Prediction of the Parental Phenotype of Commercial Tomato Hybrid (*Solanum lycopersicum* cv. Queen) using Progeny Phenotypic Ratio

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ABSTRACT

This research aimed to predict the phenotypic characteristics of the commercial hybrid parents of the tomato 'Queen' variety. In this experiment, the 'Queen' variety, a salad tomato, was cultivated as a desirable cultivar in Iran during 2022. In 2023, seeds were obtained through selfing from the ripe fruits of this variety. The seeds were planted alongside hybrid seeds, and their traits were recorded. Based on phenotypic similarity to the parent plants, genotypes 10, 22, 23, 32, and 48 were identified for component 1, while genotypes 1, 2, 11, 14, 15, 21, 28, 38, 42, 43, 45, 47, 53, 54, 58, and 74 were selected for component 2. It was hypothesized that the 'Queen' hybrid could be obtained by crossing individual plants resembling these groups. From the candidate genotypes, simulations of qualitative traits and genetic distance calculations (considering maximum distance for effective hybrid production) identified genotype 22 as most similar to one parent, and genotypes 1 and 2 as most similar to the others. These progenies were selected for further development to produce recombinant inbred lines and, ultimately, hybrids closely resembling the 'Queen' variety.

Introduction

Tomatoes are the second most economically important vegetable worldwide. They play a vital role in human health due to their rich content of vitamins, lycopene, carotene, phenolic compounds, beneficial acids, sugars, and mineral salts (Nosoohi and Davazdah Emami, 2012). Studies have shown that tomatoes and their derivatives can help prevent numerous diseases, particularly cancer and cardiovascular conditions (Arab et al., 2000). Extensive research has been conducted on tomato cultivar improvement, focusing on traits such as disease resistance, fruit quality, size, and color (Mirshamsi Kakhki et al., 2006; Hannan et al., 2007; Mohsenifard et al., 2011; Sekhar et al., 2010; Chiwina et al., 2024;

Khan et al., 2024). Genetic diversity serves as the cornerstone of any breeding program, with its success heavily dependent on the type and extent of diversity present in the genetic material. Maximizing diversity increases the likelihood of successful selection. The primary factors contributing to genetic diversity and crop evolution are Mendelian diversity, interspecies hybridization, and polyploidy (Saeidi et al., 2004). Understanding population diversity is a fundamental prerequisite in plant breeding. To develop new cultivars, it is essential to identify existing genotypes based on their genetic potential and desirable traits (Kia-Mohamadi et al., 2012), followed by selection of traits that best

align with breeding goals. Traits with higher heritability typically exhibit greater similarity between parents and offspring (Houshmand, 2003). However, due to limited resources, breeders must transfer as few progenies as possible to the next generation. Selection methods tailored to the heritability of traits are crucial for improving plant characteristics. Moreover, combining heritability with the expected genetic progress index is more effective than relying on heritability alone (Johnson et al., 1955).

Quantitative traits, characterized by continuous and measurable variation, lack distinct phenotypic classes. Their inheritance is complex, involving multiple genes and significant environmental influence, further complicating genetic analysis (Khodambashi Emami and Rabiei, 2012). To estimate genetic diversity in plant species, multivariate statistical methods are widely employed. These methods allow for the simultaneous analysis of morphological, biochemical, and molecular data, aiding in the classification of germplasm and determination of genetic distances for crossbreeding programs. Among the various multivariate techniques, cluster analysis and principal component analysis are the most critical (Mohamdi and Boddupalli, 2003). While high heritability demonstrates the effectiveness of phenotypic selection, it does not necessarily equate to substantial genetic improvement. Combining heritability with genetic progress provides a more accurate estimation of selection outcomes (Kanouni et al., 2012). Interestingly, high heritability does not always guarantee significant genetic progress (Ogunniyan and Olakogi, 2014). Conversely, traits with high heritability but low genetic progress may reflect dominance and epistatic interactions among controlling genes (Tyagi and Kahn, 2010). Selection for traits exhibiting both heritability and genetic progress is generally more successful (Gul et al., 2013).

