Genetic Analyses of Seed Yield in Onion

Eduardo Villanueva-Mosqueda

Department of Horticulture, 1575 Linden Drive, University of Wisconsin, Madison, WI 53706

Michael. J. Havey

Vegetable Crops Unit, Agricultural Research Service, U.S. Department of Agriculture, Department of Horticulture, 1575 Linden Drive, University of Wisconsin, Madison, WI 53706

ADDITIONAL INDEX WORDS. general combining ability, heritability, hybrids, specific combining ability, *Allium cepa*

ABSTRACT. Development of two-way onion (*Allium cepa* L.) hybrids is difficult due to poor seed yields on inbred female parents. Seed yield of onion is affected by inbreeding depression and the seed-production environment. A standard diallel was used to estimate combining abilities for seed yield among seven inbred onion lines. Males and hybrids differed significantly ($P < 0.05$) for seed yields. Combinations of relatively high-by-high seed-yielding inbred parents were not always the best combinations; combinations of medium-by-medium or medium-by-high seed yielders also produced good F_1 seed yielders. For the seven inbred lines, significant correlations ($P < 0.05$) were observed between mean seed yield per bulb and scape height. Parent–offspring regressions revealed no significant relationship between seed yields of randomly selected, open-pollinated bulbs and their S_1 families. Results indicate that relative seed yields of individual bulbs after self-pollination cannot be used to predict seed yields of progeny families. However, the seed yield of inbred lines of onion may reflect the potential seed yield of F_1 male-sterile lines.

Cytoplasmic male sterility (CMS) is used extensively to produce hybrid onion (*Allium cepa*) seed (Janick, 1998). Most onion cultivars in the United States are three-way hybrids, produced using F_1 male-sterile females. Two-way hybrids are desirable because of greater uniformity, as well as less time and fewer resources required for hybrid seed production. However, the development of two-way hybrids in onion is difficult due to relatively poor seed yields of inbred female parents (Davis, 1966; Erickson and Gabelman, 1954), presumably due to inbreeding depression (Jones and Davis, 1944). The degree of inbreeding depression depends on the number of segregating loci, level of directional dominance, and allelic frequencies (Benson and Hallauer, 1994). In addition to inbreeding depression, onion seed yield is affected by sizes of mother bulbs (Jones and Emsweller, 1939; Mondol and Choudhury, 1980), number of flowers per inflorescence (Erickson and Gabelman, 1954), sugar concentration of flower nectar (Lederhouse et al., 1968), potassium concentration in onion nectar (Hagler, 1990; Hagler et al., 1990; Waller et al., 1972), receptivity of flowers (Moll, 1954), pollen viability and attractiveness (Nye et al., 1971; Ockendon and Gates, 1976), and synchrony in pollination (Atkins and Davis, 1954). Other environmental factors affecting pollination are soil fertility (Brewster, 1983; Stuart and Griffin, 1946), soil moisture (Hawthorn, 1951), vernalization and spacing of mother bulbs (Hesse et al., 1979; Jones and Emsweller, 1939), distances between male-fertile and male-sterile rows (Erickson and Gabelman, 1956), ratios of male-fertile to male-sterile rows (Franklin, 1958), and temperature or humidity (Chang and Struckmeyer 1976a, 1976b; Lederhouse et al., 1968). These studies demonstrate that seed yield cannot be measured as a single event, but as

combinations of several events under genetic or environmental control. However, when the indirect factors are optimized and pollination is adequate, seed yield may be a function of genetic differences (Nye et al., 1973).

Heritability estimates allow breeders to predict gain by selection in a particular population. Well planned experimental designs control environmental and error factors. Analyses of variances (ANOVAs) from mating designs have used the performance of parental material to estimate the number of segregating loci, directional dominance, and allelic frequencies (Hallauer and Miranda, 1988). One of the most useful analyses is the diallel mating design. Random models estimate the additive and nonadditive genetic components of the total genetic variance. Fixed models estimate combining abilities of inbred parents based on average performance among all possible crosses (Griffing, 1956). Sughrue and Hallauer (1997) compared the two models and concluded that fixed models cannot be used to determine variance components due to the bias implied in the analysis. Parent–offspring (PO) regressions have been used frequently to estimate heritabilities (Hallauer and Miranda, 1988). A significant regression coefficient reveals additive genetic variance for the trait and a relatively high heritability. A trait showing significant additive genetic variance is expected to respond to recurrent selection by increasing the frequencies of beneficial alleles.

The genetics of seed yield has been studied in crops for which seed is not the primary product of commerce, e.g., alfalfa (*Medicago sativa* L.) (Peterson and Barnes, 1983). However, the genetic analysis of seed yield for onion has not been reported. In this study, a standard diallel was performed to determine combining ability for seed yield among seven inbred lines of onion. PO regressions were also used to estimate narrow-sense heritability for seed yield in three open-pollinated populations.

