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Genetic Variation among Melon Hybrids: Morphological Traits and Molecular Detection of *Fom-1* and *Fom-2* Resistance Genes

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ABSTRACT

To determine the ideal melon hybrids, genotypes by environment interaction, and *Fom-1* and *Fom-2* resistance genes, 23 melon hybrids were investigated in two locations. Which-won-where polygon plot showed that hybrids 1507, 1782, 1404, and 2109 for E1, and hybrids 1486, 1491, 1323, and 2131 for E2 were ideal. Hybrids 1623, 2077, 1323, 1491, 1486, 2109, 1507, 1404, 1782, and 2131 were close to the ideal hybrid, respectively, and they were ideal hybrids in terms of higher fruit yield ability and stability compared to others. According to the results of the TOPSIS index, six hybrids (1623, 1323, 1507, 2077, 1404, and 1491) were common between the two fields. Based on the GGE biplot results, these hybrids were close to the ideal hybrid in terms of higher fruit yield ability and stability. These hybrids also had genes for resistance to *Fom-1* or *Fom-2*, and can be introduced as promising hybrids in terms of simultaneous selection for yield and desirable traits, yield stability, and resistance to *Fom-1* or *Fom-2* genes.

Introduction

Melon (*Cucumis melo* L.) is one of the most valuable commercial crops in the Cucurbitaceae family worldwide. The important objectives of melon breeding programs include resistance to disease and insect pests, improvement of fruit quality, and increase in yield. The best characteristics of melons are tolerance to diseases and pests, average fruit weight >1.5 kg, net density (>85%), and fruit sweetness (12–15 °Brix). Melon breeding programs aim to develop cultivars adapted to different environments. Genotype-by-environment interaction (GEI), as a primary source of variation in genotype performance, is fundamental for developing superior cultivars with either broad or specific adaptation. Maximizing selection gain in the final phase of plant breeding is achieved by matching genotypes to their optimal environments based on GEI evaluation (Adiredjo et al., 2024). The GGE Biplot methodology is a powerful tool for multi-environment trial data analysis. As demonstrated by Yan and Kang (2002), its key applications include ranking test environments by their discriminatory power, delineating mega-environments, and visually assessing genotype performance and stability.

The current study used the TOPSIS (Technique for Priority Ordering Based on Similarity) index, a multi-criteria decision analysis, to select the best hybrids based on fruit yield and other studied traits. This method chooses the alternative with the least and the most distance from the positive and negative ideal solution, respectively (Hwang and Yoon, 1981).

Melon, like many crops, is susceptible to various leaf and root fungal diseases. *Fusarium* wilt is one of the most important fungal diseases of the root (Oumouloud et al., 2013). The soil-borne

ascomycete *Fusarium oxysporum* is a devastating pathogen for a wide range of crops globally. Infection typically results in a lethal vascular disease, characterized by symptoms such as yellowing, wilting, and ultimately, plant death (Sabahi and Banihashemi, 2020). Risser et al. (1976) identified four races, 0, 1, 2, and 1.2 for *Fom*, based on pathogenesis activity in muskmelon cultivars according to *Fom-1* and *Fom-2* resistance genes. Race 1 is more widespread in Iran, especially in the northern provinces of the country, and the damage to this race to the crop is more than race 2 (Banihashemi, 2010). *Fom-1* and *Fom-2* are known as dominant single resistance genes, showing stable resistance against races 0, 2, and 0, 1, respectively (Risser et al., 1976). The ability of known races to overcome resistance genes (*Fom-1* and *Fom-2*) is currently used for identification. In this way, races 0, 1, 2, and 1.2 are able to pathogenicity in cultivars without resistance genes. So race 0 can infect cultivars without *Fom-1* or *Fom-2*, race 1 is virulent to cultivars without *Fom-2*, race 2 can infect cultivars with *Fom-2* (without *Fom-1*) and 1.2 race has pathogenicity in cultivars carrying *Fom-1* and *Fom-2* (Sabahi and Banihashemi, 2020). Isolates that are known as race 1.2 can be pathogenic in melons with resistance genes. This race has two variants, 1.2W and 1.2Y, which cause wilting and yellowing, respectively. The 1.2Y variant is more common in Iran (Banihashemi, 1989; Herman and Perl-Treves, 2007). In the Perlita–FR cultivar was identified a third resistance gene, *Fom-3* confers resistance to races 0 and 2 (Zink and Gubler, 1985). The *Fom-4* gene has recently been reported as a recessive gene that is related to *Fom-1* and confers resistance to races 0 and 2 in Tortuga melon (Spanish melon accession). In the Ptherlita–FR cultivar, was identified a third resistance gene, *Fom-3* that confers resistance to races 0 (Oumouloud et al., 2010).

Iran's ranking as the third producer of this product in the world shows the country's high ability to produce this product, but unfortunately, the cultivars cultivated in the country are still indigenous cultivars that do not have high performance and quality. Therefore, it seems necessary to use classical and molecular breeding methods for the breeding of this plant.

The main objective of this study was to comprehensively evaluate melon hybrids in terms of agronomic performance and genetic resistance. To this end, we pursued the following objectives: 1) to investigate genetic diversity and select superior hybrids by employing the TOPSIS index for a simultaneous assessment of fruit yield and morphological traits, 2) to evaluate the performance stability of 23 hybrids of melon under two environmental conditions via GGE biplot analysis, 3) to screen the hybrids for the presence of the *Fom-1* and *Fom-2* resistance genes using molecular markers.

Materials and Methods

Plant materials

To investigate genetic diversity and evaluate hybrids, a set of 23 melon hybrids (belonging to Negin Bazr Danesh Company) was evaluated. This experiment was carried out using a randomized complete block design with three replications in the research fields of Khatunabad (E1) (latitude of 51° and 66′ and a longitude of 32° and 65′ and an altitude of 1517 meters above sea level) and Mahmudabad (E2) (latitude of 51° and 78′ and a longitude of 32° and 49′ and an altitude of 1575 meters above sea level) of Isfahan province. The seeds of the studied hybrids were planted on April 4, 2022. For planting, two seeds from each hybrid were sown in trays with 112 cells, filled with cocopit and perlite substrate. The germination of seedlings was carried out in protected conditions in a greenhouse. When three leaves appeared, the seedlings were transferred to the field. Ten seedlings were planted in every replicate. The distance between the plants in- and between-rows was 40 and 150 cm, respectively. Drip irrigation was used to irrigate the plants.

Plant measurements

Morphological traits were measured based on the melon's description (IPGRI, 2003). The measured traits were as follows: fruit shape, fruit length (cm), fruit diameter (cm), skin color, fruit surface armor, fruit surface tracking, flesh color, flesh thickness, flesh texture, vigor, fruit taste, brix, cavity size (cm), fruit count per plant, fruit weight (kg), and fruit yield per plant (kg).