When the goal of breeding is to produce a hybrid resembling an existing market variety, the ideal candidates for selection among diverse progenies are plants most similar to the hybrid's parents. However, a significant challenge lies in the lack of detailed information about these parent plants. Using basic genetic principles, it is possible to simulate the characteristics of the parents to a considerable extent. This approach, termed *phenotypic reverse breeding* in the present study, aims to overcome this limitation. The objective of this research is to simulate the parents of the commercial Queen tomato hybrid by applying inheritance laws and multivariate analysis. This method is designed to streamline the selection process in tomato breeding programs, enabling the development of hybrids closely resembling the 'Queen' variety.

Materials and Methods

This experiment was conducted over two consecutive summers, from 2022 to 2023. During the first year, operations included cultivating commercial hybrids and performing self-pollination among them. In the second year, commercial hybrids and the progeny resulting from their self-pollination were cultivated, and their traits were evaluated. The 'Queen' tomato variety, used in this study, typically produces 6–7 fruits cluster^{-1}, with an average fruit weight ranging from 180 to 240 g, significantly influenced by management practices and environmental conditions. The marketable fruits are red. The seeds of the 'Queen' greenhouse tomato variety were sourced from Polaris, a U.S.-based company. After ripening, the fruits were collected, and the seeds were extracted via fermentation to separate them from the gelatinous covering, resulting in second-generation seeds. The experiment involved evaluating 24 hybrid plants and 130 progeny plants grown in a greenhouse. The plants were transplanted with a spacing of 50 cm within rows and 50 cm between rows (Hochmuth, 2008). To enhance plant growth, 30 t ha^{-1} of animal manure, along with macro and micro fertilizers, were applied to the planting beds (Khosh-Khui et al., 2019). Various traits were measured, including morphological characteristics such as plant height, internode length, leaf length, leaf width, leaflet length, flower cluster length and width, fruit peduncle length and width, fruit length-to-width ratio, pericarp thickness, leaf angle with the main stem, and the angle between two leaves. Color traits, including fruit color at maturity and flesh color, were assessed using an image processing technique. Yield-related traits, such as the number of fruits cluster^{-1}, number of fruits plant^{-1}, number of clusters plant^{-1}, and single fruit weight, were also recorded.

Statistical tests were conducted to ensure data normality. Metrics such as kurtosis, skewness, mean, and variance were calculated separately for the first and second generations using the univariate procedure in SAS software. An independent *t*-test was applied to compare the average traits between the first and second generations (Soltani, 2012). To investigate the inheritance of traits in the studied populations, phenotypic ratios of the second generation were analyzed. For qualitative diversity, chi-square tests were performed using Microsoft Excel 2013

to compare populations or genetic ratios. Phenotypic components and heritability for all traits were estimated by comparing the genetic diversity of the second generation with the genetic uniformity of the first generation (Khodambashi and Rabiei, 2013). Principal component analysis (PCA) was conducted using the PrinComp procedure in SAS software, while cluster analysis was performed using the cluster procedure with Ward's grouping method (Soltani, 2013). Based on the results from all statistical analyses, second-generation plants that most closely resembled the simulated parent samples were selected. These selected plants can undergo further purification for use in future breeding programs.

Results

Analysis of the skewness and kurtosis indices for the trait data in the first generation revealed that the data followed a normal distribution (Table 1). Similarly, examining the skewness and kurtosis indices for the traits in the second-generation progeny of the 'Queen' variety confirmed that these data also followed a normal distribution. The means of these traits, along with their statistical indices, are presented in Table 2. The variance observed in the second generation increased for all traits compared to the first generation, indicating a sufficient level of genetic diversity within the existing population.

