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Project Title: Broccoli Breeding, Evaluation and Seed Production 2014
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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 but 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 2014, six experimental hybrids were planted in a replicated yield trial, which also included two commercial check hybrids and a new exserted commercial hybrid from Seminis. S446 x S458B had the highest net T/A head weight of the trial but this hybrid was also the latest in maturity. Fourteen advanced generation inbreds were selfed for seed production and an additional 15 early generation inbreds were advanced a generation. Two inbreds may have some level of drought tolerance compared to other inbreds. Fifteen CMS selections were backcrossed to various inbreds. Discussions were conducted with seed company representatives for commercializing the material in the OSU breeding program.

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 even 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

- Develop broccoli varieties adapted to western Oregon with suitable quality, high yields, and disease resistance including concentrated and uniform yield potential, large heads that are well exserted and have minimal leaf development on stems, firm, uniform florets of dark green color, and fine beads with short pedicels, which are retained after freezing.
- Develop seed production systems using cytoplasmic male sterility (CMS) or self-incompatibility (SI) to produce field scale quantities of F1 hybrid seed.
- Scale up seed production to facilitate wider testing of OSU hybrids.

3.c. SIGNIFICANT FINDINGS OSU broccoli hybrids perform similarly to commercial cultivars in terms of yield and have better processing quality.

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 F1 hybrid seed. In May and June, selfed and crossed seed is collected and is used to start transplants for planting yield trials and the breeding nursery. After evaluation, the cycle repeats with harvest of cuttings in the fall. New inbreds are derived by repeated self-pollination of F1 hybrids. Four to five generations of selfing is required before inbreds can be tested for hybrid seed production. The Inbreds, experimental

hybrids and commercial hybrids grown in the main fall planting are evaluated for head size, shape, and exsertion, segmentation, floret texture and color, maturity and disease resistance. The size of the replicated main fall season trial varies with the number of crosses that can be made (which is a function of the amount of labor available to make hand crosses) but up to 15 of the most promising OSU experimental hybrids and two to four check varieties are included. These are transplanted in one row plots 30 feet in length and replicated four times. Data recorded in addition to yield data includes plant and leaf height, exsertion, dome shape, bead size, head color and uniformity. Heads from entries in the yield trial are taken to the OSU pilot processing plant for blanching and freezing. Frozen material is evaluated at the OSU winter cutting and will be displayed at the NWFPA Expo in Portland in January.

Hybrid seed production is facilitated on a commercial scale using either self-incompatibility or cytoplasmic male sterility (CMS). The use of self-incompatibility is problematic in a program our size because of a lack of diversity in incompatibility alleles; therefore, CMS seems the best option for commercial hybrid seed production. Two sources of CMS (Anand and Ogura) have been used in the program. Anand CMS was transferred into broccoli from *Brassica tournefortii*, and has been available as germplasm but has never been used commercially. Ogura CMS came from radish and has the primary means of commercial hybrid seed production in several types of Cole crops. The patent on Ogura CMS expired about two years ago and can now be used without a license. We compared the performance of Anand and Ogura CMS and found that Ogura had fewer flower abnormalities, with showier petals and possibly higher seed set. We had been backcrossing several inbreds (S411, S445, S446 and S454) into the Anand background. In 2013, we will continue at least one more generation of backcrossing to Anand, but will initiate a backcross program with Ogura. CMS lines will be planted as females with fertile inbreds used as males. In the field, males and the associated array of females are grown in isolation or in cages on the Vegetable Farm. Seed production is evaluated based on number of siliques that set at least one viable seed, and weight of seed per female line. Another aspect of commercial scale up is identifying commercial partners who can take our inbreds and produce our hybrids or combine with their inbreds to produce a composite hybrid.

3.e. RESULTS & DISCUSSION *Greenhouse inbred and hybrid seed production:* Cuttings were taken from inbreds and breeding lines grown in the field in 2013 to establish material for crossing and hybrid seed production in the greenhouse during the winter of 2013-2014. A total of 6 cross combinations (tables 1-2) were obtained. The six experimental hybrids were planted in a replicated yield trial with four reps, which also included two commercial check hybrids and a new exserted commercial hybrid from Seminis. Twelve inbreds were selfed for seed production (table 3).

All of the trials exhibited high levels blind and multishooted plants (table 1). The large number of blind plants was most likely insect caused with flea beetle damage being the most likely proximate cause.

