
Identification of genes controlling fruit shape in Thai pumpkin (*Cucurbita moschata* Duch.)

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Abstract The genetic control of fruit shape in pumpkins was determined in the fields during 2015 and 2017. The breeding design consisted of seven crosses derived from inbred pumpkin lines. The parents originated from Thai landrace cultivars maintained by continuous selfing at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. Different qualitative characters of fruit shapes of parents were chosen, including three elongated, two flattened and two globular shaped lines. The fruit shape and length/width (L/W) ratio of all parents and derived cross-progenies (F₁ and F₂) were evaluated. The results showed that all genotypes controlling the fruit shape hypotheses resulted from the influence of three genes. The genotype Ff, Gg, and Ee resulted in a globular fruit shape, whereas ff, gg, and ee led to an elongated shape. A flattened shape was controlled by FF, GG, and EE. In terms of gene action, the results supported ee dominance over ffG- and F-gg. The elongated shape phenotype was due to the action of ffggee, ffG-ee, and F-ggee genes. This research suggests that the analysis of fruit shape using the L/W ratio could be a useful approach for selecting other characteristics of pumpkin shapes.

Keywords: Epistasis, Multiple genes, Incomplete dominance

Introduction

Cucurbita species, including pumpkins and squashes, are among some of the most important vegetable crops worldwide in terms of their economic value. They are grown in 177 countries, covering an area of 1,992,003 hectares, with 26,486,616 ton produced (FAOSTAT, 2016). Although the terms pumpkin and squash are sometimes used interchangeably, they are not the same. Edible, round *Cucurbita* fruits are usually called pumpkins, whereas those that are not round are called squashes (Paris, 2005). *Cucurbita moschata* has diverse fruit shapes, such as globular, round, flattened, dumbbell, disc, oblong, cylindrical, oval, acorn, heart shaped, pyriform, crookneck, or elongated (Hazra *et al.*, 2007). In Thailand, *C. moschata* with a flattened and round shape accounts for the

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majority of cultivated pumpkin varieties. Based on differences in fruit shapes, pumpkins are given alternative names, such as butternut, cheese pumpkin, musky ground and winter crookneck squash. Previous studies reported that watermelon fruit shape was controlled by a polygene and that a single incompletely dominant gene had a major effect. Not only the typical individuals had the homozygous recessive gene but also the homozygous dominant gene was detectable in their phenotypes (Jos é *et al.*, 2005; Tanaka *et al.*, 1995). Quantitative traits of fruit shape, ovary shape and fruit width in melon were detected near the pentamerous locus (*p* locus) and major QTL showed variations in fruit length and fruit shape linkages among andromonoecious individuals, in which fruit shape and ovary shape were co-segregation (P érin *et al.*, 2002). A study that investigated fruit shape traits using diallel crosses of sponge gourd (*Luffa cylindrica*) reported that these traits were affected by genes and the environment with fruit shape in particular controlled by direct genes and maternal plant genes (Zhang *et al.*, 2008). Hazra *et al.* (2007) reported five pumpkin fruit shapes (globular, flattened, acorn, elongated, and oval) which depended on the shape of the ovary. An oval ovary shape produced flattened or oval-shaped fruit, whereas globular, elongated, or acorn-shaped ovaries gave rise to globular elongated, and acorn-shaped fruits (Mandal, 2005). According to studies on the genetic control of fruit shape, the *Di* gene controlled fruit shape (disc versus pyriform or spherical shapes) in *C. pepo*, whereas the *Bn* gene, a dominant gene, controlled fruit shape (bell shape) in butternut (Paris and Brown, 2005; Wang *et al.*, 2012). In contrast, a homozygous recessive gene controlled the elongated crookneck shape in *C. moschata* (Paris and Brown, 2005; Wang *et al.*, 2012). Paris and Padley (2014) reported that the butternut fruit shape gene (*Bn*) in New Hampshire butternut was dominant over *bn* in terms of crookneck fruit shape in Canada crookneck and in scallop squash, the disc fruit shape trait was dominant over the spherical or pyriform shape. In addition, Hazra *et al.* (2007) reported an overdominant effect for fruit shape.