Materials and Methods

A complete diallel mating design was used to evaluate seed yields of seven inbred lines of onion and their F_1 hybrids in three environments. Cytoplasmic male sterile lines (A) and their maintainers (B) were available for all inbreds. Parental inbred lines were selected based on relative seed yields as recommended

Received for publication 13 Nov. 2000. Accepted for publication 4 May 2001. Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. We thank Mark Petrashek for technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Former graduate research assistant. Present address: Gilroy Foods, 9301 E. Lacey Blvd., Hanford, CA 93230-4765.

²USDA research geneticist and professor of horticulture; to whom correspondence should be addressed.

Table 1. Mean seed yield per flowering bulb and general combining ability (GCA) for seven inbred lines of onion measured in three environments.

Inbreds	Environment ^a					
	WM94		WM99		WSGH99	
	Seed yield (g)	GCA	Seed yield (g)	GCA	Seed yield (g)	GCA
MSU 611-1B	0.2	1.30	1.5	0.50	1.9	0.25
W52B	1.1	0.20	4.4	0.15	3.4	0.80
W101B	0.1	-0.10	2.7	-1.20	3.3	-0.70
W205B	0.1	-0.20	3.0	0.30	3.2	-0.45
W404B	0.4	-0.30	2.4	-0.80	3.4	-0.40
B2117B	0.1	-0.40	5.3	0.25	6.2	0.45
B2133B	0.2	-0.50	3.3	0.80	3.7	0.01
LSD _{0.05}	0.7		2.0		2.5	

^aEnvironments were at West Madison Experiment Station in 1994 (WM94) and 1999 (WM99) and near Walnut Street Greenhouses in 1999 (WSGH99).

by commercial onion breeders. Relatively poor seed yielding inbreds were MSU611-1A&B, W101A&B, W205A&B, and W404A&B; relatively good seed yielding inbreds were B2117A&B, B2133A&B, and W52A&B. Origins of these inbred lines were described previously (Havey 1991). Parental inbred bulbs were produced in 1991 at Palmyra, Wis., harvested, and vernalized in cold storage over the winter at $\approx 6^{\circ}\text{C}$. In April 1992, the top one-third of each bulb was removed to induce uniform flowering (Wilson, 1932) and bulbs were planted within 2 weeks. All inbred A lines were placed in 3.6 m by 7.2 m cages with a single male-fertile B line from the above listed inbreds to generate all possible male-sterile F_1 hybrids. Flowers of all inbred lines were examined for pollen production and crossing was completed using honey bees.

Inbred and male-sterile hybrid bulbs were produced, vernalized, and prepared for planting as described previously. In 1994, 10 bulbs per plot of parental inbreds and F_1 hybrids were planted in the field at West Madison Experimental Station, Madison, Wis., in a completely randomized design (CRD) with three replications. To provide pollen, male-fertile inbred parents of the diallel and other available male-fertile bulbs were planted into the randomization at a ratio of two male to six female rows. Nuclei of honey bees were used as pollinators. Umbels from all flowering bulbs of parental inbred lines and F_1 seed parents were harvested by hand and numbers of flowering bulbs counted. Seed was threshed, weighed on a plot basis, and mean seed yield in grams per flowering bulb calculated.

In 1999, 10 bulbs per plot of parental inbreds and F_1 hybrids were planted at two locations (a field adjacent to Walnut Street Greenhouses and the West Madison Experimental Station) on 19 and 22 April, 1999, respectively. In both 1999 environments, a randomized complete block design (RCBD) was used with three replications. The male-fertile inbred lines and random male-fertile bulbs were included in a ratio of two male to six female rows. Cages (3.6 m by 7.2 m) were placed over plots just after spathes opened. Blue bottle flies (*Calliphora sp.*) were used per cage at an average of 4500 pupae per week over 4 weeks after the first anthers started to dehisce. Mean seed yield in grams per flowering bulb was measured as described previously.

Experimental design differences did not allow combination of the 1994 and 1999 environments for ANOVAs. Individual analyses were performed for the 1994 versus 1999 environments and combined 1999 locations. General (males) and specific (females by males) combining abilities (GCA and SCA, respectively) among inbred lines (Hallauer and Miranda, 1988) were calculated using SAS (SAS Inst. Inc., Cary, N.C.).

