Reduced Seed Count Improves Versatility and Propagation of Small-fruited Peppers (*Capsicum annuum* L.) for Specialty Markets

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Abstract. **Small/miniature sweet and hot peppers (*Capsicum annu*um L.), such as snack peppers, are a rapidly growing class of specialty peppers. Low seed count is an important attribute for consumer acceptance of small-fruited specialty peppers. Four inbred U.S. Department of Agriculture (USDA) *C. annuum* breeding lines exhibiting uniformity for pod type and size and normal or reduced seed count were selected for producing F_1 and segregating F_2 and backcross generations. Seed content of F_1 hybrids and progeny produced from the backcross of F_1 hybrids to normal seed count parents exhibited unimodal frequency distributions and skewed toward the parent with normal seed count. Progeny produced from backcrosses to the reduced seed count parent exhibited bimodal population distributions representative of the respective parental phenotypes. F_2 populations approximated 3:1 frequency distributions skewed toward normal-seeded parental phenotypes. Chi-square tests supported a single recessive gene model with potential modifiers controlling inheritance of reduced seed count. Genetic variants with reduced seed count facilitate seed production and propagation of specialty market class peppers.**

Parthenocarpy has been the subject of numerous investigations to increase fruit quantity and quality in pollinator-dependent crops by removing or reducing the need for pollination in fruit set and growth development. Fruit set in the absence of fertilization can be desirable to increase fruit yield when conditions prevail that are adverse for fertilization. The seedless nature of parthenocarpic fruit can also improve fresh or processed fruit quality in crops when seedlessness is a desirable trait.

Environmental stress conditions, including high or low temperature, humidity, high or low light intensity, heavy rain or drought, and strong wind, negatively affect fruit set and growth. These conditions can also induce facultative parthenocarpy, resulting in fruit that are seedless or with significantly reduced seed (Dhatt and Kaur, 2016). In numerous crops, parthenocarpy may occur naturally or can be induced by exogenous hormone application or enhanced synthesis of endogenous hormones (Pandolfini et al., 2009). Early observations of parthenocarpy involved natural mutations affecting phytohormones. Auxin

was first identified as capable of inducing parthenocarpic fruit development in citrus (*Citrus sinensis*) (Gustafson, 1939). Gibberellic acid (GA) was subsequently demonstrated to induce parthenocarpy in rose (*Rosa arvensis*) and apple (*Malus domestica*) (Davison, 1960; Prosser and Jackson, 1959). More recently, cytokinins were shown to induce parthenocarpy in watermelon (*Citrullus lanatus*) and kiwi (*Actinidia deliciosa*) (Hayata and Niimi, 1995). Similar to *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*), auxin is the major inducer of fruit set in pepper (*Capsicum annuum*) and acts in part by inducing gibberellin biosynthesis (Tiwari et al., 2012). In pepper, gibberellin appears to be essential for fruit set, whereas auxin functions in both fruit set and development.

Induction of parthenocarpy is a common agricultural practice for some horticultural crops. Selective breeding for parthenocarpy has demonstrated the utility of seedlessness for improved yield and quality in selected environments. Parthenocarpic pepper (Honda et al., 2012; Tiwari et al., 2007), papaya (*Carica papaya*) (Rimberia et al., 2007), summer squash (*Cucurbita pepo*) (Robinson and Reiners, 1999), and numerous other crops have been the subjects of considerable research (Vardi et al., 2008; Varoquaux et al., 2000). Biotechnology-related approaches have also been used to modify endogenous phytohormones for parthenocarpic fruit set. Modification of auxin synthesis, sensitivity, and content, plus auxin signal transduction

and gibberellin signal transduction, have been reported for inducing parthenocarpic-like phenotypes (Joldersma and Liu, 2018; Pandolfini et al., 2009).