Fruit shape is a very important quantitative trait, which is closely related to 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 objective of this study was to determine the inheritance of fruit shape characteristics in a cross between Thai pumpkin inbred lines with elongated, globular, and flattened fruit shapes.

Materials and Methods

Plant materials

The experiment was conducted between 2015 and 2017. Seven crossed mating designs were Thai pumpkin inbred lines derived from Thai landrace pumpkin cultivars maintained by continuous selfing at Kasetsart

University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand (Table 1). The parental lines comprised three varieties with an elongated shape (KAN(or), KAN, and CM2), two with a flattened shape (Sri 8/5-T and KAN3), and two with a globular shape (KAN1 and KPS1). After the parental crosses, the fruit shapes of F₁ and F₂ progeny were evaluated.

Table 1. The mating design in the experiment

| Crossing | Shape |
|--------------------|-----------------------|
| KAN × CM2 | Elongated × Elongated |
| CM2 × KPS1 | Elongated × Globular |
| CM2 × KAN3 | Elongated × Flattened |
| Sri8/5-T × KAN(or) | Flattened × Elongated |
| Sri8/5-T × KAN1 | Flattened × Globular |
| Sri8/5-T × KPS1 | Flattened × Globular |
| Sri8/5-T × KAN3 | Flattened × Flattened |

To determine the fruit shape, the ratio of the polar length (peduncle to blossom end), referred to as the fruit length, was measured and from the polar point to the equatorial line of the fruit was classified as the width of the fruit (measured half way between the peduncle and blossom end) (Fig. 1). The ratio of fruit length (L) to fruit width (W) (L/W ratio) was recorded. An L/W ratio less than 0.55 was classified as a flattened type, whereas an L/W ratio between 0.55 and 1.50 was considered a globular type or intermediate long fruit type. An L/W ratio of more than 1.50 was classified as an elongated type.

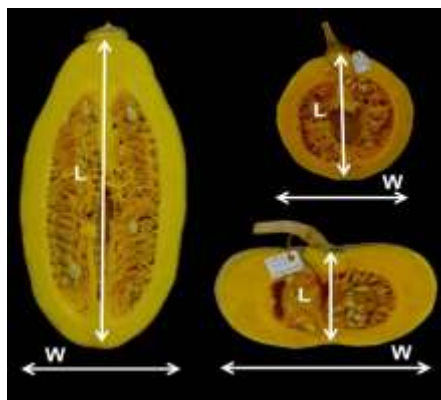


Figure 1. Fruit shape index measurement. The L/W ratio of the polar diameter (peduncle to blossom end) referred to as fruit length (L), to the equatorial width (W) of the fruit

Data analysis

The segregations of allele pairs for fruit shapes were observed and recorded, and the most appropriate segregation ratio was chosen. The normal distribution of the fruit shape ratio in F₂ populations was tested using the Shapiro–Wilk test (Shapiro and Wilk, 1965). The goodness of fit of a range of segregation patterns to a Mendelian ratio was analyzed using chi-square tests at 5% level of significance. The null hypothesis was that there would be no differences in segregation patterns. An alternative hypothesis was that there would be a difference between the predicted and observed results. Calculating the chi-square was done using the following formula:

$$x^2 = \sum \frac{(O-P)^2}{P},$$

where \sum was the sum, O was the observed value, and P was the predicted value.

