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Advancing Vegetable Crop Selection: The Superiority of the ASIIG Method for Multi-Trait Evaluation in Pepper Hybrids

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ABSTRACT

To evaluate the desirability of yellow pepper hybrids, 81 experimental hybrids along with 4 commercial hybrids as controls were assessed using a randomized complete block design with three replications. Correlation analysis revealed that the number of fruits per plant, plant height, and fruit weight were the primary traits contributing to fruit yield, indicating their importance for selection in breeding programs aimed at yield improvement. Using the ASIIG index, six hybrids were classified within the “completely ideal” category, while 31 hybrids fell into the “ideal” category, highlighting their superior overall performance. Furthermore, genotype-by-trait (GT) biplot analysis divided the 85 hybrids into eight sectors, each representing clusters of hybrids and associated traits. Notably, hybrids 382, 130, 352, 153, 38, 376, 298, 175, 358, 197, 101, 380, 66, and 99 exhibited superior performance across key traits including fruit yield, fruit count, fruit weight, final fruit set, plant height, and stalk diameter. Except for hybrid 99, all these hybrids were positioned within the “completely ideal” or “ideal” quarters according to the ASIIG index. Cluster analysis using Ward’s method grouped the 85 hybrids into four distinct clusters based on 17 evaluated traits, providing further insight into their genetic diversity and trait associations. Overall, this study demonstrates that combining GT biplot analysis with the ASIIG index is an effective approach for identifying superior yellow pepper hybrids with simultaneous improvement in multiple agronomic traits.

Introduction

Bell pepper (*Capsicum annuum* var. grossum L.), commonly known as Shimla Mirch or sweet pepper, is a widely cultivated vegetable valued for its pleasant flavor, delicate texture, high nutritional content, and the presence of anti-inflammatory compounds (Marin et al., 2004). The identification of superior pepper hybrids is crucial for advancing breeding programs, as it facilitates the selection of parental lines based on key morphological traits (Sharma et al., 2017). xploiting heterosis represents a promising strategy for sweet pepper improvement, enabling the development of hybrids that combine high yield potential with desirable fruit characteristics such as attractive color and blocky shape, and traits favored by growers in tropical and subtropical regions (Banerjee et al., 2024). Numerous studies have employed factor analysis and other multivariate techniques to explore trait relationships and their influence on yield. However, these investigations often focus on trait-performance correlations without providing clear guidance for selecting optimal genotypes. This highlights the need for robust methods that integrate multiple traits to facilitate informed genotype selection. The Adjusted Selection Index of Ideal Genotype (ASIIG) is an effective tool designed to evaluate genotypes by simultaneously considering multiple traits, assigning appropriate weights to each, and supporting decision-making regarding overall genotype performance. This index enables the comparison, ranking, and selection of superior genotypes, as well as the assessment of genetic distances and groupings among them (Hemadesh et al., 2021). Complementing this approach, the Genotype by Trait (GT) biplot (an extension of the GGE biplot methodology) is a powerful visual and analytical tool for multi-trait data analysis. It helps elucidate the relationships between genotypes and traits, facilitating the identification of genotypes that perform well across multiple desirable characteristics. In GT biplot analysis, genotypes are represented as vectors (lines), while traits are treated as testers, enabling simultaneous evaluation (Yan and Rajcan, 2002). GT biplot has been successfully applied across diverse crops, including soybean (Yan and Rajcan, 2002), pepper (Abu et al., 2011), white lupin (Atnaf et al., 2017), common bean (Oliveira et al., 2018), sunflower (Shojaei et al., 2022), cocoa (Araújo et al., 2024), olive (Dadras et al., 2024), and cowpea (Nuryati et al., 2024), demonstrating its versatility and effectiveness in genotype evaluation and selection.

Iran is rich in pepper plant diversity. However, there has been limited breeding work conducted on this crop in Iran, and the existing native populations are exposed to genetic erosion. The fruit yield and especially the yield that can be offered to the market in native populations is low due to the lack of uniformity in fruit quality. In addition, the product of native cultivars is not marketable according to international standards and they cannot compete with foreign cultivars. Therefore, it is necessary to plan and conduct breeding and agronomical research on pepper cultivars (Keshavarz et al., 2019). The objectives of the present study were: 1) to identify and select the best and most suitable yellow pepper hybrids based on the various evaluated traits using GT biplot, 2) to investigate the genetic diversity of hybrids in terms of fruit yield, and several morphological traits and integrate several important morphological traits to better evaluate hybrids using the ASIIG index and introduce superior hybrids.

Material and methods

Plant materials and growth conditions

An entire collection of 81 hybrids of yellow pepper (belonging to innovative company Negin Bazr Danesh) along with 4 commercial hybrids as controls was evaluated (Table 1). All parental lines of these hybrids were produced in Negin Bazr Danesh Co. This experiment was carried out using a randomized complete block design with 3 replications in the research greenhouses of the Faculty of Agriculture of Islamic Azad University, Isfahan branch (Khorasgan), located in the east of Isfahan, Iran, with a latitude of 51° and 46′ and a longitude of 32° and 44′ and an altitude of 1555 meters above the sea level in sandy clay loam soil, in 2021-2022. The average annual rainfall and temperature of the region were 120 mm and 16 ˚C, respectively. The temperature of the greenhouse was between 25-30 ˚C during the day and 16-18 ˚C at night, and the average humidity level of the greenhouse was 60%. The seeds of the studied hybrids were planted on November 2, 2022. For planting, two seeds from each hybrid were sown in polystyrene trays with 112 cells, filled with cocopit andperlite. The germination of seedlings was carried out in protected conditions in a greenhouse. The plants were irrigated once a day, in the morning. Seedlings were transplanted to soil on December 2 (50 d old). Two rows of seedlings were planted on each bed, in and between-row spacing were 40 and 50 cm, respectively, at a density of 2 plants per square meter. The results of the physical and chemical analysis of greenhouse soil are shown in Table 2. During the harvesting period (almost 5 months), the fruits were harvested.

Plant measurements

Morphological traits were measured based on the description of pepper (IPGRI, 1995). The measured traits are as follows: mature fruit color (light, medium, dark), stalk length (cm), stalk diameter (cm), lobe number, fruit length (cm), fruit width (cm), fruit length to width ratio, fruit shape (asymmetric and symmetrical), pericarp diameter (cm), fruit surface cracking (No and Yes), plant length (m), final fruit set (very low, low to intermediate, intermediate, intermediate to high, high), internode distance (cm), vigor (low, intermediate, high), fruit firmness (low, intermediate, high), the average number of fruits per plant, the average weight of a fruit (g), and fruit yield per plant (g).