Yield Trial: Stands were relatively uniform and percent blind ranged from about 11 – 25%. S446 x S458B had significantly higher net T/A than several experiment lines, but was not significantly different from the two check cultivars. The Seminis experimental had yields similar to Arcadia (table 1, fig. 1). Days from transplanting to harvest ranged from 65 to 80 days with the highest yielding hybrid also being the latest. This hybrid (S446 x S458B) also had the lowest percent leaves around the head. Maturity across reps was somewhat variable this year, which along with multi-stemmed plants explains some of the variation observed in yield.

The canopy heights were similar, but experimental hybrids generally had greater head height compared to the check hybrids (table 2, figure 2). All entries in the trial had fine to medium bead size and heads of

acceptable size. In general, experimental hybrids had better color, and were more exserted and segmented than check hybrids (table 2). SVO 097BL had very flat heads and light stem color but bead size was quite small.

Samples from the yield trial were blanched and frozen in the OSU department of food science pilot plant. They were evaluated in an in-house cutting in late November, and samples were displayed at the Northwest Food Processors Association meetings in mid-January.

Observation Trials: In addition to the advanced inbred lines, 15 selections at S4 to S6 generation of inbreeding were grown and evaluated, and selections were made for the next cycle of self-pollination (table 3). The OSU OP materials have been selected under organic production and may be able to set heads with lower fertilizer levels. Other selections are from experimental hybrid combinations that looked particularly good when previously evaluated in yield and observation trials. An interesting observation in 2014 was that some inbreds (such as ((S454/RS2)-2-1-1) had thin wax and were prone to wilting under water stress whereas two of the OSU OP lines had thick leaves and a heavy wax layer, and were more resistant to water stress.

Table 4 shows yield of hybrid cross combinations that have been made since 2001 compared to recurring check hybrids. In general, yields of check hybrids have remained constant over the years, and there seems to have been an increase in yields of experimental hybrids over time. Yields for 2014 appear to be lower than those observed in recent years.

Cytoplasmic Male Sterile (CMS) Facilitated Hybrid Seed Production: Fifteen selections (11 Anand and 4 Ogura CMS background) were grown in 2014 (table 5). Backcrossing continued to eight inbreds that are generally self-compatible. We continued to select for more normal flower morphology in the Anand CMS. Three isolation cages were established at the Vegetable Research Farm. Each cage contained fertile inbred plants in the S445, S446 and S545 backgrounds. Within these cages, CMS lines were grouped to obtain crossed seeds. Honeybees were introduced to the cages at flowering and remained for about one month. During this time period when presence of honeybees prevented use of pesticides on the plants, an infestation of flea beetles developed and greatly reduced seed set.

We met with seed company representatives to discuss potential for commercialization of materials from the OSU broccoli breeding program.

4. BUDGET DETAILS

1) Breeding (Myers)

Salaries and wages	
Faculty Research Assistant, field, full time	$2,591
OPE @ 69%	$1,786
Student Wages	$1,000
OPE @ 8%	$80
Services and Supplies	$2,004
Total	$7,461

2) Processing (Yorgey)

Senior Faculty Research Assistant	$2,547
OPE @ 60%	$1,528
Student Wages	$200
OPE @ 8%	$16
Services and Supplies	$187
Total	$4,478
Grand Total	$11,909

Budget Explanation: Approximately 0.10 FTE Salary and OPE for a full time faculty research assistant. The remainder of salary paid from other sources. For the senior faculty research assistant, approximately 0.05 FTE allocated to process broccoli samples; the remainder of salary to come from other sources. $1,000 was allocated to summer undergraduate students to assist in plot maintenance and harvest operations. The SFRA also supervised an undergraduate student in broccoli processing. Undergraduate student OPE was 8%. Funds for services and supplies included land use fee (0.25 hectare at $2800/ha = $700), greenhouse use fee ($1.55*500 sq. ft. = $775), and various materials used for plot work.