Results

Genes controlling pumpkin fruit shape in seven inbred lines with diverse fruit shapes (three elongated lines, two flattened lines, and two globular lines) were investigated. The parents and each hybrid fruit shape, in addition to the L/W ratio, are shown in Tables 1 and 2. The mean L/W ratios in the elongated parental lines CM2, KAN(or), and KAN were 2.09 ± 0.14 , 1.92 ± 0.24 , and 1.63 ± 0.09 , respectively. In the globular shaped parental lines (KPS1 and KAN1), the mean L/W ratios were 0.64 ± 0.17 and 0.62 ± 0.07 , respectively. The mean L/W ratios in the flattened shaped parental lines (KAN3 and Sri 8/5-T) were 0.47 ± 0.05 and 0.39 ± 0.03 , respectively. Seven crossing pairs were designed: elongated \times elongated, elongated \times globular, elongated \times flattened, flattened \times elongated, flattened \times globular, and flattened \times flattened (Table 1). When KAN (elongated shape) was crossed with CM2 (elongated shape), all F₁ and F₂ progenies had an elongated fruit shape. Similar results were found for the flattened shape, with all F₁ and F₂ progenies of flattened \times flattened crosses producing fruit with a flattened shape. There were three fruit shape groups of F₁ populations. All elongated fruits were F₁KAN/CM2, which belonged to elongated shape parent lines. The intermediate long shape (globular) lines were F₁CM2/KAN1, F₁CM2/KPS1, F₁CM2/K, and F₁Sri8/5-T//KAN(or). The flattened fruits were F₁Sri8/5-T//KPS1 and F₁Sri8/5-T//KAN1. Hybrids of the elongate \times flattened and elongate \times globular fruit shapes resulted in intermediate long shape as globular showing incomplete dominance (Table 2).

Table 2. Classification of the long fruit type in parents and F₁ and F₂ populations

| Lines | Shape | L/W ratio |
|----------------------------------|---------------------------|------------------------------------|
| Parent | | |
| CM2 | Elongated | 2.09 ± 0.14 |
| KAN | Elongated | 1.63 ± 0.09 |
| KAN(or) | Elongated | 1.92 ± 0.24 |
| KAN1 | Globular | 0.62 ± 0.07 |
| KPS1 | Globular | 0.64 ± 0.17 |
| KAN3 | Flattened | 0.47 ± 0.05 |
| Sri8/5-T | Flattened | 0.39 ± 0.03 |
| Fruit type classification | | |
| Crossing | F ₁ population | F ₂ population |
| KAN/CM2 | Elongated | Elongated |
| CM2/KPS1 | Globular | Elongated and Globular |
| CM2/KAN3 | Globular | Flattened, Globular, and Elongated |
| Sri8/5-T//KAN(or) | Globular | Flattened, Globular, and Elongated |
| Sri8/5-T//KAN1 | Flattened | Flattened and Globular |
| Sri8/5-T//KPS1 | Flattened | Flattened and Globular |
| Sri8/5-T//KAN3 | Flattened | Flattened |

Distribution of fruit shape

The distribution of the long fruit shape indices in F₂ populations is shown in Figure 2. The Shapiro–Wilk test revealed a high range of variation among the three F₂ populations derived from the crosses between the elongated shape and other fruit shape lines which were F₂Sri8/5-T//KAN(or), F₂CM2/KAN3, and F₂CM2/KPS1, showing non-normal distribution ($p < 0.05$). We found about 6-fold variation for each population from maximum to minimum of L/W ratio values (Fig. 2). The L/W ratios for F₂Sri8/5-T//KAN(or) ranged from 2.47 to 0.39, whereas those for F₂CM2/KAN3 ranged from 2.86 to 0.44. The L/W ratios for F₂CM2/KPS1 ranged from 3.11 to 0.54. The frequency distributions of the fruit shape indices for F₂KAN/CM2, F₂Sri8/5-T//KPS1, F₂Sri8/5-T//KAN1, and F₂Sri8/5-T//KAN3 were normal, as shown by the Shapiro–Wilk test ($p > 0.05$). The L/W ratios for F₂KAN/CM2, F₂Sri8/5-T//KPS1, F₂Sri8/5-T//KAN1, and F₂Sri8/5-T//KAN3 ranged from 2.85 to 1.45, 0.60 to 0.38, 0.66 to 0.39, and

0.54 to 0.34, respectively. F₂ generations from elongated × flattened or elongated × globular shape parental lines were segregated into two groups: an elongated shape and flattened/globular shape (Figs. 2A, B).

