Title:

Evaluation of Advanced Selections, Southern and Northern Highbush Blueberries for Self- and Cross-Fertility

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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 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 mechanism of self-fertility greatly varies across most flowering plants, a large number of shared structural similarities exists 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% ovules aborted and ~88% of remaining showed poor development (Huang 1997). Conversely, in cross-pollinated blueberries, only 22% 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, increasing the cost of production 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 problems 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 advance selection line 'NC3104' has high 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 in 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 (figure 1). 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 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 number of seeds were also calculated. Statistical analysis for fruit size and fruit firmness was performed using students *t*-test.

Results

- Results from this project has been bublished as an abstract (Aryal et al. 2019) and has been presented as poster in the annual meeting of National Association of Plant Breeders, Aug 25-29, Pine Mountain, GA (Appendix 1)
- A total of ten blueberry cultivars were selected for this experiment. One cultivar, 'Aurora' did not produce enough flower buds for both self- and cross-pollination. Therefore, only self-fertilization for this cultivar is reported.
- The Cultivar 'Liberty' showed some wilting symptoms while the fruit were still maturing. This cultivar was analyzed for the number of fruit set and was not included for further analyses.
- As expected pentaploids blueberry cv. 'Heintooga' did not produce any fruit after self-pollination. Self-pollination in advance selection and 'NC4992' was also highly repressed, and no fruit was developed. Therefore, it is recommended to avoid monoculture of this selection in future field trials. However, the cross-pollination efficiency in the 'NC4992' was similar to other cultivars (74.77%)
- In Heintooga, cross-pollination was also impaired with only 31.25% of the pollinated buds developing into fruit.

- Six of the ten cultivars produced fruit with both self and cross-pollination but with different efficiencies.
- Self- and cross-fertilization in cultivars 'Duke', 'Liberty', 'Reveille' and the advance selection 'NC3104' were not significantly different for the number of fruit set, as well as the total fruit weight (figure 2A, 2B).
- 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 2C).
- We observed a significant differencein berry size due to cross and self-fertilization. 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 3A).
- Overall we observed that the self-fertilized fruit tend to have higher firmness. This may be because the self-fertilized fruit are smaller. However the difference infirmness of 'Duke' and 'Star' was significantly higher on the self-fertilized fruits (figure 3B).

Discussion and Conclusions

Based on our study, the cultivars 'Duke', 'Liberty', 'Reveille', and the advanced selection 'NC3104' performed well after self-pollination in terms of fruit weight and size, which are the two main components of yield. However, the seed counts of all tested genotypes weresignificantly different between self- and cross-pollinated flowers, indicating that there is still some barriers in embryo development of self-fertilized flowers. Berry size was also significantly different between four out of six cultivars tested. Two cultivars showed higher fruit firmness upon self-pollination, however this may be due to the confounding effect of smaller berry size. We plan to repeat this experiment in the next flowering season as well as expanding the panel to include more blueberry genotypes. Once most and least self-compatible genotypes were identified, we will conduct genetic study to identify the self-incompatibility locus in Vaccinium corymbosum.

Table 1: Summary of data obtained from self and cross pollination

Cross	Female parent	Male parent	#Cross	# Fruit set	% Fruit set	Total weight	Weight/berry (gm)	Total seed	Seeds/berry	Berry size (mm^3)	Firmness (gm/mm)
1	Aurora[‡]	Aurora	105	23	21.9	27.6	1.2	173	7.52	1103.22	213.61
2	Duke	Duke	109	75	68.81	126.89	1.69	2527	33.69	1327.67	207.42
3	Duke	O'Neal	110	66	60	120.28	1.82	2992	45.33	1525.87	183.19
4	Elliott	Elliott	93	23	24.73	23.62	1.03	594	25.83	1436.76	130.21
5	Elliott	O'Neal	127	95	74.8	132.6	1.4	3561	37.48	1215.17	139.27
6	Heintooga[†]	Heintooga	107	NA	NA	NA	NA	NA	NA	NA	NA
7	Heintooga	O'Neal	112	35	31.25	40.66	1.16	10	0.29	1357.67	191.21
8	Liberty[††]	Liberty	121	70	57.85	NA	NA	NA	NA	NA	NA
9	Liberty[††]	O'Neal	103	63	61.17	NA	NA	NA	NA	NA	NA
10	NC3104	NC3104	116	91	78.45	122.8	1.35	844	9.27	945.49	178.69
11	NC3104	O'Neal	104	83	79.81	119.4	1.44	1103	13.29	929.4	170.8
12	NC4992[†]	NC4992	127	1	0.79	NA	NA	NA	NA	NA	NA
13	NC4992	O'Neal	107	80	74.77	97.77	1.22	2404	30.05	1061.58	254.69
14	O'Neal	O'Neal	104	47	45.19	64.84	1.38	1859	39.55	967.76	164.81
15	O'Neal	Star	117	90	76.92	176.38	1.96	4857	53.97	1279.28	159.33
16	Reveille	Reveille	120	71	59.17	113.02	1.59	1622	22.85	1552.9	242.96
17	Reveille	O'Neal	132	97	73.48	182.1	1.88	4853	50.03	1857.98	239.62
18	Star	Star	95	23	24.21	24.59	1.07	68	2.96	1546.69	246.6
19	Star	O'Neal	97	76	78.35	143.95	1.89	4105	54.01	1173.64	246.99