Table 1. Yield data from a replicated hybrid broccoli trial, OSU Vegetable Research Farm, Corvallis, 2014.[z]

Hybrid	Days to Harvest	No. Plts/ Plot	% Blind Plants	Gross T/A	Young Heads T/A	Culls T/A	Net T/A	Net Heads/A	Head Diameter (cm)	% Leaves
Arcadia	72	29.5	19.5	3.7	0.3	0.14	2.60	10,164	12.1	18.7
Emerald Pride	65	27.8	12.8	4.7	0.3	0.43	2.93	11,180	12.9	23.5
S446xS458B	80	27.8	25.3	4.1	0.1	0.28	3.51	10,309	13.5	3.8
S446xS460	76	29.0	11.9	3.3	0.3	0.01	2.69	11,471	12.2	11.3
S454 x S458B	66	29.0	21.6	3.4	0.6	0.26	1.83	7,986	11.5	22.9
S454x S445	66	28.8	20.3	3.5	0.7	0.27	1.89	7,986	11.6	19.0
S454xS446	69	29.5	16.9	2.8	0.3	0.00	2.11	8,857	11.4	14.8
S454xS463	72	27.5	10.8	3.3	0.4	0.09	2.32	9,728	12.4	15.4
SVO 097BL	66	27.5	22.0	3.7	0.4	0.11	2.62	9,583	12.4	16.6
LSD 0.05[y]		1.9	12.0	0.8	0.2	0.33	0.96	3,076	1.2	9.3

[z]Transplanted July 14 in 30' plots, r1ows 30" apart, 12" between plants. Mean of 4 replications.

Table 2. Observation notes from a broccoli replicated yield trial, OSU Vegetable Research Farm, Corvallis, 2014.

Entry	Plant Ht (cm)	Head Ht (cm)	Head Shape[y]	Bead Size[x]	Stem color[w]	Exser-tion[w]	Segment-ation[w]	Unifor-mity[w]	Branch-ing[w]	Overall[w]
Arcadia	77	68	5	M	5	6	4	3	3	5
Emerald Pride	68	46	4	F-M	5	5	4	7	3	7
S446xS458B	74	70	6	F	7	7	5	5	5	7
S454 x S458B	55	56	7	M	3	6	6	6	5	6
S454x S445	62	53	6	F	6	7	8	5	5	6
S454xS446	64	60	7	F-M	7	8	5	5	5	7
S454xS463	55	57	7	M-C	5	8	3	3	3	7
SVO 097BL	73	58	3	F	2	5	7	5	7	5

[z]Transplanted July 22 in 30' plots; rows 30" apart, 12' between plants. [y]Scale of 1 - 9 where 1 = flat and 9 = extreme dome. [x]F = fine; M = medium; C = coarse. [w]Scale of 1-9 where 1 = poor and 9 = excellent.

Table 3. Observation for broccoli inbreds grown at the OSU Vegetable Research Farm, Corvallis, 2014.[z]

Entry	No. plts	% blind	Maturity (days)	Head Shape[y]	Stem Color[x]	Exer-tion[x]	Segment-ation[x]	Unifor-mity[x]	Bead Size[w]	Branch	Overall Score[x]	Notes
S411	12	25	76	7	8	8	5	5	M	5	5	
S438	14	14	76	7	7	9	9	7	F	5	7	
S442	23	17	76	5	5	6	8	7	F	5	6	
S443	10	10	78	5	5	7	5	3	F	3	3	Soft heads
S445	12	17	78	5	7	7	9	7	F	3	7	
S446	13	0	78	6	7	7	3	3	M	1	7	
S454	14	21	73	6	8	8	3	7	M	3	7	
S457	13	31	73	6	4	7	5	5	F	3	5	Soft heads, small florets
S458	13	15	78	6	6	8	5	7	F	3	7	
S459	14	57	78	6	8	9	9	3	F	7	5	Too much branching
S460	4	25	-	-	5	-	-	-	F	5	-	
S462	1	100	80	5	5	5	3	-	F	7	5	Uneven bead development
S463	10	30	85	5	7	7	5	3	F	5	8	
S465	14	7	87	4	7	8	3	7	M	1	8	
(S446/S463)-2-1-1	12	8	-	7	-	-	-	-	-	-	-	Very late; leafy blond heads, small florets, severe catseye
(S446/S457)-1-1	13	23	78	6	7	7	5	1	F	var	7	
(S446/S460)-1-1-1	15	13	76	5	7	8	3	3	F-M	1	8	
(S454/RS2)-2-1-1	12	0	71	var 3-5	7	8	3	8	M	1	8	Very wilty, very uniform genetically
(S454/S445)-1	16	19	78	6	8	7	7	3	F-M	3	7	

Table 3. (continued)

