GMR

The Original by FUNPEC-RP

Genetic control of internode length in winter squash (*Cucurbita moschata*)

C.F. Almeida, R.S. Gomes, R. Machado Junior, R.L. Oliveira, R.D.F. Laurindo, R.R. Chagas and D.J.H. da Silva

Departamento de Agronomia, Universidade Federal de Viçosa, Viçosa, MG, Brasil

Corresponding author: C.F. Almeida
E-mail: cleverson_freitas02@yahoo.com.br

Genet. Mol. Res. 19 (3): gmr18660
Received June 12, 2020
Accepted August 11, 2020
Published August 31, 2020
DOI http://dx.doi.org/10.4238/gmr18660

ABSTRACT. Understanding the genetic control of internode length is essential to develop more compact winter squash genotypes. Our objective was to elucidate the genetic control of internode length before and after emergence of the first female flower in winter squash, *Cucurbita moschata*. This was done by estimating the linear and quadratic genetic components and using the maximum likelihood estimation function. The parents used were the long-vined accession BGH 7319 (P_1) and the compact cultivar 'Tronco Verde' (P_2). The F_1 plants from this cross were self-fertilized to obtain the F_2 generation, and then they were backcrossed with P_1 and P_2 to obtain generations BC_1 and BC_2, respectively. By examining the linear and quadratic genetic components of variations in internode length, we found evidence of dominance effects both before and after flowering, with a reversal in dominance after flowering. Using maximum likelihood, we observed that the internode length before flowering was controlled by one major gene with additive and dominance effects, while the internode length after flowering was controlled by multiple genes with additive and dominance effects, plus environmental effects. Based on these results, strategies using backcrosses for introgression of the major gene controlling this trait before flowering and recurrent selection for introgression of the polygenes involved in trait control after flowering are recommended.

Key words: Backcrossing; Brachitic plants; *Cucurbita moschata;* Genetic components of variance; Generation means; Heritability

INTRODUCTION

Winter squash (*Cucurbita moschata*) is one of the most socioeconomically important species in the genus *Cucurbita*, and it is a staple food for large portions of human populations in tropical and subtropical regions (FAOSTAT, 2019). Currently, the pulp of *C. moschata* fruits is the most important part of this vegetable used as food and is used in various ways in human nutrition. The pulp of its fruits is one of the most promising sources of carotenoids, such as *β*-carotene, a precursor of pro-vitamin A (Rodriguez-Amaya et al., 2008, Carvalho et al., 2012; Gomes et al., 2020).

A particularly promising aspect of *C. moschata* is the high potential for the oil from its seeds to be used for food purposes. Indeed, the seeds of this species have very high oil content, with the lipid fraction of the seeds reaching up to 49% of their composition (Patel, 2013). Associated with this, about 70% of the lipid profile of the oil in the seeds of this species is made up of unsaturated fatty acids (Sobreira, 2013; Jarret et al., 2013; Veronezi and Jorge, 2015), with a preponderance of such fatty acids as linoleic C18:2 (Δ9,12) and oleic C18:1 (Δ9) acids. The predominance of these fatty acids in the oil of *C. moschata* seeds means that they could provide a diet in which there is low production of free radicals, in addition to providing high stability to the oil during storage (Jakab et al., 2003). Additionally, *C. moschata* seed oil is also rich in bioactive components, such as vitamin E, carotenoids, and tocopherols, which protect the oil from oxidation and are components with important antioxidant activity (Veronezi et al., 2012).

C. moschata plants occupy a very large area due to their creeping stems and indeterminate growth habit (Filgueira, 2007; Laurindo et al., 2017), which is an aspect of them that limits the potential for the productivity of their culture to be increased. Given this, increasing the productivity of this vegetable depends on strategies that can increase the number of plants grown per area, for example, by developing more compact genotypes.

It should be highlighted that some previous studies of *Cucurbit* species have reported plants with reduced internode lengths, which they called brachitic plants (Kilen, 1977). According to Maynard et al., (2002), these plants have a bush-type growth habit that allows a larger plant population to be grown per unit area compared to what can be obtained using plants with long vines.

The existence of genotypes with shorter internode lengths suggests the possibility of transferring alleles associated with this trait across generations to ensure that genotypes with more compact sizes are obtained. However, for this to be attained, it is crucial to study the genetic control of this trait in *C. moschata*, including elucidating the magnitude and nature of this trait's variability in this species.

The study of the inheritance of a certain characteristic is fundamental in defining selection strategies for the characteristic and predicting the behavior of segregating generations (Cruz and Regazzi, 2001). Such information is needed to determine the criterion for and the intensity of selection for this trait, as well as the breeding methods used to conduct segregating populations (Ramalho et al., 2000).

Generation means analysis has been the most frequently used method in the study of the genetic control of traits of agronomic interest. This method has been widely used in

studies of the genetic control of quantitative traits in a variety of crops, such as wheat (Said, 2014), corn (Almeida et al., 2018), melon (Zalapa et al., 2006), and watermelon (Kumar and Wehner, 2013), among others.

Although the genetic control of the internode length in *Cucurbita* spp. has not been explored much, a few studies have investigated this topic. Some studies reported that reduced internode length in some species, such as *C. maxima* and *C. pepo*, is conferred by a single dominant gene, the *Bush* (*Bu*) gene, which has a dominant effect in the development of plants of these species (Shifriss, 1947, Robinson et al., 1976). However, other studies reported that the *Bu* gene only has a dominant effect in the initial phase of plant development, but then acts recessively during the reproductive phase, representing a reversal of dominance (Paris and Edelstein, 2001).

