Investigation of gene effects on Fruit Shape Index and Seed Size in Generations Resulting from The Crossing of Zucchini and Hull-Less Seed Pumpkin

Sima Davoodi
  Guilan University Faculty of Agriculture

Jamal-Ali Olfati (✉ jamalaliolfati@gmail.com)
  Guilan University Faculty of Agriculture   https://orcid.org/0000-0002-5485-8884

Babak Rabiei
  Guilan University Faculty of Agriculture

Atefeh Sabouri
  Guilan University Faculty of Agriculture

Research Article

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1   **Investigation of gene effects on fruit shape index and seed size in generations resulting from the**
2   **crossing of Zucchini and hull-less seed Pumpkin**

3   Sima Davoodi [1], Jamalali Olfati [2*], Babak Rabiei [3], Atefeh Sabouri [4]

4   1- Ph.D. student, Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of
5   Guilan
6
7   2- Associate Professor, Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of
8   Guilan
9
10   3- Professor, Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, University of
11   Guilan
12
13   4- Associate Professor, Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences,
14   University of Guilan
15

16   * Corresponding Author: jamalaliolfati@gmail.com

17   **Abstract**

18   Fruit shape index (round shape) and seed size have important effects on pumpkin yield. To investigate

19   these traits and create the most desirable state, a cross was made between two pumpkin cultivars. The

20   objective of this study was to estimate the main gene effects (additive, dominant and di-genic epistasis)

21   and to determine the mode of inheritance for fruit shape and seed size by generation mean analysis. Six

22   generations, namely P_1, P_2, F_1, F_2, BC_1, and BC_2 from a cross between Zucchini and hull-less seed

23   Pumpkin, S10×P25, were constructed and evaluated for fruit length, fruit width, fruit shape index, and

24   some seed-related traits (seed length, seed width, seed thickness). The experiment was conducted in the

25   research field of the Faculty of Agricultural Science, University of Guilan, Rasht, Iran in 2019. Results

26   showed a significant difference between generations in terms of fruit and seed traits. Scale and joint scale

27   tests showed the presence of epistasis for some traits. According to the results of the average traits of

28   different generations, standard heterosis and hetrobeltiosis were observed. Concerning the fruit shape

29   index and seed width, there was an over-dominance effect. The broad-sense heritability of the traits was

30   relatively high for all traits and between 52 and 92%. Narrow sense heritability was between 26 and 86%

31   and relatively low for the fruit shape index and seed width. Therefore, selection of elite lines and

32   production of their hybrids are recommended as two methods suitable for breeding to achieve the round

33   shape index and larger seed size.

34   **Keywords:** *Cucurbita pepo* **var.** *styriaca*, *Cucurbita pepo* **var.** *pepo*, **Additive effect, Dominant effect,**
35   **Epistasis, Generation mean analysis**

Introduction

Pumpkin is a plant of the Cucurbitaceae family, which according to the latest classification statistics includes 118 genera and 825 species (Jeffrey et al. 1990). In Cucurbita, five cases of *C. argyrosperma*, *C. ficifolia*, *C. maxima*, *C. moschata*, and *C. pepo* have been domesticated (Schaefer and Renner 2011). Pumpkin has a rich and long history in terms of cultivation and domestication and unique diversity in terms of morphological characteristics, particularly the shape of the fruit and has a wide range of adaptation in cultivation (Savage et al. 2015). Pumpkins have a long history of consumption and are used for many purposes, especially for seed. Despite the production of vegetables in Iran and its ranking in terms of production in global markets and due to the area under cultivation and abundant production of these products in the country, thus far, no significant activity has been performed in the field of seed production, and most of the required seeds have been supplied from abroad (Hosseinzadeh and Amjadi Souraki 2012).