Statistical analysis

Analysis of variance (ANOVA), correlation, and regression were done, using SPSS 26 software, TOPSIS index was calculated by Hwang and Yoon (1981) using Excel, and GGE biplot with GenStat 12th edition. According to the expected mean squares in ANOVA the phenotypic and genotypic variances were calculated using following formula:

$$V_e = \frac{MS_e}{r} \quad (1)$$

Where V_e is the error variance, MS_e is the error mean squares, and r is the number of replications.

$$V_g = \frac{(MS_g - MS_{gl})}{rl} \quad (2)$$

Where Vg is the genotypic variance, MS_g and MS_{gl} is the mean squares of genotypes and genotype by location interaction, and l is the number of locations.

$$V_P = V_g + V_e \quad (3)$$

Where Vp is the phenotypic variance. Broad-sense heritability (h^2) for each trait was calculated as following:

$$h^2 = \frac{V_g}{V_P} \times 100 \quad (4)$$

DNA extraction

In the molecular evaluation, two commercial hybrids were used as controls (Isabel and Charentains T containing resistant *Fom-2* and *Fom-1* genes, respectively). To determine the plant genotypes with markers, all the plants were sampled in the 2-3 stage. Total DNA was extracted from the sample using the CTAB protocol (Murray and Thompson, 1980). The quantity and quality of the extracted DNA were measured by the NanoDrop set and were used as a template in the PCR assay**.**

Molecular markers based on PCR

In the current study, Fom2-R_{409}, Fom1, and Fom2-S_{253} markers were utilized to identify *Fom-2*, *Fom-1* resistance genes, and *Fom-2* susceptible ones, respectively. The primer sequences and annealing temperatures are detailed in Table 1. Amplification reactions were performed in an Eppendorf Master cycler Gradient, using a 15 µL reaction mixture, comprising 7.5 µL *Taq* DNA Polymerase (2x Master Mix RED (Amplicon)), 1 µL of each specific primer (10 µM), 4 µL $_{dd}H_2O$, and 1-2 µL of genomic DNA (50 ng μL^{-1}). Total DNA was amplified using a BIO-RAD T100 Thermal Cycler following the manufacturer's recommendations. The thermal profile was as follows: initial denaturation at 94 °C for 180 s, followed by 35 cycles of 94 °C for 60 s, 54 °C for 60 s, and 72 °C for 120 s, with a final extension at 72 °C for 5 min. Then 3 µL of PCR product was tested in 1.2% gel agarose electrophoresis. To determine fragment size, a 100 bp DNA marker was used.

Table 1. Molecular marker-specific primer information used in the current study.

Marker ID	Marker Type	Sequence	Annealing Temperature	Fragment size (bp)
FOM1	CAPS	F: ATGAGTTTTGATAGTTTCATAAG R: GAACACTCCCTTAGATACTT	54 °C	568 (Oumouloud et al., 2015)
FOM2-R_{409}	SCAR	F: GAGAAATTTGCAATGGGTGG R: TTACACTATTATTGCTCAACTTGC	54 °C	409 (Sousaraei et al., 2018)
FOM2-S_{253}	SCAR	F: GACTTTACAGAAAGGCGTTTA R: ATTGCTCTAAGTTGATCATATTCTG	54 °C	253 (Sousaraei et al., 2018)

Results

Analysis of variance

The results of ANOVA in different locations showed that there is a significant difference between the investigated hybrids in each location and the significant difference between hybrids indicated genetic variability between melon hybrids (data not shown). To summarize the results related to the comparison of hybrid performance in two experimental locations, a combined analysis of variance was needed so that in addition to comparing the performance of genotypes, the interaction effect of genotype × location could be found. Therefore, after Hartley's F-max test (0.01, 44, 44) and the uniformity of error variances was determined, a combined analysis of variance experiment was conducted (combined analysis was conducted on traits that did not exhibit a significant Hartley's F-max result). The results of combined analysis showed that the significance of the main effects of location (except brix and fruit yield traits), hybrids, and hybrid × location interaction (Table 2). The significant effect of location for most traits could be the result of factors such as distinct pedoclimatic conditions of each site, including variations in soil physicochemical properties, microclimate, and agronomic management practices, which collectively modified the phenotypic expression of the hybrids. The significant effect of hybrids indicated genetic diversity among the experimental materials. The findings indicated that there was significant genetic variation among the hybrids. Although the average yield was not significantly different between the two experimental sites, the observation of a significant hybrid × environment interaction indicated that the hybrids responded differently to the specific conditions of each site (Table 2).

Based on the results of this study, it can be concluded that although the yield potential of the studied hybrids is different, the expression of this potential is strongly influenced by the specific environmental conditions of each region. Therefore, introducing a single superior hybrid for both regions is not recommended and it is necessary to select superior hybrids for each region separately.

In most traits (except vigor and fruit count), a large part of the phenotypic variance was explained by the genetic component, and the contribution of genetic variance to the phenotypic variance was high. The heritability results showed that high heritability values were estimated for flesh thickness, fruit weight, fruit yield, and brix traits (Table 2). The hybrids selected in this study had a wide range of variation for the target traits (e.g., yield), resulting in high genetic variance and, consequently, enhanced heritability.

Table 2. Combined analysis of variance and genetic parameters for some traits in melon hybrids grown during two locations.

Source of variance	df	Mean Square						
		Flesh thickness	Vigor	Fruit taste	Brix	Fruit count	Fruit weight	Fruit yield
Location	1	4.80**	3.79**	2.71**	2.80^{ns}	2.62**	0.20**	0.20^{ns}
Replication (Location)	4	0.07^{ns}	0.57^{ns}	3.91**	8.65**	0.03^{ns}	0.07*	0.47^{ns}
Genotypes	22	2.87**	3.27**	7.36**	24.16**	1.71**	0.95**	6.98**
Genotypes × Location	22	1.68**	2.63**	5.33**	14.29**	0.83**	0.51**	3.69**
Error	88	0.05	0.47	0.32	0.98	0.35	0.02	0.22
Coefficient of variation (CV%)	-	5.56	12.02	8.98	9.83	20.50	11.57	12.39
Hartley's F-max		1.70	1.32	1.37	1.23	1.13	1.29	1.01
Environmental variance (V_e)	-	0.02	0.16	0.11	0.33	0.12	0.01	0.07
Genotypic variance (V_g)	-	0.20	0.11	0.34	1.65	0.15	0.07	0.55
Phenotypic variance (V_p)	-	0.21	0.26	0.44	1.97	0.27	0.08	0.62
Broad sense heritability (h^2%)	-	92.43	40.65	76.28	83.43	55.40	90.31	88.17

^{ns}, *, and **; non-significant, significant at 5% and 1% probability levels, respectively. Hartley's F-max (0.01, 44, 44) = 2.114.

TOPSIS method

TOPSIS index was used to select the best hybrids based on fruit yield and other traits. The value of the TOPSIS index varies from 0 to 1, whenever the TOPSIS value is closer to one, the hybrid will be more favorable. The best hybrid was the hybrid that had the smallest distance from the ideal and was far from the undesirable hybrid. In the current research, the amount of the TOPSIS index was between 0.13 and 0.74, and 0.25 to 0.79 for E1 and E2, respectively.