In light of the conflicting information regarding the genetic control of internode length in winter squash plants from past studies, the objective of this study was to evaluate the genetic control of this characteristic in *C. moschata*. This was done with the aim of identifying alleles associated with reduced internode length so that these can be transferred across generations for the production of promising new accessions to improve seed oil production.

MATERIAL AND METHODS

Site and field trials

The experiment was conducted at the Horticulture Research Farm belonging to the Department of Agronomy of the Federal University of Viçosa (UFV), located in Viçosa, Minas Gerais (MG), Brazil (20°45′14″ S; 42°52′53″ W; 648.74 m altitude), from August to December 2016.

Seeds were initially sown in polystyrene trays, and then seedlings were transplanted to the cultivation field when they had developed their first fully expanded final leaf. The soil was previously prepared by harrowing and digging and a spacing of 4.0 m between rows and 4.0 m between plants (4×4) was adopted. All agricultural treatments, such as fertilization and irrigation, were performed whenever necessary following the recommendations of Filgueira (2007). Harvest was carried out when the fruits reached the point of commercial maturity, indicated by them having a dry stalk.

In the experiment, we characterized 17 winter squash plants from accession BGH 7319 (long-vined parent, P_1), 19 from the cultivar 'Tronco Verde' (compact parent, P_2), 23 F_1 plants, 75 and 89 plants from backcrosses BC_1 ($F_1 \times P_1$) and BC_2 ($F_1 \times P_2$), respectively, and 183 plants from the F_2 segregating generation.

The study was performed through the simultaneous analysis of plants from six generations, namely P_1, P_2, F_1, F_2, BC_1, and BC_2. The P_1 parent was accession BGH-7319, belonging to the Germplasm Bank of Vegetables of the Federal University of Viçosa (BGH-UFV), which has a long-vined phenotype. This accession produces a high mass of seeds per fruit, and seeds with a high oil content and a lipid profile with a predominance of unsaturated fatty acids (Sobreira, 2013); it is thus being considered as a promising candidate for use in programs aimed at increasing the concentration of functional fatty acids in winter squash seeds. The P_2 parent was the cultivar 'Tronco Verde', which has a relatively compact phenotype and possesses the gene responsible for reduced internode length in

homozygous plants (Figure 1). The F_1 generation was obtained from the crossing of these parents, and the F_2 generation was obtained from the self-fertilization of F_1 plants. The BC_1 and BC_2 backcrosses were obtained by crossing the F_1 plants with P_1 and P_2 plants, respectively.

Figure 1. Parents used in the crosses performed with the aim of studying the genetic control of the internode length in winter squash plant. The photographs were taken 52 days after transplantation.

Trait measurements

The plant traits evaluated herein to study the genetic control of the internode length included the average internode length before the emergence of the first female flower (ILBF) and the average internode length after the emergence of the first female flower (ILAF), obtained for the ratio of the length of the vine to the number of internodes in each of these stages of plant development.

Genetic and statistical analyses

Initially, we performed an analysis to ascertain the normality distribution of data regarding individuals of the F_2 generation (Shapiro and Wilk, 1965). The ILBF and ILAF data were subjected to analysis of variance (ANOVA) to test whether there were significant differences in these traits among generations. Post-hoc comparisons of the means of these descriptors between specific pairs of generations were performed using the LSD test, with the significance threshold set at 5% ($P < 0.05$) (Steel and Torrie, 1980).

Subsequently, estimates of the additive (σ^2_a), phenotypic (σ^2_p), genetic (σ^2_g), and environmental (σ^2_e) components of the variance in these traits were obtained, as well as dominance deviations, according to the methodology of Cruz et al., (2013). In addition, tests of different genetic models were carried out using the maximum likelihood approach.

The variance estimates were obtained using the following equations:

Phenotypic variance in F_2:

$$\hat{\sigma}^2_{f(F2)} = \hat{\sigma}^2_{(F2)} \quad \text{(Eq. 1)}$$

Environmental variance in F_2:

$$\hat{\sigma}^2_{e(F2)} = \frac{2\hat{\sigma}^2_{(F1)} + \hat{\sigma}^2_{(P1)} + \hat{\sigma}^2_{(P2)}}{4} \quad \text{(Eq. 2)}$$

$$\hat{\sigma}^2_{e(RC1)} = \frac{\hat{\sigma}^2_{F1} + \hat{\sigma}^2_{P1}}{2} \quad \text{(Eq. 3)}$$

$$\hat{\sigma}^2{}_{e(RC2)} = \frac{\hat{\sigma}^2{}_{F1} + \hat{\sigma}^2{}_{P2}}{2} \quad \text{(Eq. 4)}$$

Genetic variance in F_2:

$$\hat{\sigma}^2{}_{g(F2)} = \hat{\sigma}^2{}_{p(F2)} - \hat{\sigma}^2{}_{e(F2)} \quad \text{(Eq. 5)}$$

Additive variance in F_2:

$$\hat{\sigma}^2{}_a = 2\hat{\sigma}^2{}_{p(F2)} - [\hat{\sigma}^2{}_{p(RC1)} + \hat{\sigma}^2{}_{p(RC2)}] \quad \text{(Eq. 6)}$$

Variance due to dominance in F_2:

$$\hat{\sigma}^2{}_d = \hat{\sigma}^2{}_{g(F2)} - \hat{\sigma}^2{}_a \quad \text{(Eq. 7)}$$