Stewed squash (*Cucurbita pepo* L. var. *pepo*) is one of the species whose plants have limited growth. The seeds of this squash have a uniform cream color; its leaves are multi-lobed with rough hairs on the leaves. The tail of the fruit is hard, woody, and polygonal (angular). Its flowers are characterized by short, thick, and conical flags (Decker-Walters and Walters 2000). In general, the seeds of the edible-fruited cultivar-groups of *C. pepo* subsp. *Pepo* (Pumpkin, Vegetable Marrow, Cocozelle, Zucchini) are longer, wider, and thicker than those of other species of *C. pepo. (Paris and Nerson 2003).*

Hull-less seeded pumpkin (*Cucurbita pepo* L. var. *pepo* subsp. *styriaca*) is a species that has found an important place in the medicine and food industries owing to fatty acids in its seed oil and high protein content (Hosseinzadeh and Amjadi Souraki 2012). It has round fruit and more seeds per fruit. The fruits of hull-less seeded pumpkins are yellowish-orange in color, and the seeds are olive green. One of the most important characteristics of this plant is its skinless seeds (Mitra 2001).

One of the most diverse traits related to squash, which is also important to the consumer, is the shape of the fruit. Fruit shape can vary from round to disc-shaped to very long (Paris 2008). *Cucurbita pepo* L. is highly polymorphic for fruit shape and size. Fruit shape and size are polygenically controlled, and it has proven difficult to identify individual genes affecting these characteristics (Emerson 1910; Sinnott 1936). Fruit shape is a highly important quantitative trait, which is closely associated with fruit quality. There are no genetic models of fruit shapes (Hazra et al. 2007; Paris and Brown 2005; Wang et al. 2012), and no reports are available on the genetic control of fruit shapes in pumpkins. The analysis of fruit shape using the L/W ratio could be a useful approach to select other characteristics of pumpkin shapes (ketsakul et al. 2020). Seed size is an important trait in a hull-less seeded pumpkin, both from seed yield and consumer acceptance viewpoints. As earlier investigations indicated, the highest seed yields

70 could be obtained in small-fruited strains due to the association of fruit size with the seed size (Carle et

71 al. 1994).

72   The hybrid production method can be used to achieve the desired traits, including fruits with a

73 round shape containing larger seeds. In addition, one of the methods to evaluate the results in hybrids is

74 to use the method of generation average analysis and calculation of genetic parameters. Generation means

75 analysis is an efficient technique to estimate important gene effects, such as additive effects, dominance,

76 and interaction in the development of quantitative traits (Amaefula et al. 2014). Generation means

77 analysis method was developed by Jinks and Jones (1958), Hayman (1958), and Mather and Jinks (1982).

78 In general, knowing nature genes involved in the development of traits act is the first step in advancing

79 breeding goals, so that if most of the gene effects are additive, it is recommended that the breeding

80 process be continued through direct selection for the trait. If the genetic effects are predominant, it is

81 recommended that the breeding route be continued to produce hybrid cultivars (Bernardo 2002; Azizi et

82 al. 2006; Rebolloza 2016).

83   According to researchers, crosses were made between PI 285611 and a small-fruited hull-less

84 seeded strain (NH29-13-5, F6) with a moderately large seed (av. 14 g per 100) to study the heritability of

85 fruit and seed traits and the relationship between fruit and seed size. Heritability of fruit weight was

86 relatively low (61.7%). Heritability for seed weight, seed length, and seed thickness was 76.4, 78.7, and

87 85.2%, respectively. A lower heritability for seed width (68.1%) can probably be due to the effect of

88 different degrees of expression of the hull-less trait on seed coat development along the seed margins.

89 Seed thickness and fruit weight did not correlate with each other. Seed weight and fruit weight as well as

90 seed length and fruit weight were positively correlated with each other. Therefore, it was possible to

91 obtain small fruits with large seeds by selections (Carle et al. 1994).

92   Al-Hamadany and Al-Lelah (2010) studied broad sense heritability (BSH), narrow-sense

93 heritability (NSH), and dominance degree for some fruit characteristics (fruit length, fruit diameter, and

94 average fruit weight) in summer squash. Broad sense heritability estimates were moderate to high for the

95 studied traits. Narrow sense heritability was low for the studied characteristics. Over dominance

96 controlled the inheritance of the studied traits. Narrow sense heritability was low and valued 13% (fruit

97 length), 21% (fruit diameter) and 27% (average fruit weight).