Small/miniature sweet peppers are a rapidly growing class of specialty peppers (Burfield, 2016). The peppers are popular among consumers because of their versatility, snackability, vibrant red, orange and yellow mature fruit colors, and nutrition attributes. Mature fruit comprises the majority of the snack pepper market segment due to enhanced sweetness and the aroma of ripe fruit. Fruit are most often nonpungent. Although characteristically pungent, no-heat habanero cultivars within the snack market class provide unique fruity and floral attributes of the habanero without fruit pungency. As the product line availability grows, snack peppers may constitute a pepper commodity, much like the cherry tomato (Pullman, 2017). Seasonal field production is supplemented by year-round greenhouse production. Although field production of traditional pepper commodities is in decline in parts of the country, other parts are expanding greenhouse acreage for high-value specialty pepper production (Halleck, 2015). A relatively small number of commercial snack pepper cultivars have been developed, many of which lack uniformity and quality attributes such as low seed count, which enhances culinary convenience for product end users. Shifriss and Eidelman (1986) proposed use of male sterility for production of obligatory parthenocarpic genotypes wherein acceptable fruit weight could be maintained independent of seed production. That technology has since been granted patent protection for development of true seedless pepper (Bar et al., 2013; Gorguet et al., 2017) and used for specialty pepper cultivars including snack peppers. This article characterizes the inheritance of reduced seed count in pepper genotypes with parthenocarpic-like behavior but selected for stability of reduced seed count, thus facilitating propagation while maintaining quality attributes for the snack pepper market.

Materials and Methods

Plant materials. Four inbred USDA *C. annuum* breeding lines exhibiting uniformity for pod type and size and normal or reduced seed count were selected for producing F_1 and segregating F_2 and backcross generations. The respective F_1 and segregating F_2 and backcross populations were developed by crossing greenhouse-grown plants using standard emasculation practices. All parental lines (P_1, P_2) used in these crosses were true breeding for blocky three- to four-lobed pods typical of sweet bell pepper. Fruit of lines G15C98 and G15C104 were uniformly seedy with seed set typical for bell pepper. Lines G15C99 and G15C105 produced fruit of comparable size in comparison with G15C98 and G15C104 but were uniform for reduced seed count relative to G15C98 and G15C104. Pods were small (G15C98, 25.2 ± 1.6 g,

mean ± SE; G15C99, 23.2 ± 1.5 g; G15C104, 26.7 ± 1.2 g; G15C105, 29.6 ± 2.0 g) and typified fruit size for the snack pepper market class. These snack pepper lines are descended from segregating populations originally developed from crosses between USDA ornamental Tabasco-type pepper breeding lines and inbred sweet bell pepper germplasm. Individuals with reduced fruit seed count arose spontaneously in descendants from these crosses.

Progeny of each population were grown in the greenhouse using standard production practices. Six-week old plants of each genotype were transplanted to field plots for summer production at the Beltsville Agricultural Research Center, Beltsville, MD, into Keyport fine loam soil, a clayey, mixed, mesic Acquic Hapludult. Field-grown plants were spaced at 0.45-m intervals in single rows on polyethylene-covered raised beds, with beds positioned on 1.5-m centers with trickle irrigation. Pest control and fertilizer regimes followed standard horticultural practices for pepper production in Maryland (University of Maryland, 2018).

Fruit evaluation. Seed counts were recorded for mature pepper fruit harvested from individual field grown plants (n = 24 for individual P_1, P_2, and F_1 generations; n = 99 to 100 for BCP_1 and BCP_2; n = 185 to 197 for respective F_2 generations). The results for individual plants from respective generations are reported as the mean and SE of five replicate fruit samples collected from the same plant. Designation between reduced vs. normal seed number in respective populations were determined by seed count range for parental lines (P_2) with reduced seed (G15C99, n ≤ 40; G15C105, n ≤ 60). Natural divisions in frequency distributions for reduced seed number in segregating F_2 and backcross generations mirrored those of the low-seed parent for respective populations. Chi-square was used to test

goodness of fit for reduced seed count inheritance models.

