

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

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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**

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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 P₁, P₂, F₁, F₂, BC₁, and BC₂ 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**

37 **Introduction**

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

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

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

60 One of the most diverse traits related to squash, which is also important to the consumer, is the
61 shape of the fruit. Fruit shape can vary from round to disc-shaped to very long (Paris 2008). *Cucurbita*
62 *pepo* L. is highly polymorphic for fruit shape and size. Fruit shape and size are polygenically controlled,
63 and it has proven difficult to identify individual genes affecting these characteristics (Emerson 1910;
64 Sinnott 1936). Fruit shape is a highly important quantitative trait, which is closely associated with fruit
65 quality. There are no genetic models of fruit shapes (Hazra et al. 2007; Paris and Brown 2005; Wang et
66 al. 2012), and no reports are available on the genetic control of fruit shapes in pumpkins. The analysis of
67 fruit shape using the L/W ratio could be a useful approach to select other characteristics of pumpkin
68 shapes (ketsakul et al. 2020). Seed size is an important trait in a hull-less seeded pumpkin, both from seed
69 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 (F₁). 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, F₁ seeds were planted in the field and were self-pollinated
119 to produce second-generation seeds (F₂). Furthermore, many other F₁-generation plants were backcrossed
120 with both parental lines to produce backcrossing generations (BC₁ and BC₂). In the third year, the seeds
121 of the six generations, including two parental lines (P₁ and P₂), along with F₁, F₂, BC₁ and BC₂ 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 \times dominance epistasis and dominance \times dominance epistasis, respectively,
 138 and α , β , α^2 , $2\alpha\beta$ and β^2 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) $t_A = \frac{A}{\sqrt{V\overline{P}_1 + V\overline{F}_1 + 4V\overline{BC}_1}}$

146 (7) $t_B = \frac{B}{\sqrt{V\overline{P}_1 + V\overline{F}_1 + 4V\overline{BC}_2}}$

147 (8) $t_C = \frac{C}{\sqrt{V\overline{P}_1 + V\overline{P}_2 + 4V\overline{F}_1 + 16V\overline{F}_2}}$

148 (9) $t_D = \frac{D}{\sqrt{4V\overline{F}_2 + V\overline{BC}_1 + V\overline{BC}_2}}$

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) $m = 0.5\overline{P}_1 + 0.5\overline{P}_2 + 4\overline{F}_2 - 2\overline{BC}_1 - 2\overline{BC}_2$

153 (11) $d = 0.5\overline{P}_1 - 0.5\overline{P}_2$

154 (12) $h = 6\overline{BC}_1 + 6\overline{BC}_2 - 8\overline{F}_2 - \overline{F}_1 - 1.5\overline{P}_1 - 1.5\overline{P}_2$

155 (13) $i = 2(\overline{BC}_1 + \overline{BC}_2) - 4\overline{F}_2$

156 (14) $j = 2(\overline{BC}_1 - \overline{BC}_2) - \overline{P}_1 + \overline{P}_2$ or $j = 2\overline{BC}_1 - \overline{P}_1 - 2\overline{BC}_2 + \overline{P}_2$

157 (15) $l = \overline{P}_1 + \overline{P}_2 + 2\overline{F}_1 + 4\overline{F}_2 - 4\overline{BC}_1 - 4\overline{BC}_2$

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_{P1} is
 169 the first parent variance, V_{P2} is the first parent variance, V_{F2} is the second generation of variance, V_{F1} is
 170 the first generation of variance, and V_{BC1} and V_{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 $(H/D)^{1/2} = \left(\frac{2V[H]^{1/2}}{V[D]} \right)$

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 \times 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 \times 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 \times
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 \times 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 \times 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 \times 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 \times dominant interaction.
246 Conc Long & ambiguous. Please rewrite more clearly. uring 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.

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

263 Regarding most of traits, broad-sense heritability was relatively high. Furthermore, high narrow-
 264 sense heritability in some traits, such as fruit length and seed length, indicated that selection of the best
 265 individuals based on these traits could be successful, since the phenotype expresses almost the same
 266 genotype (Javanmard et al. 2018). These results were consistent with the findings obtained by Saad
 267 (2003), showing that the values of broad- and narrow- sense heritability were high for fruit length; this
 268 demonstrates the importance of additive genetic variance in the inheritance of this trait.