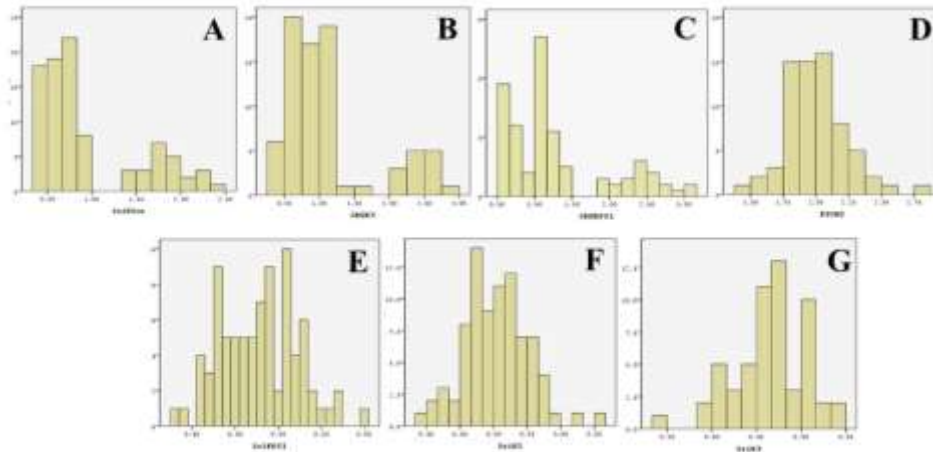


Figure 2. Frequency distribution of the L/W ratio in seven F₂ populations; F₂Sri8/5T//KAN(or) (A), F₂CM2/KAN3 (B), F₂CM2/KPS1 (C), F₂KAN/CM2 (D), F₂Sri8/5T//KPS1 (E), F₂Sri8/5T//KAN1(F), and F₂Sri8/5T//KAN3(G)

The fruit shape index (L/W ratio) was used to classify F₂ individuals by a chi-square analysis for the goodness of fit to determine the segregation pattern (Table 3).

Table 3. Distribution of fruit shape in seven F₂ populations

| F ₂ populations | Parent shape | L/W ratio | | | No. of F ₂ plants | | | Ratio | χ^2 |
|----------------------------|--------------|-----------|------|------|------------------------------|----|----|-------|-----------------------------|
| | | Mean | Max | Min | F | G | E | | |
| KAN/CM2 | E x E | 2.00±0.05 | 2.85 | 1.62 | 0 | 0 | 68 | | |
| CM2/KPS1 | E x G | 1.28±0.47 | 3.11 | 0.56 | 0 | 78 | 23 | G:E | 3:1 0.27 ^{ns} |
| CM2/KAN3 | E x F | 1.13±0.46 | 2.86 | 0.44 | 10 | 53 | 15 | F:G:E | 3:9:4 4.40 ^{ns} |
| Sri 8/5T//KAN(or) | F x E | 0.95±0.33 | 2.47 | 0.39 | 22 | 45 | 21 | F:G:E | 21:32:11 3.94 ^{ns} |
| Sri 8/5T//KAN1 | F x G | 0.50±0.00 | 0.66 | 0.39 | 69 | 14 | 0 | F:G | 3:1 2.93 ^{ns} |
| Sri 8/5T//KPS1 | F x G | 0.48±0.00 | 0.60 | 0.38 | 78 | 5 | 0 | F:G | 15:1 0.01 ^{ns} |
| Sri 8/5T//KAN3 | F x F | 0.46±0.00 | 0.54 | 0.34 | 57 | 0 | 0 | | |

$\chi^2_{(1, 0.05)} = 3.841$, $\chi^2_{(2, 0.05)} = 5.991$, ^{ns} = nonsignificant, * = significant at 5% probability; Fruit shape was classified as flattened (F), globular (G), or elongated (E). F indicates an L/W ratio < 0.55, G denotes an L/W ratio of 0.55–1.50, and E indicates an L/W ratio of > 1.50.