Statistical analysis

Data pertaining to morphological traits and fruit characteristics were collected. Analysis of variance (ANOVA), correlation, stepwise regression, and cluster analysis was done using SPSS 26 software, ASIIG index and heterosis by Microsoft Excel, and genotype by trait biplot with GenStat 12th edition. The amount of heterosis and ASIIG index were calculated by Sood and Kumar (2010) and Hemadesh et al. (2021), respectively.

$$\text{Heterosis (\%)} = \frac{\overline{F}_1 - \overline{\text{Control}}}{\overline{\text{Control}}} \times 100$$

Results

Analysis of variance

The result of ANOVA showed that there was a significant difference between hybrids on all the traits, except for pericarp diameter, fruit surface cracking, and final fruit set (data not shown). The significant difference between hybrids indicated genetic variability between pepper hybrids.

Heterosis

Heterosis breeding represents a promising approach to enhancing the production and productivity of sweet peppers, achieving results that may not be possible using traditional methods (Sharma et al., 2013). In the current study, a wide range of heterosis over control varieties was observed in hybrids for marketable fruit yield and its attributing traits (Table 3). The highest amount of heterosis (216.59%) was observed in hybrid 411 for the fruit count trait compared to the C2 control. Results indicated that hybrids No. 200, 369, 172, 415, 142, 69, 380, 265, 370, and 330 showed positive heterosis over commercial checks for fruit weight and 411, 382, 343, 408, 376, 410, 175, 178, 197, 358, 266, and 153 overall control varieties except C4 for fruit counts. only three hybrids (411, 382, and 343) showed positive heterosis over C4. Hybrids of 382, 343, 408, and 175 showed the highest positive heterosis over all control varieties for yield, which showed the genetic capacity of the parents to produce a commercial hybrid. These hybrids offer a high scope for exploiting heterosis to improve horticultural traits and can also be released as superior and stable hybrids after further field trials.

Table 1. List and pedigree of yellow pepper hybrids and controls used in this research.

Hybrid	Pedigree	Hybrid	Pedigree
5	434-9-4*202-4-5	200	75-4-2*440-4-2
20	404-3-4*472-2-2	203	400*472-2-2
21	40-2-3*74-4	212	402-2-4*74-3
26	40-4-4*74-3	218	400*85-4-4
29	83-2-4*74-3	265	403-2-2*85-4-4
30	40-4-40*72-4-3	266	400*85-4-4
32	42-2-2*204-5	268	85-4-4*400
35	40-2-3*42-2-2	298	79-4-4*47-3-5
38	40-2-3*403-2-2	300	472-2-2*79-4-4
41	72-2-4*403-2-2	330	75-4-2*24-4-4
42	40-4-40*42-2-2	343	79-4-4*40-2-4
43	83-2-4*72-4-3	350	92-2-2*40-4-40
49	84-3*74-3	351	472-2-2*74-3
51	72-4-3*472-2-2	352	440-2-4*79-4-4
66	42-2-2*472-2-2	356	204-4*79-4-4
68	79-4-4*74-3	357	98-4-4*79-4-4
69	74-3*403-2-2	358	99-4-4*79-4-4
75	42-2-2*72-4-3	359	472-2-2*85-4-4
85	83-2-4*74-3	367	40-4-40*93-3
94	40-4-4*72-4-3	369	440-2-4*98-4-4
96	40-2-3*83-2-4	370	444-2-8*98-4-4
99	74-4*472-2-2	371	79-4-4*98-4-4
100	72-4-3*204-5	372	403-4-5*98-3
101	79-4-4*403-2-2	373	440-2-4*98-3
110	74-4*204-5	376	472-2-2*99-4-4
111	403-2-2*204-5	378	440-2-4*99-2-2
116	79-4-4*408-4-5	380	47-4-4*402-2-4
126	72-2-4*74-3	381	40-4-40*402-2-4
128	42-2-2*400	382	79-4-4*403-2-4
130	79-4-4*403-2-2	389	472-2-2*444-2-8
137	95-5*42-2-2	407	403-2-2*74-4
142	40-2-3*72-2-4	408	402-4-4*79-4-4
146	40-4-40*74-4	410	79-4-4*93-3
151	404*472-2-2	411	200-2*95-4-4
153	403-2-2*79-4-4	412	79-4-4*95-4-4
172	40-4-4*79-4-4	413	472-2-2*98-3
174	99-4-4*74-3	414	79-4-4*99-4-4
175	99-4-4*92-7	415	79-4-4*99-2-2
178	84-4*79-4-4		
179	74-4*72-4-3		
189	40-4-40*74-3		
193	40-4-40*79-4-4		
197	442-2-2*455-4-4		

Table 2. Some of the physical and chemical properties of the studied greenhouse soil.

Physical characteristics	Amount
Soil sampling depth (cm)	0-30
Saturation percentage	56
Electrical conductivity (dS m^{-1})	4
Acidity (pH)	7.75
Total neutralizing value (%)	36
Organic carbon (%)	1.81
Gypsum (%)	0.0
Nitrogen (%)	0.18
Phosphorus (ppm)	71.4
Potassium (ppm)	938
Ferrum (mg kg^{-1})	14
Zinc (mg kg^{-1})	10.9
Manganese (mg kg^{-1})	11
Copper (mg kg^{-1})	4.6
Boron (mg kg^{-1})	2.7
Sand (%)	45
Clay (%)	29
Loam (%)	26
Soil texture	Sandy Clay Loam

Table 3. Heterosis range over the standard check for studied traits in pepper hybrids.