Results

True breeding pepper lines selected for reduced fruit seed count exhibited significantly reduced seed count relative to breeding lines with seed set typical for pepper. Mean fruit seed counts for G15C99 and G15C105 (reduced seed count) were only 10.2% and 20.5% of seed counts for corresponding lines G15C98 and G15C105 (normal) in the respective crosses (Table 1). Fruit mean seed counts for F_1 hybrids and BCP_1 progeny were skewed toward normal seed counts with population distributions characteristic of those observed for the normal seed count parent, P_1, (G15C99, G15C105) in both crosses (Table 1, Figs. 1 and 2). Population distributions for F_2 and BCP_2 generations derived from crosses of G15C98 × G15C99 and G15C104 × G15C105 exhibited distinct bimodal population distributions spanning the distributions of respective normal (P_1) and reduced (P_2) seed count parents from both crosses.

F_2 progeny from the cross of G15C98 × G15C99 displayed a population frequency distribution for fruit seed count that approximated a 3:1 normal:reduced distribution consistent with the presence of a single recessive gene conditioning reduced fruit seed count (Table 2). A unimodal (1:0) normal seed count frequency distribution for the backcross of the F_1 to the normal (P_1) seed count parent (BCP_1 generation) and 1:1 normal to reduced fruit seed count frequency distribution for the backcross of the F_1 to the reduced (P_2) seed count parent (BCP_2 generation) further supported a single gene model for reduced vs. normal seed count. Chi-square goodness-of-fit test for the F_2 and BCP_2 generations supported sufficiency of a single gene model to explain the observed segregation in this cross.

Similar to the G15C98 × G15C99 cross, F_1 hybrid progeny derived from the cross of G15C104 × G15C105, backcrossed to the normal (P_1) seed count parent (BCP_1 generation) and reduced (P_2) seed count parent (BCP_2 generation), exhibited unimodal (1:0) and 1:1 bimodal population frequency

Table 1. Mean values and standard errors for pepper fruit seed count in parental, F_1, F_2, and backcross generations from the *Capsicum annuum* L. crosses G15C98 × G15C99 and G15C104 × G15C105, respectively.

Generation	Mean	SE
G15C98 × G15C99		
P_1[z]	150.0	6.2
P_2	15.3	1.1
F_1	154.6	5.8
F_2	106.7	5.1
BCP_1	185.3	3.8
BCP_2	94.9	8.1
G15C104 × G15C105		
P_1[y]	123.7	6.0
P_2	25.3	1.4
F_1	178.7	8.1
F_2	99.6	4.3
BCP_1	146.5	3.5
BCP_2	94.6	7.2

[z]P_1 = G15C98; P_2 = G15C99; BCP_1 and BCP_2 are the backcross of the F_1 to G15C98 (P_1) and G15C99 (P_2), respectively.

