

Inbreeding and Gene Action in Butternut Squash (*Cucurbita moschata*) Seed Starch Content

Endocría y Acción Génica para el Contenido de Almidón en Semilla de Zapallo (*Cucurbita moschata*)

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Abstract. The effect of inbreeding and gene action on butternut squash (*Cucurbita moschata* Duch.) seed production and seed starch content was evaluated at two locations in the department of Valle del Cauca, Colombia, using six accessions (S_0) and their inbred lines S_1 and S_2 . Significant differences were found between the accessions and inbred lines, but not between localities. The seed production and seed starch content showed no significant effects of inbreeding depression. An additive-type gene action predominated, suggesting that recurrent selection could be the best strategy to increase the frequencies of genes promoting seed production and seed starch content.

Key words: Butternut squash, genetic improvement, nutrition, genes.

Resumen. Se evaluó el efecto de la endocría y la acción génica en la producción de semilla y en el contenido de almidón en la semilla de zapallo (*Cucurbita moschata* Duch.) en dos localidades del departamento del Valle del Cauca, Colombia, utilizando seis accesiones (S_0) y sus respectivas líneas endocriadas S_1 y S_2 . Se detectaron diferencias significativas entre accesiones y líneas endocriadas pero no entre localidades. La producción de semilla y el contenido de almidón no presentaron efectos significativos de depresión endogámica. Predominó la acción génica de tipo aditivo, sugiriendo que la selección recurrente podría ser la mejor estrategia para incrementar las frecuencias de los genes que favorecen la producción de semilla y contenido de almidón en la semilla.

Palabras clave: Ahuyama, mejoramiento genético, nutrición,

Only the pulp of butternut squash (*Cucurbita moschata* Duch.) is used in human and animal nutrition. The seeds are discarded, which could represent a potential loss of valuable nutrients because little information is available on the potential value of these seeds as a source of nutrients (Karaye *et al.*, 2012).

Each squash fruit can contain between 30–150 g of seed, adding up to 500–1000 kg/ha (Ortiz, 2009). These chewable seeds have a sweet, nutty flavor, attributable to their ethereal extract content, which also confers a seed oil content higher than 45% (Applequista *et al.*, 2006; Ortiz *et al.*, 2009). The only commercial value generally given to squash seeds is, however, its use as grain (Criollo *et al.*, 1999). Squash seed cakes have 50% crude protein (CP) and a gross

energy (GE) above 4.5 megacalories per kg (Ortiz *et al.*, 2009).

Seed starch yield should be measured quantitatively to identify the S_0 populations and their inbred S_1 and S_2 lines, presenting greater consistency in overall seed starch production and their response to inbreeding (Ortiz, 2009).

Inbreeding has been used in breeding programs to fix phenotypes of agronomic interest, identify favorable genotypes and reduce the percentage of heterozygotes in populations (Ortiz *et al.*, 2008). Inbreeding, however, not only reduces the population mean, causing a loss of vigor (fitness), particularly in allogamous plants, but also increases genetic variance

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between families and reduces it within families (Falconer and Mackay, 1996), with a gradual increase of additive variance (σ_A) at the expense of dominance (σ_D) in completely homozygous lines (Ceballos, 1998).

In the case of butternut squash, inbreeding should be considered as mandatory when selecting good parental material. Although inbreeding depression may occur, as in most allogamous species, it is almost imperceptible in cucurbits (Ortiz *et al.*, 2008) but has been recorded in advanced lines of the cucumber, squash, melon and watermelon (Robinson, 2000; Cardoso, 2004).

In cucurbits, particularly in butternut squash, inbreeding depression depends on the genetic structure of each population, so it is possible to select inbred lines S_0 , S_1 and S_2 that outperform S_0 , attributable to the accumulation of favorable, homozygous genes (Ortiz *et al.*, 2009), or lines that can be used as parental material in hybridization breeding programs.

Although some information is available on the effect of inbreeding on traits related to fruit yield and quality (dry matter, starch, carotene and protein in pulp), little is known about the impact it has on seeds and seed components.

This research aimed to study the performance of six accessions of butternut squash in three generations of

inbreeding (S_0 , S_1 and S_2) and the resulting impact on seed production and seed starch content.

MATERIALS AND METHODS

Location. The study was conducted at two locations in the department of Valle del Cauca, Colombia. The first, the Experiment Center of the Universidad Nacional de Colombia–Palmira Campus (CEUNP, its Spanish acronym), is located in the municipality of Candelaria (03°25' N latitude, 76°25' W longitude) at an altitude of 973 m above sea level, with a mean annual temperature of 26 °C, an annual precipitation of 1,100 mm, and 76% relative humidity (Ortiz, 2009). The second, a facility of the Servicio Nacional de Aprendizaje (SENA), is located in the municipality of Buga (3°53' N latitude, 76°18' W longitude) at an altitude of 969 m above sea level, with a mean annual temperature of 23 °C, an annual precipitation of 980 mm, and 74% relative humidity (Ortiz, 2009).