98   Paris and Nerson (2003) investigated the seed samples of 174 accessions of *Cucurbita pepo* for

99 the seed length, width, and thickness. They revealed that the mean seed length ranged from 8.8 to 23.3

100 mm, the mean seed width from 5.0 to 12.5 mm, and the mean seed thickness from 1.2 to 3.8 mm. The

101 length-to-width ratio ranged from 1.5 to 2.5, the length-to-thickness ratio from 3.7 to 8.2, and the width-

102 to-thickness ratio from 2.0 to 4.9. Based on the results in *C. pepo*, seed size is positively associated with

103 fruit size, and seed shape is inversely associated with fruit shape.

104   Therefore, in this study, an attempt was made to create a combination through intersection of two
105   pumpkin species having a round shape in terms of fruit shape and a longer length, width and thickness in
106   terms of seed quality. Therefore, appropriate methods should be employed to improve the traits to be
107   determined by examining the effect of the desired genes.

108   **Materials and methods:**

109   Zucchini and hull-less seeded Pumpkin pure lines (P25 and S10) were selected for hybridization to
110   improve fruit shape (round) and seed size (large). Field experiments to generate populations and record
111   field data were performed during three consecutive planting seasons in the spring and summer of 2017-
112   2019 in the research field of the Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran. In the
113   first year, two parental lines, Pumpkin (S10) and Zucchini (P25), which were previously purified by
114   Davoodi et al. (2015), were crossed to produce the seeds of the first generation (F_1). At this stage, the
115   field cultivation system was considered in rows, and planting distance of two meters between rows and
116   one meter on the rows (Sajed et al. 2002; Elizabeth 2001; Latifi et al. 2012) and the steps were performed
117   by implementing the drip irrigation system, using cover mulch, and exercising necessary and appropriate
118   care in all stages of growth. In the second year, F_1 seeds were planted in the field and were self-pollinated
119   to produce second-generation seeds (F_2). Furthermore, many other F_1-generation plants were backcrossed
120   with both parental lines to produce backcrossing generations (BC_1 and BC_2). In the third year, the seeds
121   of the six generations, including two parental lines (P_1 and P_2), along with F_1, F_2, BC_1 and BC_2 were
122   cultivated in a randomized complete block design with three replications in the research field of the
123   Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran. The desired traits, such as fruit
124   length, fruit width, fruit shape index, seed length, seed width, and seed thickness were measured. Length
125   along the axis of each fruit from the place of the peduncle to the tail of the fruit and width at the
126   equatorial region of the fruit were measured. Measurements were performed on each seed using a digital
127   caliper based on millimeters. Seed length along the seed axis, seed width from the widest part of the seed,
128   and seed thickness from the thickest part of the seed were considered.

129   Random samples were selected from each generation, so that from each parent and the first
130   generation, 10 plants per replication and 30 plants, and from each of the backcrosses and the second
131   generation 30 plants per replication and 90 plants, were considered.

132   To analyze the data and evaluate the genetic effects of controlling the studied traits, the method
133   employed by Mather and Jinks (1982) according to Equation (1) was used:

134   (1) $Y = m + \alpha [d] + \beta [h] + \alpha^2 [i] + 2\alpha\beta[j] + \beta^2[l]$

135   Where Y is the mean of one generation, m is the mean of all generations in a cross, [d], [h], [i], [j] and [l]
136   are the effects of genetic parameters of Mather and Jinks' model (1982) (additive, dominance, additive

137 ×additive epistasis, additive × dominance epistasis and dominance × dominance epistasis, respectively,

138 and α, β, α², 2αβ and β² are the coefficients of these genetic parameters, respectively). Equations (2) to (5)

139 were used to test the presence of epistatic effects, and t-Student based on equations (6) to (9) was used to

140 test their significance (Mather and Jinks 1982):

141 (2) $A = 2\overline{BC}_1 - \overline{F}_1 - \overline{P}_1$

142 (3) $B = 2\overline{BC}_2 - \overline{F}_1 - \overline{P}_2$

143 (4) $C = 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2$

144 (5) $D = 2\overline{F}2 - \overline{BC}1 - \overline{BC}2$

145 (6) $t_A = \dfrac{A}{\sqrt{V\bar{P}1 + V\bar{F}1 + 4V\,\overline{BC}1}}$

146 (7) $t_B = \dfrac{B}{\sqrt{V\,\bar{P}1 + V\,\bar{F}1 + 4V\,\overline{BC}2}}$

147 (8) $t_C = \dfrac{C}{\sqrt{V\bar{P}1 + V\bar{P}2 + 4V\,\bar{F}1 + 16V\bar{F}2}}$