Table 1. Normality indices of the first generation of the 'Queen' variety.

| | | | | | | Traits | | | | | |
Statistical indices	Fruit brightness	fruit pedicel width	fruit pedicel length	flower cluster width	flower cluster length	Leaflet width	Leaflet length	leaf width	leaf length	Internode length	Plant height
skewness	-0.88	-0.07	1.49	0.29	-1.11	0.45	-0.05	-0.07	0.04	0.75	-0.47
kurtosis	-0.03	-0.18	2.00	-1.78	1.18	1.2	-1.64	-1.00	0.08	0.63	-0.22
mean	5.5	0.58	5.16	0.62	2.99	7.1	14.4	31.2	37.3	9.6	175.7
variance	0.235	0.007	0.24	0.011	0.04	0.87	2.32	1.49	1.64	1.69	53.05

Table 1. Continued.

| | | | | | | | | | Traits |
Statistical indices	The angle of two leaves together	The angle of the leaf with the stem	Fruit diameter	Fruit width	Pericarp thickness	Fruit weight	Number of fruits cluster^{-1}	Number of fruits plant^{-1}	Number of clusters plant^{-1}	Red color intensity
skewness	-0.59	0.66	0.29	1.4	-0.45	-1.4	-0.66	-0.68	-0.17	1.23
kurtosis	1.22	-0.86	-0.62	1.6	-1.37	1.9	-1.9	0.19	-2.3	1.11
mean	87.7	72.8	6.5	5.1	0.91	128.3	4.1	20.5	4.8	0.09
variance	23.2	56.8	0.24	0.18	0.008	68.08	0.25	8.8	0.26	0.0001

Table 2. Normality indices of the second generation of the 'Queen' variety.

| | | | | | | Traits | | | | | |
Statistical indices	fruit brightness	fruit pedicel width	fruit pedicel length	flower cluster width	flower cluster length	leaflet width	Leaflet length	leaf width	leaf length	Internode length	plant height
skewness	0.43	-0.38	-0.02	0.06	-0.36	-0.5	-0.25	-0.23	-1.06	-0.49	-0.27
kurtosis	-0.84	-0.09	0.24	-0.26	-0.47	0.12	-0.94	0.43	0.82	0.21	-0.61
mean	4.5	0.47	4.2	0.51	2.9	4.6	8.8	22.9	30.4	7.74	143.01
variance	0.57	0.009	0.48	0.01	0.14	0.94	2.4	17.2	24.2	2.29	394.9

Table 2. Continued.

	Traits									
Statistical indices	The angle of two leaves together	The angle of the leaf with the stem	Fruit diameter	Fruit width	Pericarp thickness	Fruit weight	Number of fruits cluster^{-1}	Number of fruits plant^{-1}	Number of clusters plant^{-1}	Red color intensity
skewness	-0.15	-0.04	0.60	0.32	-0.46	-0.43	0.83	0.29	-0.36	0.35
kurtosis	1.88	-0.12	0.94	-0.3	0.11	0.47	-0.6	-0.82	-0.71	-0.96
mean	101.3	66.08	5.3	5.02	0.67	106.4	4.3	20.6	5.1	0.071
variance	187.1	95.1	0.25	0.34	0.01	185.1	0.7	20.3	0.72	0.0002

For traits such as the angle between two leaves, the number of fruits cluster^{-1}, the number of fruits plant^{-1}, and the number of clusters plant^{-1}, no significant differences were observed between the two generations. This suggests a strong resemblance to the parental characteristics in these traits for the investigated hybrid (Table 3).

It appears that the breeder's objective was to develop high-yielding hybrids by selecting parents with superior fruit production. Accordingly, the parental plants exhibited an angle of 87–102 degrees between two leaves, an average of four fruits cluster^{-1}, and 20 fruits plant^{-1}.

Table 3. t-test between first (F1) and second (F2) generations.

	Traits										
Statistical indicators	Fruit brightness	fruit pedicel width	fruit pedicel length	flower cluster width	flower cluster length	Leaflet width	Leaflet length	leaf width	leaf length	Internode length	plant height
Mean F$_1$	5.5	0.58	5.16	0.62	2.99	7.1	14.4	31.2	37.3	9.6	175.7
Mean F$_2$	4.5	0.47	4.2	0.51	2.98	4.63	8.83	22.9	30.4	7.74	143.0
t-test	4.3**	3.5**	6.2**	2.5**	0.1**	7.7**	7.9**	6.9**	5.6**	2.9**	4.1**

Table 3. Continued.

