OPVC CONTINUING PROJECT REPORT: YEAR 2016

1. OPVC REPORT COVER PAGE (maximum 2 pages)

OPVC Project Number:

Project Title: Broccoli Breeding, Evaluation

PI: James R. Myers
Organization: Oregon State University
Telephone: 541-737-3083
Email: james.myers@oregonstate.edu
Address: ALS 4017, Department of Horticulture
City/State/Zip: Corvallis, OR 97331

Total Project Request (all years):
Year 1: $12,000

Other funding sources: none

2. EXECUTIVE SUMMARY (ABSTRACT): Processors need broccoli with better quality traits than what is available in cultivars developed for California and Mexico fresh markets. Farmers need to reduce labor costs of broccoli production by mechanizing harvest. Most contemporary commercially available cultivars are not suitable for either mechanical harvest or processing. The objective of the OSU broccoli breeding program is to develop broccoli varieties adapted to western Oregon with suitable quality and high yields. The program operates on a one year cycle where cuttings from the field are taken into the greenhouse in the fall where they are rooted and hand crossed and self-pollinated to produce seed for the next generation. Seed is harvested in May and June and used to plant trials for fall evaluation.

In 2016, a major shift in program emphasis occurred. We did not conduct a replicated trial of experimental hybrids as we have in past years. The processing and sample display and evaluation of experimental hybrids was temporarily discontinued. We contracted with P & R Seeds to scale up hybrid seed production of one hybrid (O446/S454) for eventual field production in Oregon. Seed of the inbreds (S446, S454 and O446) was provided to P & R Seeds, who contracted with a grower in Chile to conduct increase of the inbreds in 2016, to be followed by hybrid seed production in 2017. This work is also complementary to an ODA Specialty Crop Block Grant that was obtained in 2016 to develop a robotic harvesting system for direct harvest of broccoli for processing.

In the field program at the OSU Vegetable Research Farm near Corvallis, We did grow a full set of inbred lines and acquired observational data. In addition, three isolation plots were established for testing various inbred combinations for hybrid seed production. An excellent increase of O446 x S454 hybrid was obtained.

3. FULL REPORT (no maximum)

3.a. BACKGROUND Because of the high labor input into broccoli harvest, much of the acreage of broccoli grown for processing and fresh market has shifted out of the United States to countries where labor costs are lower. Broccoli production in western Oregon continues to face stiff competition, and to be competitive, growers need to produce broccoli more efficiently and affordably. Machine harvest of broccoli could lower production costs. A limitation has been the lack of broccoli varieties suitable to mechanical harvest. From a crop production standpoint, the two key factors in developing varieties that are suitable for mechanical harvest are uniform heading and appropriate plant architecture.

Most commercially available broccoli hybrids are high yielding but have short plants with heavy but poorly exserted heads. Short plants have high fiber in the portion of the stem subtending the head that must be used to achieve a normal-length cut. The lack of height as well as the high fiber makes them unsuitable for machine harvest.

In addition to direct harvest characteristics, processors need broccoli that makes a high quality pack. Florets and stems need to be dark green in color and should be uniform in color and shape; beads should be small, and retained during the blast freezing process. An added benefit to dark green color that we recently discovered is that darker color is associated with higher carotenoid (compounds such as pro-vitamin A) levels. Heat tolerance, and resistance to bacterial head rot, downy mildew, and club root is desirable. Inbred lines from the Oregon State University breeding program have the genetic potential to create hybrids with greatly improved head exsertion and segmentation, better color, and low fiber. The OSU hybrids are suitable for machine harvest, and some inbreds possess some of the already discussed disease resistance characteristics.

Many OSU hybrids are high quality and have shown stable, high yields over several years and it appears now that the major limitation to achieving commercial seed production of hybrids is the scaling up of hybrid seed production using cytoplasmic male sterility or self-incompatibility. There is also a need to derive new inbreds with improved disease resistance

3.b OBJECTIVES

- Increase seed supply of OSU inbreds and backcross selected inbreds into an Ogura CMS background
- Evaluate inbreds for hybrid seed production.
- Scale up to commercial production of F1 hybrid seed.

3.c. SIGNIFICANT FINDINGS .

3.d. METHODS The broccoli breeding program follows a one year breeding cycle. Cuttings from hybrids and inbreds are brought from the field in the fall and rooted in the greenhouse. Upon flowering during the winter, inbreds are bud pollinated to self-pollinate them and crossed with other inbreds to produce

Transplants of inbreds and breeding lines are started from seed produced in the greenhouse and planted in the field for observation and selection. Pair-wise inbred combinations are also planted in isolation to evaluate seed production potential for F1 hybrid seed production. Seed of S454 S446 inbreds

was also provided to P & R Seeds to oversee increase and production of a hybrid at a commercial scale for development and evaluation of a mechanical broccoli harvester for Oregon growers.