Traits	Heterosis range (%) over controls			
	C1	C2	C3	C4
Mature fruit color	-50-16.67	-57.14-0.00	-50-16.67	-50.00-16.67
Stalk length (cm)	-20-56.19	-18.45-59.22	-13.40-69.07	-8.70-78.26
Stalk diameter (cm)	-44.72-13.89	-31.38-41.38	-41.47-20.59	-28.93-46.43
Lobe number	-50-116.67	-70.00-30.00	-66.67-44.44	-75.00-8.33
Fruit length-to-width ratio	-47.28--3.79	-20.06-45.89	-8.97-66.12	-28.11-31.20
Fruit shape	0.00-100	-25.00-50.00	-50.00-0.00	-25.00-50.00
Pericarp diameter (cm)	-6.25-62.5	-34.78-13.04	-34.78-13.04	-16.67-44.44
Fruit surface cracking	-16.67-0.00	-16.67-0.00	-16.67-0.00	-16.67-0.00
Plant height (m)	-34.44-11.11	-15.71-42.86	-30.99-16.96	-26.25-25.00
Final fruit set	-44.44-44.44	-50.00-30.00	-37.5-62.5	-58.33-8.33
Internode distance (cm)	-27.78-44.44	-27.78-44.44	-38.09-23.81	-43.48-13.04
Vigor	-55.56-0.00	-33.33-50.00	-33.33-50.00	-42.86-28.57
Fruit firmness	-33.33-50.00	-55.56-0.00	-42.86-28.57	-55.56-0.00
No. fruits per plant	-45.16-65.76	4.74-216.59	-56.32-32.02	-40.11-81.03
Fruit weight (g)	-32.77-56.77	-43.66-31.39	-42.94-00.06	-46.66-24.38
Harvest number	-10.00-10.00	-6.90-13.79	-18.18-0.00	0.00-22.22
Fruit yield (g)	-31.59-50.60	4.65-130.41	-49.22-11.81	-47.45-15.70

Adjusted selection index of ideal genotype (ASIIG)

To select the best hybrids in terms of fruit yield and other traits using the ASIIG method, all the traits entered/un-entered in the regression model along with the standardized regression coefficient for each trait were used as the optimal weight, and the value of ASIIG for each hybrid was calculated (Table 4). The value of ASIIG varies from 0 to 1, the best hybrid was the hybrid that had the smallest distance from the ideal and was far from the undesirable hybrid. Whenever the ASIIG value is closer to one, the hybrid will be more favorable (Hemadesh et al., 2021).

In the current study, the amount of ASIIG index was between 0.18 and 0.73, and the highest amount of ASIIG index belongs to hybrids 382 (0.7-0.8), 343, 408, C4, 411, 175 (0.6-0.7), 178, 358, 410, 376, 352, 330, 407, 200, 378, 153, 298, 203, 415, 197, 357, 266, 172, 130, 351, and 38 (0.5-0.6), respectively.

The fruit yield of these selected hybrids (except 411) was higher than the average yield of all studied hybrids. On the other hand, according to the current study, hybrids 179 and C2 with the lowest ASIIG values (0.1-0.2) were the weakest in terms of fruit yield and other yield-related traits (Table 4). These hybrids had a lower yield compared to other studied hybrids.

Table 4. Amounts of ASIIG indices for 85 hybrids of pepper.

Hybrid	d^+	d^-	ASIIG	ASIIG rank	Hybrid	d^+	d^-	ASIIG	ASIIG rank
382	0.047	0.125	0.728	1	369	0.105	0.079	0.428	4
343	0.052	0.119	0.698	2	96	0.094	0.069	0.421	4
408	0.053	0.116	0.686	2	414	0.097	0.069	0.416	4
C4	0.054	0.112	0.676	2	151	0.099	0.071	0.416	4
411	0.073	0.133	0.646	2	C1	0.096	0.067	0.413	4
175	0.068	0.102	0.602	2	300	0.100	0.069	0.408	4
178	0.073	0.092	0.557	3	193	0.100	0.067	0.402	4
358	0.075	0.094	0.555	3	51	0.099	0.066	0.400	4
410	0.074	0.090	0.546	3	128	0.102	0.067	0.397	5
376	0.074	0.089	0.545	3	367	0.100	0.065	0.394	5
352	0.077	0.092	0.544	3	29	0.099	0.064	0.393	5
330	0.079	0.094	0.543	3	30	0.100	0.065	0.392	5
407	0.078	0.091	0.539	3	110	0.102	0.063	0.380	5
200	0.085	0.098	0.536	3	356	0.103	0.062	0.378	5
378	0.078	0.090	0.534	3	389	0.110	0.066	0.375	5
153	0.078	0.089	0.533	3	94	0.102	0.061	0.372	5
298	0.080	0.089	0.528	3	85	0.103	0.061	0.371	5
203	0.079	0.088	0.528	3	146	0.107	0.063	0.370	5
415	0.083	0.092	0.526	3	371	0.105	0.061	0.367	5
197	0.080	0.084	0.513	3	268	0.109	0.061	0.358	5
357	0.081	0.086	0.513	3	99	0.112	0.062	0.356	5
266	0.081	0.084	0.508	3	35	0.108	0.058	0.351	5
172	0.088	0.091	0.508	3	69	0.119	0.062	0.344	5
130	0.087	0.088	0.503	3	218	0.112	0.057	0.335	5
351	0.086	0.087	0.502	3	413	0.117	0.058	0.330	5
38	0.084	0.085	0.500	3	373	0.111	0.054	0.329	5
C3	0.088	0.086	0.494	4	42	0.113	0.055	0.328	5
412	0.084	0.082	0.493	4	370	0.122	0.059	0.325	5
32	0.086	0.082	0.489	4	21	0.117	0.054	0.316	5
359	0.088	0.082	0.484	4	142	0.129	0.058	0.311	5
265	0.090	0.084	0.480	4	381	0.123	0.055	0.308	5
380	0.093	0.083	0.472	4	20	0.121	0.052	0.300	6
66	0.090	0.077	0.461	4	41	0.116	0.048	0.293	6
101	0.090	0.076	0.458	4	26	0.118	0.048	0.289	6
372	0.091	0.076	0.456	4	350	0.123	0.050	0.288	6
212	0.093	0.077	0.455	4	137	0.123	0.046	0.275	6
111	0.091	0.076	0.454	4	5	0.121	0.044	0.268	6
116	0.093	0.074	0.444	4	49	0.135	0.044	0.248	6
68	0.093	0.074	0.442	4	43	0.130	0.037	0.221	6
100	0.093	0.073	0.440	4	126	0.147	0.037	0.200	6
174	0.094	0.071	0.433	4	C2	0.154	0.034	0.183	7
189	0.094	0.071	0.430	4	179	0.144	0.031	0.179	7
75	0.095	0.071	0.429	4					

ASIIG: Adjusted selection index of ideal genotype, di^+: Distance from ideal, di^-: Distance from anti -ideal.

By evaluating the efficiency of the ASIIG index in selecting the best hybrids in terms of all studied traits simultaneously, the studied hybrids were segregated into seven groups. It is important to note that, since the ASIIG index in this study was less than 0.8, the first group consisted of hybrids whose ASIIG values were greater than 0.7 and less than 0.8. hybrids whose index was greater than 0.6 and less than 0.7 were in the second group, and other hybrids were also grouped accordingly (Table 5). The results of the grouping of hybrids based on the ASIIG index (Table 5) showed that in terms of mature fruit color, fruit length-to-width ratio, fruit shape, fruit surface cracking traits, plant height, vigor, fruit firmness, fruit count per plant, fruit weight, and fruit yield significant differences were observed among most groups. On the other hand, no significant differences were observed for stalk length, stalk diameter, lobe number, pericarp diameter, final fruit set, internode distance, and harvest number in any of the groups with each other.