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

Figure 1. Self- and cross-pollination experiment in the greenhouse: Top panel-experimental plants growing inside the greenhouse; Bottom panel depicts floral buds before emasculation, after emasculation, and mature fruits right before harvesting.
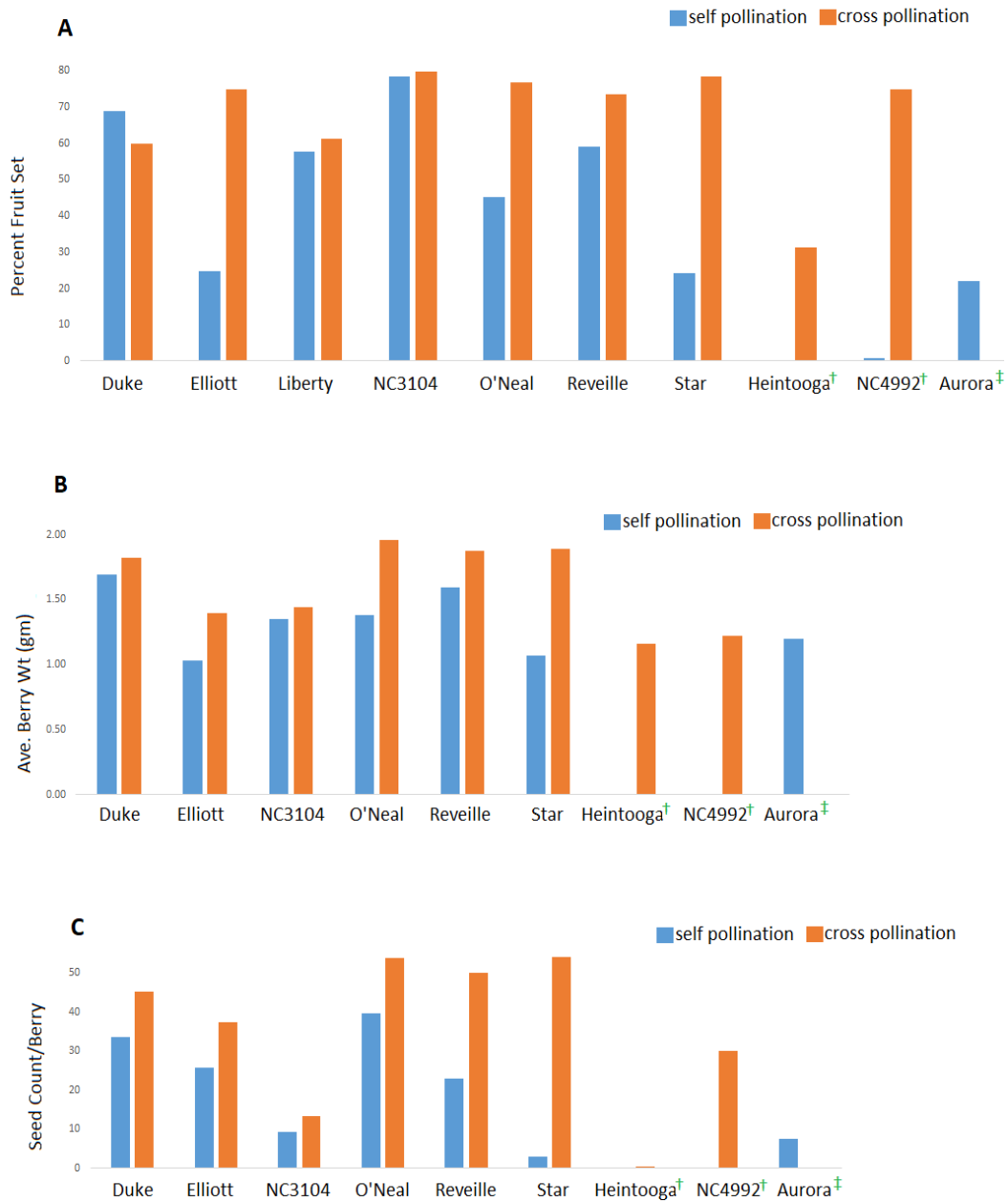
Figure 2. *fertilization efficiency of self- and cross-pollinated flowers: A) percentage of pollinated buds that developed into fruit, B) Weight of self- and cross-pollinated berries, C) Seed count on self- and cross-pollinated berries*
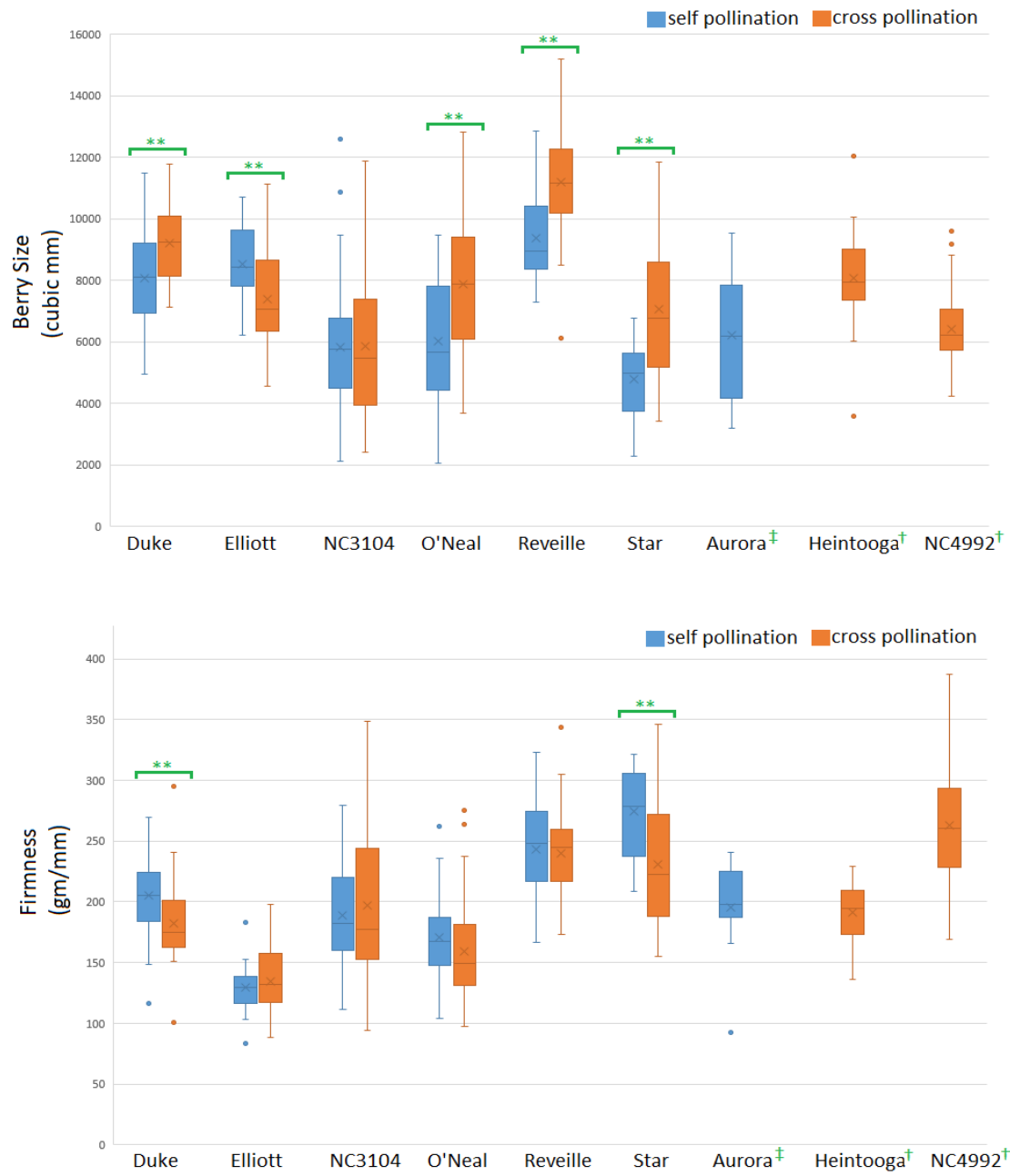
Figure 3. *Variation on berry size and fruit firmness in self and cross-pollinated flowers. Top panel-berry size, Bottom panel- fruit firmness ** indicates the statistically significant difference between the pairs*