	Traits									
Statistical indicators	The angle of two leaves together	The angle of the leaf with the stem	Fruit diameter	Fruit width	Pericarp thickness	Fruit weight	Number of fruits bunch^{-1}	Number of fruits plant^{-1}	Number of clusters plant^{-1}	Red color intensity
Mean F$_1$	87.7	72.8	6.5	5.18	0.91	128.3	4.1	20.5	4.8	0.09
Mean F$_2$	101.3	66.08	5.31	5.02	0.67	106.4	4.3	20.6	5.1	0.07
t-test	-0.8^{ns}	2.08**	8.4**	0.2**	7.5**	4.8**	-0.05^{ns}	-0.1^{ns}	-1.4^{ns}	2.4**

^{ns}, **: non-significant and significant at $P \leq 0.01$ respectively.

The mean values of traits such as plant height, internode length, leaf length and width, flower cluster length and width, fruit peduncle length and width, fruit brightness, leaf angle with the main stem, fruit length, fruit diameter, pericarp thickness, fruit weight, and color intensity were higher in the first generation compared to the second generation, with significant differences

observed between the two generations. This suggests that the parents of the hybrid were not uniform in traits where the first generation exhibited higher averages, and it is also possible that self-fertilization contributed to the reduction of these traits in the second generation. The phenotypic, environmental, and genetic variance, along with the heritability estimates for various traits, are provided in Table 4. For qualitative traits in the second generation of the 'Queen' variety, chi-square tests comparing observed and expected data for a 3:1 ratio indicated no significant differences (Table 5). This result confirms that the observed frequencies align with the expected frequencies, maintaining the desired ratio for all the studied traits.

Table 4. Phenotypic, environmental, and genetic variance, as well as the amount of heritability for different traits.

Statistical indices	Fruit Brightness	fruit peduncle width	fruit peduncle length	flower cluster width	flower cluster length	leaflet width	Leaflet length	leaf width	leaf length	Internode length	Plant height
Phenotypic variance	0.57	0.009	0.48	0.015	0.14	0.94	2.44	17.2	24.2	2.29	533.05
Genetic variance	0.34	0.007	0.24	0.004	0.1	0.07	0.12	4.73	6.59	0.59	296.7
Environmental variance	0.23	0.002	0.24	0.011	0.04	0.87	2.32	12.5	17.6	1.69	236.2
Heritability	0.59	0.77	0.49	0.07	0.69	0.07	0.05	0.27	0.27	0.26	0.55

Table 4. Continued.

	Traits									
Statistical indices	The angle of two leaves together	The angle of the leaf with the stem	Fruit diameter	Fruit width	Pericarp thickness	Fruit weight	Number of fruits cluster⁻¹	Number of fruits plant⁻¹	Number of clusters plant⁻¹	Red color intensity
Phenotypic variance	187.1	95.15	0.25	0.34	0.01	185.1	0.46	16.3	0.52	0.0002
Genetic variance	163.8	38.3	0.002	0.15	0.006	117.4	0.2	7.49	0.25	0.0001
Environmental variance	23.2	56.8	0.24	0.18	0.005	68.08	0.25	8.89	0.26	0.0001
Heritability	0.87	0.4	0.009	0.46	0.54	0.63	0.45	0.45	0.48	0.514

Based on the results of the principal components analysis, three components were selected according to eigenvalues higher than one, with cumulative variability higher than 90%, to explain the population in terms of independent characteristics (Fig. 1).

The parental lines differed in their components, and the progeny displayed significant diversity. In conventional selection methods, plants are selected from a region that is favorable in terms of all components. Based on this approach, genotypes 3, 5, 6, 12, 13, 27, 35, 41, 49, 52, 56, 57, 64, and 70 should be selected for components 1, 2, and 3. In contrast, our proposed method focuses on selecting genotypes based on their similarity to the parents. Using this approach, genotypes 10, 22, 23, 32, and 48 were identified for component 1, while genotypes 1, 2, 11, 14, 15, 21, 28, 38, 42, 43, 45, 47, 53, 54, 58, and 74 were selected for component 2 (Table 6). It is hypothesized that the 'Queen' hybrid was derived from the crossing of individual plants resembling these groups. Among the candidate genotypes, and considering the simulation of qualitative traits and the genetic distance between individuals—defined as the maximum allowable distance for hybrid production (Fig. 2)—genotype 22 is expected to be the plant most similar to one parent, while genotypes 1 and 2 are likely to resemble the other parent.