In E1, the highest amount of TOPSIS index belongs to hybrids 1507, 1623 (0.7-0.8), 1782, 1323, 2077, 1404 (0.6-0.7), and 2109 (0.5-0.6), respectively. The fruit yield of these selected hybrids was higher than the average yield of all studied hybrids. According to the current results, hybrid 2113 with the lowest TOPSIS value (0.127) was the weakest in terms of fruit yield and other yield-related traits (Table 3). After hybrid 2128, this hybrid had a lower yield than other studied ones.

In E2, hybrids 1623, 1323 (0.7 - 0.8), 2131, 1491, 2128, 1486, 2112 (0.6-0.7), and 2113, 2077, 1507, 1398, 1404, 2070, 1441 (0.5-0.6) had the highest amount of TOPSIS index, respectively. The fruit yield of these selected hybrids (except 1507, 1404, 2070, and 1441) was higher than the average yield of all studied hybrids. Hybrid 2092 with the lowest TOPSIS value (0.252) was the weakest in terms of fruit yield and other yield-related traits (Table 3). This hybrid had a lower yield than other studied ones.

By evaluating the efficiency of the TOPSIS index in selecting the best hybrids in terms of all studied traits simultaneously, the studied hybrids were segregated into seven and six groups for E1 and E2, respectively. It is also worth noting that since the TOPSIS index in this study was less than 0.8, the first group consisted of hybrids whose TOPSIS 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 (Tables 4 and 5).

Table 3. Amounts of TOPSIS indices for 23 hybrids of melon.

Khatunabad field (E1)					Mahmudabad field (E2)				
Hybrid	d^+	d^-	TOPSIS index	TOPSIS rank	Hybrid	d^+	d^-	TOPSIS index	TOPSIS rank
1507	0.131	0.367	0.738	1	1623	0.069	0.267	0.794	1
1623	0.123	0.321	0.724	1	1323	0.095	0.240	0.717	1
1782	0.161	0.287	0.641	2	2131	0.099	0.229	0.698	2
1323	0.159	0.249	0.611	2	1491	0.109	0.248	0.695	2
2077	0.174	0.272	0.610	2	2128	0.126	0.212	0.627	2
1404	0.176	0.263	0.600	2	1486	0.152	0.231	0.604	2
2109	0.207	0.214	0.509	3	2112	0.145	0.220	0.603	2

2046	0.226	0.212	0.484	4	2113	0.139	0.187	0.574	3
1491	0.223	0.196	0.468	4	2077	0.181	0.235	0.565	3
2092	0.258	0.165	0.389	5	1507	0.146	0.182	0.554	3
2112	0.271	0.162	0.374	5	1398	0.152	0.181	0.544	3
2131	0.275	0.157	0.364	5	1404	0.170	0.193	0.531	3
1486	0.275	0.145	0.346	5	2070	0.187	0.197	0.513	3
2132	0.283	0.142	0.334	5	1441	0.192	0.195	0.504	3
2070	0.309	0.142	0.315	5	2080	0.210	0.204	0.493	4
1441	0.360	0.153	0.299	6	2081	0.188	0.153	0.448	4
1363	0.325	0.126	0.279	6	2132	0.230	0.178	0.436	4
2080	0.362	0.129	0.262	6	2109	0.186	0.142	0.433	4
2081	0.337	0.099	0.227	6	1782	0.223	0.123	0.355	5
1948	0.358	0.104	0.225	6	1363	0.227	0.122	0.349	5
1398	0.356	0.097	0.214	6	1948	0.223	0.112	0.334	5
2128	0.376	0.102	0.214	6	2046	0.232	0.114	0.329	5
2113	0.388	0.056	0.127	7	2092	0.263	0.089	0.252	6

TOPSIS: Technique for Priority Ordering Based on Similarity, di^+: Distance from ideal, di^-: Distance from non-ideal.

Table 4. Grouping of 23 hybrids of melon based on TOPSIS index and mean of different traits in each group, in the E1.

Trait	Group average based on TOPSIS 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	
Fruit shape	3.000	2.500	1.000	4.000	2.000	2.429	1.000	2.391
Fruit length	20.000	19.858	17.367	13.667	15.300	13.019	10.000	15.525
Fruit diameter	16.500	16.500	17.000	13.750	13.411	12.990	13.800	14.291
Skin color	2.500	2.000	3.000	3.000	2.833	3.000	3.000	2.739
Fruit surface armor	2.000	2.000	2.000	2.000	1.833	2.000	2.000	1.957
Fruit surface tracking	2.000	1.750	2.000	2.000	2.000	1.714	2.000	1.870
Flesh color	1.500	4.250	4.000	2.500	4.000	3.429	2.000	3.435
Flesh thickness	4.500	4.813	5.000	2.750	3.589	3.445	2.250	3.767
Flesh texture	3.000	3.500	4.000	3.000	3.500	3.429	4.000	3.435
Vigor	7.083	5.642	3.700	5.800	5.028	5.748	4.333	5.512
Fruit taste	5.417	6.375	7.667	7.875	5.472	6.148	5.000	6.114
Brix	7.617	10.267	12.800	12.267	9.156	10.476	6.400	9.926
Cavity size	7.500	6.875	7.000	6.750	5.694	5.586	6.000	6.186
Fruit count	3.000	2.667	3.000	2.833	2.500	3.000	2.333	2.768
Fruit weight	2.350	2.117	1.550	1.383	1.328	0.765	0.650	1.368
Fruit yield	7.050	5.175	4.650	4.150	3.450	2.257	1.950	3.748

Table 5. Grouping of 23 hybrids of melon based on TOPSIS index and mean of different traits in each group, in the E2.

Trait	**Group average based on TOPSIS index**						**Total**
	1	**2**	**3**	**4**	**5**	**6**	
	0.7-0.8	**0.6-0.7**	**0.5-0.6**	**0.4-0.5**	**0.3-0.4**	**0.2-0.3**	
Fruit shape	1.000	2.200	2.571	2.250	3.500	3.000	2.478
Fruit length	15.200	15.527	15.086	12.692	14.725	14.667	14.694
Fruit diameter	14.633	14.453	13.719	12.433	13.800	11.200	13.639
Skin color	2.500	3.000	2.286	2.750	2.750	3.000	2.652
Fruit surface armor	2.000	1.800	2.000	2.000	2.000	2.000	1.957
Fruit surface tracking	2.000	2.000	1.857	2.000	1.500	2.000	1.870
Flesh color	3.000	3.400	3.000	4.000	3.500	5.000	3.435
Flesh thickness	4.900	3.887	4.686	3.567	3.842	3.567	4.141
Flesh texture	4.000	3.000	3.000	3.750	4.000	4.000	3.435
Vigor	5.653	6.663	5.465	5.864	5.747	5.067	5.843
Fruit taste	7.475	6.885	6.539	7.166	4.508	5.223	6.394
Brix	12.053	11.008	10.469	11.360	7.178	8.267	10.211
Cavity size	5.633	6.880	5.414	5.267	5.900	4.067	5.752
Fruit count	3.667	3.400	3.048	3.083	2.500	2.000	3.043
Fruit weight	1.500	1.427	1.386	0.888	1.308	1.100	1.292
Fruit yield	5.150	4.707	3.905	2.913	3.233	2.200	3.824

The results of the grouping of the studied hybrids based on the TOPSIS index showed that in E1 (Table 4), two hybrids (1507 and 1623) were placed in group one, and their average fruit yield, fruit weight, and fruit count were 7.5 kg, 2.5 kg, and 3.3, in hybrid 1507 and 6.6 kg, 2.2 kg, and 2.7, in hybrid 1623, respectively. Therefore, Hybrids 1507 and 1623 are most similar to the ideal hybrid. In E2 (Table 5), two hybrids (1623 and 1323) were placed in group one, their average fruit yield, fruit weight, and fruit count were 5.2 kg, 1.3 kg, and 4, in hybrid 1623 and 5.1 kg, 1.7 kg, and 3.3, in hybrid 1323, respectively. Therefore, Hybrids 1623 and 1323 are most similar to the ideal hybrid.

