Final Report:

Evaluation of Advanced Selections, Southern and Northern Highbush Blueberries for Self- and Cross-Fertility (SRSFC grant 2020 R-20)

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This project is a continuation of our previous project funded by SARFC grant 2019 R-13. This year we have added four more blueberry cultivars in our pollination experiment and have also repeated last year's experiment on 10 cultivars and advanced selection lines with different pollen sources. We measured the fruit set efficiency and fruit quality parameters in the 2019 experiment. For the 2020 experiment, we measured the fruit set parameters; however, other traits that required laboratory instrument and could not be measured due to COVID-19. Table 1 and Table 2 below summarizes the findings of the blueberry self- and cross-pollination experiments conducted in 2019 and 2020.

Abstract

Self-incompatibility (SI) is the condition where pollen grains of one plant cannot fertilize the ovule(s) of the same plant to produce a viable embryo(s). SI in the plant species is under strict genetic control. Although the mechanism of self-fertility greatly varies across most flowering plants, a large number of shared structural similarities exist among them. The SI in blueberries varies from partially self-fertile (Highbush blueberries) to complete self-incompatible (low-bush blueberries). To achieve a higher pollination rate, and hence a higher fruit production, blueberry growers are required to grow patches of different cultivars in their field and complement the pollination process with several beehives to provide enough pollinators. The introduction of the self-fertility trait in the blueberry cultivar will reduce the production cost to growers by eliminating obligatory outcrossing conditions. In this project, we studied the variation in self- and cross-fertility in four Northern Highbush, four Southern Highbush cultivars, and two advance selection lines at NC State University. A set of ~ 100 flowers from each genotype was hand-pollinated with the pollen of the same plant, while another set of ~100 flowers was pollinated with the pollen from a different cultivar. The self-fertility score for each cultivar was

determined in terms of the percentage of successful fruit set, fruit weight, fruit size, and the number of seed development. Our initial analysis shows that the Northern Highbush cultivar 'Duke' is highly self-fertile with the self/cross ratio of 1.14, 0.87, and 0.92 for each of percentage fruit set, fruit size, and fruit weight, respectively. Similarly, the advance selection line 'NC3104' of the Southern Highbush blueberry was performed better than other cultivars with the self /cross ratio of 0.70, 1.01, and 0.93 for the percentage fruit set, fruit size, and fruit weight, respectively.

Introduction

Self-incompatibility (SI) is the condition where pollen grains of one plant cannot fertilize the ovule(s) of the same plant to produce a viable embryo(s). Successful embryo formation is necessary for the development of fruit from the ovary of a flower. Thus, self-incompatible species usually require appropriate means of pollen transfer to set fruit. SI is reported in ~ 39% of the plant species (Igic 2008).

SI in plants is under strict genetic control. Although the self-fertility mechanism greatly varies across most flowering plants, many shared structural similarities exist among them. For instance, they contain at least two polymorphic determinant genes surrounded by highly polygenic sequences (Takayama and Isogai 2005). In the model plant Arabidopsis, the SI is controlled by two tightly linked S gene loci, S locus receptor kinase (SRK) and S locus cysteine-rich protein (SCR). A mutation on these genes confers a self-fertile mating system in *A. thaliana,* while a functional copy of the genes in *A. lyrata* renders it completely self-incompatible (Kusaba 2001).

The SI in blueberries varies from partially self-fertile to complete self-incompatible. Highbush blueberries can somewhat tolerant self-pollination, while low-bush blueberries are strictly self-incompatible (Meader and Darrow 1944, Meader and Darrow 1947). The molecular mechanism of self-incompatibility in blueberry is not known to date. A line of research shows that the incompatibility in blueberry is post-zygotic, where the ovule abortion occurs between 5-10 days after pollination (Huang 1997). In self-pollinated highbush blueberry, ~35% of ovules aborted, and ~88% of remaining showed poor development (Huang 1997). Conversely, in cross-pollinated blueberries, only 22% of ovaries were aborted, and 33% had poor development (Huang 1997). This indicates that cross-pollination is required for the optimum fruit setting and higher yield in blueberries. Enough cross-pollination can increase the berry yield by 10-20% (Issacs 2016).