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

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

284

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

285
286

287

Table 2. Analysis of variance for fruit and seed traits

Mean squares							
Source of Variation	df	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.

289

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

Generation	Fruit length (cm)	Fruit width (cm)	Shape index	Seed length (mm)	Seed width (mm)	Seed thickness (mm)
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	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

291

292 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 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}
χ^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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300

301

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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305 **REFERENCES:**

306 Al-Ballat, IA (2008) Breeding studies on summer squash crop (*Cucurbita pepo* L.). Dissertation, Faculty
 307 of Agriculture, Tanta University.

308 Al-Hamadany SYH, Al-Lelah WBA (2010) Estimating of heterosis and genetic variability in summer
 309 Squash (*Cucurbita pepo* L.). MJA 38(4): 27-37

310 Amaefula C, Agbo CU, Nwofia GE (2014) Hybrid vigor and genetic control of some quantitative traits of
 311 tomato (*Solanum lycopersicum* L.). OJGen 4: 30-39. DOI:[10.4236/ojgen.2014.41005](https://doi.org/10.4236/ojgen.2014.41005)

312 Aruah C, Uguru M, Oyiga B (2010) Variations among some Nigerian Cucurbita landraces. Afr. J. Plant
 313 Sci 4: 374-386

314 Al-Zabae HA (2006) Estimating genetic parameters by diallel crossing in summer squash. AJAS 4(1):
 315 169-180

316 Azizi F, Rezaie A, Saeidi G (2006) Generation means analysis to estimate genetic parameters for
 317 different traits in two crosses of Corn inbred lines at three planting densities. J. Agric. Sci. Technol 8:
 318 153-169

319 Bernardo R (2002) Breeding for quantitative traits in Plants, 3rd edn. Stemma Press. 369 p.

320 Carle BR, Loy JB (1994) Heritability of seed size in hull-less seeded strains of *Cucurbita pepo* L. CGC
 321 Report 17:125-27. <https://doi.org/10.21273/HORTSCI.29.4.245c>

322 Decker-Walters D, Walters T (2000) Squash. In: Kiple KF, Ornelae KC (ed) The Cambridge world
 323 history of food. Cambridge University Press, pp 335-351 .

324 Davoodi S, Olfati JA, HamidaOghli Y, Sabouri A (2015) Standard hetrosis in *Cucurbita moschata* and
 325 *Cucurbita pepo* interspecific hybrids. Int. J. Veg. Sci. 22(4):383-388.
 326 <https://doi.org/10.1080/19315260.2015.1042993>

327 Elizabeth TM (2001) Plant spacing demonstration plot with Jack-o- Lantern and giant pumpkins. In:
328 Morales MR. (ed.) Midwestern vegetable variety trial report for 2001. Purdue, pp 225–227.

329 Emerson RA (1910) The inheritance of sizes and shapes in plants. Am. Nat 44: 739–746.
330

331 Falconer DS, Mackey TFC (1996) Introduction to quantitative genetics 4rd edn. Department of Genetics
332 Society of America, 479p.
333

334 Hazra P, Mandal AK, Dutta A K, Ram HH (2007) Breeding pumpkin (*Cucurbita moshata* Duch. ex
335 Poir.) for fruit yield and other characters. Int. J. Plant Breed. 1:51-64
336

337 Hussien AH (2015) Nature of gene action and heterotic performance for yield and yield components in
338 summer squash (*Cucurbita pepo* L.). Int. J. Plant prod, Mansoura University 6(1): 29 – 40.
339 DOI:[10.21608/jpp.2015.49274](https://doi.org/10.21608/jpp.2015.49274)

340 Hosseinzadeh Colagar A, Amjadi Souraki O (2012) Review of Pumpkin Anticancer Effects. Quran
341 Medicine, MJIRI 1(4): 93-104. DOI:[10.5812/QURANMED.8923](https://doi.org/10.5812/QURANMED.8923)
342

343 Hayman BI (1958) The separation of epistasis from additive and dominance variation in generation
344 means. Heredity 12(3): 371-390

345 Javanmard T, Soltani Saleh-Abadi F, Bihamta MR (2018) Estimation of some genetic parameters through
346 generation mean analysis in melon. Indian J. Agric. Res. 52: 619-624. DOI: [10.18805/IJARE.A-355](https://doi.org/10.18805/IJARE.A-355)

347 Jinks JH, Jones RM (1958) Estimation of components of heterosis. Genetics 43(2): 223-234.
348

349 Jeffrey C (1990) Systematic of the Cucurbitaceae. In: Bates DM, Robinson RW, Jeffrey C (ed) Biology
350 and Utilization of the Cucurbitaceae, Cornell University Press, Ithaca, pp 3-9.