In addition to the ranking of the experimental hybrids, there is a need to somehow group the hybrids in terms of desirability. By determining the distance between ideal and non-ideal, hybrids can be grouped into four groups: completely ideal (TOPSIS index = 0.584 to 0.738), ideal (TOPSIS index = 0.432 to 0.584), non-ideal (TOPSIS index = 0.279 to 0.432), and completely non-ideal (TOPSIS index = 0.127 to 0.279). Therefore, hybrids 1507, 1623, 1782, 1323, 2077, and 1404, and hybrids 2109, 2046, and 1491 were placed in the completely ideal and ideal quarters, respectively. In addition to having high yield, these hybrids had other favorable traits such as fruit length, fruit diameter, flesh thickness, vigor, brix, fruit count, and fruit weight. Hybrids 2113, 2128, 1398, 1948, 2081, 2080, and 1363 were placed in the completely non-ideal quarter, in E1. In E2, grouping included: completely ideal (TOPSIS index = 0.658 to 0.794), ideal (TOPSIS index = 0.523 to 0.658), non-ideal (TOPSIS index = 0.387 to 0.523), and completely non-ideal (= 0.252 to 0.387). According to the desirability quadrants based on the TOPSIS index, hybrids 1623, 1323, 2131, and 1491, and hybrids 2128, 1486, 2112, 2113, 2077, 1507, 1398, and 1404 were placed in the completely ideal and ideal quarters, respectively. In addition to having high yield, these hybrids had other favorable traits such as fruit length, fruit diameter, flesh thickness, brix, fruit count, and fruit weight. Hybrid 2092, 2046, 1948, 1363, and 1782 were placed in the completely non-ideal quarter.

Correlation analysis

In E1, the highest significant positive correlations were observed between fruit yield and fruit weight ($r = 0.89^{**}$), fruit length ($r = 0.74^{**}$), fruit diameter ($r = 0.60^{**}$), cavity size ($r = 0.55^{**}$), flesh thickness ($r = 0.42^{*}$), respectively. TOPSIS index showed significant positive correlations with fruit yield ($r = 0.94^{**}$), fruit weight ($r = 0.94^{**}$), fruit length ($r = 0.77^{**}$), fruit diameter ($r = 0.74^{**}$), cavity size ($r = 0.60^{**}$), and flesh thickness ($r = 0.59^{**}$), respectively. TOPSIS index showed significant negative correlations with skin color ($r = -0.41^{*}$) (Table 6).

In E2, the yield had significant positive correlations with fruit count ($r = 0.50^{*}$), fruit diameter ($r = 0.50^{*}$), fruit weight ($r = 0.49^{*}$), and fruit length ($r = 0.72^{*}$), respectively. TOPSIS index showed significant positive correlations with fruit yield ($r = 0.75^{**}$), fruit count ($r = 0.61^{*}$), brix ($r = 0.50^{*}$), and fruit taste ($r = 0.48^{*}$), respectively. TOPSIS index showed significant negative correlations with fruit shape ($r = -0.43^{*}$) (Table 7).

The findings of this research showed that fruit quality traits did not show a correlation with fruit yield traits. Therefore, any of the hybrids with more fruit weight, fruit length, fruit diameter, cavity size, flesh thickness, and fruit count will have more fruit yield.

GGE biplot analysis

According to the GGE biplot method, the first and second principal components explained 74.78 and 25.22% of the changes, respectively. This means that the biplot of these two components justifies 100% of the hybrid changes and the interaction between the hybrid and the environment, which indicates the high validity of the biplot diagram in explaining the hybrid changes along with the interaction between hybrids and the environments. In general, the lack of explanation of the existing changes in the sum of the first and second main components will indicate the complex nature of the interaction between hybrid and environment (Yan and Tinker, 2005), but this does not mean that the biplot is invalid (Yan et al., 2007). If the biplot can explain at least 60% of the data variation, it can be applied to determine the mega environments (Yang et al., 2009).

Discriminating ability of environments

Figure 1 shows the correlation between environments (test locations) using the angle between them. The location vectors are the lines that connect the origin of the biplot and the markers of the test locations, and the cosine of the angle between them approximates the correlation coefficient between ones (Yan, 2002). According to Figure 1, an angle of nearly 90 degrees between two environmental vectors indicated the absence of a significant correlation between them, meaning that performance in one environment was independent of the other.

Table 6. Correlation analysis among different morphological traits and TOPSIS index in the E1.

traits	Fruit shape	Fruit length	Fruit diameter	Skin color	Fruit surface armor	Fruit surface tracking	Flesh color	Flesh thickness	Flesh texture	Vigor	Fruit taste	Brix	Cavity size	Fruit count	Fruit weight	Fruit yield
Fruit length	0.097	1														
Fruit diameter	0.076	0.592**	1													
Skin color	-0.038	-0.268	-0.330	1												
Fruit surface armor	0.067	-0.088	0.481*	-0.092	1											
Fruit surface tracking	-0.398	-0.052	-0.178	-0.380	-0.083	1										
Flesh color	-0.022	0.066	0.018	-0.169	0.213	0.117	1									
Flesh thickness	0.201	0.554**	0.773**	-0.189	0.223	-0.457*	0.081	1								
Flesh texture	-0.273	-0.187	0.082	-0.169	0.512*	0.166	0.288	0.067	1							
Vigor	0.314	0.089	0.214	-0.141	0.429*	-0.060	-0.251	0.287	0.168	1						
Fruit taste	0.199	-0.046	0.356	-0.119	0.403	0.013	0.104	0.195	-0.107	-0.032	1					
Brix	0.133	-0.135	0.221	-0.012	0.290	-0.071	0.182	0.202	-0.178	-0.126	0.937**	1				
Cavity size	-0.133	0.393	0.689**	-0.376	0.453*	0.321	-0.142	0.296	0.027	0.273	0.501*	0.329	1			
Fruit count	-0.288	-0.159	-0.231	0.092	0.045	0.263	-0.086	-0.276	0.029	0.107	-0.197	-0.117	0.043	1		
Fruit weight	0.074	0.827**	0.742**	-0.406	0.012	-0.019	-0.093	0.609**	-0.077	0.261	-0.001	-0.124	0.518*	-0.164	1	
Fruit yield	0.061	0.745**	0.604**	-0.363	-0.035	0.184	-0.219	0.423*	-0.096	0.252	-0.077	-0.187	0.549**	0.172	0.892**	1
TOPSIS	0.212	0.775**	0.739**	-0.414*	0.075	0.041	-0.104	0.588**	-0.129	0.296	0.155	0.042	0.602**	0.001	0.936**	0.945**