3.e. RESULTS & DISCUSSION *Greenhouse inbred and hybrid seed production:* Cuttings were taken from inbreds and breeding lines grown in the field in 2015 to establish material for crossing and hybrid seed production in the greenhouse during the winter of 2015-2016. Two hundred ninety nine cuttings were taken for rooting with 72% of these surviving to be potted for crossing. These were bud pollinated by hand to self the inbreds and produce seed for the 2016 growing season. A list of the inbreds and breeding lines (along with pedigrees) that were advanced in the greenhouse and established in the field is shown in table 1. Most lines are highly inbred but a few are still segregating and showing significant variation in the field.

Observation Trials: The observation trial included 18 highly inbred lines, 16 advanced lines still undergoing inbreeding and selection, and four Ogura cytoplasmic male sterility (CMS) lines at various stages of backcrossing to selected inbreds (Table 2). These were evaluated at heading for various traits important to processing including number of blind plants, various head characteristics (color, bead size, segmentation) and plant characteristics (head exsertion, branching, uniformity and overall performance). Because of heavy rains in October when heads were maturing, we were also able to rate the lines for head rot, and did observe several inbreds with apparent resistance. We also made selections for cuttings for greenhouse production, and in part, selected single plants on the basis of reduced head rot.

Hybrid Seed Production: Three isolation plots were established, two at the Vegetable Research Farm and one at the Lewis Brown Farm. Two of the inbred pairs had O446, the CMS version of S446, as the female parent (Table 3). The third pair included S454, which is a self-fertile inbred, and S462, which in our previous tests, has been mostly self-incompatible (not setting selfed seed unless bud pollinated). Seed collected from S462 as the maternal parent, should, in principle, be hybrid with S454 being the material parent. In previous years, we have used cages that exclude pollinators to achieve isolation but have had varied results in obtaining good seed set. This year, we relied on distance from any other sources of *B. oleracea* pollen to maintain purity. In general, we had good success, particularly with the O446/S454 cross combination in producing seed with about 251 g of seed obtained (enough to plant almost 80,000 plants or enough to plant two or more acres).

Off-season commercial seed production: Approximately 200 seeds each of S454, S446 and O446 Seeds were provided to Peter Mes of P & R Seeds, who contracted with a custom seed production firm in Chile to first increase inbred lines, then to produce hybrid seed using the cross combination of O446 x S454. In 2016 the seeds were sown in small caged plots (Figure 1) and hand pollinated to provide maximum yield of parent seed stock. Plants were sown in April, Transplanted in May, pollinated in November, and seed harvest is expected to begin January 8, 2017. Seed yield estimates based on obtaining approximately 1 gram per plant are 216 grams of O446 x S446, and 96 grams of S454. The seeds harvested from these plots will be used for a subsequent large production of hybrid seed in 2017. Large caged plots will be pollinated using honeybees to facilitate crossing. Harvest of the F_1 hybrid seed will occur in January of 2018. These seeds will be available for both small scale testing and large scale production in Oregon for the 2018 growing season.

4. BUDGET DETAILS

Salaries and benefits	
Faculty Research Assistant (2% FTE)	$782
OPE @ 70%	$547
Student Wages	$2,508
OPE @ 10%	$251
Total Salary and Wages	$3,290
Total OPE	$798
Contractual	$6,335
Supplies	$100
Land use and greenhouse rental	$1,477
Total	$12,000

BUDGET NARRATIVE

Salary and OPE, faculty research assistant: FRA will commit approximately 1% FTE to overseeing broccoli breeding activities in the greenhouse and field. OPE for FRA is 70%.
Undergraduate student labor: Two undergraduate students will bud pollinate and cross broccoli inbreds in the greenhouse with time required of approximately 20 hr/wk for six weeks. A student will assist in field activities (planting, cultivating, hoeing, managing isolation cages), averaging 1.5 hr/wk for 16 wk. Undergraduate student OPE is 10%.
Services and supplies: Includes $100 for field and greenhouse supplies ((fertilizer, pots, labels, stakes, tags, crossing supplies, envelopes, paper bags, etc.).
Contractual: Hybrid seed production and inbred line maintenance will be outsourced to P & R Seeds.
Facilities rental fees: Charges include land use rental (0.25 acre at $1,259 per acre = $315), and greenhouse rental ($1.55*750 sq. ft. = $1,163).