Table 5. Chi-Square test results between the expected and observed ratios of different traits of the 'Queen' variety.

| Traits	Phenotype progeny	Expected ratios	Observed ratios
Plant height	X- (very tall)	57	60
	xx (tall)	19	16
	All progeny	76	
	Chi-square test result	0.42^{ns}	
The angle of two leaves together	X- (obtuse)	57	50
	xx (acute)	19	26
	All progeny	76	
	Chi-square test result	0.06^{ns}	
Red color intensity	X- (red)	57	63
	xx (reddish orange)	19	13
	All progeny	76	
	Chi-square test result	0.11^{ns}	
Fruit brightness	X- (gloss)	57	59
	xx (opaque)	19	17
	All progeny	76	
	Chi-square test result	0.59^{ns}	
Pericarp thickness	X- (very thick)	57	56
	xx (thick)	19	20
	All progeny	76	
	Chi-square test result	0.7^{ns}	
Fruit diameter	X- (wide)	57	51
	xx (round)	19	27
	All progeny	76	
	Chi-square test result	0.11^{ns}	
fruit peduncle width	X- (thick)	57	53
	xx (thin)	19	23
	All progeny	76	
	Chi-square test result	0.09^{ns}	

^{ns}: non-significant at $P \leq 0.05$.
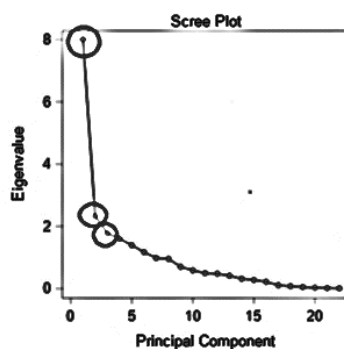
Fig. 1. A scree plot for selecting the best number of components. Components that have eigenvalues higher than 1 were selected.

Table 6. Estimated component values for tomato segregated genotypes.

Genotype	Component 1	Component 2	Component 3	Genotype	Component 1	Component 2	Component 3
1	95.9	34.03	41.5	39	113.02	35.2	28.6
2	94.9	32.4	39.3	40	93.3	28.1	37.2
3	117.4	30.7	40.3	41	100.3	35.8	36.6
4	111.2	33.4	32.1	42	97.8	34.6	34.7
5	110.1	34.6	38.4	43	86.9	39.1	33.4
6	106.01	37.4	44.6	44	82.5	24.7	29.7
7	94.4	29.9	28.9	45	87.9	33.8	33.3
8	123.3	38.8	30.7	46	68.9	22.9	30.1
9	105.3	31.9	33.2	47	98.3	37.7	38.1
10	101.2	29.2	38.1	48	106.6	25.2	37.3
11	87.6	33.2	36.2	49	100.04	33.8	36.2
12	110.7	39.1	43.9	50	85.1	25.9	35.9
13	101.2	37.7	36.6	51	91.3	29.7	33.4
14	85.2	36.9	43.8	52	110.5	31.8	40.5
15	85.3	36.2	42.1	53	99.4	37.2	37.4
16	79.1	23.3	34.5	54	83.3	34.5	34.4
17	99.8	28.2	37.1	55	80.8	27.4	31.9
18	100.3	31.2	31.8	56	111.6	32.1	35.5
19	91.6	29.3	39.9	57	109.5	36.7	36.05
20	80.4	28.7	36.6	58	98.8	32.1	31.1
21	73.3	34.02	27.2	59	76.05	23.2	28.6
22	125.6	23.3	37.9	60	73.3	20.9	24.6
23	108.9	28.6	37.9	61	134.5	41.2	33.2
24	83.1	22.9	32.03	62	132.9	41.3	27.2
25	83.9	24.9	27.01	63	115.2	39.4	32.7
26	54.3	25.5	45.7	64	109.2	31.8	38.4
27	108.5	34.9	35.6	65	124.9	36.4	29.4
28	96.08	34.3	37.6	66	137.01	40.3	30.9
29	81.4	26.8	37.4	67	151.9	47.7	32.7
30	98.07	29.2	34.2	68	150.3	46.5	33.4
31	82.6	26.6	30.2	69	115.3	35.3	29.5
32	109.2	27.7	32.03	70	124.3	35.8	38.5
33	122.4	36.2	31.8	71	123.6	38.7	33.1
34	113.2	35.7	32.1	72	115.8	30.3	27.6
35	106.03	39.8	35.6	73	114.02	32.3	34.1
36	107.6	39.7	33.3	74	98.4	30.8	28.9
37	99.3	29.9	36.5	75	123.5	36.8	30.2
38	89.3	37.2	37.2	76	90.6	26.07	29.5