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

Table 7. Correlation analysis among different morphological traits and TOPSIS index, in the E2.

traits	Fruit shape	Fruit length	Fruit diameter	Skin color	Fruit surface armor	Fruit surface tracking	Flesh color	Flesh thickness	Flesh texture	Vigor	Fruit taste	Brix	Cavity size	Fruit count	Fruit weight	Fruit yield
Fruit length	0.213	1														
Fruit diameter	0.180	0.762**	1													
Skin color	-0.060	-0.500*	-0.291	1												
Fruit surface armor	0.084	-0.409	-0.145	-0.106	1											
Fruit surface tracking	-0.380	0.177	-0.047	-0.378	-0.083	1										
Flesh color	0.055	-0.087	-0.285	-0.196	0.213	0.117	1									
Flesh thickness	0.067	0.364	0.588**	-0.159	-0.018	-0.148	-0.311	1								
Flesh texture	-0.240	-0.365	-0.220	-0.216	0.512*	0.166	0.288	-0.258	1							
Vigor	-0.333	-0.213	-0.115	0.340	-0.264	0.105	-0.220	-0.385	0.063	1						
Fruit taste	-0.258	-0.310	-0.077	0.275	0.147	0.203	0.148	0.090	-0.109	0.258	1					
Brix	-0.265	-0.246	-0.041	0.237	0.142	0.264	0.179	0.093	-0.126	0.236	0.988**	1				
Cavity size	0.081	0.595**	0.729**	-0.292	-0.093	-0.081	0.042	0.039	-0.098	0.167	-0.097	-0.067	1			
Fruit count	-0.540**	-0.374	-0.300	0.056	0.105	0.360	-0.044	-0.155	0.200	0.275	0.122	0.110	-0.116	1		
Fruit weight	0.296	0.742**	0.779**	-0.284	-0.343	-0.176	-0.233	0.607**	-0.485*	-0.360	-0.191	-0.161	0.460*	-0.414*	1	
Fruit yield	-0.269	0.422*	0.498*	-0.218	-0.350	0.203	-0.357	0.334	-0.192	0.084	-0.176	-0.171	0.396	0.500*	0.490*	1
TOPSIS	-0.432*	0.128	0.342	-0.029	-0.131	0.346	-0.149	0.348	-0.195	0.200	0.484*	0.496*	0.254	0.611**	0.260	0.753**

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

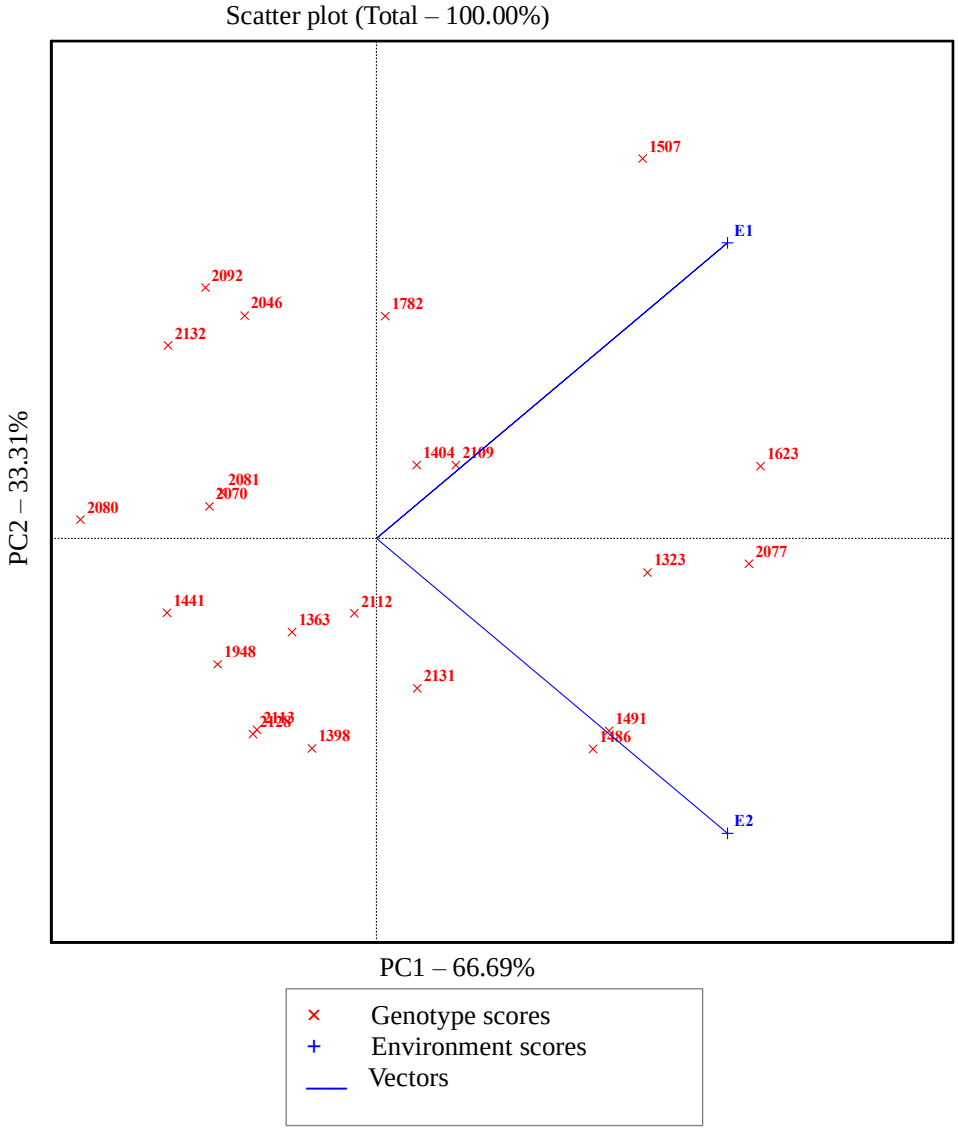

Fig. 1. GGE biplot shows the "relationship between environments" in 23 melon hybrids at two locations for fruit yield trait (E1: Khatunabad field and E2: Mahmudabad field).

The length of the environmental vectors shows an estimate of the standard deviation within each environment and also reveals a measure of the power of discrimination of the hybrids in experimental environments (Yan and Tinker, 2006). The environmental vectors for E1 and E2 exhibited approximately equal lengths, indicating that these two environments demonstrated similar discriminating ability and selective power for evaluating hybrid performance.

Which-won-where polygon view of GGE biplot

In the GGE biplot, the polygon is formed by connecting the vertex hybrids with straight lines, and the rest of the hybrids are placed within the polygon. The vertex hybrids were the best or the worst due to the fruit yield in some or all of the test locations due to their distance from the origin of the biplot.