Entry	No. plts	% blind	Maturity (days)	Head Shape[y]	Stem Color[x]	Exer-tion[x]	Segment-ation[x]	Unifor-mity[x]	Bead Size[w]	Branch	Overall Score[x]	Notes
(S454/S446)-1	12	8	78	3-7	7	6-8	5	3	F-C (var)	3	7	
(S454/S458A)-1	12	33	76	3-5	8	6-7	3-9	1	F-C	3	7	
(S454/S465)-1	14	21	78	6	7	7	5	3	F-M	3	5	
(S454/RS2)-1-1-1	14	50	73	5	8	9	7	7	F	7	5	Small heads
(S458A/S446)-1	15	27	78	7	8	7	9	5	F-M	3	7	Tight heads
(S462/S460)-1	17	35	80	5	5	8	5-7	3	F	1	7	
(S463/S446)-1-1	17	24	80	4	8	8	7	3	F	5	7	Rough heads
OSU OP1-1-3-1-1	14	14	78	5	7	8	8	3	VF	1	8	Very firm heads, variable height, thick leaves
OSU OP1-1-3-1-2	15	20	80	6	4	7	5	3	F	3	7	Thick leaves
OSU OP1-3-1-1-1	16	25	65		8	8	5	7	F-M	3	5	Soft heads, small?

[z]Transplanted July 22 in 30" rows, at 12" within row spacing. [y]Scale of 1-9 where 1 = flat and 9 = extreme dome. [x]Scale of 1-9 where 1 = poor and 9 = excellent. [w]VF = very fine, F = fine, M = medium, C = coarse.

Table 4. Yield data (net T/A) from 14 years of broccoli yield trials, Corvallis, 2014

Entry[z]	2001	2002	2003	2004	2005	2006[y]	2007	2008	2009	2010	2011	2012[y]	2013	2014
S445 x S454	4.1	4.3	6.1	3.4			3.3	3.6				3.9		1.9
S446 x S454			3.7	4.8		3.7	4.1	2.8	3.6	2.1	3.1			2.1
S446 x S455										3.9				
S446 x S458B														3.5
S446 x S460										3.4				2.7
S446 x S462										3.4				
S454 x S457										3.1				
S454 x S458									2.2		2.6[x]		4.2	1.8
S454 x S459									2.2	2.9				
S454 x S460													3.1	
S454 x S462									2.0		2.2	2.7	4.8	
S454 x S463											3.5			2.3
S454 x S465											3.3		4.3	
S456 x S446											3.8[x]			
S456 x S454									4.3					
S459 x S446									2.3					
S462 x S446									6.0			3.3		
S462 x S460													4.3	
S465 x S411											3.6[x]	1.9		
S463 x S446												3.3		
S465 x S446												2.5	3.1	
USVL 093 x S446													4.3	
Arcadia							4.1	3.9		3.1	4.6	3.6	3.9	2.6
Excelsior	3.8	5.0	3.9	3.4	2.3	4.5								
Legend		4.7												
Regal	3.6		5.3	4.2	3.3	3.6	5.2							
Emerald Pride			3.4	4.5	4.3	3.9	4.2		3.2	3.5	3.3	3.1	4.7	2.9
Imperial									3.9	4.0	4.8	4.8		
LSD 0.05	0.8	1.4	0.9	0.9	0.8		1.0	0.9	1.1	1.0	1.1		1.6	1.0

[z]Cross and reciprocal combined; crosses involving discontinued inbreds not shown. [y]Uneven number of reps required calculation of LS means and pair-wise comparisons rather than LSD. [x]Entries consisted of three reps, instead of four, requiring the use of a different LSD (1.13) and comparison among only marked entries.

Table 5. Cytoplasmic male sterile selections grown in an observation trial at the Vegetable Research Farm, Corvallis, 2014.[z]

Entry	No. plts	No. Blind	% blind	Maturity (days)	Head Shape[y]	Stem Color[x]	Exer-tion[x]	Seg-ment[x]	Uniform-ity[x]	Bead Size[w]	Branch	Overall Score[x]
A411*3-1/S411[v]	15	2	13.3	73	6	7	7	3	8	M	1	7
A411*3-1/S459	17	3	17.6	73	5-7	7	8	3	3	M	3	7
A411*3-1/S460	14	4	28.6	76	5-7	5	5-7	3	3	M-C	3	7
A411*3-1/S463	2	1	50.0	78	5	5	5	3	3	M	7	5
A411*3-1/S465	1	-	0.0	80	4	7	4	1	-	M-C	5	3
A411*3-2/S411	15	2	13.3	73	6	8	6	1	5	C	3	3
A411*3-2/S460	13	3	23.1	78	5	4	8	3	3	F-M	3	7
A411*3-2/S465	18	7	38.9	80	5	7	7	7	3	F-M	3	5
A446*2-1/S446	13	5	38.5	76	3-5	7	7	7	3	F-M	1	7
A454*3-3/S454	13	4	30.8	78	5	7	7	5	3	M	3	5
A454*3-4/S454	10	3	30.0	73	5	8	7	5	1	M	5	5
O446*1-1/S462	16	6	37.5	78	7	7	7	3	7	F	1	7
O446*1-1/S463	15	6	40.0	78	5	5	7	1	3	F-M	1	5
O446*1-1/S446	13	4	30.8	80	5	5	5	7	3	F-M	1	7
O446*1-1/S465	14	5	35.7	78	5	3	3	7	3	M	3	7