PO regressions were performed to estimate narrow-sense heritability of seed yield. A total of 32 N-cytoplasmic bulbs from the open-pollinated populations 'Brigham Yellow Globe' (BYG), 'Mountain Danvers' (MD), and 'Sapporo-Ki' (SKI) were randomly selected in 1992. These bulbs and populations were considered to be a random sample from long-day storage germplasm. In 1993 parental bulbs were self-pollinated and seed yields measured. At least 10 bulbs from at least 22 S_1 families were evaluated for seed yield using a RCBD with three replications over at least 3 years (Madison, Wis., from 1996 to 2000). S_1 families were planted in 3.6 m by 7.2 m cages (blocks) and blue-bottle flies were used as pollinators. Seed yield of the family was measured, number of flowering bulbs counted, and seed yield in grams per flowering bulb calculated. Progeny means were regressed on parental values to estimate narrow-sense heritability for seed yield (Hallauer and Miranda, 1988).

Results and Discussion

DIALLEL ANALYSIS OF SEED YIELD. We undertook a diallel analysis to systematically test the performance of seven onion inbred lines for seed yield across three environments. Because inbred parents were selected for their relative seed yields and not randomly from an open pollinated population in gametic phase linkage equilibrium, additive and nonadditive variances were not estimated to avoid biased results and conclusions (Cockerham, 1956; Sughroue and Hallauer, 1997). Seed yields in the 1994 environment were generally poor, likely because bees were used as the primary pollinators. We noticed that bees much preferred nearby fields of alfalfa and observed relatively few visits to onion flowers. ANOVAs from the 1994 and 1999 experiments showed significant ($P < 0.05$) differences for mean seed yield among inbreds (Table 1). Mean seed yield of inbreds did not always agree with estimates of GCA. For example, inbred MSU611-1 yielded the lowest quantities of seed, yet it was one of the best general combiners in 1994 (Table 1) and produced relatively high seed-yielding hybrids (Table 2). SCA in 1994 was only significant at $P < 0.1$. Combinations of relatively high-by-high seed-yielding inbred parents were not always the best combinations; combinations of medium-by-medium or medium-by-high seed yielders also produced good F_1 seed yielders (Table 2).

In 1999, flies in cages were used for pollinations. The cages were necessary to keep the pollinators confined to onions. As expected, seed yields of all inbreds and hybrids was greater than observed in 1994 (Tables 1 and 3). Locations in 1999 were not significantly different for mean seed yield per bulb. Significant

Table 2. Mean seed yield (grams per flowering bulb) for single-cross male-sterile hybrids at West Madison Experiment Station in 1994.

Female parent	Male parent						
	MSU611-1B	W52B	W101B	W205B	W404B	B2117B	B2133B
				Seed yield (g)			
MSU611-1A		2.2	ND ^a	0.9	2.2	1.6	ND
W52A	2.3		2.5	0.2	1.0	1.1	1.3
W101A	2.3	1.2		1.9	0.2	0.8	0.2
W205A	3.6	0.3	1.0		0.7	0.3	0.1
W404A	3.1	1.8	0.4	0.9		0.8	1.1
B2117A	2.4	0.5	0.6	0.3	0.8		0.9
B2133A	1.5	2.4	0.7	1.4	1.0	0.5	
LSD _{0.05} ^y	1.6						

^aND = no data due to insufficient bulb production or flowering.^yLSD = least significant difference for separating means across columns or rows.

Table 3. Mean seed yield (grams per flowering bulb) for single-cross male-sterile hybrids across two environments in 1999.

Female parent	Male parent						
	MSU611-1B	W52B	W101B	W205B	W404B	B2117B	B2133B
				Seed yield (g)			
MSU611-1A		5.9	6.1	6.4	6.3	6.1	6.5
W52A	5.6		5.0	ND ^a	4.3	6.4	6.3
W101A	6.6	5.8		6.3	3.7	6.7	5.0
W205A	5.8	8.0	4.8		3.2	6.5	6.3
W404A	5.2	5.0	2.4	4.5		5.0	6.6
B2117A	6.9	7.4	8.1	6.3	6.6		7.0
B2133A	7.4	6.1	3.0	5.5	7.2	7.0	
LSD _{0.05} ^y	2.0						

^aND = no data due to insufficient bulb production or flowering.^yLSD = least significant difference for separating means across columns or rows.

differences ($P < 0.05$) among males (GCA) and hybrids (SCA) were observed within each location and across both locations in 1999 (Tables 1 and 3). No significant interactions were detected between GCA or SCA and locations. Inbred lines B2117, B2133, and W52 yielded the most seed per bulb in 1999; lines B2117, B2133, MSU611-1, and W52 showed positive GCA for seed yields per bulb (Table 1). The best F_1 combinations for seed yield were with MSU611-1 and B2133 (Table 3). High specific combining ability for seed yield was found for the crosses between B2117A x W101B and W205A x W52B (Table 3).