In the present research, the vertex hybrids were 1507, 1623, 2077, 1486, 1398, 2128, 2080, and 2092. These hybrids had the largest vector length in their respective directions, which indicated their greater interaction with the environment and were specifically adapted to the environment in their respective sectors. However, other hybrids had less interaction in response to the environmental changes. The hybrids close to the origin of the biplot (2112, 1404, and 2109) showed wider adaptation, more stability, and less response to environmental variation than the others (Fig. 2).

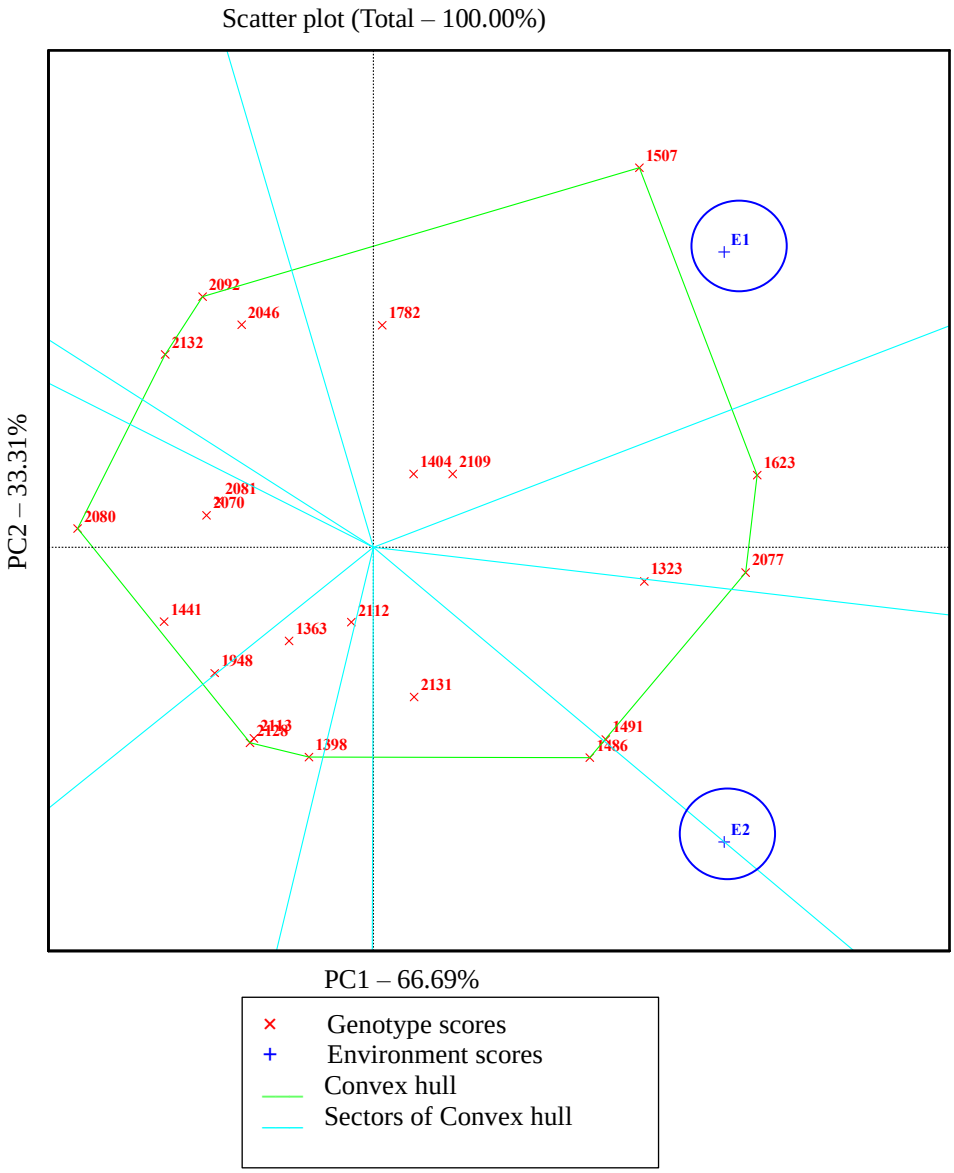

Fig. 2. Polygon of GGE biplot method for determining mega-environments and the appropriate hybrids for each environment.

In the Khatunabad field (E1), hybrids 1507, 1623, 2077, 1323, 1782, 2109, 1404, 1491, 2046, 1486, and 2092 had a higher average than the average total fruit yield, and hybrids 2128, 2113, 2080, 1441, and 1948 had the lowest fruit yield, respectively. In the Mahmudabad field (E2), hybrids 1486, 1491, 2077, 1623, 1323, 2131, 1398, 2112, 2113, 2128, and 2109 were assigned a higher mean than the mean total fruit yield, and hybrids 2092, 2132, 2046, 2080, and 2081 had the lowest fruit yield, respectively.

Which-won-where polygon plot showed that hybrids 1507 (the most favorable) and then hybrids 1782, 1404, and 2109 were ideal for E1, and hybrids 1486 (the most favorable) and then hybrids 1491, 1323, and 2131 were ideal for E2. Conversely, hybrids 2128 and 2113, which are located at the farthest distance from the origin on the opposite side of the environments, had the lowest fruit yield in E1, but in E2, they had a higher fruit yield than the total average, suggesting a high contribution to the existing genotype by environment interaction.

Ranking hybrids relative to the ideal hybrid

An ideal hybrid has both high mean fruit yield and high stability. The center of the concentric circles (Fig. 3) represents the position of an ideal hybrid, which is defined by a projection onto the mean-environment axis that equals the longest vector of the hybrids that had above-average mean fruit yield and by a zero projection onto the perpendicular line (zero variability across environments). A hybrid is more

desirable if it is closer to the ideal hybrid. Although such an ideal hybrid may not exist in reality, it can be used as a reference for hybrid evaluation (Yan and Tinker, 2006).