Heritability estimates were obtained using the following equations:
Broad-sense heritability (h_b^2):

$$h_a^2 = \frac{\hat{\sigma}^2{}_{g(F2)}}{\hat{\sigma}^2{}_{p(F2)}} \quad \text{(Eq. 8)}$$

Narrow-sense heritability (h_n^2):

$$h_n^2 = \frac{\hat{\sigma}^2{}_a}{\hat{\sigma}^2{}_{p(F2)}} \quad \text{(Eq. 9)}$$

For the complete model, the mean effects of all possible homozygotes (m), and the additive (a), dominance (d), and epistatic effects of genes, were estimated as follows: additive × additive (aa), additive × dominant (ad), and dominant × dominant (dd). For the dominant × additive model, only the additive, dominance and mean effects were estimated. Both the additive effects (a) and the nonadditive effects (d) were estimated from the generation means using the weighted least squares method (Mather and Jinks, 1977).

Generation means analysis was performed using the following complete model:

$$\hat{m} = \frac{1}{2}\overline{P_1} + \frac{1}{2}\overline{P_2} + 4\overline{F_2} - 2\overline{RC_1} - 2\overline{RC_2} \quad \text{(Eq. 10)}$$

$$\hat{a} = \frac{1}{2}\overline{P_1} - \frac{1}{2}\overline{P_2} \quad \text{(Eq. 11)}$$

$$\hat{d} = -\frac{3}{2}\overline{P_1} - \frac{3}{2}\overline{P_2} - \overline{F_1} - 8\overline{F_2} + 6\overline{RC_1} + 6\overline{RC_2} \quad \text{(Eq. 12)}$$

$$a\hat{a} = -4\overline{F_2} + 2\overline{RC_1} + 2\overline{RC_2} \quad \text{(Eq. 13)}$$

$$a\hat{d} = -\overline{P_1} + \overline{P_2} + 2\overline{RC_1} - 2\overline{RC_2} \quad \text{(Eq. 14)}$$

$$d\hat{d} = \overline{P_1} + \overline{P_2} + 2\overline{F_1} + 4\overline{F_2} - 4\overline{RC_1} - 4\overline{RC_2} \quad \text{(Eq. 15)}$$

The effects using one model rather than another on predicted trait variances were assessed using the *t*-test (with the significance threshold set at 5%), in which each parameter was compared to a null value (H_0: $\beta_i = 0$). All statistical analyses were performed using the computer program Genes (Cruz, 2013), with the exception of genetic model tests, for which the likelihood estimators and the computer program Monogen v.0.1 developed by Silva (2003) were used.

Based on the likelihood values obtained for each model, it was possible to compare models of interest to test different hypotheses. These likelihood tests were performed using likelihood ratio (LR) statistics, based on the following equation:

$$LR = -2 \ln \frac{L(M_i)}{L(M_j)} \quad \text{(Eq. 16)}$$

where $L(M_i)$ and $L(M_j)$ correspond to the likelihoods of models i and j, respectively, and where model i must be hierarchical in relation to model j.

The maximum likelihood tests are applied to hierarchical models, therefore the contrast between two models under consideration may or may not lead to the rejection of one of them. Thus, the significance of the contrast shows that there is a significant difference between the models, and that the most complete model is the one that best explains the characteristic under study. If the contrast is not significant, the reduced model is the one that best explains the characteristic under study (COPATI, 2019). The Models developed by Silva (2003) and implemented in the Monogen Program are shown in Table 1.

Table 1. Genetic models used in Monogen software (Silva 2003) and their respective parameters.

Models	Estimated parameters								
1: Major gene with additive and dominance effects + polygenes with additive and dominance effects	μ	A	D	[a]	[d]	V_a	V_d	S_{ad}	σ^2
2: Major gene with additive and dominance effects + polygenes with additive effects only	μ	A	D	[a]	V_a	σ^2			
3: Major gene with additive effect only + polygenes with additive and dominance effects	μ	A	[a]	[d]	V_a	V_d	S_{ad}	σ^2	
4: Major gene with additive effect only + polygenes with additive effects only	μ	A	[a]	V_a	σ^2				
5: Polygenes with additive and dominance effects	μ	[a]	[d]	V_a	V_d	S_{ad}	σ^2		
6: Polygenes with additive effects only	μ	[a]	V_a	σ^2					
7: Major gene with additive and dominance effects	μ	A	D	σ^2					
8: Major gene with additive effect only	μ	A	σ^2						
9: Environmental effects only	μ	σ^2							

μ: reference constant; A: additive effect of the major gene; D: dominance effect of the major gene; [a]: additive polygenic component; [d]: polygenic dominance component; V_a: additive variance; V_d: variance attributed to deviations in the dominance of the polygenic effects; S_{ad}: component of the variation due to the product of the additive polygenic effects and the polygenic effects of dominance; σ^2: environmental variance.

RESULTS

When analyzing data from the F_2 segregating population, no discrete distribution was observed in the trait data, and it was thus possible to accept that these data were normally distributed. Given this, analyses were performed on the estimates of generation means, variances, and heritabilities obtained based on the quantitative approach, as well as on estimates of different genetic effects on the control of the assessed traits.

Significant differences in ILBF and ILAF were observed among generations ($P < 0.05$), with low coefficients of variation (13.02 and 7.00%, respectively) found for these data (Table 2).

Table 2. Generation means and average degree of dominance of the traits ILBF and ILAF assessed in the P_1, P_2, F_1, F_2, BC_1, and BC_2 generations derived from the crossing of the BGH 7319 (P_1) accession with the cultivar 'Tronco Verde' (P_2) of winter squash.

