. Instrumental analyses were performed including puncture tests to examine skin toughness, and compression tests to examine berry firmness. Sensory panels were also conducted. Overall, toughness was not shown to increase with later season harvest intervals on a cultivar. The study concluded that the effect of cultivar has a larger significance than time, and that overall environmental variation has a large effect.

Our Own Preliminary Results: 2014 season

During the 2014 harvest season, fruit was harvested from the University of Georgia Blueberry Research Farm near Alapaha, GA from seven highbush cultivars: 'Rebel', 'Star', 'Emerald', 'Farthing', 'Meadowlark', 'Legacy', and 'Camellia'; two advanced highbush selections: TH-1111 and TH-1125; and seven rabbiteye cultivars: 'Vernon', 'Alapaha', 'Brightwell', 'Powderblue', 'Tifblue', 'Ochlockonee', and 'Premier'. Genotypes represented an array of fruit ripening times throughout the season. Some of the major differences identified among blueberry types and genotypes are reported below.

Texture - An Instron testing machine was used to run puncture (skin strength) and Kramer shear press (fruit firmness) tests. Genotypes were significantly different ($P<.0001$) for max load value (N) across southern highbush and rabbiteye genotypes for puncture and Kramer test. Blueberry type was not significantly different for skin strength ($P=0.861$) ranging from 1.24N (TH-1111) to 0.80 N ('Rebel'), and was significantly different for fruit firmness ($P<.0001$) ranging from 722.2N ('Powderblue') to 285.4N ('Rebel') (Table 1). Results suggest that skin strength in highbush and rabbiteyes are similar; however, rabbiteye fruit are firmer than highbush fruit overall.

Chemical Quality Components- Fruit were frozen for approximately seven to eight months before chemical analyses. Measurements included total titratable acids (TTA), soluble solids content (°brix), total monomeric anthocyanin concentration (mg/L cyanidin-3-glucoside equivalents), sugars, organic acids, antioxidants, and sugar acid ratio (°brix /TTA and total sugars/total acids). Total sugar, organic acid, and antioxidant content were also determined. Overall, highbush genotypes were significantly higher ($P<0.05$) for total titratable acids (0.69% vs. 0.43%), and rabbiteye genotypes were significantly higher for soluble solids (13.4% vs. 11.6%) and sugar acid ratio (°brix /TTA) (34.5 vs. 23.3) (Table 1). Sucrose was significantly higher in rabbiteyes (2.54 mg/g vs. 0.10 mg/g). Total sugar content was not significantly different between types, and sugar acid ratio (total sugar/total acid) was significantly different between types. Rabbiteyes were significantly higher for three of the six organic acids (oxalic, succinic, and malic acid) and were over 60% higher for total acids (12.2 mg/g vs. 7.5 mg/g). For antioxidants, rabbiteyes were 37% higher for total antioxidants (20,482 µg/g vs. 14,934 µg/g) in comparison to highbush blueberries. Results show variation within genotypes for fruit quality and suggest that rabbiteye varieties are quite high in many important compounds.

Fiber - Fruit were frozen for ten to eleven months before fiber analyses. Measurements included neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin; and hemicellulose and cellulose were derived from equations using NDF, ADF and lignin (Silva, 2005). Genotypes were significantly different across blueberry types for all five parameters. Rabbiteyes were significantly higher for all five parameters ($P\leq0.05$) in comparison with highbush (Table 2).

Seed - Fruit were kept frozen at -15°C until seed was extracted in June 2015. Individual berry weight and diameters were taken, and extracted seed were classified into plump and shriveled categories and counted. Total seed number and total seed weight were recorded. Genotypes were significantly different for all traits examined (P<0.001). Highbush were significantly higher than rabbiteyes for all berry and seed traits measured, except for total seed weight (highbush were not significantly different than rabbiteyes) and for percent seed weight / berry weight (rabbiteyes were significantly higher than highbush, P<0.0001; Table 2). Results suggest that, contrary to subjective perception, rabbiteyes are not seedier than highbush.