The macromolecular analysis of the squash fruits was performed at the Animal Nutrition and Agricultural Prospective Laboratory of the Universidad Nacional de Colombia-Palmira campus.

Genetic material. Six open-pollinated S_0 accessions were used as well as their S_1 and S_2 inbred lines (Ortiz *et al.*, 2009) and the commercial check variety UNAPAL-Bolo Verde (Table 1).

Table 1. Origin of the S_0 accessions of butternut squash used in the study.

Accession	Origin (municipality, department)
2, 6	Pradera, Valle del Cauca
28, 34	Patía, Cauca
79, 80	Santa Marta, Magdalena
UNAPAL-Bolo Verde	Commercial check

Source: Ortiz *et al.*, 2009.

Experimental methodology. The accessions and their inbred lines were planted in a field using a randomized complete block experimental design with four replicates and five plants per replicate. At harvest, three plants were gathered from the center of each plot and one fruit was selected from each of these plants to submit to laboratory analysis.

Test variables. Seed production per plant (in grams) and seed starch content (%) were measured using the method proposed by the American Association of Cereal Chemists (BeMiller and Low, 1998; Peris-Tortajada, 2000).

Statistical analysis. The $1-w_s/w_0$ model was used to estimate inbreeding depression, where w_s is the mean

of the trait in inbred plants and w_0 the mean trait in non-inbred plants (Hayes *et al.*, 2005). The positive or negative relationship determines the type of gene action that controls the expression of the quantitative trait (Fox, 2005). The statistical significance of inbreeding depression in S_1 and S_2 was estimated using Student's t-test at the 0.05 and 0.01 levels of probability.

The model proposed by Mather and Jinks (1982) was used to estimate the genetic effects associated with additivity and dominance, as described below:

Mean of the zero generation of inbreeding (\bar{S}_0):

$$\bar{S}_0 = \mu + a + d$$

Mean first generation of inbreeding (\bar{S}_1):

$$\bar{S}_1 = \mu + a + \frac{d}{2}$$

Therefore:

$$2\bar{S}_1 - \bar{S}_0 = \mu + a$$

$$2(\bar{S}_1 - \bar{S}_0) = d$$

Where:

μ : Overall mean of trait.

- a: Additive contribution of genes involved in trait expression $a = [\sum a(-) + \sum a(+)]$.
- d: Deviations of dominance resulting from intralocus interactions contributing to trait expression $d = [\sum d(-) + \sum d(+)]$.

Based on the above expressions, it can be deduced that:

- If $\mu + a$ is **positive**, then the additive effects of the genes favor trait expression. If $\mu + a$ is **negative**, then the contrary occurs.
- If d is **positive**, dominance is in the sense of the genes favoring trait expression; if d is **negative**, then the contrary occurs.

The model proposed by Gardner and Lonnquist (1959) was used to estimate the average degree of dominance (a.d.d.):

$$a.d.d. = \frac{2(\bar{S}_1 - \bar{S}_0)}{2\bar{S}_1 - \bar{S}_0} = \frac{d}{\mu + a} = \frac{D}{A}$$

When:

- a.d.d. = 0 Additive gene action (D=0).
- a.d.d. < 1 Partial dominance.
- a.d.d. = 1 Complete dominance.
- a.d.d. > 1 Overdominance.

Table 2. Means per generation (S_0 , S_1 , S_2) and locality (Candelaria, Buga) for the evaluated traits.

Generation	Seed production /plant (g)		Seed production/hectare (kg)		Seed starch content (%)	
	Candelaria	Buga	Candelaria	Buga	Candelaria	Buga
S_0	277.4	266.5	743.2	775.2	12.7	12.5
S_1	349.9	302.9	1051.1	942.8	13.8	13.7
S_2	280.1	297.3	836.9	838.6	13.4	13.0
Mean	302.5	288.9	877.1	852.2	13.3	13.1
CV (%)	38.7	33.2	35.8	30	9.1	7.1
LSD _{5%}	162.4	132.9	435.1	353.8	1.7	1.3

RESULTS AND DISCUSSION

Table 2 shows the mean for each trait in the three generations of inbreeding (S_0 , S_1 and S_2) and at both localities.

No significant differences were found between the generations of inbreeding or between the locations.