Generation	ILBF	ILAF
BGH7319 (P_1)	18.35a	14.84a
'Tronco Verde' (P_2)	4.08e	6.05d
Mean of parents (X_{12})	11.22b	10.445bc
F_1	8.79cd	9.34c
F_2	7.15d	11.05b
BC_1	9.94bc	14.51aa
BC_2	4.98e	10.08bc
CV(%)	13.02	7.00
Gmd	-0.34	-0.25

Means followed by the same letter in the same column did not differ significantly according to the LSD test ($P > 0.05$). ILBF: average internode length (cm) before emergence of the first female flower; ILAF: average internode length (cm) after emergence of the first female flower; BC: Backcrossing; BC_1: P_1 x F_1; BC_2 : P_2 x F_1; CV (%): coefficient of variation; gmd: average degree of dominance, calculated based on generation means; $X_{12} = (P_1 + P_2)/2$.

Generation means

Variability was observed between the parents BGH-7319 and 'Tronco Verde' in the characteristics under analysis. Specifically, significant differences ($P < 0.05$) were detected between the averages of ILBF and ILAF of the two parents. For the parent 'Tronco Verde', the averages of ILBF and ILAF were lower than those of the parent BGH-7319 (Table 2).

The mean trait values of the F_1 generation differed from those of both parents, thus demonstrating the absence of complete dominance effects on the traits studied. For ILBF, there was incomplete dominance, since the mean of the F_1 generation was lower and significantly different from the means of both parents, although it was closer to the mean of the parent with the lower value for this trait. However, for ILAF, there was no dominance or additive interaction, since the mean of the F_1 generation for this characteristic was between the estimated mean values of the parents, and was not significantly different from their mean values (Table 2).

The mean ILBF values of the F_1 and F_2 generations were not significantly different. In contrast, the mean ILAF values of these generations were significantly different, with the F_2 generation having an average of ILAF higher than the F_1 generation. (Table 2)

The values of the average degree of dominance (gmd) estimated from the mean values of the analyzed traits were between 0 and 1 (Table 2). When the gmd values of the internode length before (ILBF) and after flowering (ILAF) were compared, it was observed that the gmd value for the former was apparently larger in magnitude than that of the latter. Regarding transgressive segregators, minimum values were observed in the F_2 generation for both trait descriptors.

Genetic components of means

When the dominant × additive model was used, all effects were significant except for dominance effects for ILAF (Table 3). There were significant epistatic effects on the control of the studied traits, meaning that they had to be analyzed using the complete model (Table 3). In addition to the effects of dominance (d) and additive effects (a), the complete

model also considers the epistatic effects of the additive × additive (aa), additive × dominant (ad), and dominant × dominant (dd) interactions. When the data were analyzed with the complete model, it was observed that all effects were significant, with the exception of the epistatic additive × additive effect, which was not significant for ILBF (Table 3).

Table 3. Dominant × additive and complete models of variation of ILBF and ILAF among winter squash generations.

Dominant × additive model				Complete model						
Trait	**m**	**a**	**d**	**m**	**a**	**d**	**aa**	**ad**	**dd**	**Epistasis**
ILBF	9.11**	5.14**	-2.67**	10.47**	7.05**	-11.39**	0.71ns	-4.62**	9.69**	Duplicate
ILAF	11.29**	4.80**	-0.47ns	6.35**	3.45**	16.30**	3.20*	3.91**	-13.74**	Duplicate

m: homozygous mean; a: additive effect; d: dominance effect; aa: additive × additive epistatic effect; ad: additive × dominant epistatic effect; dd: dominant × dominant epistatic effect; *t*-test results: **, significant at $P < 0.01$, * significant at $P < 0.05$, ns, not significant ($P > 0.05$); ILBF: average internode length (cm) before emergence of the first female flower; ILAF: average internode length (cm) after emergence of the first female flower.

When the simple and complete models for ILAF were compared, disagreements were observed. For example, the dominance effect was non-significant in the simple model, whereas the dominance effect was significant in the complete model (Table 3).

In the complete model, a difference was observed in both the sign and magnitude of the effect of dominance on the two studied traits, with this effect being negative before flowering (-11.39 for ILBF) and positive after flowering (16.30 for ILAF) (Table 3).

Genetic components of variance

In this study, the broad-sense heritability values estimated for ILBF (56.2%) and ILAF (57.22%) were similar, and the estimated narrow-sense heritability was greater than the broad-sense heritability for both traits (Table 4). Negative variance estimates for these traits are possible, but were not found herein (Table 4), meaning that we found evidence for additive variance, but not dominance, in these traits. The variance due to dominance could be obtained by subtracting the additive from the genetic variance; however, since the additive variance estimate was larger than the genetic one, this would result in a negative, and therefore biased, estimate (Kozik et al., 2013).

Table 4. Estimation of genetic parameters to the traits ILBF and ILAF in the F_2 population derived from crossing the BGH 7319 accession (P_1) with the cultivar 'Tronco Verde' (P_2) of winter squash.

Trait	$\hat{\sigma}^2_f$	$\hat{\sigma}^2_e$	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_a$	$\hat{\sigma}^2_d$	$\hat{h}^2_{b\,(\%)}$	$\hat{h}^2_{n\,(\%)}$
ILBF	15.5565	6.8123	8.7442	20.2445	-	56.20	130.13
ILAF	17.4503	7.4643	9.9860	13.6340	-	57.22	78.13

σ^2_f: phenotypic variance; σ^2_e: environmental variance; σ^2_g: genotypic variance; σ^2_a: additive variance; σ^2_d: variance due to dominance; h_b^2: broad-sense heritability (%); h_n^2: narrow-sense heritability (%); ILBF: average internode length (cm) before emergence of the first female flower; ILAF: average internode length (cm) after emergence of the first female flower; -: negative dominance variance values are omitted.