148 (9) $t_D = \dfrac{D}{\sqrt{4V\bar{F}2 + V\overline{BC} + V\overline{BC}2}}$

149 To estimate the effects of genes controlling the studied traits, Equations (10) to (15) were used (Mather

150 and Jinks 1982):

151

152 (10) $m = 0.5\overline{P1} + 0.5\overline{P2} + 4\overline{F2} - 2\overline{BC1} - 2\overline{BC2}$

153 (11) $d = 0.5\overline{P1} - 0.5\overline{P2}$

154 (12) $h = 6\overline{BC1} + 6\overline{BC2} - 8\overline{F2} - \overline{F1} - 1.5\overline{P1} - 1.5\overline{P2}$

155 (13) $i = 2(\overline{BC}1 + \overline{BC}2) - 4\overline{F2}$

156 (14) $j = 2(\overline{BC}1 - \overline{BC}2) - \overline{P1} + \overline{P2}$ or $j = 2\overline{BC1} - \overline{P1} - 2\overline{BC2} + \overline{P2}$

157 (15) $l = \overline{P}1 + \overline{P}2 + 2\overline{F1} + 4\overline{F2} - 4\overline{BC}1 - 4\overline{BC}2$

158 To evaluate the best genetic model controlling each of the studied traits, joint scaling tests based on the

159 chi-squared test were used, and the degree of correspondence between the observed and expected means

160 (obtained from the significant values of genetic parameters) of the respective generations were

161 statistically tested.

162 The additive (V_D), dominance (V_H), and environmental (V_E) variance components controlling the

163 phenotypic diversity of each of the studied traits were also calculated based on Equations (16) to (18)

164 (Kearsey and Pooni 1996):

165   (16) $V_D = 2 V_{F2} - (V_{BC1} + V_{BC2})$

166   (17) $V_H = V_{F2} - V_D - V_E$

167   (18) $V_E = \frac{1}{3} (V_{P1} + V_{P2} + V_{F1})$

168   Where V_D is the additive variance, V_H is the dominance variance, V_E is the environment variance, $V_{\overline{P1}}$ is

169   the first parent variance, $V_{\overline{P2}}$ is the first parent variance, $V_{\overline{F2}}$ is the second generation of variance, $V_{\overline{F1}}$ is

170   the first generation of variance, and $V_{\overline{BC1}}$ and $V_{\overline{BC2}}$ are the first and second back-crossing, respectively.

171   To determine the dominance deviations in different gene loci, the mean degree of dominance was

172   estimated based on Equation (19): (Mather and Jinks 1982):

173        $(H/D)^{1/2} = (\frac{2V[H]^{1/2}}{V[D]})$

174   Broad- and narrow-sense heritability were calculated using Equations 20 (Falconer and Mackay 1996)

175   and 21 (Mather and Jinks 1982):

176    $h_b^2 = \frac{V_{F2} - V_E}{V_{F2}} = \frac{V_D + V_H}{V_D + V_H + V_E}$

177    $h_n^2 = \frac{V_D}{V_{F2}} = \frac{V_D}{V_D + V_H + V_E}$

178   The data normality test was performed using the SPSS software, and all other statistical and genetical

179   analyses were conducted using the SAS software (Kang 2003). To compare the means of the studied

180   generations, Tukey's test was used at 5% and 1% probability levels.

181   **Results and discussion:**

182   Examination of the normal distribution in the measured data based on skewness and kurtosis tests

183   using the SPSS software indicated that the data related to all studied traits had a normal distribution

184   (Table 1).

185   The results of the analysis of variance demonstrated a significant difference between the six

186   generations for all studied traits at the probability level 1% (Table 2); in other words, the genetic analysis

187   and review of their inheritance were possible.