Inbreeding had no significant effect on the average performance of the accessions. Its effect depended on the genetic complexity of the trait being studied, the geographical origin of the accession and the level of heterozygosity, combined with the ability of each accession to respond differently to the inbreeding process, which could be attributed to the fact that the starch synthesis pathway is not only controlled

genetically but is also influenced by the metabolic phase during which sugar conversion occurs (Tofiño *et al.*, 2006). This corresponds to a physiological response of the genotype to the environment as indicated by Falconer and Mackay (1996), where inbred individuals are highly sensitive to the effects of environmental variation.

Response to inbreeding. The significant negative values obtained by the model $ID = [1 - (W_s/W_o)]100$, where ID is inbreeding depression, are associated with favorable responses to the inbreeding process. Significant positive values correspond to evident depressive effects while non-significant values,

both positive and negative, indicate insensitivity to inbreeding (Table 3).

ID values, such as -75.7** (S_0-S_1) and -60.4** (S_0-S_2) for the trait seed production/hectare in the case of accession 2, indicate a favorable response to inbreeding as a result of a higher concentration of favorable homozygous genes, which increases seed production/hectare, as compared with values of 39.7** (S_1-S_2) and 37.1** (S_0-S_2) in the case of accession 34, which are associated with depressive effects and could be attributed to the manifestation of homozygous genes that reduce seed production/hectare. This

Table 3. Response to inbreeding (% ID) for seed production and seed starch content in different butternut squash accessions.

Accession	Generation	Inbreeding depression		
		Seed production/ plant	Seed production/ hectare	Seed starch content
2	S_0-S_1	-18.9	-75.7 **	4.8
	S_1-S_2	-18.1	8.7	-6.5
	S_0-S_2	-40.4 *	-60.4 **	-1.5
6	S_0-S_1	-23.9	-25.7 *	-15.2 *
	S_1-S_2	-9.3	NA	-8.0
	S_0-S_2	-33.8 *	-37.3 **	-9.7
28	S_0-S_1	-45.3 *	-17.3	-8.1
	S_1-S_2	27.9 *	7.5	9.7
	S_0-S_2	-4.8	-8.4	2.3
34	S_0-S_1	26.2	-4.4	-24.7 **
	S_1-S_2	37.1	39.7 **	17.3
	S_0-S_2	53.6 **	37.1**	-3.1
79	S_0-S_1	-21.6	-41.3 **	-7.5
	S_1-S_2	28.4 *	33.3 **	1.8
	S_0-S_2	12.9	5.7	-5.6
80	S_0-S_1	-74.8 **	-64.4 **	-14.5 *
	S_1-S_2	16.5	23.4 *	4.6
	S_0-S_2	-46.0 *	-26.0	-9.3

* Significant difference at a level of significance of 0.05.

** Highly significant difference at a level of significance of 0.01.

NA: Not available.

indicates that there is no pattern of response to inbreeding in the case of these accessions and traits (Ortiz *et al.*, 2009).

According to the differential response of S_0 accessions and their inbred lines, three groups were defined:

G1 (-, -) Negative response to inbreeding in S_1 and S_2 . Inbreeding depression is moderate in S_1 and S_2 , for example in the case of the trait seed production/plant in accession 34 (Table 4).

G2 (+, -) Positive response to inbreeding in S_1 but presenting inbreeding depression when passing from

Table 4. Classification of butternut squash accessions based on mean performance to inbreeding by groups G_i (S_1 , S_2) based on information presented in Table 3.

Descriptor	G1(-, -)	G2(+, -)	G3(+, +)
Seed production/plant	34	28, 79, 80	2, 6
Seed production/hectare		2, 28, 34, 79, 80	6
Seed starch content		34, 80	6

S_1 to S_2 , as occurs in accessions 6, 34 and 80 for seed starch content and in accessions 2, 28, 34, 79 and 80 for seed production/hectare. The positive response to inbreeding assumes highly significant negative values; in other words, inbreeding does not suppress a trait *per se*, which characterizes the performance of butternut squash (Ortiz, 2009).

G3 (+, +) Positive response to inbreeding in S_1 and S_2 . Inbreeding helped improved the mean performance of accessions in S_1 and S_2 , for example seed production/plant in accessions 2 and 6 and seed production/hectare in accession 6 (Table 4).