Genetic control of internode length before (ILBF) and after emergence of the first female flower (ILAF) analyzed via maximum likelihood

The results of the inheritance tests performed using maximum likelihood are shown in Table 5. For both variables, model 1 was first contrasted with model 5 (1×5) and with model 7 (1×7). The first of these contrasts tested for the existence of one major gene controlling each trait, and the second tested for the existence of polygenes influencing the trait. After these comparisons, the other models shown in Table 1 were also tested.

Table 5. Hypothesis testing of genetic models for the inheritance of the average internode length before (ILBF) and after emergence of the first female flower (ILAF) in winter squash, evaluated in generations P_1, P_2, F_1, F_2, BC_1, and BC_2 derived from the crossing of BGH-7319 (P_1) and 'Tronco Verde' (P_2).

	ILBF (a)				ILAF (b)		
Models	**DF**	**χ^2**	**P**	**Models**	**DF**	**χ^2**	**P**
1×2	**3**	**29.7301***	**0.00000179**	1×2	3	4.0426	0.256897983
1×3	**1**	**17.4045***	**0.000030131**	1×3	1	0.8884	0.345886140
1×4	4	97.6792	0.000000538	1×4	4	4.3176	0.364721211
1×5	**2**	**17.4045***	**0.000166334**	**1×5**	**2**	**1.014ns**	**0.602274651**
1×6	5	97.6792	0.000000400	1×6	5	1.0597	0.957603564
1×7	**5**	**80.124***	**0.000000387**	**1×7**	**5**	**12.9788***	**0.023577726**
1×8	6	139.1305	0.000000646	1×8	6	17.4705	0.007701124
1×9	**7**	**279.507***	**0.000000806**	1×9	7	106.2393	0.000000624
2×4	1	67.9491	0.000000092	2×4	1	0.275	0.599986480
2×6	2	67.9491	0.000000333	2×6	2	< 0	-
2×7	2	50.3939	0.000000390	2×7	2	8.9362	0.011468981
2×8	3	109.4004	0.000000654	2×8	3	13.4279	0.003797024
2×9	4	249.7769	0.000000989	2×9	4	102.1967	0.000000552
3×5	1	< 0	-	3×5	1	0.1255	0.72347703
3×6	4	80.2746	0.000000297	3×6	4	0.1712	0.996536359
3×8	5	121.726	0.000000412	3×8	5	16.552	0.005364719
3×9	6	262.1024	0.000000806	3×9	6	105.3508	0.000000591
4×6	1	< 0	-	4×6	1	< 0	-
4×8	2	41.5413	0.000000181	4×8	2	13.1528	0.001392880
4×9	3	181.8277	0.000000859	4×9	3	101.9217	0.000000361
5×6	3	80.2747	0.000000407	**5×6**	**3**	**0.0456ns**	**0.997439366**
5×9	5	262.1025	0.000000976	5×9	5	105.2252	0.000000421
6×9	2	181.8277	0.000000658	**6×9**	**2**	**105.1796***	**0.000000519**
7×8	1	59.0065	0.000000214	7×8	1	4.4916	0.034060057
7×9	2	199.3829	0.000000644	7×9	2	93.2604	0.000000509
8×9	1	29.7301	0.000000113	8×9	1	4.0426	0.044364603

DF: degrees of freedom; Chi-square test results: χ^2, Chi-square value, P, probability, *, significant at $P < 0.05$, ns, not significant ($P > 0.05$); ILBF and ILAF: internode length (cm) before and after flowering, respectively; < 0: negative values, perhaps due to convergence problems.

The most adequate model to explain variations in ILBF was determined by contrasting all of the possible explanatory models for this characteristic. For ILBF, the contrast made between models 1 and 5 (1×5) indicated whether or there was a gene that had a greater effect on this trait than others (Table 5a). This contrast allowed us to compare the model with a major gene plus polygenes (model 1) with that including only polygenes (model 5). The null hypothesis in this test was rejected, suggesting the existence of a major gene, since the inheritance of the trait could be more adequately explained by the complete model.

In the contrast of model 1 with 7 (1×7) for ILBF, which compared trait variation due to a gene with a greater effect than others plus polygenes (model 1) against variation

only due to a gene with a greater effect (model 7), the H_0 was also rejected. This shows that polygenes were also involved in the control of this characteristic.

To verify the existence of dominance effects of the major gene on ILBF, model 1 was contrasted with model 3 (1×3). The null hypothesis of no difference was rejected, which verified that the major gene had dominance effects on this trait.

To test for dominance effects of polygenes on ILBF, model 1 was contrasted with model 2 (1×2). In this contrast, the null hypothesis was rejected, showing that the dominance effects of polygenes also influenced the variation in this characteristic. In light of this, we last tested for environmental effects on ILBF by contrasting models 1 and 9 (1×9). As the result of this contrast was significant, environmental effects could be concluded to have had meaningful impacts on this characteristic. Given this, the model most suitable for explaining the internode length before flowering was concluded to be the complete model (model 1), in which ILBF was influenced by a major gene with additive and dominance effects, polygenes with additive and dominance effects, and environmental effects.