188   Evaluation of the mean and standard error of the traits (Table 3) revealed a significant difference

189   between the means of parents and offspring for some traits, such as fruit length and width and seed length

190   and width. Furthermore, the phenomenon of standard heterosis was observed for these traits, meaning

191   that the average of the first generation (F1) for these traits was higher than the average parent. In the case

192   of seed length, the value of the F1 generation obtained from the crossing of two parents was higher than

193   the mean of the parents and even more valuable than the parents. Therefore, the phenomenon of

194   heterobeltiosis (heterosis compared to the best parent) was observed for this trait. These results are very

195   favorable considering the increase of fruit width and seed size. The value of this trait in F2 and BC1

196   generations was higher than that of both parents, while in the BC2 generation (backcrossing with the

197   second parent), it was higher than the average of the parents but lower than the best parent value.

198   According to the results, it is possible that the back-cross method transfers the desired characteristics to

199   progenies. The average of the F2 generation compared to the F1 generation showed a decrease, which can

200   be attributed to the effects of self-pollination and the existence of the phenomenon of inbreeding

201   depression. Saad (2003) also confirmed these results by reporting the presence of heterosis in the fruit

202   and seed traits of squash.

203   For the fruit shape index (fruit length to width ratio), considering that the optimal breeding goal

204   is the round shape (i.e. shape index near to one), the offspring of the new generation has values of the

205   shape index lower than those of the parents, which would be better due to higher yield (Ketsakul et al.

206   2020). Observation of the measurement results of this trait showed that the fruit shape index in the F1

207   generation (1.39) was decreased compared to the average of the parents (1.69), which was favorable

208   according to the intended purpose. On the contrary, the value of this trait did not considerably decrease in

209   the F2 generation compared to the F1 generation. In fact, in terms of achieving fruits with a round to oval

210   shape, a favorable population was obtained. This superiority of offspring over the average of parents can

211   indicate the existence of dominance or over-dominance effects in controlling this trait. The results of

212   backcross generations demonstrated that the fruit shape index in BC1 was less than that in mid-parents;

213   however, in BC2, it was more than that of mid-parents (Table 3). The fruit length of the first and second

214   generations corresponded to the average of the parents. The value of this trait in the offspring of BC1 and

215   BC2 was also less than that of mid-parents but more than that of parents with a lower value. Regarding

216   the fruit width trait, the average of F1 generation was higher than that of parents and higher than that of

217   the best parents. Therefore, the phenomenon of heterobeltiosis was observed for this trait in the current

218   experiment. Moreover, in the offspring of the F2 generation not only there was no decrease compared to

219   the F1 generation but also a higher value was obtained than the average of parents and even higher than

220   parents with a higher value. This result is highly desirable considering that we aimed to reduce the length

221   and increase the width of the fruit to reach the round shape of the fruit. This increase in value was also

222   observed about BC1, while the results of BC2 were decreased compared to both parents.

223   In the case of seed thickness, the mean F1 obtained from the crossing of two parents was lower

224   than the mean of the parents. Furthermore, the value of the F1 generation was less than the average of the

225   parents, but it was close to the average and higher than that of parents. The value of this trait in the results

226   of the F2 generation and the BC1 and BC2 was lower than the average of the parents and more than the

227   value of the parents with less seed thickness.

228   The results of the scaling tests showed that none of the scaling tests A, B, C and D was

229   significant for the fruit shape index, seed length, seed width, and seed thickness traits, demonstrating that

230   the effects of epistasis (non-allelic effects) did not play a crucial role in controlling the diversity of the

231   mentioned traits. However, to ensure the results, it is better to calculate and test the effects of epistasis.

232   On the contrary, for fruit length and width, the B-scaling test was significant at the level of 5%

233   probability, which probably indicates the presence of an additive × dominance interaction (Table 4).

234   Table 5 presents the genetic parameters controlling each of the studied traits. The results

235   indicated that all genetic parameters were significant for fruit length, so that the dominance and

236   dominance × dominance interaction effects and the other components were significant at 5% and 1%

237   probability levels, respectively. In addition, the negative dominance effect and the positive dominance ×

238   dominance interaction effect for fruit length exhibited the presence of the duplicate epistasis effect of

239   reducing dominance alleles. On the contrary, for fruit width, except for the additive× dominance

240   interaction, which was significant at 1% probability level, the other effects were not significant. For the

241   fruit shape index, the additive and the additive × dominance interaction effects were significant at 1%

242   probability level, and the other components were not significant.