Methodologies

For the 2016 harvest season, we collected fruit from a large array of southern highbush and rabbiteye advanced breeding selections. Breeding germplasm selected represented a range of fruit ripening times throughout the season (early, middle and late season ripening). Fruit from both southern highbush and rabbiteye were harvested when plants were approximately 50% ripe at the University of Georgia Blueberry Research Farm near Alapaha, GA from May to July. Fourteen breeding selections were harvested: TH-1091, TH-1111, TH-1241, TH-1331, TH-1332, TH-1334, TH-1351, TH-1352, TH-1356, TH-1362, TH-1409, TH-921, TH-944, and TH-948. Four cultivars were also harvested in addition to the breeding selections: 'Camellia', 'Rebel', 'Star', and 'Suziblue'. Cultivars were added for use as a point to which to compare breeding selections to evaluate potential improvements offered by new selections on various fruit quality traits. Fruit was harvested from three plants (reps) for each genotype, frozen and held at -15°C, and transported frozen to the Griffin campus, Griffin, GA on dry ice. Fruit is currently being held at -15ºC until all tests are completed. Fruit was not evaluated for fresh texture due to the spread in ripening times of multiple genotypes. Instead, fruit will be evaluated for frozen texture traits as an estimate of overall skin toughness and fruit firmness. An Instron universal testing machine (Model 1122, Instron Corp., Canton, MA) will be used to estimate puncture in and out (skin toughness) and Kramer shear press (fruit firmness) as outlined in Silva et al., 2005. Berries will be brought to room temperature of approximately 20ºC for two hours and tests will be arranged in order of most to least sensitive. Twelve berries of similar size per plant per cultivar will be used for puncture analyses, and two samples of 50.0+/-1.0g of berries will be used for the Kramer Shear tests.

In addition to the above planned textural analyses, blueberry juice has been extracted from frozen fruit was measured for total soluble solids (ºBrix), pH, and titratable acidity (TTA). Blueberry juice will also be evaluated for total anthocyanin content (cyanidin-3-glucoside equivalents in mg/L), sugar acid ratio, sugar profile, organic acid profile, and antioxidant profile. The addition of an HPLC machine to the lab in spring semester 2017 will allow for the measurement of the additional fruit chemical quality traits. Juice samples are currently prepared and are being held at -15°C until HPLC processing. Frozen fruit will also be analyzed for fiber traits (NDF and ADF) at the University of Georgia Agricultural and Environmental Services Lab. Lastly, frozen fruit were examined for seed traits including number of plump and shriveled seed, total number of seed, seed weight (g), and percent seed weight/berry weight (g).

Additional fruit quality traits listed above will be collected in the upcoming months. Results presented below are seed trait measurements and juice chemical quality traits of TTA, ºBrix, and ºBrix/TTA. Data were examined using the GLM procedure in SAS (v.9.4) to examine genotype and replicate differences. Genotype differences were examined using the Tukey HSD ($P \leq 0.05$) within the GLM procedure.

Results

All genotypes were screened for berry size and seed traits (Table 1). Overall, the breeding selections generally have a larger fruit than cultivar standards 'Rebel' and 'Suziblue' for fruit size based on berry weight, berry diameter of the equator, and berry diameter of the stem to calyx end. TH-1331, TH-1351, and TH-1334 were the only breeding selections with larger berry size as compared with the large-fruited cultivar, 'Camellia'. Out of these four breeding selections, TH-1332 was the largest genotype for berry weight and both diameters, and was significantly higher than all other genotypes examined for both berry weight and diameter of the stem to calyx end of the fruit.

For seed number, number of plump seed, shriveled seed, and total seed number were measured. For plump seed number, breeding selections TH-1241 and TH-1409 had significantly lower plump seed numbers as compared with 'Camellia', 'Suziblue' and 'Rebel', yet these genotypes did not have lower seed numbers than 'Star'. This suggests that there is the potential for parentage in the breeding germplasm to reduce plump seed number as compared with three out of the four commonly grown cultivars that were examined. Similarly, for shriveled seed number, total seed number, and total seed weight, our current data suggest that the genetic potential exists in the breeding program to either move towards, or surpass the lowered seed levels in 'Camellia', 'Suziblue', and 'Rebel' by using these genotypes as parental material. Additionally, % seed weight / berry weight was examined, as this is currently being examined for usefulness in consumer acceptability of blueberry fruit seediness. The breeding selections TH-1241, TH-1334, and TH-921 all have significantly lower ratios for seed weight to berry weight than do 'Rebel', 'Suziblue', and 'Camellia'. TH-1409 is the only breeding selection that is significantly lower than the lowest cultivar, 'Star'. This suggests that using genotype TH-1409 may be the best parent in achieving lower seed weight to berry weight levels.