The information given in Tables 3 and 4 indicates that, unlike other self-pollinated species such as maize, inbreeding depression in cucurbits is uncertain, at least for the traits being evaluated in this study, and depends not only on the accession's degree of heterozygosity in S_0 , but also on the "remnant" genetic load manifested in S_1 and S_2 . As a hypothesis, it can be assumed that, in the case of accessions that have been geographically isolated for a long time, undesirable genes responsible for inbreeding depression may have been removed through a natural process of inbreeding in related crosses so that these accessions, contrary to what is assumed, have a high degree of homozygosity when collected and entered into the program's work collection. As a result, when these accessions are submitted to inbreeding they are either insensitive or respond favorably or unfavorably to the process, as found by Espitia *et al.* (2006) regarding fruit traits.

Type of gene action. If S_0 accessions are heterozygous, inbreeding is expected to depress their performance because a certain number of heterozygous loci acquire the recessive homozygous condition. If these recessive homozygous genes are part of the genetic load of the inbred accession, then the depressive effect on the population mean will be evidenced (Sahagún and García, 2009).

When $S_1 < S_0$, parameters **d** and **D/A**, which are associated with deviations in dominance and a.d.d., are less than zero as a result of loss of heterozygosity, which, in addition to the residual heterozygosity in S_1 , reduces the mean value of the inbred accession (effect of reverse dominance) as in group G (-, -) (Table 4).

When $S_1 \approx S_0$, parameters **d** and **D/A** are approximately zero and the gene action that controls trait expression is additive in nature or, if not, then there is a balance between positive and negative deviations of dominance ($d^+ \approx d^-$), for example in the case of the trait seed production/hectare in accession 80.

When both **d** and **D/A** present positive values, the partial dominance significantly increases the means of the evaluated traits in inbred accessions as occurred for most of the traits under study, with a.d.d up to 0.55, as occurred for the variable seed production/hectare in accession 79 (Table 5).

The parameter $\mu + a$ was positive in all accessions (Table 5), attributable to a higher proportion of alleles with favorable additive effects for trait expression in loci acquiring the homozygous condition after selfing.

Table 5. Dominant gene action (**d**), additive gene action ($\mu + a$) and average degree of dominance (a.d.d.=**D/A**) associated with inbreeding in butternut squash.

Accession	Parameter	Trait		
		Seed production/ plant	Seed production/ hectare	Seed starch content
2	$2S_1-S_0 = \mu+a$	381.96	1078.37	12.00
	$2(S_1-S_0)=d$	114.18	625.11	-1.69
	D/A ¹	0.30	0.58	-0.14
6	$2S_1-S_0 = \mu+a$	490.71	1458.57	15.49
	$2(S_1-S_0)=d$	144.82	484.04	3.58
	D/A	0.30	0.33	0.23
28	$2S_1-S_0 = \mu+a$	473.92	1269.76	15.04
	$2(S_1-S_0)=d$	225.38	325.84	2.11
	D/A	0.48	0.26	0.14
34	$2S_1-S_0 = \mu+a$	577.68	1892.58	14.54
	$2(S_1-S_0)=d$	195.33	872.90	2.04
	D/A	0.34	0.46	0.14
79	$2S_1-S_0 = \mu+a$	334.88	1264.70	14.70
	$2(S_1-S_0)=d$	191.40	692.44	3.32
	D/A	0.57	0.55	0.23
80	$2S_1-S_0 = \mu+a$	121.21	808.29	19.43
	$2(S_1-S_0)=d$	-134.01	71.62	6.44
	D/A	-1.11	0.09	0.33

¹ D/A = (d/ $\mu+a$) = a.d.d. = average degree of dominance.

The performance of the trait seed production/plant in accession 80, with a.d.d. of -1.11, should be highlighted because inbreeding in this particular case creates an imbalance in the dominance relationships in heterozygous loci so that intralocus interactions favor the alleles depressing the expression of said trait.

Different breeding strategies for seed production and starch content can be derived from the above results such as the following: advancing to S_2 those accessions suffering inbreeding depression for subsequent crossbreeding between inbred lines to obtain commercially valuable hybrids; selection of S_1 or S_2 inbred populations presenting a mean performance superior to that of the S_0 accession; or simply selection of S_0 accessions for traits insensitive to inbreeding.

CONCLUSIONS

The parameter D/A indicates that, with the exceptions already mentioned, inbreeding in butternut squash helps increase additive gene action as a result of the increase in the number of homozygous loci after selfing, which in turn significantly favors the mean response of inbred accessions. Similarly, the loci remaining heterozygous in S_1 (residual heterozygosity) express levels of dominance in favor of traits associated with seed production and seed starch content.

The response to inbreeding in butternut squash varies among accessions, generations of inbreeding (S_1 , S_2) and traits, with no clearly defined pattern.

The predominance of additive gene action over the dominance type for the traits under study suggests that a recurrent selection program could serve as a strategy to increase the frequencies of genes that promote the expression of traits associated with seed production and starch content in butternut squash.

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