Discussion

Diversity observed in the F1 generation is purely environmental. Environmental diversity arises from the impact of various environmental factors on plants with identical genotypes and is not transmitted to their progeny (Khodambashi and Rabiei, 2012). Traits such as internode length, leaf length, leaf width, leaflet length, leaflet width, flower cluster width, fruit peduncle length, leaf angle with the main stem, fruit diameter, and fruit weight showed high contributions from environmental variance. This indicates a strong influence of environmental factors on these traits, attributable to their low heritability. In contrast, phenotypic diversity in the second generation results from both genetic and environmental diversity (Khodambashi and Rabiei, 2012). Traits such as plant height, the angle between two

leaves, fruit diameter, flower cluster length, fruit peduncle width, pericarp thickness, intensity of red color, and fruit brightness exhibited high genetic variance and heritability. These traits were less influenced by environmental factors, suggesting a strong inheritance pattern.

Based on these findings, traits such as plant height, flower cluster length and width, fruit peduncle length and width, leaf angle with the main stem, fruit width, pericarp thickness, fruit diameter in cross-section, number of fruits cluster^{-1}, fruit weight, and number of fruits plant^{-1} are likely qualitative. However, for traits where the second-generation mean exceeded that of the first generation, the role of heterozygosity in controlling these traits appears minimal (Spenani et al., 2015). For qualitative diversity, the chi-square test (χ^2) is used to compare two populations or genetic ratios. If the calculated chi-square value exceeds the table value for the corresponding degrees of freedom, it indicates a lack of agreement between observed and expected frequencies (Khodambashi and Rabiei, 2012). Using this test, we simulated parental traits as follows: very tall and tall for plant height, obtuse and acute for the angle between two leaves, red and reddish-orange for color, glossy and opaque for fruit brightness, very thick and thick for pericarp thickness, wide and round for fruit diameter, and thick and thin for fruit peduncle width. However, the specific arrangement and interaction of these traits remain unclear and require further analysis.