351 Kang MS (2003). Handbook of formulas and software for plant geneticists and tree breeders. Food
352 Products Press. 69p.

353 Kearsey MJ, Pooni HS (1996) The genetic analysis of quantitative traits. Chapman and Hull.
354 <http://dx.doi.org/10.1007/978-1-4899-4441-2>
355

356 Ketsakul S, Imsabai W, Tangtrakulwanich K, Auvuchanon A (2020) Identification of genes controlling
357 fruit shape in thai pumpkin (*Cucurbita moschata* Duch.). IJAT 16(3): 629-640

358 Latifi M, Barimavandi A, Sedaghatoor S Lipayi SR (2012) Sowing date and plant population effects on
359 seed yield of *Cucurbita pepo*. Int J Agric Biol 14: 641–644

- 360 Mather K, Jinks JL (1982) Biometrical Genetics. (Third Ed.).Champan and Hall. 398p.
361 <https://doi.org/10.1002/bimj.4710260111>
- 362 Mitra J (2001) Genetics and genetic improvement of drought resistance in a crop plant. Curr. Sci. 80(6):
363 758-763.
- 364 Navjot K (2018) Genetic studies of yield and its component traits using generation mean analysis in
365 Summer squash (*Cucurbita pepo* subsp. *pepo*). Veg. Sci. 45(2): 154-160
- 366 Paris HS (2008) Summer Squash, pp 351-379.In: J. Prohens, and F. Nuez (eds.). Handbook of Plant
367 Breeding.Vegetables I. Springer, Heidelberg.
- 368 Paris HS, Brown RN (2005) The Genes of Pumpkin and Squash. Hort Science 40:1620-1630.
369 <https://doi.org/10.21273/HORTSCI.40.6.1620>
- 370 Paris HS, Nerson H (2003) Seed **Dimensions in the Subspecies and Cultivar-groups of *Cucurbita***
371 ***pepo*. Genetic. Resour. Crop Evol 50:** 615–625. DOI:[10.1023/A:1024464831595](https://doi.org/10.1023/A:1024464831595)
- 372 Rebolloza-Hernández H, Castillo-Gutiérrez A, Carapia-Ruíz VE (2016) Estimation of genetic parameters
373 and selection of S1 lines in a segregating population of tropical maize. Rev. Mexicana Cienc. Agríc
374 7(8):8, 1893–1904.
- 375 Saad MS (2003) Inheritance of some economical traits in squash (*Cucurbita pepo* L.). Dissertation,
376 Faculty of Agriculture, Mansoura University, Egypt.
- 377 Schaefer H, Renner SS (2011) Phylogenetic relationships in the order Cucurbitales and a new
378 classification of the gourd family (Cucurbitaceae). *Taxon*, 60: 122-138. DOI:[10.1002/tax.601011](https://doi.org/10.1002/tax.601011)
- 379 Savage JA, Haines DF, Holbrook NM (2015) The making of giant pumpkins: how selective breeding
380 changed the phloem of *Cucurbita maxima* from source to sink. Plant, Cell & Environment 38
381 (8):1543–1554. DOI: [10.1111/pce.12502](https://doi.org/10.1111/pce.12502)
- 382 Sajed MA, Hosseini-Moghaddam H, Yazdani D, Ahmadi-Aval P (2002) Effect of plastic mulch of soil,
383 plant spacing and P and K fertilization level on growth and seed and oil yield of medicinal Squash.
384 Proceedings of Iranian Conference on Medicinal Herbs. 13-15 Feb. Tehran, Iran. pp.188.
- 385 Sinnott GW (1936) A developmental analysis of inherited shape differences in cucurbit fruits. Am.Nat.
386 70: 245–254. DOI:[10.1086/280661](https://doi.org/10.1086/280661)
- 387
388 Wang YH, Behera TK, Kole C (2012) Genetics, Genomics and Breeding of Cucurbits. Taylor & Francis
389 Group. Boca Raton, FL. <https://doi.org/10.1201/b11436>