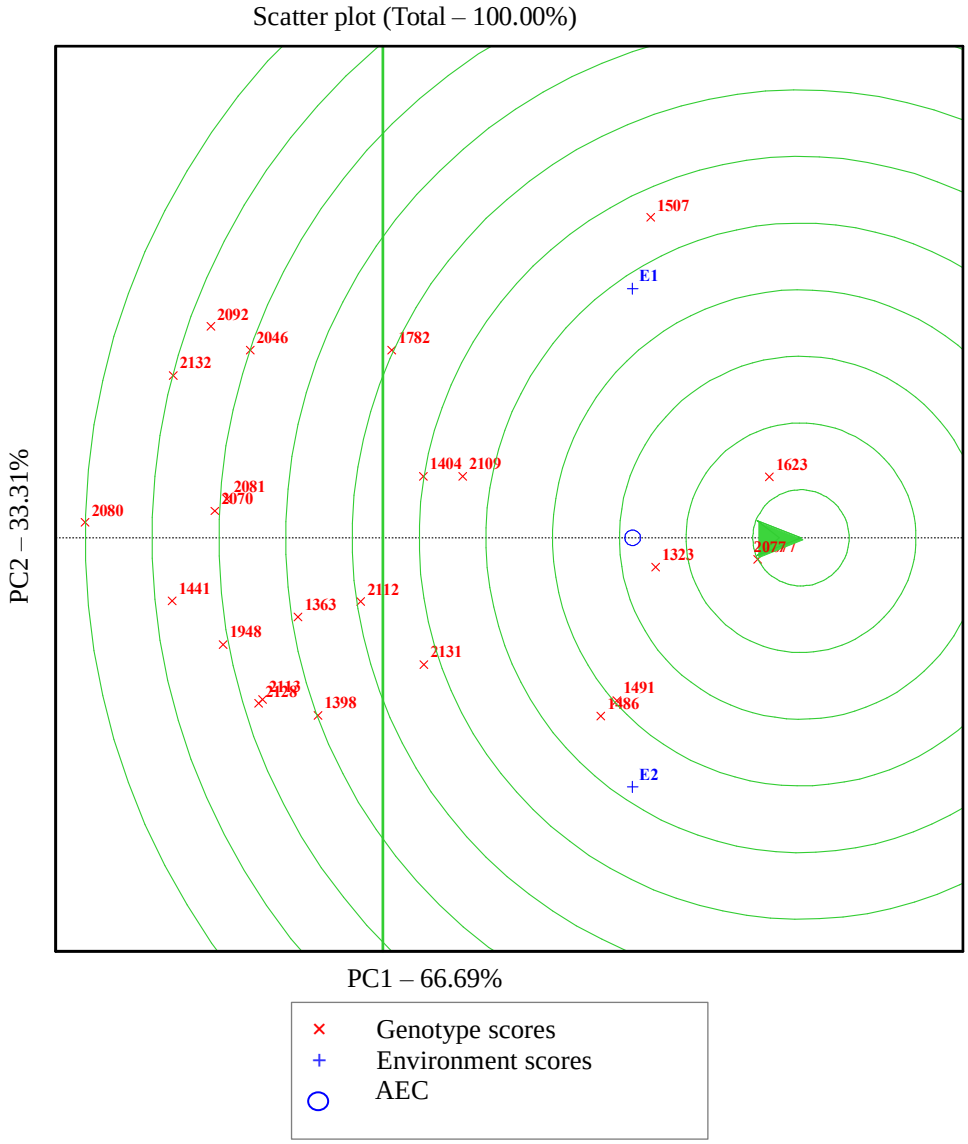

Fig. 3. GGE-biplot for comparison of the melon hybrids with the ideal hybrid based on fruit yield and stability (the arrow is the best point that the software determines to identify the best hybrids and environments).

Therefore, hybrids 2077 and then 1623 and 1323 which were very close to the ideal hybrid, were ideal hybrids in terms of higher fruit yield ability and stability compared to others. In addition, hybrids 1491, 1486, 2109, 1507, 1404, 2131, and 1782 located on the next concentric circle, may be regarded as desirable ones.

Ranking environment relative to the ideal environment

In the GGE biplot, the ideal environment, indicated by the small circle with an arrow pointing to it, is the most discriminating of hybrids and yet representative of the other experimental environments. Environments E1 and E2 were the same distance from the center of the circle, so they had the same discriminating power to identify hybrids adapted to that area (Fig. 4).

Molecular markers

In the current study, three specific markers were utilized to detect *Fom-1* and *Fom-2* resistance, as well as *Fom-2* susceptibility genes, in 23 melon hybrids. According to the protocol established by Sousaraei et al. (2018), the *Fom-2* primer amplified a 409 bp fragment in pure homozygous genotypes via PCR. Among the 23 hybrids analyzed, 13 hybrids and the Isabel variety displayed the band associated

with the *Fom* -2 resistance gene. Specifically, four hybrids were heterozygous, and nine were homozygous for the *Fom-2* resistance gene. The Isabel cultivar served as a control for the *Fom-2* resistance gene. Another genotype lacking the 409 bp band was identified, which did not have the *Fom-2* resistance gene.

PCR product electrophoresis for the *Fom-1* resistance gene revealed a 568 bp band in 13 hybrids and the Charentais-*Fom-1* cultivar (Fig. 5A). The PCR assay identified seven hybrids with a 253 bp fragment indicative of the *Fom-2*-susceptible gene. The presence of this single 253 bp band confirms that these hybrids are homozygous for the susceptibility gene. For the markers of *Fom-2* resistance and *Fom-2* susceptibility, three hybrids (2113, 1441, and 1404) displayed both the 409 bp and 253 bp bands, indicating heterozygosity (Fig. 5B and C). Detection of resistance and susceptibility genes in the 23 hybrids along with the control cultivars Isabel and Charentais *Fom-1* were shown in Table 8. The results indicated that 13 out of the 23 hybrids harbor the resistance gene and exhibited genetic resistance to *Fom* races 0 and 1. The presence of both resistance genes, *Fom-1* and *Fom-2* resistance genes, in seven hybrids, as detected using markers Fom2-R_{409} and Fom1, is particularly significant. These genes provide robust resistance against the predominant pathogenic races of *Fom*, demonstrating genetic resistance to races 0, 1, and 2 in melon hybrids developed by NBD Seeds Company.