The most adequate model to explain variation in ILAF was also determined by performing contrasts between the possible explanatory models for this characteristic. However, the initial contrast between models 1 and 5 (1×5) for this trait was non-significant, which provided evidence that there was no effect of a major gene on the control of this characteristic (Table 5b).

The contrast of models 1 and 7 (1×7) for ILAF was significant, showing the existence of effects of polygenes on this trait. In light of this, we next tested for the existence of dominance effects by these polygenes on the trait. As the existence of a major gene controlling this trait had already been rejected, model 5 was contrasted with model 6 (5×6) for ILAF, and not model 1 with model 2 (1×2), as previously performed for ILBF.

The contrast of model 5 with model 6 (5×6) was not significant for ILAF, demonstrating the absence of dominance effects by polygenes on this trait. Thus, the simplest model was the one that best explained variations in ILAF. Given this, environmental effects were next tested by comparing models 6 and 9 (6×9). Since the result of this contrast was significant, it could be concluded that ILAF is controlled by polygenes with additive effects, and it is also influenced by environmental effects.

DISCUSSION

The traits examined in this study were evaluated with good to excellent experimental precision, since values of coefficients of variation below 15% were obtained. This showed that there was good experimental precision and supports the reliability of the results obtained (Table 2).

Generation means

The statistically significant differences found between the parents are of great importance because, according to some authors, the genetic and statistical parameter estimates of the genetic control of certain characteristics can be estimated with greater precision when there is a divergence between the parents used (Cruz et al., 2005).

The mean ILBF of the plants in the F_1 generation was lower than the mean ILBF of their parents, with a tendency for dominance to occur in the transmission of this trait across generations. Therefore, the dominant alleles of the parents responsible for reducing the value of this characteristic (internode length) can consequently be interpreted as exerting partial or incomplete dominance. The occurrence of incomplete dominance demonstrates that the evaluation of and selection for this characteristic must be carried out by means of progeny tests so that heterozygous individuals can be differentiated from homozygous ones (Table 2).

For ILAF, there was only additive allelic interaction. Therefore, it may be possible to optimize the selection gains obtained in breeding individuals with desirable values of this characteristic, since the progeny of selected individuals would produce superior progeny (Ramalho, 2012).

The superiority of the F_2 generation over the F_1 generation for ILAF indicates that the dominant alleles were responsible for increasing the internode length after flowering. From this, it can be assumed that there was a reversal of dominance, since before flowering the dominant alleles were responsible for the reduction of the internode length, while this reduction was governed by recessive alleles after flowering.

When the gmd estimates made for the abovementioned characteristics are considered, the occurrence of a partial dominance interaction can be inferred because, according to Ramalho (2012), when the average degree of dominance is between 0 and 1 this means that this type of interaction has occurred. This makes it difficult to select superior individuals for these traits since their offspring will perform less well than themselves. For these characteristics, selection can be performed using hybrid combinations. The higher gmd found for ILBF shows that there were greater dominance effects on ILBF than ILAF (Table 2).

The presence of transgressive segregants among the minimum values of both traits found in the F_2 generation demonstrates that the crosses performed are a promising method for reducing the internode lengths in winter squash. They also show that there is good complementation between the parents, pointing to the possibility of being able to select individuals with a greater number of effective alleles than the parents for targeted breeding (Ramalho, 2012).

Genetic components of means

The absence of significant dominance effects for ILAF in the dominant × additive model can be explained by the manifestation of epistatic effects in the control of the evaluated characteristics. According to Viana (2000), epistatic interactions tend to increase the biases in estimates of additive and dominant genetic components, and thus should be studied using the complete model rather than partial models.

The inconsistencies we found when comparing the complete model to simpler ones can be explained by the occurrence of such a bias. Specifically, there was likely duplicated epistasis in the significant additive × additive and dominant × dominant effects found for both ILAF and ILBF. According to Viana (2000), duplicated epistasis can cause problems in estimating additive and dominance effects because it introduces bias into such estimates. In addition, the dominant × dominant epistasis had a high-magnitude and negative parameter value in the complete model, thus contributing to decreases in internode length.

Additive effects are important in predicting the expression of quantitative characters, while the effects of dominance can be explored through the identification of hybrids due to the dominant nature of the character (Lyimo et al., 2011). If the additive effects of genes on a trait are significant, then the mean trait values of backcrosses tend to be more closer to those of the recurrent parent. As a result, performing repeated backcrosses followed by selection can increase or decrease the value of the desired characteristic, depending on the recurrent parent used (Zewdie and Bosland, 2000). On the other hand, the presence of dominance hinders the selection process in segregating generations due to the difficulty in differentiating dominant homozygous individuals from heterozygotes. In this latter case, selection must be carried out through the evaluation of progenies (Cruz et al., 2001).

The signs of the estimates of genetic effects can vary, with negative values of dominance effects denoting the contribution of dominant alleles to the reduction of the characteristic, while positive values denote their contribution to increases in the trait value (Mather and Jinks, 1982). Therefore, in this case, it can be assumed that the dominance effects observed increased the internode length after flowering, and decreased it before flowering, confirming the reversal of dominance already noted above for this trait.

Genetic components of variance

The main importance of estimating genetic parameters lies in their ability to make predictions that can contribute to improving selection gains. Obtaining estimates of the genetic and environmental components of variance in a trait of interest is of fundamental importance in the evaluation of segregating populations and guides the definition of successful strategies for the selection process (Pimentel et al., 2013). When estimates of a trait's heritability are high, then selection can be carried out on individual plants and in early generations, allowing genetic gains to be obtained quickly; otherwise, selection must be based on tests repeated in several locations and in more advanced generations (Kumar and Wehner, 2013).