The results of the grouping of the studied hybrids based on the ASIIG index (Table 5) showed that one hybrid (382) was placed in group one. Its average fruit yield, fruit weight, and fruit count was 28245.21, 167.40, and 34.80 g, respectively. Therefore, Hybrid 382 is most similar to the ideal hybrid. The average fruit yield, the number of fruits, plant height, and pericarp diameter of the first group were higher than all the control cultivars. The average fruit weight of this group was only lower than the C3 cultivar.

In addition to the ranking of the experimental hybrids, it is necessary to group them based on their desirability. By determining the distance between ideal and non-ideal, hybrids can be grouped into four groups: completely ideal (ASIIG = 0.591 to 0.728), ideal (ASIIG = 0.453 to 0.591), non-ideal (ASIIG = 0.316 to 0.453), and completely non-ideal (ASIIG = 0.179 to 0.316). According to the desirability quadrants based on the ASIIG index, hybrids 382, 343, 408, C4, 411, and 175 in the completely ideal quarter, hybrids 178, 358, 410, 376, 352, 330, 407, 200, 378, 153, 298, 203, 415, 197, 357, 266, 172, 130, 351, 38, C3, 412, 32, 359, 265, 380, 66, 101, 372, 212, and 111 in the ideal quarter, and Hybrids 179, C2, 126, 43, 49, 5, 137, 350, 26, 41, 20, 381, 142, and 21 were placed in the completely non-ideal quarter.

Table 5. Grouping of 81 hybrids along with 4 controls based on ASIIG index and mean of different traits in each group.

Trait	Group average based on ASIIG index							Total
	1	2	3	4	5	6	7	
	0.7-0.8	0.6-0.7	0.5-0.6	0.4-0.5	0.3-0.4	0.2-0.3	0.1-0.2	
Mature fruit color	1.33[b]	1.80[ab]	1.52[b]	1.79[ab]	1.71[ab]	1.85[ab]	2.17[a]	1.72
Stalk length (cm)	2.00[a]	1.87[a]	1.83[a]	1.90[a]	1.88[a]	1.70[a]	2.00[a]	1.86
Stalk diameter (cm)	2.00[a]	1.67[a]	1.80[a]	1.81[a]	1.65[a]	1.67[a]	1.67[a]	1.74
Lobe number	3.67[a]	2.53[a]	3.35[a]	3.40[a]	2.61[a]	2.93[a]	2.83[a]	3.06
Fruit length-to-width ratio	1.33[a]	1.00[b]	1.18[ab]	1.11[ab]	1.13[ab]	1.15[ab]	1.17[ab]	1.13
Fruit shape	2.00[a]	1.60[ab]	1.75[ab]	1.67[ab]	1.61[ab]	1.59[ab]	1.50[b]	1.66
Pericarp diameter (cm)	0.80[a]	0.70[a]	0.71[a]	0.70[a]	0.72[a]	0.72[a]	0.78[a]	0.71
Fruit surface cracking	2.00[a]	1.93[ab]	1.75[b]	1.81[ab]	1.84[ab]	1.89[ab]	2.00[a]	1.83
Plant height (m)	3.13[a]	2.80[ab]	2.76[ab]	2.72[b]	2.50[b]	2.54[b]	2.43[b]	2.66
Final fruit set	3.67[a]	3.67[a]	3.60[a]	3.31[a]	3.29[a]	3.07[a]	3.67[a]	3.38
Internode distance (cm)	7.00[a]	6.93[a]	6.48[a]	7.29[a]	6.12[a]	6.33[a]	6.50[a]	6.64
Vigor	2.67[a]	2.13[ab]	2.36[ab]	2.31[ab]	2.34[ab]	2.19[ab]	1.83[b]	2.30
Fruit firmness	3.00[a]	2.27[b]	2.47[ab]	2.49[ab]	2.55[ab]	2.48[ab]	2.83[ab]	2.50
No. fruits per plant	34.80[a]	34.99[a]	26.97[b]	24.69[bc]	21.74[cd]	19.35[d]	15.33[e]	24.37
Fruit weight (g)	167.40[ab]	150.75[b]	180.16a	173.28[ab]	172.98[ab]	162.06[ab]	160.70[ab]	171.94
Harvest number	10.33[a]	10.27[a]	10.30[a]	10.09[a]	9.97[a]	9.78[a]	9.83[a]	10.08
Fruit yield (g)	28245.21[a]	25114.85[b]	23423.26[b]	21180.65[c]	18633.56[d]	15939.10[e]	12544.00[f]	20575.44

Means with similar letters in each row are not significantly different at 5% probability level, according to Duncan's multiple range test.

Correlation Analysis

The highest significant positive correlations were observed between yield and the fruit count ($r = 0.71^{**}$), plant height ($r = 0.41^{**}$), fruit shape ($r = 0.27^{*}$), lobe number ($r = 0.26^{*}$), fruit weight ($r = 0.24^{*}$), and stalk diameter ($r = 0.23^{*}$), respectively. Therefore, any of the hybrids with more fruit count, plant height, and fruit weight will have more fruit yield, as well as hybrids with more lobe number, symmetrical fruit shape, and optimal stalk diameter (between 1-1.5 cm), will have more fruit yield. In contrast, the significant negative correlations were observed between yield with the mature fruit color ($r = -0.25^{*}$) as well as fruit surface cracking ($r = -0.22^{*}$). ASIIG index showed significant positive correlations with fruit yield ($r = 0.94^{**}$), fruit count ($r = 0.89^{**}$), plant height ($r = 0.43^{**}$), final fruit set ($r = 0.27^{*}$), and harvest number ($r = 0.24^{*}$), respectively (Table 6).