CORRELATION BETWEEN SEED YIELD AND HEIGHT OF SCAPE. Significant differences were observed among the parental inbreds for scape height (Table 4). Correlations between mean seed yield per bulb of the seven inbred lines and scape height were signifi-

cant (Table 5). Inbred lines with short scapes tended to yield less seed; those with taller scapes tended to produce more seed. Scape height may be related to the level of inbreeding, i.e., shorter scapes result from more inbreeding and may produce fewer seeds. Selection during inbreeding for single centers may produce inbred lines with fewer scapes per bulb, also reducing seed yield. Correlations between inbred scape height and seed yield across all cross combinations (GCA) was significant ($P < 0.05$) for each of the two 1999 locations and nonsignificant for their combination (Table 5). Inbred lines B2117 and B2133 were good combiners for scape height (Table 4). We observed no significant correlations between scape heights in the F_1 hybrids and seed yields (means reported in Villanueva-Mosqueda, 1999). For example, short scape height for MSU611-1 was not associated

Table 4. Mean scape height (ht) and general combining ability (GCA) for scape height for seven inbred lines of onion measured in two environments.

Male parent	Environment ^a			
	WSGH99		WM99	
	Scape ht (cm)	GCA	Scape ht (cm)	GCA
MSU611-1B	69.0	-3.5	62.2	-2.1
W52B	80.6	0.9	75.6	-0.5
W101B	83.6	-4.2	69.9	-2.3
W205B	83.9	0.0	75.9	0.0
W404B	78.1	-3.6	70.8	-5.4
B2117B	102.4	3.8	97.6	3.1
B2133B	91.6	7.1	93.0	7.2
LSD _{0.05}	5.0		4.9	

^aEnvironments were in 1999 near Walnut Street Greenhouses (WSGH99) and at West Madison Experiment Station (WM99).

Table 5. Correlation coefficients between mean seed yield and scape height over environments for seven inbred onion lines and their cross combinations (GCA²).

Environments	Inbreds	GCA
Walnut Street Greenhouse 1999	$r = 0.93^*$	$r = 0.45^*$
West Madison 1999	$r = 0.79^*$	$r = 0.69^*$
Both	$r = 0.78^*$	$r = 0.12^{ns}$

²GCA= General combining ability across all cross combinations.

^{ns}, *Nonsignificant or significant at $P < 0.05$.

with lower seed yield in any of its F₁ combinations (Tables 2, 3, and 4). This analysis suggests that scape height of the F₁ was independent of seed yield. Inbred lines with short scape heights produced F₁ hybrids with higher seed yields and inbreds with taller scapes did not necessarily yield more seed in F₁ combinations.

PARENT–OFFSPRING REGRESSION ANALYSIS. Onion is inbred by self-pollination of individual bulbs or by mass pollination among a small number of bulbs. After a generation of self-pollination, seed yield of an individual bulb is known. We used PO regressions to determine if seed yields of parental bulbs were an indicator of the performance of inbred progenies, as suggested by Erikson and Gabelman (1956). PO regressions revealed no significant relationship between seed yields of parental plants and S₁ progenies ($b = -0.06$ in 1996, $b = 0.04$ in 1997, and $b = -0.04$ in 2000), suggesting that there is little to no additive genetic variance for seed yield in these populations and that seed yield is highly affected by the environment. In contrast, significance for GCA obtained from the diallel mating design among inbred onion lines suggested additive genetic components for seed yield. The plants and inbred lines used in both of these studies were sampled from long-day storage germplasm (Bark and Havey, 1995; Havey, 1991). Results indicate that relative seed yields of individual bulbs after self-pollination cannot be used to predict seed yields of progeny families. However, seed yields of inbred lines may reflect potential seed yields of hybrid male-sterile lines (Tables 1, 2, and 3).

Hybrid onion cultivars in the United States are primarily three-way hybrids generated using a male-sterile F₁ seed parent to increase seed yields. Meiosis in the male-sterile F₁ seed parent reduces uniformity in the three-way hybrid. To achieve greater uniformity, some male-sterile F₁ seed parents are crosses of sister lines. An example is MSU611-1A x MSU611B, the seed parent of the hybrid ‘Spartan Banner 80’. One obstacle to development of superior two-way hybrid onion cultivars is the unacceptably low seed yield of inbred parents. Our results indicate that higher seed-yielding parents may not produce superior seed-yielding progenies. However, the significant GCA for seed yield indicates that phenotypic recurrent selection may increase seed yields (Table 1). Selection for traits correlated with higher seed yields, such as bulb vigor and size (Erikson and Gabelman, 1954), may produce better seed-yielding inbreds, but potential correlations with undesirable characteristics, such as multiple centers, could be problematic.

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