[y]P_1 = G15C104; P_2 = G15C105; BCP_1 and BCP_2 are the backcross of the F_1 to G15C104 (P_1) and G15C105 (P_2), respectively
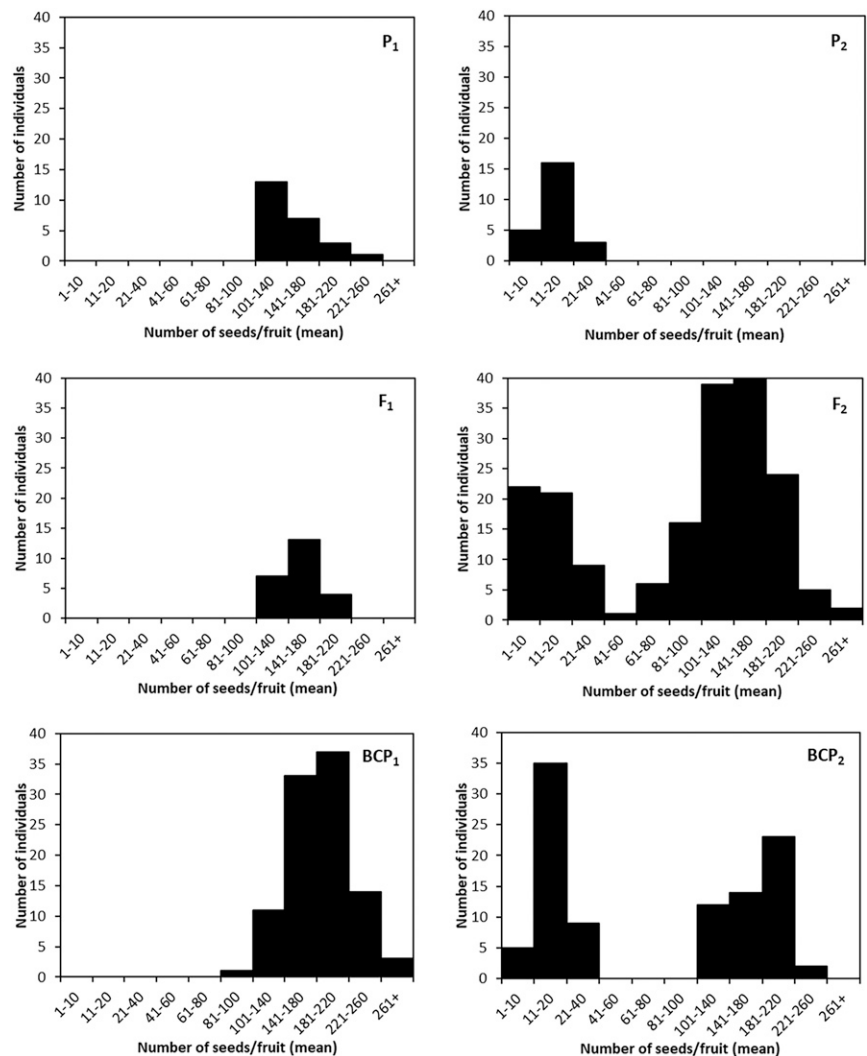

Fig. 1. Population distributions for pepper fruit seed count in parental (P_1 = G15C98; P_2 = G15C99), F_1 hybrid, F_2, and backcross generations from the cross of G15C98 × G15C99. BCP_1 and BCP_2 are the backcross of the F_1 to G15C98 (P_1) and G15C99 (P_2), respectively.
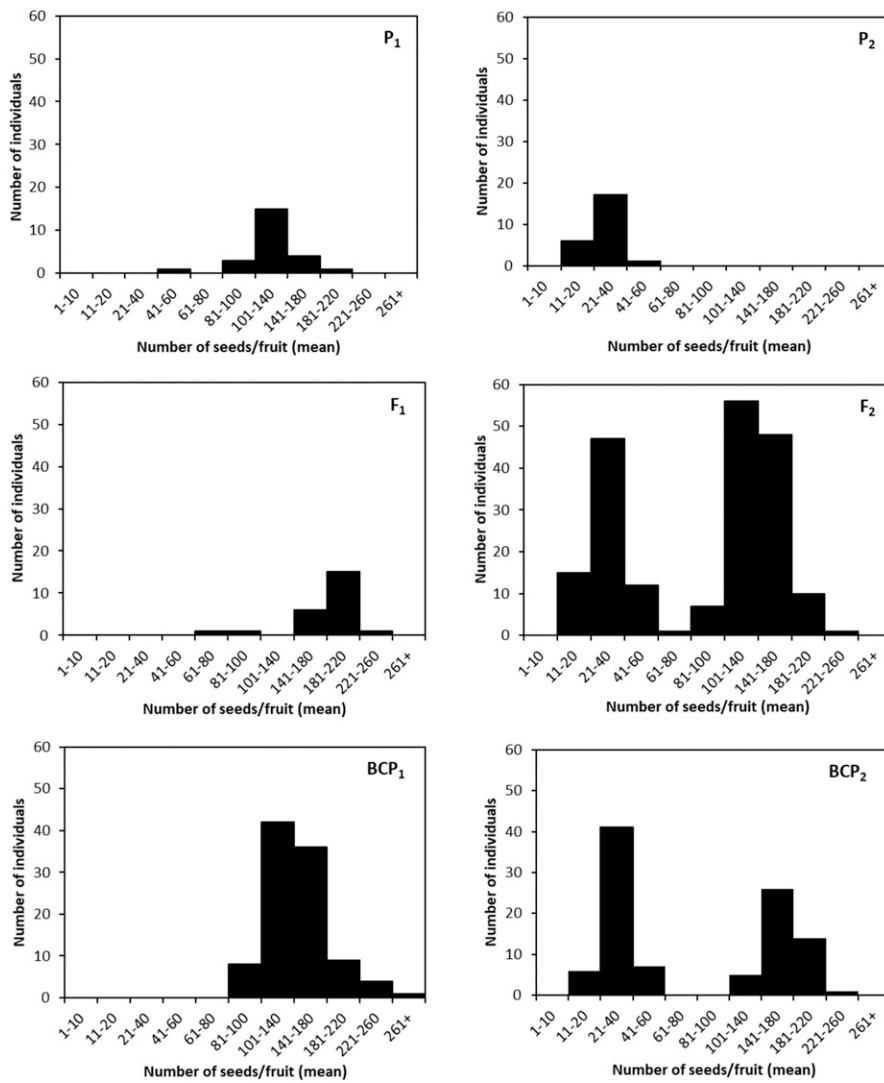
Fig. 2. Population distributions for pepper fruit seed count in parental (P_1 = G15C104; P_2 = G15C105), F_1 hybrid, F_2, and backcross generations from the cross of G15C104 × G15C105. BCP_1 and BCP_2 are the backcross of the F_1 to G15C104 (P_1) and G15C105 (P_2), respectively