Stepwise regression

The stepwise regression model was designed to eliminate ineffective or less significant traits while identifying those that accounted for significant variation in fruit yield.In the current study, the stepwise regression model was used to determine the effective components in the fruit yield of 85 hybrids based on 17 traits. A total of five traits entered the model and fruit count was the first trait and explained 51% of the fruit yield changes. The following entered traits in the model were as follows: fruit weight, harvest number, stalk diameter, and final fruit set, which explained a total of 95% of the changes in fruit yield. The contribution of other traits in justifying the changes in fruit yield was minimal. In other words, other traits had no significant effect on the regression model, so the difference of hybrids in terms of fruit yield in this research can be attributed to the difference of these traits. The obtained regression relationship was in the form of the following equation:

$$Y = -315.49 + 75.83\,X_1 + 12.77\,X_2 - 202.07\,X_3 + 138.00\,X_4 + 37.60\,X_5$$

In the above equation, Y is the fruit yield, X_1 to X_5 including the traits fruit count, fruit weight, harvest number, stalk diameter, and final fruit set, respectively and the number 315.49 is the regression constant.

Table 6. Correlation analysis among different morphological traits and ASIIG index.

Trait	Mature fruit color	Stalk length (cm)	Stalk diameter	Lobe number	Fruit length-to-width ratio	Fruit shape	Pericarp diameter	Fruit surface cracking	Plant height	Final fruit set	Internode distance	Vigor	Fruit firmness	No. fruits per plant	Fruit weight	Harvest number	Fruit yield
Mature fruit color	1																
Stalk length (cm)	0.098	1															
Stalk diameter (cm)	-0.211	0.073	1														
Lobe number	-0.030	-0.024	0.226*	1													
Fruit length-to-width ratio	0.000	-0.186	-0.018	0.246*	1												
Fruit shape	0.107	0.119	.256*	0.247*	0.244*	1											
Pericarp diameter (cm)	-0.064	-0.179	-0.085	-0.074	-0.055	0.153	1										
Fruit surface cracking	0.039	0.004	-0.096	-0.077	-0.064	-0.257*	0.043	1									
Plant height (m)	-0.152	-0.087	0.321**	0.132	0.121	0.059	-0.029	-0.091	1								
Final fruit set	0.096	0.039	-0.240*	0.119	0.070	0.047	-0.036	0.045	0.044	1							
Internode distance (cm)	0.077	0.088	-0.123	0.043	0.107	0.127	-0.094	-0.130	-0.165	0.047	1						
Vigor	0.097	-0.071	0.087	-0.016	0.133	0.013	0.117	-0.070	0.102	0.081	-0.176	1					
Fruit firmness	0.168	-0.147	-0.008	0.093	0.164	0.145	0.391**	0.100	-0.120	0.028	-0.085	0.429**	1				
No. fruits per plant	-0.079	-0.009	0.068	-0.032	-0.060	-0.025	-0.139	-0.089	0.442**	0.257*	0.042	0.126	-0.091	1			
Fruit weight (g)	-0.281**	0.098	0.081	0.188	0.053	0.282**	0.148	-0.159	-0.129	-0.088	-0.035	-0.114	-0.134	-0.372**	1		
Harvest number	-0.134	0.029	-0.014	-0.252*	-0.154	-0.149	-0.006	-0.027	0.103	-0.038	-0.140	-0.192	-0.382**	0.262*	0.250*	1	
Fruit yield (g)	-0.249*	0.053	0.231*	0.259*	0.059	0.270*	-0.025	-0.225*	0.407**	0.262*	0.077	0.147	-0.068	0.714**	0.243*	0.074	1
ASIIG	-0.204	0.047	0.168	0.141	-0.006	0.152	-0.083	-0.173	0.433**	0.269*	0.052	0.113	-0.104	0.892**	0.036	0.240*	0.939**

* and **: significant at 5% and 1% probability levels, respectively.

GT biplot

To identify the desired hybrids in terms of the studied traits, a GT biplot polygon diagram was drawn. GT biplot showed that the two main components explained a total of 99.99% (PC1 = 99.67 and PC2 = 0.31%) of the changes (Fig. 1). Yang et al. (2009) stated that the first two components should explain more than 60% of the variation in the data. The results of the present research showed that most of the total variation was explained by the first component, indicating that the biplot diagrams efficiently represent the variability of the data. In this diagram, hybrids that are farthest from the origin of the diagram are connected by lines and form a polygon so that other hybrids are placed inside this polygon (Yan and Kang, 2002). Hybrids that are in a section with one or more specific traits show good performance for that trait. Also, hybrids close to the origin of the graph do not respond well to trait changes (Yan and Rajcan, 2002). Based on the drawn multidimensional diagram, hybrids of 407, 350, C2, 137, 268, 408, and 382 had the greatest distance from the origin (located at the vertices of the biplot) and may be investigated in breeding programs to help develop hybrids that respond to traits of interest. Therefore, hybrids located at the apex of the biplot, also known as apex hybrids, show the best performance in one or more traits. Also, in each section hybrids 382, 130, 352, 153, 38, 376, 298, 175, 358, 197, 101, 380, 66, and 99 in terms of fruit yield, fruit count, fruit weight, final fruit set, plant height, and stalk diameter traits, hybrids 407, 111, 32, 172, 414, 351, 100, 68, C3, 212, 414, 20, 96, and 371 in terms of vigor, fruit length-to-width ratio, fruit shape, lobe number, and internode distance traits, hybrids 41, 26, 21, 35, 413, 94, 75, 389, 110, and 193 in terms of fruit firmness and pericarp diameter traits, hybrids C2, 350, 142, 49, 381, 126, 179, 42, 146, 30, 367, 85, 189, and 29 in terms of mature fruit color trait, hybrids137, 373, 43, 218, 370, 174, 356, and 69 in terms of fruit surface cracking trait, and hybrids 268, 266, 411, 116, C1, 5, 410, 369, 128, and 51 in terms of stalk length and harvest number traits were more desirable than other hybrids. According to Figure 1, the hybrids that are placed in the parts of the polygon where no traits are included are the weakest in terms of all traits while those inside the polygon demonstrate minimal responses to the studied traits.

Cluster analysis

The dendrogram obtained from the cluster analysis of 81 pepper hybrids along with the controls based on the studied traits is presented in Figure 2. Grouping of hybrids by Ward's method (Fig. 2.) placed the hybrids in four groups (14, 33, 21, and 17 hybrids in clusters 1 to 4, respectively). Discriminant analysis was used to check the correctness of the grouping of hybrids in the cluster analysis method. The probability of accuracy of grouping with discriminant analysis for 4 clusters was 94.1% (data not shown). The first cluster hybrids were assigned the highest value in terms of the mature fruit color, pericarp diameter, fruit surface cracking, plant height, number of fruits per plant, harvest number, and fruit yield traits compared to the other clusters. The hybrids of the second cluster had the highest amount of the mature fruit color, stalk length, fruit length-to-width ratio, fruit shape, pericarp diameter, vigor, and fruit firmness traits. The third cluster hybrids were assigned the highest value in terms of the stalk diameter, lobe number, fruit length-to-width ratio, fruit shape, pericarp diameter, plant height, vigor, fruit firmness, number of fruits per plant, and fruit yield compared to the other clusters. The hybrids of the fourth cluster were allocated the highest value in terms of the stalk length, stalk diameter, fruit shape, number of fruits per plant, fruit weight, harvest number, and fruit yield compared to the other clusters (Table 7). It should be noted that the coding of qualitative traits was done in such a way that the most amount of code was given to marketable characteristics. The traits of the final fruit set and internode distance did not show statistically significant differences among the clusters.