Table 1. Inbreds and breeding lines grown in the field at the OSU Vegetable Research Farm in 2016.

Line	Previous no.	Pedigree
S411	88-76-4-1-2	HS179-1/S240-11-8
S442	91-203-2-3-1-1	S352/S240-11-8
S445	91-203-2-3-1-5	S352/S240-11-8
S446	91-203-2-3-2-1	S352/S240-11-8
S454	91-232-4-1-2-1	S233/Emerald City
S457	04-3-2-2-3	S454 /S387/S411/S446
S460	04-4-1-1-3	S411/S446/S454 /S387
S462	04-4-2-2-1	S411/S446/S454 /S387
S463	04-5-2-2-1	S454 /S446/USVL 089
S465	04-5-2-2-3	S454 /S446/USVL 089
S466	11-1-1-1-2	S446/S460
S469	11-2-1-1-1	S454/RS2
S471	09-1-1-3-1-2-2	OSU OP Selection
S472	09-1-1-3-1-2-1	OSU OP Selection
S473	09-1-3-1-1-1-1	OSU OP Selection
S474	09-1-1-3-1-1-1	OSU OP Selection
S475	09-1-1-3-1-1-2	OSU OP Selection
Not yet assigned	(S446/S457)-1-1-1-1	S446/S457
Not yet assigned	(S446/S457)-1-1-2-1	S446/S457
Not yet assigned	(S446/S457)-1-1-2-2	S446/S457
Not yet assigned	(S446/S457)-1-1-2-3	S446/S457
Not yet assigned	(S454/S445)-1-1-1	S454/S445
Not yet assigned	(S454/S446)-1-1	S454/S446
Not yet assigned	(S454/RS2)-2-1-1-1	S545/RS2
Not yet assigned	(S454/RS2)-2-1-1-2	S545/RS2
Not yet assigned	(S454/RS2)-2-1-1-2	S545/RS2
Not yet assigned	(S458A/S446)-1-1-2	S458A/S446
Not yet assigned	(S458A/S466)-1-3-1	S458A/S446
Not yet assigned	(S458A/S446)-1-2-2	S458A/S446
Not yet assigned	(S458A/S466)-1-3-1	S458A/S466
Not yet assigned	(S462/S460)-1-1-2	S462/S460
Not yet assigned	(S463/S446)-1-1-1-1	S463/S446
Not yet assigned	(S468/S446)-1	S468/S446
Not yet assigned	O446*1-1/S462-3-1/S462-1//S454	O446/S462/S462//S454
Not yet assigned	O446*1-1/S462-3-1/S462-1//S454	O446/S462/S462//S454
Not yet assigned	O446*3 S446	O446/S446
Not yet assigned	O446*1-1/S465-2//S465-2///S465	O446/S465//S465///S465

Table 2. Broccoli inbreds and breeding lines grown in an observation trial at the OSU Vegetable Research Farm in 2016. Trial was planted from transplants on 5 August.

Line	No. Plts	No. Blind	Blind (%)	Days after transplanting	Head Shape[z]	Bead Size[y]	Stem color[x]	Ex-sertion[x]	Segmentation[x]	Uniformity[x]	Branch[x]	Head rot[w]	Overall[x]	Notes
Advanced Lines														
S411	28	4	14.3	69	6	MC	7	7	5	7	5	3	6	
S442	12	2	16.7	76	7	F	5	6	9	4	3	8	5	Leafy heads, most heads water soaked with head rot
S445	7	0	0.0	71	3	F	7	6	7	8	3	0	7	Heads subdivides into small half inch florets
S446	26	1	3.8	73	6	FM	7	7	3	7	3	7	8	
S454	27	10	37.0	76	7	FM	7	8	5	3	5	4	8	
S457	26	3	11.5	65	5	F	5	6	5	7	3	4	6	
S460	6	2	33.3	80	5	F	5	8	3	8	3	9	7	Small heads, marginal leaf chlorosis
S462	27	7	25.9	69	4	FM	7	6	5	7	8	5	5	Strong marginal leaf chlorosis
S463	6	2	33.3	76	6	FM	5	7	5	7	5	5	8	Marginal leaf chlorosis
S465	23	3	13.0	90		F	7							Very late, plot varies for marginal leaf chlorosis, virus?
S466	16	4	25.0	73	5	FC	5	6	5	3	5	8	4	Plot highly variable esp bead size & maturity
S466	8	1	12.5									5		
S472	16	1	6.3	76	3	FM	5	7	4	8	3	6	5	Many leaning plants
S469	28	3	10.7	73	6	FM	7	9	5	7	3	6	8	Some lodging but not bad for such a tall plant
S471	16	0	0.0	76	4	F	3	5	7	7	1	6	9	Flat but nice dense heads, head rot not as severe
S473	15	2	13.3	69	7	FM	7	8	7	7	5	5	7	Slightly soft heads, very tall plants
S474	25	1	4.0	80	6	F	5	7	7	5	1	3	8	Tendancy for leafy heads
S475	25	6	24.0	83	4	F	5	6	8	6	1	3	7	Flat heads, some leafy