The low broad-sense heritability values we found for internode lengths possibly indicate the strong impact of environmental variance on these traits. Lower estimates of heritability indicate that there is less possibility of obtaining selection gains for and more environmental influence on these traits. This means there will be greater difficulty in obtaining gains by selection in early generations, and consequently a selection design using progeny tests is instead recommended (Maris, 1988).

The superior narrow-sense heritability we found for internode lengths compared with broad-sense heritability estimates for these traits can be also explained. According to Kumar and Wehner (2013), this discrepancy is inherent to the generation means analysis method, which is based on measurements obtained from individual plants.

Analyses of the genetic control of internode length before (ILBF) and after emergence of the first female flower (ILAF) via maximum likelihood

The results obtained in this study are similar to those reported in a previous study (Wu et al., 2007), in which reductions in the internode length of winter squash were concluded to be under monogenic control, and with complete dominance. The results of

another previous study also confirmed the occurrence of dominance reversal in squash plants, in which a gene that initially acted as dominant acted as recessive after flowering (Paris and Edelstein, 2001).

A candidate gene that may be the major gene acting before the opening of the first female flower in winter squash is the *Cma_004516* gene that encodes gibberellin (GA) 20-oxidase, an enzyme involved in the GA biosynthesis pathway. According to Zhang et al. (2015), in plants growing with a bush-type habit there was a deletion in the promoter region of this gene, and the expression of this gene was greater in vine-type than in bush-type plants. This shows that *Cma_004516* is a possible candidate gene controlling the growth of the vine in winter squash (Zhang et al., 2015).

The results found in this study provide fundamental information that can be used in defining the selection strategies to be adopted to reduce the internode length of winter squash. Since there is a difference in the genetic control mechanism of this characteristic before and after flowering, the selection strategies for the improvement of this characteristic in each of these phases must be different.

Based on the results of likelihood analyses, the absence of dominance effects in the control of internode length can be attributed to the unavoidable influence of the environment on the expression of this trait. This is because the information assessed in a study of generation means is obtained from measurements of individual plants, with the absence of an experimental design to control for the effects of environmental variations. An alternative method that can be used to reduce such errors in studies of genetic control is to obtain measurements from families derived from F_2, BC_1, and BC_2 plants.

Given the above results, possible strategies that could be used to reduce winter squash internode length include backcrosses for the introgression of the major gene and recurrent selection for the introgression of the polygenes. The selection of winter squash for shorter internodes must be performed both before and after flowering. This selection strategy must consider the possibility of the plants being both heterozygous and homozygous for the major gene, and thus self-fertilization of the selected plants must be performed to target the selection of homozygotes for the major gene in the next generation.

Among the plants that express shorter internode lengths before flowering, one should select those that also express shorter lengths after flowering, since these plants will express higher allelic dosages of genes that can reduce the target characteristic at both developmental stages, and thus provide greater selection gains.

CONCLUSIONS

Analysis of average internode length via the estimation of linear and quadratic genetic components demonstrated the occurrence of dominance effects both before and after flowering in winter squash. A dominance reversal was also noted, in which dominant alleles conferred reduction in internode length before the opening of the first female flower, whereas after the opening of the first female flower this reduction was instead conferred by the recessive alleles of polygenes. Model selection via maximum likelihood analyses showed that the average internode length before the opening of the first female flower in winter squash is controlled by one gene with a greater effect than the others (a major gene) with additive and dominance effects, multiple other polygenes with additive and dominance effects, and environmental effects. On the other hand, the average internode length after the

opening of the first female flower is controlled by additive polygenes plus environmental effects. Based on the results in this study, we suggest that performing backcrosses for the introgression of the gene with the greatest effect and recurrent selection for the introgression of the other polygenes are suitable strategies for selection in segregating populations of winter squash targeting reduced internode lengths.

ACKNOWLEGDMENTS

We thank the Agronomy and general biology Department of the Federal University of Viçosa-UFV and the Brazilian Federal Agency for Support and Evaluation of Graduate Education (Capes) for providing the MSc. fellowship for the first author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Almeida VC, Viana JMS, Risso LA, Ribeiro C, et al. (2018). Generation mean analysis for nitrogen and phosphorus uptake, utilization, and translocation indexes at vegetative stage in tropical popcorn. *Euphytica*. 214: 103.

Carvalho LMJ de, Gomes PB, Godoy RL de O, et al. (2012). Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. *Food Res Int*. 47: 337-340.

Cruz CD and Regazzi AJ (2001). Modelos biométricos aplicados ao melhoramento genético. 2.ed.rev. Viçosa: UFV, 390p.

Cruz CD (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci*. 35: 271-276.

Cruz CD (2005). Princípios de genética quantitativa. Viçosa, UFV, 394p.

Copati MGF, Alves FM, Dariva FD, Pessoa HP, et al. (2019). Resistance of the wild tomato Solanum habrochaites to *Phytophthora infestans* is governed by a major gene and polygenes. *An. acad. bras. ciênc.* 91: e20190149.

Food and Agriculture Organization of the United Nations- FAOSTATE. Food and agriculture Date. Available at: http://www.fao.org.faostat/em/#home>. Accessed: May 20, 2020.

Filgueira FAR (2007). Novo manual de olericultura: agrotecnologia moderna na produção e comercialização de hortaliças. 3. Ed. Viçosa: Universidade Federal de Viçosa, 421p.

