Breeding Classic *Cucurbita maxima* Buttercup Squash for Increased Genetic Diversity

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Introduction: The unique ability for cucurbits, naturally outcrossers, to withstand inbreeding has allowed pedigree line breeding techniques to become the industry standard for development of new squash varieties, buttercup included (1; 7). Self-fertilization is preferred because it ensures rapid homozygosity and the fixation of desirable characteristics within the variety (1). A method of recurrent selection breeding was developed with the goal of creating a new variety of *C. maxima* buttercup squash that competed with or outperformed other market varieties, yet maintained maximum heterozygosity. This would be achieved by avoiding self-pollination at critical stages of the breeding project and maintaining adequate numbers of progeny over the selection process.

Materials & Methods: The seed from the original population was developed in 1994-96 by John Navazio and John Schneeberger at Garden City Seeds in Hamilton, Montana (5). The project sought to combine the characteristics of cold soil vigor, classic buttercup shape, early fruit maturity, and high fruit quality by combining a small-sized, well-maintained, market version of the standard variety Buttercup (Cooper's Seeds, Auckland, New Zealand) with the vigorous, early maturing farmer variety Selma's Buttercup, bred by Selma Gunderson of Buffalo, South Dakota. The latter was segregating for shape, epidermal color, and fruit quality. A minimum of a dozen plants of each of the two varieties were allowed to open-pollinate and seed was bulked. This seed was planted into cool soil at least two weeks before commercial crops were planted in the Bitterroot Valley and seedlings were selected for early, strong emergence and vigor. The resultant population of 5,400 to 6,000 plants was allowed to open-pollinate. At early harvest (Sept 1-10) plants with at least three fruit at full maturity with were marked for harvest. At frost at least 350 fruit were harvested from at least 250 selected plants. Seed was harvested after four weeks of curing in the fruit and was bulked. This process was repeated for a second season and the population named Montana Maxima Early Composite (MMEC). Starting with this population selection toward a new variety was started in Olympia Washington ten years later with a progeny test. Refinement of a promising family over a three-year period led to a possible variety that exhibited a high percent of phenotypic uniformity for classic buttercup characteristics and high quality fruit, and also demonstrated a higher potential for heterozygosity.

Field plots at The Evergreen State College Organic Farm were arranged in 6' x 6' grids with 6' between mound centers. The placement of squash full-sib families and dimensions of the plot varied from year to year with rotation of the field and available space. Three feet wide pieces of black landscape cloth covered in-row spacing for weed suppression. Between-row spacing was rototilled as needed. Stakes were placed in the mounds to indicate plant families. Full-sib families were direct seeded in early June and harvested in mid October. Fruit harvesting, curing and storage were performed according to Coleman (3). Seed processing and drying followed the methods outlined by Ashworth (2). Once seeds were completely dried, they were placed into labeled envelopes.

To achieve healthy population size 50 plants were grown in 2005, ~300 in 2006 and ~250 in 2007 (6). All pollinations were made by hand to avoid contamination from unwanted specimens and followed the procedure outlined by Ashworth (2). In 2005 male and female flowers were randomly crossed among different plants as they became mature, a method used to reinvigorate the seed through cross-pollination. Included in the field in 2005 were 39 MMEC seeds and 15 market class C. maxima varieties. The family referred to as #8 was originally a cross between 'Bonbon' (F1) (Johnny's Selected Seeds, Winslow, Maine) and a select MMEC plant. In 2006 twenty-one seeds were planted from each of the 12 full-sib families from this cross in 2005. A progeny test was performed by making random pollinations between the 21 siblings within a full-sib family. The best fruit from the four families (#107, #307, #707, and #807) that exhibited the highest performance based on cumulative characteristics were saved for seed.

There were eight superior plants from family #806. In 2007, nine seeds from each hand-pollinated fruit from these eight superior #806 plants were sown and plants within the same family were crossed amongst each other randomly, permitting possible crossings between cousins, siblings, and self-pollinations. Seed from nineteen hand-pollinations were harvested from #8 for future breeding purposes. A pedigree was kept of all male and female parents, their respective performance in the field, and progeny. Squash characteristics were recorded across a number of categories for 2005, 2006 and 2007 including days to seedling emergence, germination rate, days to first pistillate flower, vigor, yield, and fruit quality. Fruit quality was tested in 2007 with the use of taste testing and Brix readings with a refractometer (4).

Results & Discussion: Only family #8 showed a promising consecutive increase in uniformity required for the development of a new variety, with seeds saved from eight out of 23 fruits (~30% uniformity) in 2006 and 19 out of 23 fruits (~60% uniformity) in 2007. Brix readings taken for the four families grown in 2007 were by the far the strongest determining factor for the continued breeding of family #8. The average °Bx RDS for #8 was 12% with the 19 fruit saved exhibiting sweet to super-sweet flavors and an ideal texture characterized as chalky/smooth. When tested, the market variety 'Bonbon', grown as a control, averaged 13% °Bx RDS. The highest Brix reading for 'Bonbon' fruit was 17°Bx RDS and the lowest was 8°Bx RDS. The highest Brix reading for #807 was 16°Bx RDS and the lowest was 6.5°Bx RDS. The highest average percentage reached by other composite families was 10%, and all exhibited low uniformity for desirable texture and sweetness.

Of the four families tested in 2007 for field performance, #8 ranked average to low across several categories. Further field analysis can be made in the future through vigorous comparisons between the composite cross and current market varieties. Family #8 germinated eight days after direct seeding and had an 88% germination rate. It had the second highest germination rate, yet was the slowest in average days to seedling emergence, trailing the others by a half day at nine-and-a-half days. For average days to first pistillate flower #8 took the longest at 57 days, with the shortest of the other three families achieving their first female flowers after 55 days. There is a noticeable difference in average days to first pistillate flower and seedling emergence between 2006 and 2007, with numbers increasing for all MMEC derived families in 2007. Average days to seedling emergence for family #8 increased by one-and-a-half days

from 2006 to 2007, and average days to first pistillate flower increased from 55 days in 2006 to 57 days in 2007. This is likely due to a change in weather conditions and soil patterns. Family #8 ranked the second highest in yield with an average of one-and-a-quarter fruits per plant and had an average weight of 1.25 lbs a fruit, the lowest of other MMEC derivatives. The target weight for a new variety of buttercups squash is 2-4 lbs. Field results for #8 indicate the need for careful monitoring of field performance in the future combined with further selection for vigor, early seedling emergence and pistillate flower emergence.

Breeding work to further refine #807 to be released as a commercial variety will be conducted through participatory plant breeding research at the Organic Seed Alliance (OSA), Port Townsend, Washington with the cooperation of Washington farmer-breeders and Prescott College, Prescott, Arizona. Germplasm release and distibution will be handled by the Organic Seed Growers and Trade Association (information is available at the OSA website <<u>seedalliance.org</u> <<u>http://</u> seedalliance.org/>>).

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