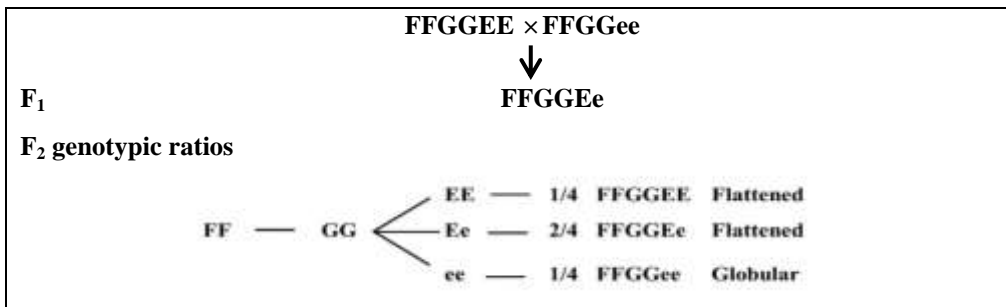


Figure 3. Genotype and genotypic ratios of F₁ and F₂ progenies derived from Sri8/5-T × KAN1

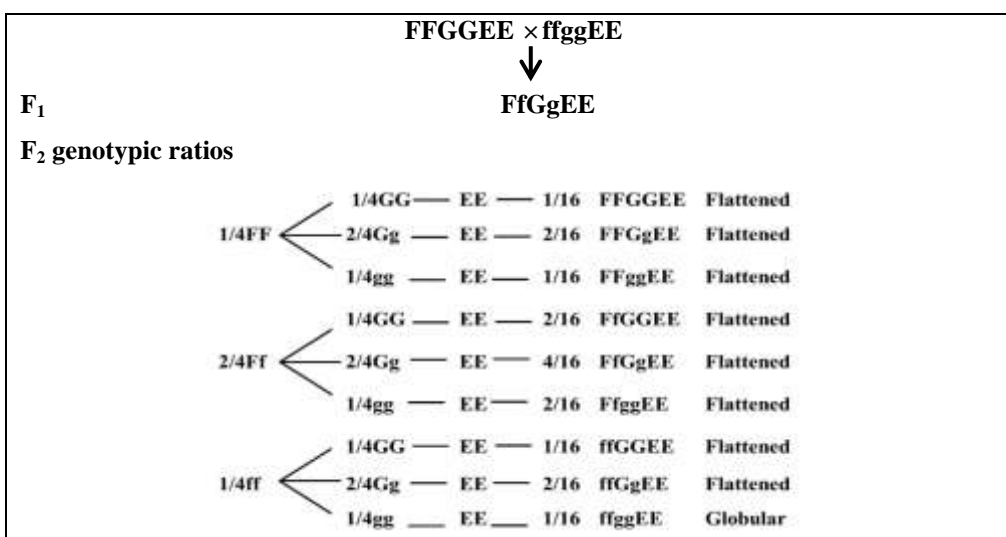


Figure 4. Genotype and genotypic ratios of F₁ and F₂ progenies derived from Sri8/5-T × KPS1