Table 2. (continued)

Line	No. Plts	No. Blind	Blind (%)	Days after transplanting	Head Shape[z]	Bead Size[y]	Stem color[x]	Ex-sertion[x]	Segmentation[x]	Uniformity[x]	Branch[x]	Head rot[x]	Overall[x]	Notes
Early generation lines														
(S446/S457)-1-1-1-1	13	0	0.0	76	7	M	7	7	8	6	3	3	7	Small leaves in head
(S446/S457)-1-1-2-1	18	1	5.6	73	5	F	5	6	8	5	3	2	7	Leafy heads
(S446/S457)-1-1-2-2	23	6	26.1	76	5	FM	5	7	5	5	3	4	6	Leafy heads
(S446/S457)-1-1-2-3	18	0	0.0	71	6	F	7	7	7	5	3	1	8	Variable bead size
(S454/S445)-1-1-1-1	27	7	25.9	76	6	FM	7	7	5	7	3	5	6	Short stature
(S454/S446)-1-1	22	2	9.1	57	6	FM	5	5	5	3	3	8	5	Discard, cat's eye, bad head rot
(S454/RS2)-2-1-1-1	18	3	16.7	76	4	FMC	7	7	7	5	3	1	7	Still variable but some nice types
(S454/RS2)-2-1-1-2	13	3	23.1	81	4	F	5	7	8	7	3	5	8	Leafy heads, taller than sister line
(S454/RS2)-2-1-1-2	19	0	0.0									5		
(S458A/S446)-1-1-2	16	1	6.3	76	5	F	3	7	5	7	3	9	6	Large heads but flat
(S458A/S466)-1-3-1	28	3	10.7	76	7	F	7	7	7	5	5	3	7	Still somewhat variable
(S458A/S446)-1-2-2	6	0	0.0	73	6	FM	3	6	3	7	1	1	6	
(S458A/S466)-1-3-1	15	1	6.7	76	4	F	5	7	5	7	3	5	6	Different from previous plot with same number, quite variable
(S462/S460)-1-1-2	12	0	0.0	73	7	FM	7	5	5	5	1	3	7	Still somewhat variable
(S463/S446)-1-1-1-1	7	0	0.0	76	7	FMC	7	8	5	7	1	7	7	
(S468/S446)-1	19	3	15.8	76	7	FMC	7	7	7	1	3	3	7	Still highly variable

Table 2. (continued)

Line	No. Plts	No. Blind	Blind (%)	Days after transplanting	Head Shape[z]	Bead Size[y]	Stem color[x]	Ex-sertion[x]	Segmentation[x]	Uniformity[x]	Branch[x]	Head rot[x]	Overall[x]	Notes
Cytoplasmic male sterile lines														
O446*1-1/S462-3-1/S462-1//S454	17	2	11.8	76	6	MC	7	7	5	3	3	3	3	Still highly variable
O446*1-1/S462-3-1/S462-1//S454	11	2	18.2	77	7	MC	7	7	5	3	3	4	3	
O446*3 S446	29	2	6.9	71	5	MC	5	4	3	3	1	5	3	Only a few plants look like S446
O446*1-1/S465-2//S465-2///S465	29	5	17.2	90		F	5							Resembles S465

[z]Scale of 1 to 9 where 1 to 3 has concave heads, 5 is a flat head, and 7 and above is a domed head. [y]F = fine, M = medium, C = coarse bead size. [x]Scale of 1 to 9 where 9 is best/highest. [w]Scale of 0-9 where 0 indicates no visible head rot and 9 indicates that entire head is water-soaked and decaying.

Table 3. Seed set in broccoli isolation plots grown at the OSU Vegetable Research and Lewis Brown Farms in 2016.

Cross combination	Seed yield (g)[z]	Est. seed no.
O446/S462	18	5,714
S462/S454	38 (S454) 13 (S462)	12,064 (S454) 4,127 (S462)
O446/S454	251	79,684

[z]Seed harvested only from cytoplasmic male sterile O446 where this inbred was the female parent; seed harvested from both parents in the S462/S454 cross.

Figure 1. OSU broccoli inbreds being grown in a screen house in Chile for off season seed production.