To achieve a higher productivity rate, blueberry growers put about 3-4 hives per acre of the blueberry farm to ensure that there are enough pollinators required for successful cross-pollination. This incurs an extra cost to blueberry producers, increases production cost, and lowers their profit margin. Furthermore, since the blueberry plants are clonally propagated, planting different plants from the same cultivar does not help for cross-pollination. Several patches of different varieties must be grown on the blueberry field to provide the pollen source from different genotypes. Additionally, the blooming time of different varieties should also be considered to ensure the synchrony of pollen and ovule maturity. In the early days of the blueberry industry, bushes were planted in alternating rows to promote cross-pollination (Shutak, 1966). However, as the industry progressed and individual farms became larger in acreage, there was more interest, and it became more common to plant varieties in larger, solid blocks composed of single cultivars. With the advent of such practices, self-fertility and self-fruitfulness of cultivars became more critical in determining the success of any given cultivar. The introduction of the self-fertility trait in the blueberry cultivar will reduce the production cost to growers by eliminating obligatory outcrossing conditions.

Self-incompatibility is a bigger problem when there are not enough pollinators. In 2018 poor fruit set was observed across NC blueberry farms, mostly due to a combination of cold injury and poor weather conditions during bloom time. The growing season in 2018 was very rainy, and honeybees will not fly when it is rainy, and some of NC native pollinators are also less active in rainy conditions (H Burrack, Pers. Comm.). The poor pollination, along with rain, resulted in a 30-40% crop loss in North Carolina (Fruit Growers News and Media). The recent problem with beehive collapse may even cause more significant issues to the blueberry industry in the long-term. The development of self-fertile cultivars will ensure sustainable production of blueberries independent of pollinators.

In this project, we studied the variation in self- and cross-fertility in blueberry with the aim of breeding new cultivars with higher success in self-fertility. Two advanced blueberry selections ('NC3104', 'NC4992'), one recently released pentaploid cultivar at NC State breeding program ('Heintooga'), three widely grown across NC ('Star', 'O'Neal', and 'Reveille'), and four popular northern highbush blueberries ('Arora'. 'Elliot', 'Liberty' and 'Duke') were evaluated for their responses to cross- and self-pollination under greenhouse conditions. Our data shows that the northern highbush 'Duke' and the advanced selection 'NC3104' had a higher success rate in self-pollination in terms of the number of fruit set, berry weight, and successful seed formation.

Materials and Methods

Plant Materials: Six-year-old and field-grown mature plants of each cultivar and advanced selection were dug out in 2017 from the field in Castle Hayne, NC, and were transplanted in large pots and maintained in the greenhouse condition. The plants were put in the cold room at 4 °C from mid-Jan to the end of Feb 2019 to provide them sufficient chilling hours for flower induction. After the chilling hour treatment, the plants were moved back to the insect-free greenhouse and maintained under the natural lights, adequate irrigation, and fertilization. The plants started setting flowers from the end of February until the end of April.

Pollination: Self and cross-pollination were performed by hand to avoid any bias to fruit size and seed size. Initial batch flowers for each genotype were used to collect pollen, which was used later for self-pollination. The O'Neal cultivar was used as the pollen source for cross-pollinating for all other genotypes (except, Star was used as a pollen source for O'Neal cross-pollination). For hand pollination, the blueberry flowers were emasculated by removing the petals and stamens from the mature floral buds. The emasculated buds were allowed to mature for 2 more days before pollination. A set of about 100 emasculated buds were pollinated with the pollen collected from the same plant (self-pollination), and another set of ~100 flowers was pollinated with the pollens from another genotype (cross-pollination)

Data collection and analysis: The first set and maturation from each experimental plant were monitored weekly. The fruits were collected after-ripening (visual analysis of commercial picking stage). The successful pollination was calculated in terms of the percentage of fruit formation in the self-pollinated flowers in comparison to the cross-pollinated flowers. In addition to the fruit formation, various phenotypes traits such as fruit weight, fruit size, fruit firmness, and the number of seeds were also calculated. Statistical analysis for fruit size and fruit firmness was performed using students *t*-test.