Fruit chemical quality traits of TTA, ºBrix, and ºBrix/TTA were also examined for all genotypes (Table 2). For TTA, the range present across all genotypes examined was 0.11% (TH-1409) to 0.85% (TH-1241). The four cultivars were spaced throughout this range: 0.78% ('Star'), 0.63% ('Camellia'), 0.30% ('Suziblue'), and 0.24% ('Rebel'). No breeding selections were identified to be significantly higher or lower than all cultivars examined. However, individual selections may be selected based upon their relationship to a particular cultivar to move either closer to or farther away from the TTA level of a cultivar of interest. When examining ºBrix, TH-1241 was the only breeding selection that had significantly higher soluble solid content as compared with the four cultivars measured. This suggests that TH-1241 may be a desirable genotype for selection to increase ºBrix levels in the fruit. For ºBrix/TTA, breeding selections TH-1409, TH-1332, and TH-1351 had exceptionally high levels. These selections would be useful as breeding parents to work to increase this level in future breeding selections. 'Star' and 'Camellia' ranked significantly lower than all but one genotype examined (TH-1241), which indicates that current selected germplasm within the breeding program is already making improvements upon this fruit quality trait.

Data for additional fruit quality traits will be compared in a similar manner as data collection is completed.

Conclusions

1. In the 2016 season, breeding selections were evaluated and compared with four widely grown cultivars and evaluated for their potential usefulness in improving, or surpassing, the current cultivars for respective fruit quality traits.
2. For berry size, breeding selections TH-1331, TH-1351, and TH-1334 have the potential to be used as breeding parents to shift population means in the direction of the large-fruited cultivar, 'Camellia'. The data suggest TH-1332 may be used to exceed the fruit size of the largest cultivar evaluated (Table 1).
3. For seed number, breeding selections TH-1409, TH-1241, TH-1334, and TH-921 may be useful as parental material for reducing seed number traits. TH-1409 may be the most as a parent in breeding for lower seed weight to berry weight levels (Table 1).
4. For fruit chemical quality traits of TTA, ºBrix, and ºBrix/TTA, there were no breeding selections identified to increase or decrease TTA levels as compared with the cultivars examined. For increasing ºBrix, TH-1241 has potential as a breeding parent. For increasing

ºBrix/TTA, breeding selections TH-1409, TH-1332, and TH-1351 may be the most useful parental material. However, due to exceptionally high levels in these three breeding selections for ºBrix/TTA as compared to the other genotypes measured, it may be best to re-test these three selections to confirm the high ºBrix/TTA levels.

Impact Statement

Blueberry is an important crop in Georgia and throughout the US, and an increased knowledge of highbush and rabbiteye advanced breeding selections' fruit quality will benefit blueberry growers, consumers, and the industry as a whole by providing material with increased quality. Phenotypic variation within and among species for fruit quality characteristics is present as shown by previous research and our preliminary results. This project allows for further identification of possible differences within and among species of advanced blueberry breeding selections to be used for possible new cultivars and as parents.

Citation(s) for any publications arising from this project:

Literature Cited

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Table 1. Seed traits for fruit from southern highbush (*Vaccinium corymbosum* L.) genotypes grown in Georgia during the 2016 season[a].