Discussion

Fruit yield is the most important economic trait of bell pepper. In the current study, Hybrid 382 exhibited the highest (130.41, 50.60, 15.70, and 11.81%) positive heterosis for fruit yield over C2, C1, C3, and C4, respectively. Hybrid 411 exhibited the highest (216.59, 81.03, 65.76, and 32.02%) positive heterosis for fruit count over C2, C3, C1, and C4, respectively. After hybrid 411, hybrid 382 had the highest number of fruits. For fruit weight trait, Hybrid 200 exhibited the highest (56.77, 31.39, 24.38, and 0.06%) positive heterosis over C1, C2, C3, and C4, respectively.

The positive heterosis observed in the present study for the traits of fruit count, fruit weight, and fruit yield compared to all the studied controls, may be a breeding advantage to obtain a higher yield. The results of this study showed that there was positive and negative heterosis over the control varieties for all studied traits. Negativeheterosis rate is a sign of the tendency of hybrids toward the parent with lower trait values. Ahmed and Muzafar (2000) also mentioned the highest heterosis of 174.52% over better parent for fruit yield in sweet pepper. Shrestha et al. (2011) reported heterosis -20.3-129.8%, heterobeltiosis -24.6-119.3%, and heterosis over standard cultivars -46.9-73.1% for fruit yield and heterosis -19.2-104%, heterobeltiosis -42.4-87.2%, and heterosis over standard cultivars -40.2-76.1% for fruit count in sweet pepper.

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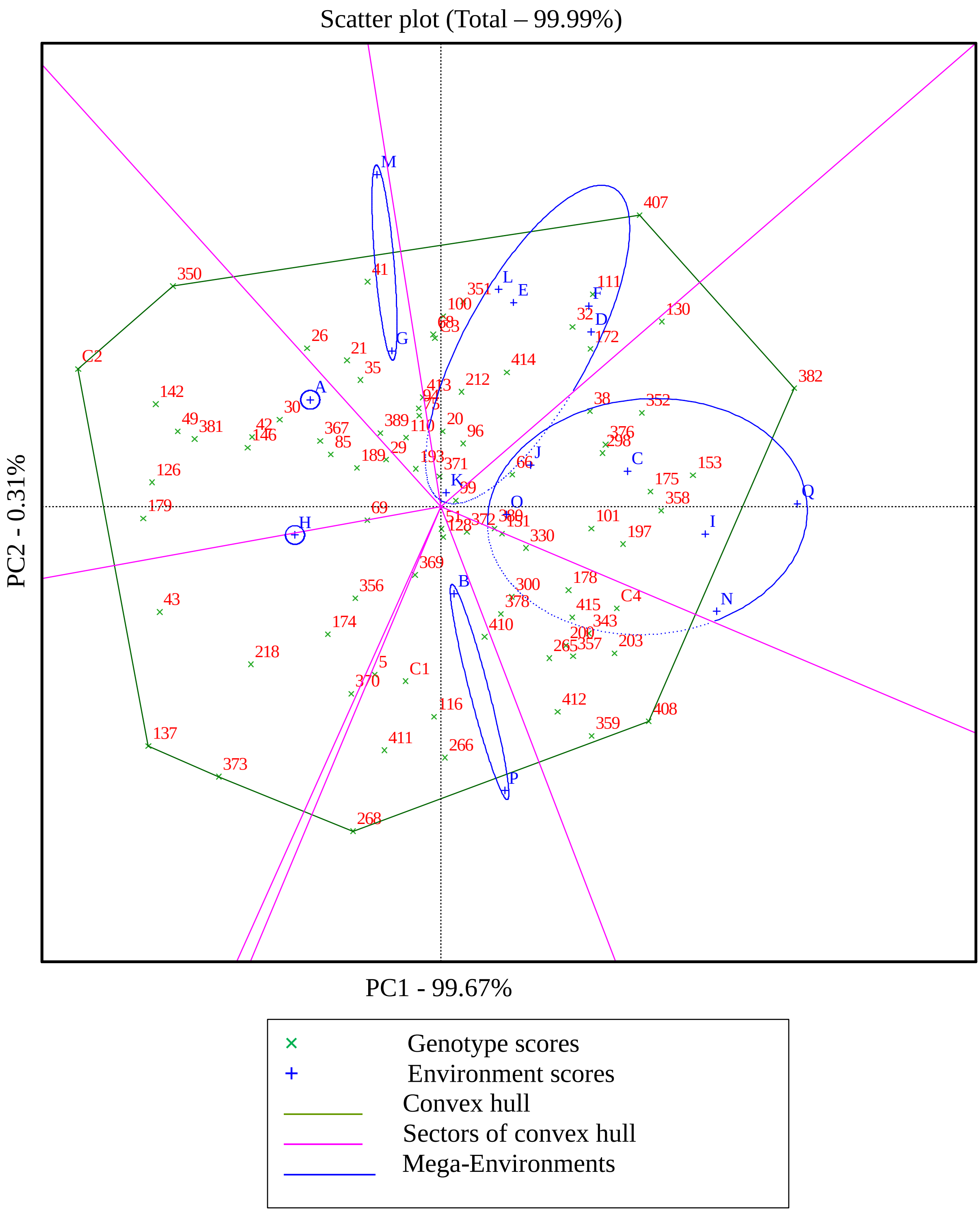

Fig. 1. Polygon view of the which-won-where biplot graph based on hybrids and traits.