Results:

1. Self-pollination efficiency in blueberry fruit formation

Fruit formation efficiency was measured in a total of 14 blueberry genotypes (12 cultivars and 2 advance selection lines). Among them, 4 cultivars were Northern Highbush Blueberry (NHB) (row 1-4 of table 1), and the remaining 10 were Southern Highbush Blueberry (SHB) (row 5-14 of table 1). Among the northern HighBush cultivars, 'Duke' and 'Liberty' showed a higher degree of self-pollination success with similar efficiency as cross-pollination (Table 1). Simultaneously, 'Elliott' and 'Aurora' had a low level of self-pollination sufficiency with only half as much flowers setting fruit than the cross-pollination.

Among the SHB cultivars and genotypes, 'Heintooga' and NC4992 completely failed to set fruit on the self-pollination flowers. Four cultivars ('Reveille', 'Rebel', 'Legacy', and 'New Hanover') and one advance selection line (NC3104) showed relatively high efficiency of fruit formation on the self-pollination flower. The cultivars 'O'Neal' and 'Star' showed a medium level of self-pollination success. The cultivar 'Pinnacle' had a medium level of fruit formation on one successful self-pollination experiment, but the cross-pollination experiments failed due to other growth relation conditions, and we could not make any comparison.

Table 1: Efficiency of cross-pollination and self-pollination (2019 and 2020) on fruit set parameter of selected blueberry genotypes

Cross#	Female Parent	Male Parent	Year	#Cross	Fruit set	% Fruit Set	Total Weight (gm)	Weight/ Berry (gm)
1	Aurora	Aurora	2019	105	23	21.9	28	1.2
		Aurora	2020	25	12	48	18	1.5
		O'Neal	2019	37	27	72.97	42	1.56
		Star	2020	NA	NA	NA	NA	NA
2	Duke	Duke	2019	109	75	68.81	127	1.69
		Duke	2020	NA	NA	NA	NA	NA
		O'Neal	2019	110	66	60.00	120	1.82
		Star	2020	NA	NA	NA	NA	NA
3	Elliott	Elliott	2019	93	23	24.73	24	1.03
		Elliott	2020	208	77	37.02	86	1.12
		O'Neal	2019	127	95	74.80	133	1.40
		Star	2020	83	56	67.47	92	1.64
4	Liberty	Liberty	2019	121	70	57.85	NA	NA
		Liberty	2020	192	134	69.79	143	1.07
		O'Neal	2019	103	63	61.17	NA	NA
		Star	2020	180	119	66.11	139	1.17
5	Heintooga	Heintooga	2019	107	0	0	0	0
		Heintooga	2020	208	0	0	0	0
		O'Neal	2019	112	35	31.25	41	1.16
		Star	2020	207	57	27.54	68	1.19
6	NC3104	NC3104	2019	116	91	78.45	123	1.35
		NC3104	2020	209	160	76.56	232	1.45
		O'Neal	2019	104	83	79.81	119	1.44
		Star	2020	198	149	75.25	244	1.64
7	NC1992	NC1992	2019	127	1	0.79	NA	NA
		NC1992	2020	NA	NA	NA	NA	NA
		O'Neal	2019	107	80	74.77	98	1.22
		Star	2020	NA	NA	NA	NA	NA
8	O'Neal	O'Neal	2019	104	47	45.19	65	1.38
		O'Neal	2020	60	47	61.67	37	1.27
		Star	2019	117	90	76.92	176	1.96
		Duke	2020	72	106	81.94	59	1.80
9	Reveille	Reveille	2019	120	71	59.17	113	1.59
		Reveille	2020	215	126	58.60	209	1.66
		O'Neal	2019	132	97	73.48	182	1.88
		Star	2020	188	123	65.43	225	1.83
10	Star	Star	2019	95	23	24.21	25	1.07
		Star	2020	NA	NA	NA	NA	NA
		O'Neal	2019	97	76	78.35	144	1.89
		Duke	2020	NA	NA	NA	NA	NA
11	Rebel	Rebel	2020	NA	NA	NA	NA	NA