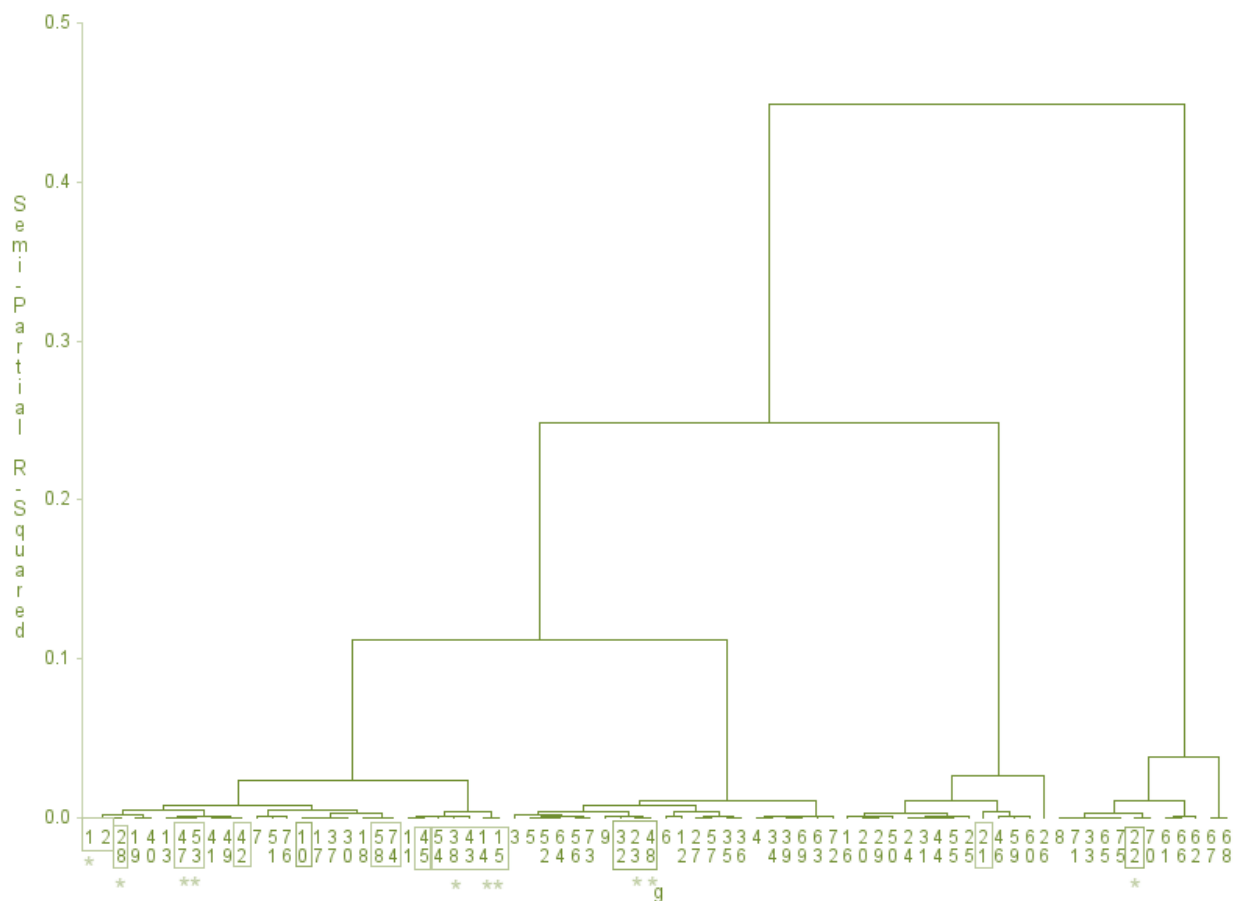

Fig. 2. Cluster analysis of genotypes.

Identifying compatibility linked with high-yield heterosis is a critical step in hybrid development. Parents with higher general combining ability and greater genetic distance are more likely to produce hybrids with superior performance. Although estimating compatibility ability is expensive and time-intensive, molecular methods are being explored to predict heterosis. The results have been inconsistent: Zhua et al. (1999) and Liu et al. (1999) reported no correlation between genetic distance based on DNA markers and heterosis, while Takatsu et al. (2001) and Smith et al. (1990) suggested that molecular markers can predict F1 hybrid performance. If this breeding method successfully identifies the desired genotypes, the cost of the breeding

project can be significantly reduced by limiting the number of plants selected in each cycle. This cost reduction is achieved through efficient resource allocation and reduced labor requirements. Moreover, it enables more precise monitoring of plants, allowing researchers to identify and cultivate superior genotypes effectively. Enhanced precision in selection and monitoring will lead to improved crop yields, better plant health, and ultimately contribute to the sustainability and success of the breeding program.

Conclusions

This study successfully explored the phenotypic characteristics and genetic diversity of the 'Queen' tomato hybrid and its progeny over two generations. The findings highlighted the influence of environmental and genetic factors on key traits, providing valuable insights into heritability, phenotypic variance, and selection criteria. The proposed phenotypic reverse breeding method demonstrated its potential to simulate parental traits and identify promising genotypes for hybrid production. Genotypes with high genetic similarity to the simulated parents were identified, offering a cost-effective and precise approach for future breeding programs. These results pave the way for developing high-yield, high-quality tomato hybrids while optimizing resource allocation and advancing breeding efficiency.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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