[z]Transplanted July 22 in 30" rows, with 12" within row spacing. [y]Scale of 1-9 where 1 = flat and 9 = extreme dome. [x]Scale of 1-9 where 1 = poor and 9 = excellent. [w]F = fine, M = medium, C = coarse. [v]A = Anand CMS and O = Ogura CMS.

Figure 1. Broccoli Yield Trial Net Tons/Acre, 4 Replications, 2014

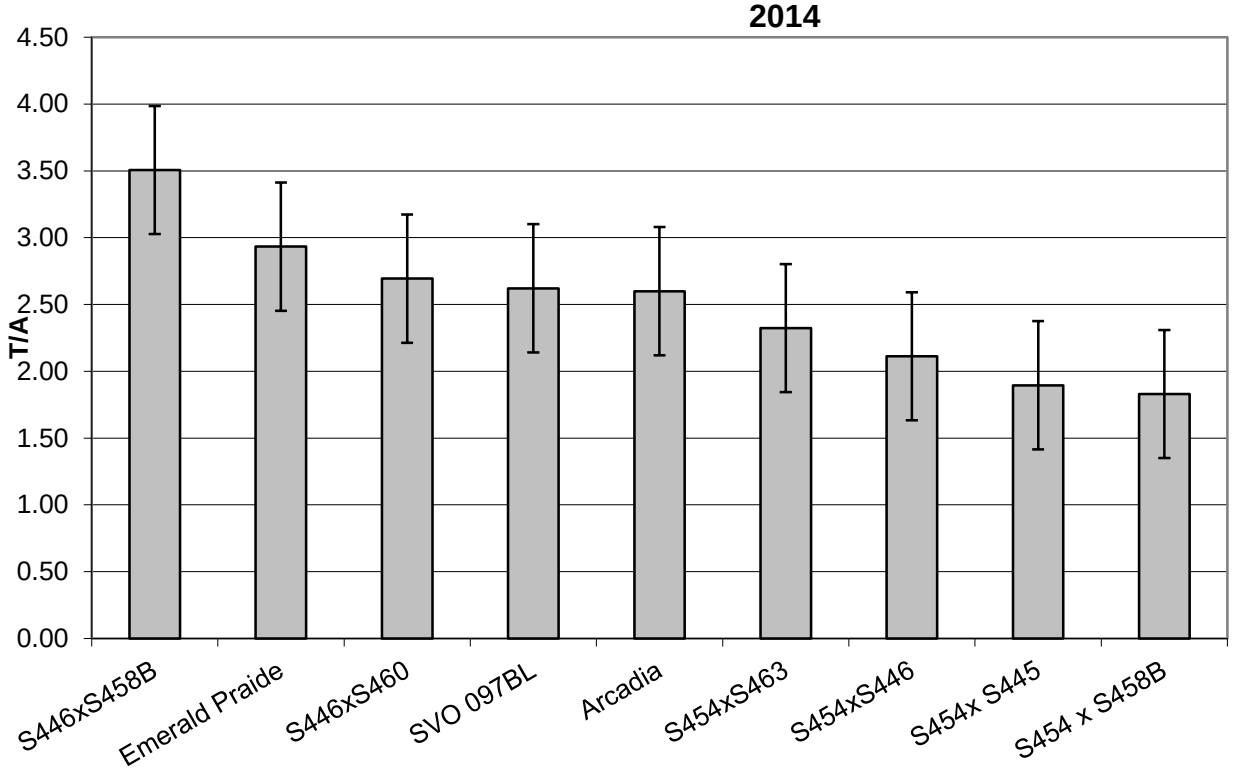

FIgure 2. Plant and Head Heights, Broccoli Trial, 2013