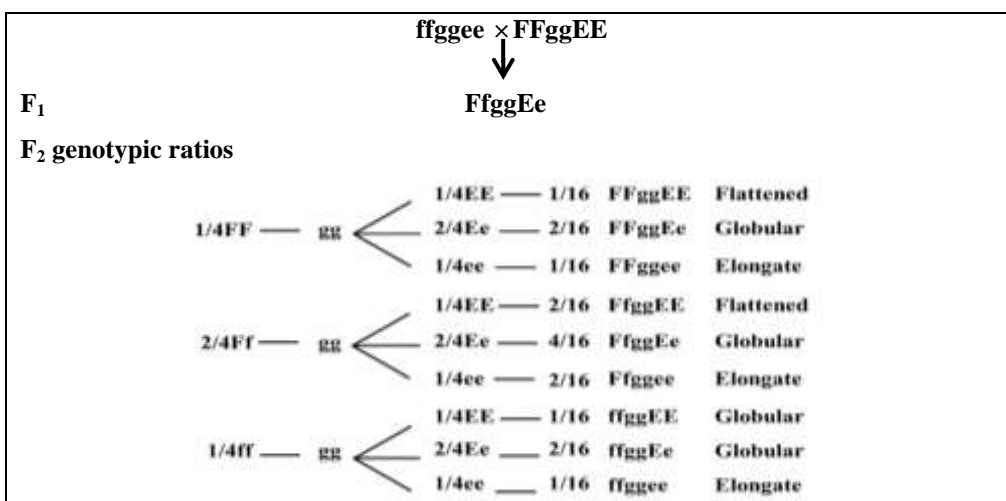


Figure 5. Genotype and genotypic ratios of F₁ and F₂ progenies derived from CM2 × KAN3

Inheritance of fruit shape

The hypothesis of this study was that the elongated fruit shape in *C. moschata* could be controlled by homozygous recessive alleles, whereas the elongated crookneck shape could be controlled by homozygous recessive alleles, as reported by Paris and Brown (2005) and Wang *et al.* (2012). The ratio of F₂Sri8/5T//KAN(or), which was a flattened × elongated, resulted in flattened globular and elongated shapes with 21:32:11, respectively, and the chi-square test was nonsignificant. The hypothesis from this cross was that characteristic of the elongated shape of KAN(or) was controlled by homologous recessive alleles (ee). The flattened shape of Sri8/5T was EE, and the globular shape was incomplete dominance Ee.

The two crosses between the flattened × globular shapes were Sri8/5-T//KPS1 and Sri8/5-T//KAN1. The F₂Sri8/5-T//KPS1 fruit shape ratio was 78:5 or 15:1 of the flattened and globular shapes. These ratios suggested that fruit shape was controlled by two genes (*F* and *G*). The flattened shape of Sri8/5-T was controlled by the FFGG genotype, and the globular KPS1 was the ffgg genotype (Fig. 4). However, the crossing of the flattened × elongated shapes between F₂Sri 8/5-T and KAN(or) showed the trait for an elongated shape was controlled by the *E* gene. The hypothesis of the genotype of flattened fruit shape Sri8/5-T was FFGGEE and globular fruit shape KPS1 was ffggEE. The second cross between the flattened and elongated shapes was Sri8/5-T//KAN1. The F₂ ratio of Sri8/5-T//KAN1 was 69:14 or 3:1, with non-significant for the chi-square test. The hypothesis of the KAN1 genotype was FFGGee (Tables 3 and 4) (Fig. 3). The F₂ segregation of two crossings as following and the crossing of Sri8/5-T//KPS1 showed duplicated dominant epistasis of three genes.

Table 4. Fruit shapes and genotypes of the seven parental lines

| Parent lines | Fruit shape | Genotype |
|---------------------|--------------------|-----------------|
| CM2 | Elongated | ffggee |
| KAN | Elongated | ffggee |
| KAN(or) | Elongated | ffggee |
| KAN1 | Globular | FFGGee |
| KPS1 | Globular | ffggEE |
| KAN3 | Flattened | FFggEE |
| Sri8/5-T | Flattened | FFGGEE |

The previous hypothesis was that the elongated fruit shape was controlled by the ee genotype, with three genes predicted to control fruit shape. When CM2, another elongated line with an ffggee genotype, was crossed with KAN3, a flattened shape line, the ratio of the F₂ population was 10:53:15 or 3:9:4. The genotype of the flattened type KAN3 was predicted to be FFggEE (Tables 3 and 4) (Fig. 5). In the CM2//KPS1 crossing, the segregation of the globular and elongated was 3:1 and the F₂ segregation of two crossings (Fig. 6).