Genotype[b]	Berry Wt(g)		Dia. 1 (mm)[c]		Dia. 2 (mm)[d]		Plump No.		Shriveled No.		Total Wt(g)		Total No.		% Seed Wt / Berry Wt	
'Camellia'	3.3	CBD[f]	20.3	BC	14.9	CB	53.8	BEDC	25.4	FDE	0.042	BAC	79.2	BAC	1.31	ECD
'Rebel'	1.8	HI	15.9	I	13.0	JI	43.9	FEDC	26.9	FDEC	0.027	EFG	70.8	EDC	1.48	BCD
'Star'	1.4	J	14.2	J	12.2	J	22.3	H	19.9	FE	0.012	H	42.2	F	0.92	EFG
'Suziblue'	2.2	HG	16.9	H	14.4	FCEBD	46.2	FEDC	32.1	BDEC	0.030	EFDG	78.3	BAC	1.36	CD
TH-1091	2.5	FG	18.5	FEG	13.5	HGI	65.0	BA	18.1	F	0.049	A	83.1	BAC	1.98	A
TH-1111	2.3	G	17.6	HG	13.6	FHGI	58.8	BAC	14.5	F	0.035	EFDC	73.3	BEDC	1.51	BC
TH-1241	1.5	JI	14.8	JI	12.3	J	25.9	HG	32.1	BDEC	0.014	H	58.0	FED	0.89	HFG
TH-1331	2.5	FG	17.7	HG	14.5	CEBD	73.0	A	22.3	FE	0.046	BA	95.3	A	1.86	BA
TH-1332	4.1	A	21.5	A	16.2	A	41.7	FEDG	48.6	A	0.029	EFG	90.3	BA	0.71	HG
TH-1334	3.6	CB	21.0	BA	15.0	B	39.4	FEG	40.3	BA	0.029	EFG	79.7	BAC	0.82	HFG
TH-1351	3.7	B	21.4	A	14.1	FCEGD	51.2	BEDC	41.3	BA	0.039	BDC	92.5	A	1.10	EFGD
TH-1352	3.1	ED	19.8	DC	13.4	HGI	41.5	FEDG	39.0	BAC	0.029	EFG	80.5	BAC	0.94	EFG
TH-1356	2.7	FE	18.7	FE	13.9	FHEGD	47.2	FEDC	36.2	BDAC	0.026	FG	83.4	BAC	0.94	EFG
TH-1362	2.5	FG	17.9	FHG	13.7	FHEGI	39.3	FEG	39.4	BAC	0.029	EFDG	78.7	BAC	1.19	EFCD
TH-1409	2.5	FG	18.1	FEG	13.2	HI	21.3	H	22.1	FE	0.012	H	43.5	F	0.50	H
TH-921	2.5	FG	17.8	FHG	14.6	CBD	34.7	FHG	22.7	FE	0.020	HG	57.4	FE	0.80	HFG
TH-944	2.8	FE	19.0	DE	13.4	HGI	56.5	BDC	18.4	F	0.033	EFDC	74.9	BDC	1.21	EFCD
TH-948	3.2	CD	20.2	BC	14.6	CBD	55.9	BDC	24.5	FDE	0.036	EDC	80.5	BAC	1.13	EFCD

[a] Fruit was harvested from UGA Blueberry Research Farm near Alapaha, GA.
[b] N = 18. For each genotype n=15, (3 plants x 5 berries/plant).
[c] Diameter measures the berry equator.
[d] Diameter measures from the stem scar to the caylx of the berry.
[f] Differences examined using Tukey HSD ($P \leq 0.05$).

Table 2. Total titratable acids (TTA), soluble solid content (°brix) and sugar acid ratio (°brix/TTA) for fruit from southern highbush (*Vaccinium corymbosum* L.) genotypes grown in Georgia during the 2016 season[a].

Genotype[b]	TTA (%)		°brix		°brix/TTA	
'Camellia'	0.63	B[c]	9.2	C	14.7	H
'Rebel'	0.24	HFGE	11.7	BC	49.7	CD
'Star'	0.78	A	11.8	BC	15.4	H
'Suziblue'	0.30	DFCE	9.2	C	31.0	FG
TH-1091	0.28	DFE	10.4	BC	38.1	FDE
TH-1111	0.37	DC	10.9	BC	30.5	FG
TH-1241	0.85	A	15.3	A	19.5	HG
TH-1331	0.26	DFGE	10.4	BC	41.1	FDE
TH-1332	0.13	HG	12.6	BA	98.8	A
TH-1334	0.42	C	11.8	BC	29.9	FG
TH-1351	0.18	HFG	12.9	BA	71.9	B
TH-1352	0.37	DC	12.4	B	33.5	FE
TH-1356	0.38	DC	11.6	BC	30.5	FG
TH-1362	0.26	DFGE	11.8	BC	45.9	CDE
TH-1409	0.11	H	12.3	B	109.9	A
TH-921	0.20	HFGE	11.5	BC	59.3	CB
TH-944	0.31	DCE	12.4	B	40.3	FDE
TH-948	0.42	C	12.0	B	29.1	FG

[a] Fruit was harvested from UGA Blueberry Research Farm near Alapaha, GA.
[b] N = 18. For each genotype n=6, (3 reps x 2 subreps/rep).
[c] Differences examined using Tukey HSD ($P \leq 0.05$).