243   The results for seed traits indicated that the additive and additive × dominance interaction effects

244   were significant at 5% and 1% probability levels, respectively, while the other components were not

245   significant. Simple effects and interactions were significant except dominant × dominant interaction.

246   Conc Long & ambiguous. Please rewrite more clearly.erning seed thickness, only the additive effect was

247   significant at 1% probability level. The additive effect is investigated as a genetic effect in plant breeding,

248   which is significant according to the researcher and corresponds to the results of the experiment. In

249   addition, dominance genetic variations were significant for all studied mature fruit traits, except for

250   average mature fruit diameter, since it was not significant.

251   Table 6 presents the evaluation of the components of variance, along with heritability and degree

252   of dominance of each of the studied traits. The results indicated that concerning fruit length, fruit width,

253   seed length and seed thickness, the amount of additive variance more than the dominant variance

254   confirms the positive effect of selection on the breeding of these traits. However, in the case of the fruit

255   shape index, the additive variance was higher than the dominant variance, and regarding the seed width,

256   the two additives and dominance variance exhibited the same value.

The results showed that about fruit length, fruit width and seed length, the environmental variance was less than the additive variance but greater than the dominance variance. The importance of additive and dominance variance regarding the fruit shape index and the seed thickness has been reported. This indicates the low effect of the environment on these traits in this experiment, but in the case of seed width, environmental variance revealed a greater effect than both additive and dominance variance did.

Regarding most of traits, broad-sense heritability was relatively high. Furthermore, high narrow-sense heritability in some traits, such as fruit length and seed length, indicated that selection of the best individuals based on these traits could be successful, since the phenotype expresses almost the same genotype (Javanmard et al. 2018). These results were consistent with the findings obtained by Saad (2003), showing that the values of broad- and narrow- sense heritability were high for fruit length; this demonstrates the importance of additive genetic variance in the inheritance of this trait.

Concerning some traits, such as fruit shape index and seed width, relatively low narrow-sense heritability indicates the effect of the dominance component in controlling these traits, and hybrid seed production can be recommended to improve these traits. In other words, low narrow-sense heritability in these traits confirms that there is less effect of additive variance and a positive effect of selection on these traits (Rebolloza 2016). According to Al-Zabaee's reports (2006), broad-sense heritability was high for their studied traits, such as fruit length and fruit diameter, while narrow-sense heritability was moderate.

The average degree of dominance for the fruit shape index and seed width was more than one, which probably indicates the presence of the over-dominance action of the genes controlling these traits; hence, selection of these traits was ineffective. In other words, it is better to use the hybrid production method to improve these traits in the studied population. Other researchers, in studying the gene action of different traits in squash, have reported similar results (Al-Ballat. 2008; Aruah et al. 2010; Najvot 2016). Moreover, the average degree of dominance for fruit length, fruit width, seed length and seed thickness varied from 0.27 to 0.94, demonstrating the relative or partial dominance gene action as well as the importance of both additive and dominance effects in these traits. Accordingly, the selection can be made in early generations (Al-Zabaee 2006; Al-Ballat 2008; Hussien 2015).

Table 1. Data normality test

	Fruit length	Fruit Width	Shape Index	Seed length	Seed width	Seed thickness
Skewness	0.623	0.342	0.918	-0.022	-0.183	1.157
kurtosis	-0.240	-1.193	0.166	-1.105	0.294	1.148
Sig (Kolmogorov-smirnov)	0.20	0.20	0.052	0.20	0.20	0.18
Sig (Shapiro-wilk)	0.17	0.81	0.079	0.22	0.19	0.04

Table 2. Analysis of variance for fruit and seed traits

| Source of Variation | df | Mean squares | | | | | |
		Fruit length	Fruit width	Shape index	Seed length	Seed width	Seed thickness
Replication	2	0.098^{ns}	0.496^{ns}	0.006^{ns}	0.850^{*}	0.198^{ns}	0.102^{ns}
Generation	5	19.163^{**}	9.462^{**}	0.297^{**}	2.312^{**}	1.199^{**}	0.515^{**}
Error	10	1.746	1.074	0.014	0.175	0.089	0.034
C.V.%		6.180	7.356	7.889	2.619	3.280	8.005

288 ns,*,**: Non-significant and significant at 5 and 1% probability levels, respectively.