		Rebel	2020	188	107	56.91	137	1.28
		Star	2020	217	150	69.12	160	1.07
		O'Neal	2020	153	109	71.24	145	1.33
12	Legacy	Legacy	2020	NA	NA	NA	NA	NA
		Legacy	2020	250	148	59.20	210	1.42
		O'Neal	2020	203	140	68.97	221	1.58
		Star	2020	202	146	72.28	215	1.47
13	New Hanover	New Hanover	2020	NA	NA	NA	NA	NA
		New Hanover	2020	197	165	83.76	252	1.53
		O'Neal	2020	204	182	89.22	290	1.59
		Star	2020	207	157	75.85	228	1.45
14	Pinnacle	Pinnacle	2020	NA	NA	NA	NA	NA
		Pinnacle	2020	191	88	46.07	165	1.88
		O'Neal	2020	NA	NA	NA	NA	NA
		Star	2020	NA	NA	NA	NA	NA

**NA indicates data not available because of insufficient flowering or other experimental failure not necessarily biological pollination barrier*

2. Self-pollination efficiency in blueberry seed development

A total of nine genotypes (7 cultivars and 2 advanced selection) were measured for seed formation efficiency in self-and cross fertilization conditions (Table 2). The number of seeds per fruit was always higher in cross-fertilized fruit compared to self-pollinated ones. However the seed number difference between self- and cross-pollinated fruit in the advance selection ‘NC3104’ was not significant compared to the other cultivars (Figure 1).

Table 2: Efficiency of cross-pollination and self-pollination (2019) on seed formation of selected blueberry genotypes

Cross	Female Parent	Male Parent	#Cross	Total seed	Seeds/berry
1	Aurora‡	Aurora	105	173	7.52
			NA	NA	NA
2	Duke	Duke	109	2527	33.69
		O'Neal	110	2992	45.33
3	Elliott	Elliott	93	594	25.83
		O'Neal	127	3561	37.48
4	Heintooga†	Heintooga	107	NA	NA
		O'Neal	112	10	0.29
5	NC3104	NC3104	116	844	9.27
		O'Neal	104	1103	13.29
6	NC4992†	NC4992	127	NA	NA
		O'Neal	107	2404	30.05
7	O'Neal	O'Neal	104	1859	39.55
		Star	117	4857	53.97
8	Reveille	Reveille	120	1622	22.85
		O'Neal	132	4853	50.03
9	Star	Star	95	68	2.96
		O'Neal	97	4105	54.01

‡ No cross-pollination due to low flowering; † self-pollination not successful; †† fruit did not mature due to an unknown disorder