Table 2. Chi-square goodness-of-fit test for a single, gene model conferring normal seed vs. reduced seed content in pepper fruit derived from the *Capsicum annuum* L. crosses G15C98 × G15C99 and G15C104 × G15C105, respectively.

	Observed					
Population	Normal seed	Reduced seed	Total	Ratio tested	Chi-square	Probability
			G15C98 × G15C99			
P_1^z	24	0	24	1:0		
P_2	0	24	24	0:1		
F_1	24	0	24	1:0		
BCP_1	99	0	99	1:0		
BCP_2	51	49	100	1:1	0.04	0.84
F_2	132	53	185	3:1	1.42	0.23
			G15C104 × G15C105			
P_1^y	23	1	24	1:0		
P_2	0	24	24	0:1		
F_1	24	0	24	1:0		
BCP_1	100	0	100	1:0		
BCP_2	46	54	100	1:1	0.64	0.42
F_2	123	74	197	3:1	16.98	<0.001

[z]P_1 = G15C98; P_2 = G15C99; BCP_1 and BCP_2 are the backcross of the F_1 to G15C98 (P_1) and G15C99 (P_2), respectively.
[y]P_1 = G15C104; P_2 = G15C105; BCP_1 and BCP_2 are the backcross of the F_1 to G15C104 (P_1) and G15C105 (P_2), respectively.

distributions, respectively, and were supported by a chi-square goodness-of-fit test for a single gene model for high vs. reduced seed count (Table 2). However, the F_2 population distribution from this cross deviated significantly ($P < 0.001$) from an expected 3:1 distribution, with over-representation of reduced seed count progeny.

Discussion

In pepper, expression of parthenocarpy exhibits genotypic variation. Facultative parthenocarpy can occur in response to high or low irradiance, water stress, or high temperature (Jaafar et al., 1994; Rylski and Spigelman, 1982). Environmentally induced parthenocarpy in pepper is often associated with fruit deformity, reduced fruit size, and poor market value (Shifriss and Eidelman, 1986). Tiwari et al. (2007) identified pepper genotypes in which parthenocarpy occurred irrespective of low night temperature stress and genotypes where parthenocarpy was expressed only at low night temperature. Genotypes were also identified in those studies for which seedlessness had little influence on fruit shape and size. Picken (1984) and Honda et al. (2012) also reported that seeds are just one of multiple factors that may influence fruit shape and size.