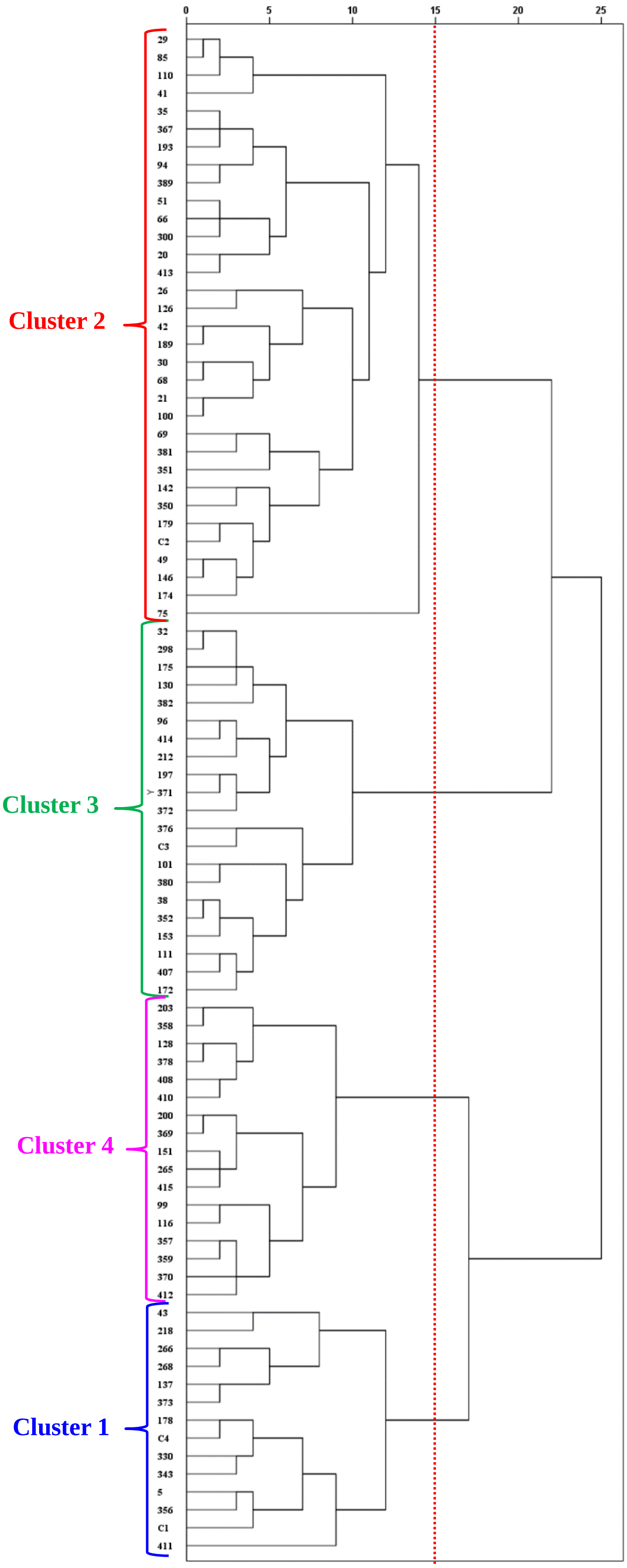

Fig. 2. Dendrogram of cluster analysis of studied hybrids using Ward's method.

Table 7. Mean of plant traits of different groups of pepper hybrids in cluster analysis.

Trait	Mean of clusters				Total mean
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	
Mature fruit color	1.74[ab]	1.90[a]	1.60[bc]	1.49[c]	1.72
Stalk length (cm)	1.79[b]	1.89[ab]	1.79[b]	1.96[a]	1.86
Stalk diameter (cm)	1.64[b]	1.68[b]	1.87[a]	1.78[ab]	1.74
Lobe number	2.14[c]	2.96[b]	3.78[a]	3.14[b]	3.06
Fruit length-to-width ratio	1.02[b]	1.17[a]	1.21[a]	1.06[b]	1.13
Fruit shape	1.36[b]	1.73[a]	1.71[a]	1.71[a]	1.66
Pericarp diameter (cm)	0.69[ab]	0.74[a]	0.73[a]	0.67[b]	0.71
Fruit surface cracking	1.98[a]	1.87[b]	1.78[c]	1.69[d]	1.83
Plant height (m)	2.78[a]	2.46[c]	2.91[a]	2.63[b]	2.66
Final fruit set	3.29[a]	3.43[a]	3.46[a]	3.25[a]	3.38
Internode distance (cm)	6.55[a]	6.89[a]	6.34[a]	6.59[a]	6.64
Vigor	2.05[b]	2.41[a]	2.45[a]	2.10[b]	2.30
Fruit firmness	2.19[b]	2.69[a]	2.73[a]	2.12[b]	2.50
No. fruits per plant	26.70[a]	21.68[b]	26.31[a]	25.25[a]	24.37
Fruit weight (g)	158.72[c]	169.52[b]	172.73[b]	186.54[a]	171.94
Harvest number	10.43[a]	9.85[b]	9.86[b]	10.53[a]	10.08
Fruit yield (g)	19909.02[b]	18533.81[b]	22953.81[a]	22149.44[a]	20575.44

Means with similar letters in each row are not significantly different at 5% probability level, according to Duncan's multiple range test.

Similar results have been reported regarding positive and negative heterosis in traits such as fruit length (-39.13 to 31.23%), fruit width (-31.79 to 20.81%), number of locules per fruit (-33.33 to 21.21%), and pericarp thickness (-75.95 to 5.44%) in bell pepper (Praveen et al., 2017). Similarly, other results have been reported positive and negative heterosis in traits such as fruit length, fruit count, number of lobes, pedicel length, pericarp thickness, plant height, pericarp thickness, and yield in pepper (Sharma et al., 2013).

In the current study, based on correlation coefficients, it may be concluded that the number of fruits, plant height, fruit shape, lobe number, fruit weight, and stalk diameter were main fruit yield contributing traits which should be considered during selection for improving fruit yield. In the previous studies, Sood et al. (2009) reported that fruit yield had a significant positive association with fruits per plant, harvest duration, and average fruit weight.

In another report, a highly significant and positive phenotypic and genotypic correlation of yield was found with fruit length, fruit width, number of fruits per plant, pericarp thickness, average fruit weight, and harvest duration (Sharma et al., 2019). Thakur et al. (2019) reported that a highly significant and positive genotypic and phenotypic correlation of yield per plant was found with fruit weight, fruit breadth, number of fruits per plant, number of lobes per fruit, and number of primary branches. The correlation results of fruit yield with other traits in the present study were in agreement with the results of previous reports. In other research, Jabbari and Darvishzadeh (2023) stated that fruit yield had a positive and significant correlation with the traits of fruit diameter, fruit circumference, pulp weight, and single fruit weight, but the traits of fruit count and fruit length had a negative and significant phenotypic correlation with fruit yield. The results of the present research showed that the number of fruits had a positive and significant correlation with the fruit yield, which was in conflict with the results of Jabbari and Darvishzadeh (2023). The results obtained regarding the correlation between traits in different experiments can be conflicting. This means that the relationship between two traits is reported to be positive and significant in an experiment and negative and significant in another similar experiment in another region. Considering that the nature of the relationships between the components is not only genetic and changes from one environment to another, for this reason different results may be reported in the experiments (Keshavarz et al., 2019).