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Aryal, R., Nelson, A., Farruggio, L., Lipscomb, D., and Ashrafi, H. (2019). Evaluation of Self-Compatibility in Ten Highbush Blueberry Cultivars by Controlled Crossing in Greenhouse. *National Association of Plant Breeders meeting, Aug 25-29, 2019. Pine Mountain, GA*

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Appendix 1

The poster presented in the annual meeting of National Asscian of Plant Breeders 2019

Evaluation of Self-Compatibility in Ten Highbush Blueberry Cultivars by Controlled Crossing in Greenhouse

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BACKGROUND

- 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).
- 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.
- Introduction of the self-fertility trait in the blueberry cultivar will reduce the production cost to growers by eliminating the obligatory outcrossing conditions.
- In this project, we studied the variation in self- and cross-fertility of four Northern Highbush, four Southern Highbush cultivars, and two advance selection accession at NC State University.
- Findings presented here will help blueberry breeders towards developing a self compatible blueberry line.

MATERIALS AND METHODS

- In winter 2017 six-year-old field grown blueberry cultivars (10) were transplanted into the 20 gallon pots and were grown on a gravel pad at Horticultural Field Lab of NC State University (Figure 1). Two plants of each cultivar were transplanted to have a second back up plant.
- The plants were moved into a cold room in December 2018 and were kept at 4 °C walking cold rooms. After accumulating 1200 chilling hours (50 days), the male parents were move into the greenhouse. After two weeks the maternal were taken out of the cold room and were placed on a greenhouse bench with average daily temperature of 22-25 °C and ambient light.
- 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 'Star' or 'O'Neal' cultivars.
- Ripe fruit from each cross was harvested at ripening stage.
- Longitudinal and latitudinal diameters of a individual berry were measured using a digital Vernier caliper. The berry volume (size) was calculated from the average diameter with the assumption of spherical shape.
- Firmness of individual berries (15-80 individuals, depending on number of harvest) were measured using FirmTech 2 (BioWorks, KS) instrument.
- The self-fertility score for each cultivar was determined in terms of percentage of successful fruit set, fruit weight, fruit size, and the number of developed seed.
- Statistical test between self-and cross-fertilization parameter was performed using a two tail t-test in excel.

Figure 1. Self- and cross-pollination experiment in the greenhouse: Top panel- experimental plants growing inside the greenhouse; Bottom panel depicts floral buds before emasculation, after emasculation, and mature fruits right before harvesting.