Our results bear similarity to those observations. Fruit weight of normal seeded parent lines and parent lines selected for reduced seed count were similar. Reduced seed count lines produced 5- to 10-fold fewer seed in open field conditions compared with conventional or normal seed count genotypes. Similar behavior has been observed when conducting controlled pollinations for seed increase under winter greenhouse conditions with relatively cooler night temperatures (data not shown). Under open field conditions, fruit with zero seed were not observed, likely due to the prevalence of pollinators. Pollinators are generally abundant under field conditions where the rate of outcrossing may reach 90% (Bosland, 1993; Tanksley, 1984). Our working observations when producing seed for propagation under winter greenhouse conditions are a low incidence of seedless fruit (<5%) or flower and fruit abscission in the absence of self- or cross-pollination. Reduced female fertility has been suggested as one of several mechanisms that result in parthenocarpic fruit and may explain reduced seed count. A low incidence of seedless fruit yield in parthenocarpic genotypes may also be explained by aberrant reduced gibberellin levels that result in flower and fruit abscission (Tiwari et al., 2012) in the absence of pollination and seed development. Additional research is required to elucidate the mechanisms responsible for our observed reduced seed count phenotypes, which are not seedless and hence not strictly parthenocarpic. In tomato, genetically modified parthenocarpic-like lines produced fruit that were seedless or with significantly reduced seed count. These tomato lines produced fruit that were seedless ≈75% of the time, with seeded fruit producing 60% to 80% fewer seed compared with non-parthenocarpic-like lines (Rotino et al., 2005). Expression of facultative parthenocarpy varies and does not condition 100% seedless fruit.

Tiwari et al. (2007, 2011) reported a positive relationship between development of carpelloid structures and parthenocarpic

potential in pepper fruit. Parthenocarpy was positively correlated with carpelloid mass in both pollinated and unpollinated fruit. Inheritance of parthenocarpy in genotypes with strong carpelloid development was inherited as a single recessive gene. Carpelloid development was not prevalent in our reduced seed count genotypes. However, similar to Tiwari et al. (2011), inheritance of reduced seed count in our genotypes was in agreement with a single recessive gene model. Deviation from this model in the F_2 generation for the cross of G15C104 × G15C105 may be due to modifier genes that influence seed count. Seed count in the F_1 hybrid exceeded that of the high seed parent in this cross and reduced seed count progeny were over-represented in the F_2 generation.

In related Solanaceous crops, production of seedless parthenocarpic fruit is heritable but does not fit a simple genetic model. In tomato, parthenocarpy is reported to be controlled by several single-gene recessives (Fos et al., 2001; Gorguet et al., 2005). In pepino (*Solanum muricatum*), a single dominant gene controlling parthenocarpy has been reported (Prohens et al., 1998). In the Cucurbitaceae, parthenocarpy in cucumber (*Cucumis sativus*) is influenced by two major additive-dominant-epistatic genes and additive-dominant polygenes (Yan et al., 2010). A single gene recessive with incomplete dominance for seedlessness has been postulated to explain three fruit classes observed in highbush blueberry (*Vaccinium corymbosum*), normally seeded fruit, diminished seed fruit, and seedless fruit (Ehlenfeldt and Vorsa, 2007).

In summary, our results demonstrate that simple genetic inheritance can explain reduced seed number in small-fruited specialty pepper lines suitable for breeding new snack pepper cultivars. Further research is required to determine the physiological basis of reduced seed count and its relationship to parthenocarpic models for seedless fruit reported in other Solanaceous crops. Low seed content is a desirable attribute to enhance consumer acceptance of snack peppers. Genetic variants with reduced seed count, vs. completely seedless fruit, facilitates seed production and propagation for public domain specialty market class peppers.

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