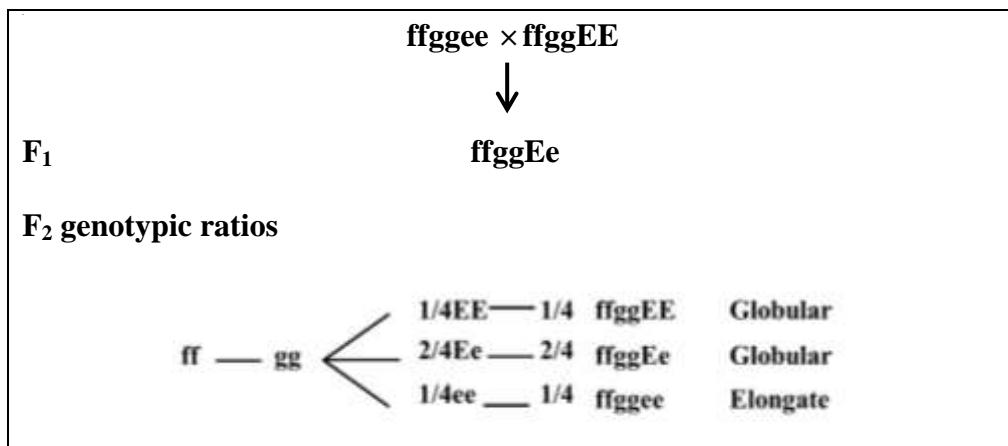


Figure 6. Genotype and genotypic ratios of F₁ and F₂ progenies derived from CM2 × KPS1

From the previous six crosses ratio, we suggested that the genotype of the seven inbred lines are: ffggee genotype of elongated lines CM2, KAN, and KAN(or); the genotypes of the flattened shaped pumpkin lines Sri8/5-T and KAN3 are FFGGEE and FFggEE, respectively; and those of the globular shaped (KAN1 and KPS1) lines are FFGGee and ffggEE, respectively (Table 4). The results suggested that the globular fruit shape was controlled by FF and GG, whereas EE controlled the expression of the flattened type. Ff, Gg, Ee and ff, gg, and ee controlled globular and elongated shapes. The hypothesis of gene action was ee dominant to ffG- and F-gg, which other elongated genotypes were ffG-ee and F-ggee.

The F₂Sri8/5T//KAN(or) population should be recalled, and the genotype of the two inbred lines are FFGGEE and ffggee, wherein the F₁ has FfGgEe genotype thus, globular in shape. The ratio of the flattened:globular:elongated shape phenotype in the F₂ population was 21:32:11. This finding was in agreement with the expected genotypes in the F₂ population. The genotypes and phenotypes are shown in Figure 7. As shown in the figure, the ratio of flattened:globular:elongated shapes was 22:45:21, respectively.