Gomes RS, Machado Júnior R, de almeida CF, Chagas RR. et al. (2020). Brazilian germplasm of winter squash (*Cucurbita moschata* D.) displays vast genetic variability, allowing identification of promising genotypes for agro-morphological traits. *Plos One.* 15: e0230546.

Jakab A, Jablonkai I and Forgacs E (2003). Quantification of the ratio of positional isomer dilinoleoyl-oleoyl glycerols in vegetable oils. Rapid Commun. *Mass Spetrom*. 17: 2295- 2302.

Jarret RL, Levy I, Potter TL, Cermak SC, et al. (2013). Seed oil content and fatty acid composition in a genebank collection of *Cucurbita moschata* Duchesne and *C. argyrosperma* C. Huber. *Genet. Resour. Crop. Ev.* 11: 149-157.

Laurindo RDF, Laurindo BS, Delazari FT, Carneiro PC De S, at al. (2017). Potencial de híbridos e populações segregantes de abóbora para teor de óleo nas sementes e plantas com crescimento do tipo moita. *Ceres*. 64: 582-591

Lyimo HJF, Pratt RC and Mnyuku RSOW (2011). Heritability and gene effect estimates for components of partial resistance to grey leaf spot of maize by generation mean analysis. *Plant Breed*. 130: 633-639.

Kozik EU, Klosinska U, Call AD and Wehner TC (2013). Heritability and genetic variance estimates for resistance to downy mildew in cucumber accession Ames 2354. *Crop Sci*. 53: 177-182.

Kumar R and Wehner TC (2013). Quantitative Analysis of Generations for Inheritance of Fruit Yield in Watermelon. *HortScience*. 48: 844-847.

Maris B (1988). Correlations within and between characters between and within generations as a measure for the early generation selection in potato breeding. *Euphytica*. 37: 205-209.

Mather K and Jinks JL (1977). Introduction to biometrical genetics. Ithaca NY. Cornell Univ.

Maynard DN, Elmstrom GW and Carle RB (2002). 'EI Dorado' and 'La Estrella': compact plant tropical pumpkin hybrids. *HortScience*. 37: 831-833.

Paris HS and Edelstein M (2001). Same Gene for Bush Growth Habit in Cucurbita pepo ssp. pepo as in C. pepo ssp. ovifera. *Cucurbit Genetics Cooperative Report*. 24: 80-81.

Patel S (2013). Pumpkin (*Cucurbita* sp.) seeds as nutraceutic: A review on status quo and scopes. *Med. J. Nutrition Metab*. 4: 51-62.

Pimentel AJB, Ribeiro G, Souza MA, Moura LM, at al. (2013). Comparação de métodos de seleção de genitores e populações segregantes aplicados ao melhoramento de trigo. *Bragantia*. 72: 113-121.

Ramalho MAP, Santos JB and Pinto CABP (2012). Genética na agropecuária. Lavras: UFLA. p.472.

Robinson RW, Munger HM, Whitaker TW and Bohn GW (1976). Genes of the Cucurbitaceae. *HortScience*. 11: 554-568.

Rodriguez-Amaya DB, Kimura M, Godoy HT and Amaya-Farfan J (2008). Updated Brazilian database on food carotenoids: Factors affecting carotenoids composition. *J. Food Compos. Anal*. 21: 445-463.

Said AA (2014). Generation mean analysis in wheat (*Triticum aestivum* L.) under drought stress conditions. *Ann. Agric. Sc*. 59: 177-184.

Shifriss O (1947). Developmental reversal of dominance in *Cucurbita pepo*. *J. Am. Soc. Hortic*. 50: 330-346.

Shapiro SS and Wilk MB (1965). An analysis of variance test for normality (completed samples). *Biometrika*. 52: 591-611.

Silva WP (2003). Estimadores de máxima verossimilhança em misturas de densidades normais: uma aplicação em genética. Masters of Science thesis. (Universidade Federal de Lavras, Lavras, 60p.

Sobreira FM (2013). Divergência genética entre acessos de abóbora para estabelecimento de coleção nuclear e pré-melhoramento para óleo funcional. 88p. Ph.D. thesis. Universidade Federal de Viçosa. Viçosa-MG.

Steel RGD and Torrie JH (1980). Principles and procedures of statistics. A biometrical approach, McGraw-Hill Book Company.

Veronezi CM and Jorge N (2015). Chemical characterization of the lipid fractions of pumpkin seeds. *Food sci. nutr*. 45: 164-173.

Viana JMS (2000). Generation mean analysis in relation to polygenic systems with epistasis and fixed genes. *Pesqui. Agropecu. Bras*. 35: 1159-1167.

Veronezi C and Jorge N (2012). Bioactive compounds in lipid fractions of pumpkin (*Cucurbita sp*) seeds for use in food. *J. Food Sci*. 77: C653-C657.

Veronezi CM and Jorge N (2015). Chemical characterization of the lipid fractions of pumpkin seeds. *Nutr Food Sci*. 45: 164-173.

Wu T, Zhou J, Zhang Y and Cao J (2007). Characterization and inheritance of a bush-type in tropical pumpkin (*Cucurbita moschata* Duchesne). *Sci. hortic*. 114: 1-4.

Zalapa JE, Staub JE and Mccreight JD (2006). Generation means analysis of plant architectural traits and fruit yield in melon. *Plant Breed. 125*: 482-487.

Zhang G, Ren Y, Sun H, Guo S, et al. (2015). A high-density genetic map for anchoring genome sequences and identifying QTLs associated with dwarf vine in pumpkin (*Cucurbita maxima* Duch.). *BMC genomics*. *16*: 1101.