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Table 3. Comparison of means of the generations for all studied traits

Generation	Fruit length (cm) Mean±SE	Fruit width (cm) Mean±SE	Shape index Mean±SE	Seed length (mm) Mean±SE	Seed width (mm) Mean±SE	Seed thickness (mm) Mean±SE
P1	18.266±0.044	13.666±0.12	1.336±0.008	14.703±0.042	7.705±0.025	1.930±0.029
P2	25.576±0.053	12.433±0.06	2.056±0.014	15.709±0.043	8.954±0.046	3.105±0.028
F1	22.113±0.409	15.950±0.579	1.390±0.035	17.152±0.142	9.477±0.196	2.247±0.039
F2	21.856±1.139	14.653±0.833	1.496±0.095	16.123±0.512	8.500±0.078	2.134±0.189
BC1	19.445±0.827	16.050±0.697	1.216±0.104	16.706±0.395	9.111±0.078	2.132±0.150
BC2	21.023±0.886	11.803±0.193	1.783±0.073	15.503±0.363	8.815±0.148	2.450±0.174
HSD(0.01)	4.904	3.847	0.452	1.554	1.068	0.693
HSD(0.05)	3.747	2.94	0.346	1.187	0.816	0.529

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Table 4. Scaling tests to assess the epistatic (non-allelic) effects

Scaling test	Fruit length	Fruit width	Shape index	Seed length	Seed width	Seed thickness
A	1.489^{ns}	-2.484^{ns}	0.294^{ns}	-1.117^{ns}	-0.907^{ns}	-0.087^{ns}
B	5.643^{*}	4.777^{*}	-0.12^{ns}	1.444^{ns}	0.846^{ns}	0.452^{ns}
C	0.678^{ns}	-0.612^{ns}	0.188^{ns}	0.062^{ns}	1.811^{ns}	0.993^{ns}
D	3.244^{ns}	1.453^{ns}	-0.007^{ns}	0.018^{ns}	-0.936^{ns}	-0.314^{ns}

293 ns,*,**: Non-significant and significant at 5 and 1% probability levels, respectively.
294 Significance levels are shown based on the t-test compared to table t.

295

296 Table 5. Estimation of genetic effects for different generations resulting from the Zucchini and Hull-less seeded
297 Pumpkin for the studied traits

Genetic parameter	Fruit length	Fruit width	Shape index	Seed length	Seed width	Seed thickness
m	28.411^{**}	15.956^{**}	1.683^{**}	15.279^{**}	6.460^{**}	1.889^{**}
d	-3.655^{**}	0.616^{ns}	-0.360^{**}	-0.503^{*}	-0.624^{**}	-0.587^{**}
h	-19.919^{*}	-5.206^{ns}	-0.453^{ns}	1.503^{ns}	5.045^{*}	0.619^{ns}
i	-6.489^{ns}	-2.906^{ns}	0.013^{ns}	-0.072^{ns}	1.870^{**}	0.628^{ns}
j	4.154^{ns}	7.260^{**}	-0.413^{**}	3.412^{**}	1.752^{*}	0.538^{ns}
l	13.622^{*}	5.200^{ns}	0.160^{ns}	0.370^{ns}	-1.929^{ns}	-0.261^{ns}
x^2	0.160^{ns}	0.035^{ns}	0.0019^{ns}	0.0004^{ns}	0.0092^{ns}	0.035^{ns}

298 ns,*,**: Non-significant and significant at 5 and 1% probability levels, respectively.
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302 Table 6. Estimation of generation variance components for different generations resulting from the cross between
303 Zucchini and Hull-less seeded Pumpkin for the studied traits

Variance component	Fruit length	Fruit width	Shape index	Seed length	Seed width	Seed thickness
Additive variance	1.698	0.719	0.002	0.356	0.009	0.028
Dominance variance	0.086	0.072	0.004	0.013	0.009	0.012
Environmental variance	0.172	0.353	0.001	0.023	0.016	0.003
Narrow-sense heritability	0.86	0.62	0.28	0.90	0.26	0.64
Broad-sense heritability	0.91	0.69	0.85	0.94	0.52	0.92
The average degree of dominance	0.31	0.44	2	0.27	1.41	0.94

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