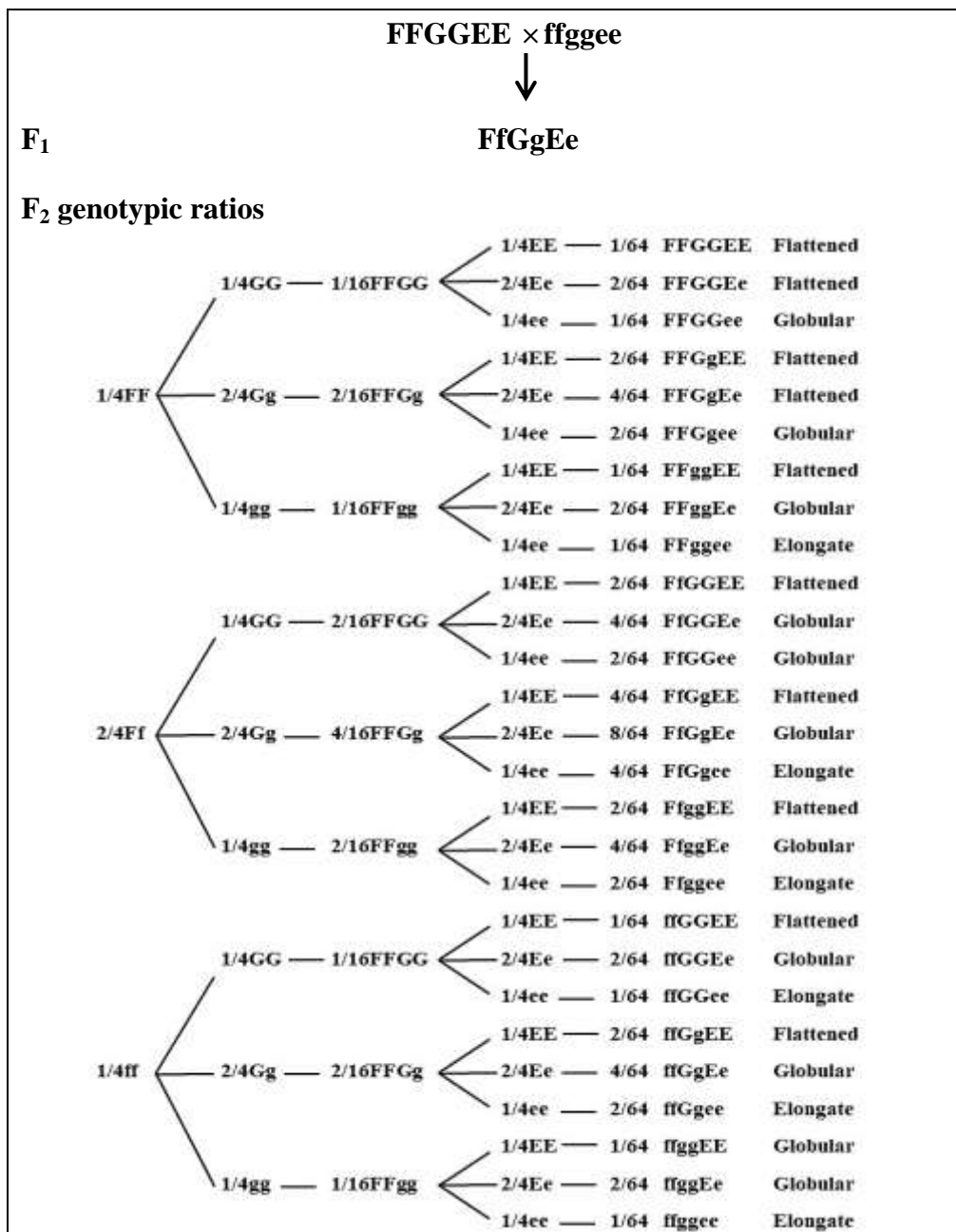


Figure 7. Genotype and genotypic ratios of F₁ and F₂ progenies derived from Sri8/5-T × KAN(or)

Discussion

The results of the present study revealed that the elongated fruit shape was controlled by homozygous recessive alleles. This finding was in accordance with that of previous research by Paris and Brown (2005) and Wang *et al.* (2012). The genotype of the elongated shaped lines CM2, KAN,

and KAN(or) was ffggee. The genotypes of the flattened shaped pumpkin Sri8/5-T and KAN3 lines were FFGGEE and FFggEE, respectively. The genotype of KAN1 (globular shape) was FFGGee, whereas that of KPS1 (globular shape) was ffggEE (Table 4). According to the data, fruit shape was clearly controlled by duplicate dominant epistasis of three genes. All genotypes were suggested the hypothesis of fruit shape controlling gene that FF, GG, and EE controlled the flatten shape trait and that Ff, Gg, Ee and ff, gg, and ee controlled globular and elongated shape traits. In terms of gene action, the results provided support for ee dominance over ffG- and F-gg and ffG-ee and F-ggee as elongated genotypes (Figs. 3–7). On the other hand, Paris and Brown (2005) reported that the fruit shape characteristics were highly polygenic and defied simple Mendelian genetics. In the future, we suggest that studies using larger populations and/or populations having different structures (e.g., backcross populations or recombinant inbred lines) would be useful for clarification of fruit shapes in pumpkins.

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