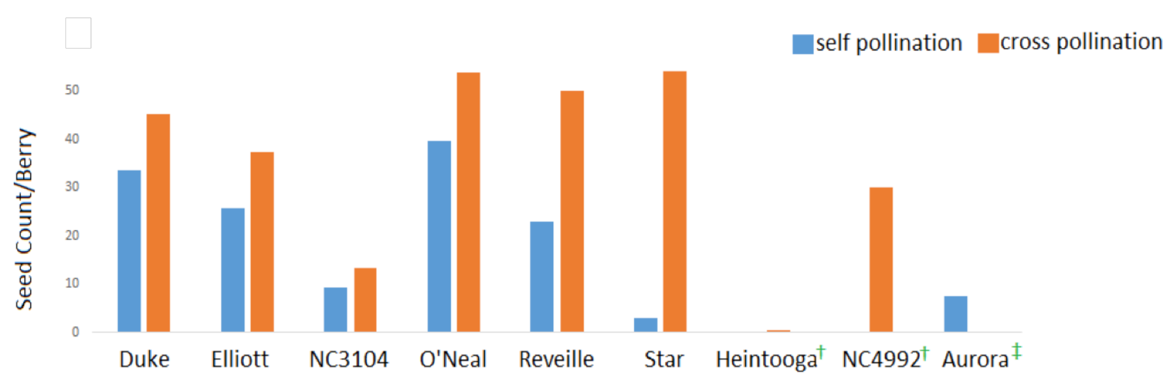

Figure 1. fertilization efficiency of self- and cross-pollinated flowers: A) percentage of pollinated buds that developed into fruit, B) Weight of self- and cross-pollinated berries, C) Seed count on self- and cross-pollinated berries

3. Self-pollination efficiency in blueberry fruit size and firmness

We measured the fruit size and fruit firmness in 9 blueberry genotypes (7 cultivars and 2 advanced selection) (Table 3). A significant difference in berry size due to cross and self-fertilization was observed in all blueberry lines that produced fruits. Four of the six cultivars showed a significantly larger fruit size after cross-pollination. There was no significant difference between self- and cross-pollinated treatments for fruit size of advance selection 'NC3104'. Interestingly, self-fertilized fruit in the northern highbush 'Elliott' was larger than in cross-fertilized fruit (Figure 2A).

The firmness of self-fertilized fruits in two blueberry cultivars ('Duke' and 'Star') was significantly higher than the cross-fertilized fruits. Although not statistically significant, all other genotypes also tend to have higher firmness in self-fertilized fruits than in cross-fertilized fruits. This may also be because of the smaller size of the self-fertilized fruit (Figure 2B).

Table 3: Efficiency of cross-pollination and self-pollination on fruit parameter of selected blueberry genotypes

Cross	Female	Male parent	#Cross	Berry size	Firmness
1	Aurora[‡]	Aurora	105	1103.22	213.61
			NA	NA	NA
2	Duke	Duke	109	1327.67	207.42
		O'Neal	110	1525.87	183.19
3	Elliott	Elliott	93	1436.76	130.21
		O'Neal	127	1215.17	139.27
4	Heintooga[†]	Heintooga	107	NA	NA
		O'Neal	112	1357.67	191.21
5	NC3104	NC3104	116	945.49	178.69
		O'Neal	104	929.4	170.8
6	NC4992[†]	NC4992	127	NA	NA
		O'Neal	107	1061.58	254.69
7	O'Neal	O'Neal	104	967.76	164.81
		Star	117	1279.28	159.33
8	Reveille	Reveille	120	1552.9	242.96
		O'Neal	132	1857.98	239.62
9	Star	Star	95	1546.69	246.6
		O'Neal	97	1173.64	246.99

‡ No cross-pollination due to low flowering; † self-pollination not successful; †† fruit did not mature due