RESULTS

- A total of ten blueberry cultivars were selected for this experiment. One cultivar 'Aurora' did not produce enough flower buds for both self- and cross- pollination. Therefore, only self fertilization for this cultivar is reported.
- The Cultivar 'Liberty' showed some wilting symptom while the fruits were still maturing. This cultivar was analyzed for number of fruit set and dropped from all other analyses.
- As expected pentaploids blueberry cv. 'Heintooga' did not produce any fruit on self pollination. Self pollination in advance selection and 'NC4992' was also highly repressed and no fruit was developed.
- In Heintooga, cross pollination was also impaired with only 31.25% of the pollinated buds developing into fruit. However the cross pollination efficiency in the 'NC4992' was similar to other cultivars (74.77%)
- Six of the 10 cultivars produced fruits with both, self and cross pollination but with different efficiency.
- Self- and cross-fertilization in cultivars 'Duke', 'Liberty', 'Reveille' and the advance selection 'NC3104' were not significantly different for the number of fruit set, and the total fruit weight (figure 2A, 2B).
- The number of seed per fruit was always higher in cross fertilized fruit compared to self pollinated ones. However the seed number of self- and cross-pollinated fruit in the advance selection 'NC3104' was not significant compared to the other cultivars (figure 2C).
- We observed a significant differences in berry size due to cross and self fertilization. Four of the six cultivars shows significantly larger fruits after cross-pollination. There was no difference in fruit size of advance selection 'NC3104'. Interestingly, self fertilized fruit in the northern highbush 'Elliott' was larger than in cross fertilized fruit (figure 3A).
- Overall we observed that the self-fertilized fruits tend to have higher firmness. This may just be because of the smaller size of the self fertilized fruits. However the difference on firmness of 'Duke' and 'Star' was significantly higher on the self fertilized fruits (figure 3B).
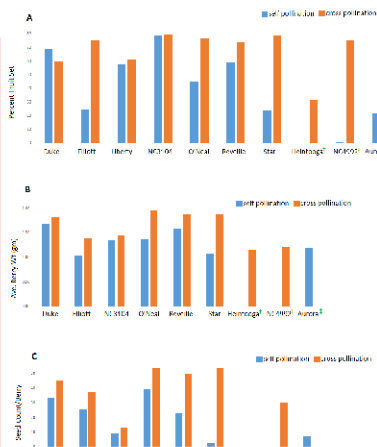
Figure 2. fertilization efficiency of self- and cross-pollinated flowers: A) percentage of pollinated buds that developed into fruit, B) Weight of self- and cross-pollinated berries, C) Seed count on self- and cross-pollinated berries
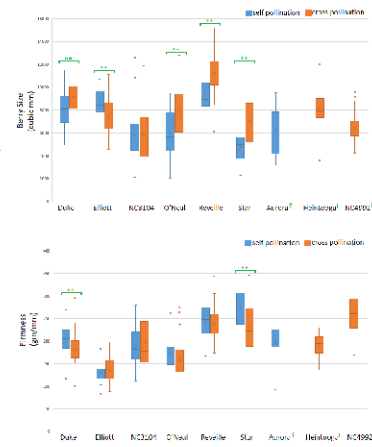
*Figure 3. Variation on berry size and fruit firmness in self and cross pollinated flowers. Top panel- berry size, Bottom panel- fruit firmness ** indicates the statistically significant difference between the pairs*

Table 1: Summary of data obtained from self and cross pollination

cross	female parent	male parent	# emasculation	# harvest	% harvest	total weight	weight/berry (gm)	total seed	seed/berry	Berry size (mm³)	firmness (gm/mm)
1	Aurora[‡]	Aurora	105	23	21.90	27.6	1.20	173	7.52	1103.22	213.61
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3	Duke	O'Neal	110	66	60.00	120.28	1.82	2992	45.33	1525.87	183.19
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6	Heintooga[†]	Heintooga	107	NA	NA	NA	NA	NA	NA	NA	NA
7	Heintooga	O'Neal	112	35	31.25	40.66	1.16	10	0.29	1357.67	191.21
8	Liberty[††]	Liberty	121	70	57.85	NA	NA	NA	NA	NA	NA
9	Liberty[††]	O'Neal	103	63	61.17	NA	NA	NA	NA	NA	NA
10	NC3104	NC3104	116	91	78.45	122.8	1.35	844	9.27	945.49	178.69
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12	NC4992[†]	NC4992	127	1	0.79	NA	NA	NA	NA	NA	NA
13	NC4992	O'Neal	107	80	74.77	97.77	1.22	2404	30.05	1061.58	254.69
14	O'Neal	O'Neal	104	47	45.19	64.84	1.38	1859	39.55	967.76	164.81
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18	Star	Star	95	23	24.21	24.59	1.07	68	2.96	1546.69	246.60
19	Star	O'Neal	97	76	78.35	143.95	1.89	4105	54.01	1173.64	246.99

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

CONCLUSION AND FUTURE DIRECTION

Based on our study, the cultivars 'Duke', 'Liberty', 'Reveille' and the advanced selection 'NC3104' perform well under self-pollination conditions in terms of fruit weight and size, which are two main components of yield. However the seed count in all tested genotypes was significantly different between self- and cross-pollinated flowers, indicating that there is still some barriers embryo development of self-fertilized flowers. Berry size was also significantly different between four out of six cultivars tested. Two cultivars showed higher fruit firmness upon self-pollination, however this may be due to the smaller berry size. We plan to repeat this experiment in next flowering season as well expand the panel to include more blueberry genotypes. Once most and least self-compatible genotypes were identified, we will conduct genetic study to identify the self incompatibility locus in *Vaccinium corymbosum*.

CONTACT INFO

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