In our research, traits fruit count, fruit weight, harvest number, stalk diameter, and final fruit set were entered into the stepwise regression model as the most effective traits on fruit yield which explained a total of 95% of the changes in fruit yield. In another research on 65 lines of pepper, results of stepwise regression showed that three traits of stem length, stem diameter, and number of fruits per plant had the greatest effect on fruit yield (Keshavarz et al., 2019). Thakur et al. (2019) reported that the maximum direct and positive effect on fruit yield was contributed by fruit weight followed by fruit count, thousand seed weight, fruit breadth, number of seeds per fruit, and fruit length. They stated that the improvement in these traits will lead to higher yield in bell peppers. In other research, Jabbari and Darvishzadeh (2023) stated that based on the results of stepwise regression for fruit yield, seven traits of pulp weight, round surrounding of bush, fruit diameter, fruit count, plant height, total seed weight and number of branches were entered into the model as the most effective traits on fruit yield, which explained 84.6% of the fruit yield changes between 30 Iranian pepper accessions.

According to the ASIIG index, hybrids 382, 343, 408, C4, 411, and 175 in the completely ideal quarter and hybrids 178, 358, 410, 376, 352, 330, 407, 200, 378, 153, 298, 203, 415, 197, 357, 266, 172, 130, 351, 38, C3, 412, 32, 359, 265, 380, 66, 101, 372, 212, and 111 in the ideal quarter were identified as the best hybrids in this study. Hemadesh et al. (2021) studied genetic diversity and selected high-yielding lines with desirable agronomic traits using the ASIIG index of 140 barely advanced lines. Their result showed that the ASIIG index, by integrating different traits and determining the appropriate weight for different traits, selects the desired lines more effectively; thus, the lines with the highest ASIIG amount were the ideal lines. They evaluated the efficiency of the ASIIG index in selecting the best lines in terms of quantitative and qualitative traits simultaneously; where they classified the studied lines into seven groups based on the ASIIG index. In previous studies, the ASIIG index was used to identify superior genotypes of apple (Ahmadi et al., 2022), olive (Taghizadeh et al., 2021), barley (Hemadesh et al., 2021), and Asian pear (Ahmadi et al., 2023) according to all the investigated morphological traits

The GT biplot in our research indicated that hybrid 382 (the vertex hybrid), along with hybrids 130, 352, 153, 38, 376, 298, 175, 358, 197, 101, 380, 66, and 99 had the highest values for various traits, including fruit yield, fruit count, fruit weight, final fruit set, plant height, and stalk diameter. In a study conducted by Abu et al. (2011), 22 morphological traits were assessed across 10 pepper genotypes for three years. They reported variation due to GT biplot of 70.7, 65.9, 70.7, and 75.3% for 2002, 2003, 2004, and combined analysis, respectively. The authors concluded that selecting high-yielding genotypes in pepper can effectively be achieved through indirect selection of yield-related components, as demonstrated by the GT biplot analysis In many researches, the GT method has been used to evaluate and select different cultivars in terms of multiple traits, and the mentioned method has been introduced as a suitable tool for multivariate data exploration and graphical representation of genotype data in traits. GT biplot has already been used in crops such as soybean (Yan and Rajcan, 2002), white lupin (Atnaf et al., 2017), common bean (Oliveira et al., 2018), sunflower (Shojaei et al., 2022), cocoa (Araújo et al., 2024), olive (Dadras et al., 2024), and cowpea (Nuryati et al., 2024).

In our research, cluster analysis by Ward's method placed 85 hybrids into four groups based on 17 investigated traits. In a study by Saleh et al. (2016), sixteen quantitative morphological traits were evaluated in local pepper germplasm. Using cluster analysis of combined data, they grouped 60 pepper genotypes into five clusters and 10 sub-clusters and stated that these clusters can be useful for the selection and source of favorable genes. In another survey by complete linkage clustering method 28 genotypes of sweet pepper classified into eight different groups (Danojević and Medić-Pap, 2018). In another research on 65 lines of pepper, cluster analysis using Ward method, classified lines into six different groups. These researchers stated that this grouping was associated with some of the morphological traits that are associated with the marketability and geographic distribution of lines (Keshavarz et al., 2019).

Based on the findings of this research, 37 hybrids in the completely ideal and the ideal quarters were distributed in clusters 3 (18 hybrids), 4 (11 hybrids), 1 (6 hybrids), and 2 (2 hybrids). The third cluster hybrids were assigned the highest value in terms of the stalk diameter, lobe number, fruit length-to-width ratio, fruit shape, pericarp diameter, plant height, vigor, fruit firmness, number of fruits per plant, and fruit yield compared to the other clusters. The hybrids of the fourth cluster were allocated the highest value in terms of the stalk length, stalk diameter, fruit shape, number of fruits per plant, fruit weight, harvest number, and fruit yield compared to the other clusters.

Conclusion

According to the findings of this research, hybrid selection based on several traits is one of the basic characteristics of vegetable crop research, and it is inevitable to present a logical and suitable method considering the simultaneous selection of several traits. Although multivariate statistical methods provide great help in the simultaneous studies of traits, in some cases, especially in vegetable products, due to the unequal weight of different traits in the selection index of the desired cultivar and the application of special breeding goals, they are not effective. The ASIIG method, considering the appropriate weight for each trait according to the selection objective, can provide a suitable method for simultaneous selection and determining the superior genotypes. In general summary, the use of the ASIIG index along with other statistical methods can be a good pattern in choosing the desired variety in terms of quantitative and qualitative traits in vegetables, so it is recommended to use the ASIIG index in addition to multivariate methods such as GT biplot to determine the favorable varieties in a wider range of other vegetables. Therefore, the efforts of researchers and breeders in choosing a correct and logical method for determining the desired genotypes can encourage farmers to choose the appropriate cultivars according to the cultivation environment. In total, based on ASIIG index, hybrids 382, 343, 408, C4, 411, and 175 in the completely ideal quarter and hybrids 178, 358, 410, 376, 352, 330, 407, 200, 378, 153, 298, 203, 415, 197, 357, 266, 172, 130, 351, 38, C3, 412, 32, 359, 265, 380, 66, 101, 372, 212, and 111 in the ideal quarter were identified as the best hybrids in this study.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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