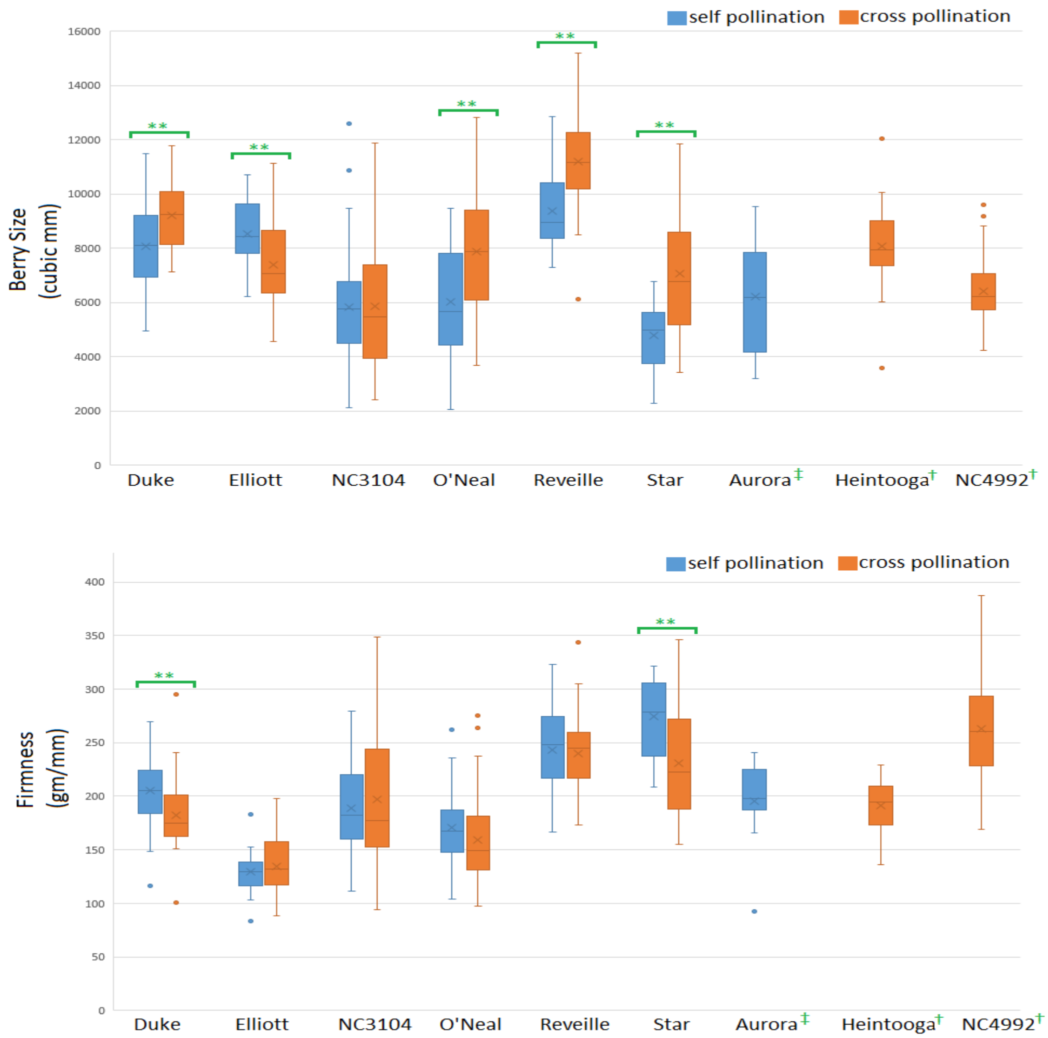

Figure 2. Variation on berry size and fruit firmness in self and cross-pollinated flowers. Top panel- berry size, Bottom panel- fruit firmness ** indicates the statistically significant difference between the pairs

Discussion and Conclusions

Based on the above study, six cultivars, 'Duke', 'Liberty', 'Reveille', 'Legacy', 'Rebel', and 'New Hanover' and one advanced selection 'NC3104' performed well after self-pollination in terms of fruit development. However, the seed counts of the selected, tested genotypes show that seed development is significantly lower in all cultivars except the advanced selection line 'NC3104'. Interestingly berry size was significantly smaller in the self-fertilized condition of all genotypes except 'Elliott' and 'NC3104'. Based on the above findings, we conclude that 'NC3104' overperformed among all cultivars tested in all self-fertilization parameters and can be used as a source parent for breeding self-fertilizing blueberries.

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