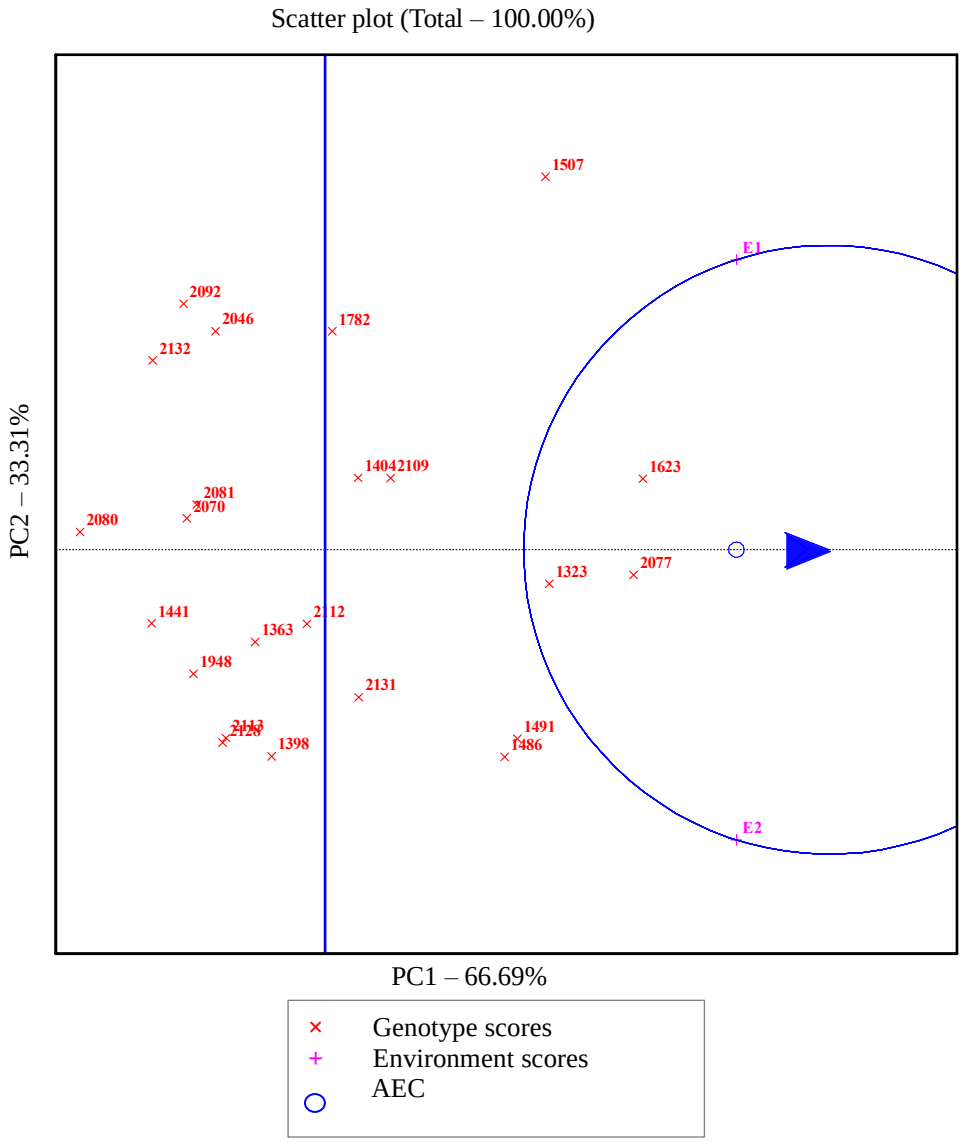

Fig. 4. Hybrid and hybrid-environment interaction biplot based on comparison of environments with the ideal environment (E1: Khatunabad field and E2: Mahmudabad field).

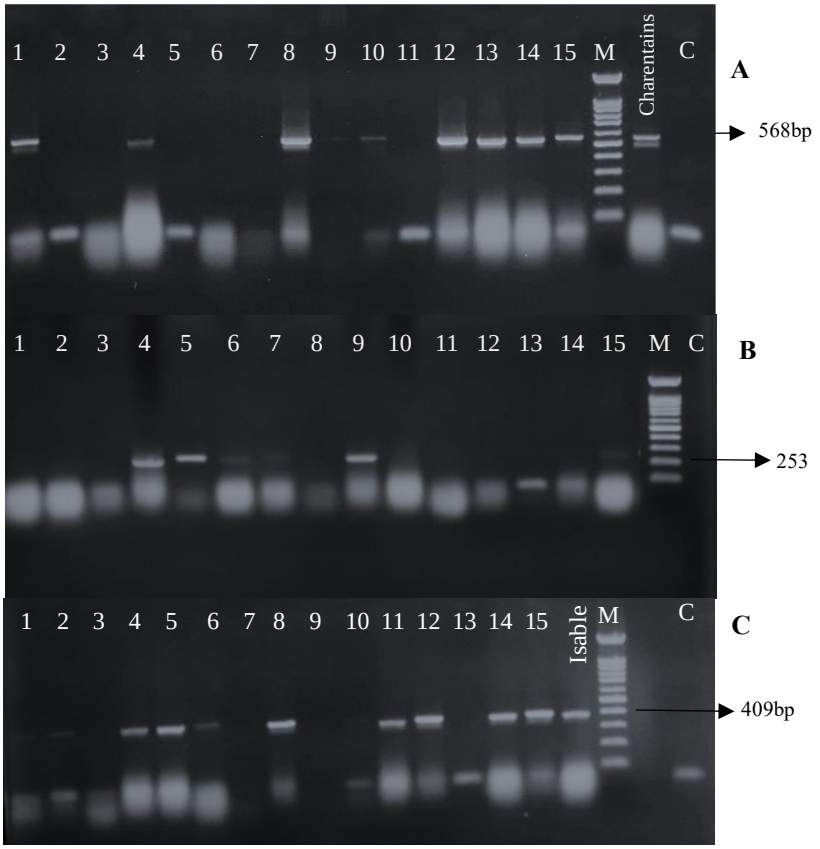

Fig. 5. Agarose gel showing amplified fragment 568 bp (*Fom-1* R), 253bp (*Fom-2* S), and 409 bp (*Fom-2* R), respectively in some hybrids (**A**, **B**, and **C**). In three pictures (**A**, **B**, and **C**): C; negative control and M; 1 kb ladder (Promega).

Table 8. PCR results with the markers related to Fom-1, Fom-2, and susceptible genes.

No.	Hybrid name	*Fom* resistance and susceptible gene markers		
		Fom2-R_{409}	Fom2-S_{253}	Fom1
1	2046	+	-	-
2	2113	-	+	+
3	1623	+	-	+
4	2132			
5	2081	+	-	-
6	1948		+	-
7	2128	+	-	+
8	2112	-	-	-
9	2070	+	-	-
10	1491	+	-	-
11	2092	+	+	+
12	2131	-	-	+
13	1363	+	-	+
14	1323	+	-	+
15	2080	-	+	+
16	1398	-	+	+
17	2077	-	-	+
18	1507	-	-	+
19	1441	+	+	+
20	1486	+		+
21	1404	+	+	-
22	1782	-	-	-
23	2109	-	-	-
Control	Isabel	+	-	-
Control	Charentains	-	-	+

Discussion

Based on the results of the current research, the combined ANOVA showed a very significant difference among the studied hybrids. The interaction effect of location and hybrids was significant in terms of all studied traits. These findings are consistent with previous research by Adiredjo et al. (2024) who similarly reported significant genotypic and genotype-by-environment interaction effects for yield and quality traits in melon across multiple locations. In the current study, based on correlation coefficients, it may be concluded that fruit weight, fruit length, fruit diameter, cavity size, flesh thickness, and fruit count were the main contributing traits that should be considered during selection for improving fruit yield. In the previous studies, Shet et al. (2024) reported that fruit yield had a significant positive association with several morphological traits including peduncle length, average fruit weight, fruit length, cavity length, flesh thickness, and fruit count per plant. In another report, a highly significant and positive correlation of fruit weight was found with flesh thicknesses, fruit length, fruit diameter, and cavity length (Bagheriyan et al., 2015). In a study by Rad et al. (2014), a negative correlation between fruit length and fruit weight was reported in the evaluation of 41 native melons, which conflicts with the findings of the present study.

Considering the negative correlation between average fruit weight and fruit count per plant (Zalapa et al., 2008; Feyzian et al., 2009), the development of cultivars capable of supporting two to three fruits, while simultaneously carrying on commercially acceptable fruit sizes in the Iranian market (3-4 kg), may be challenging. Therefore, increasing the melon yield through increasing fruit counts is not a suitable choice for breeding targets. Consequently, parental selection emphasizing high average fruit weight emerges as a more appropriate breeding strategy (Dehghani et al., 2012).

According to the results of the TOPSIS index, hybrids 1507, 1623, 1782, 1323, 2077, 1404, 2109, 2046, and 1491, in E1 and hybrids 1623, 1323, 2131, 1491, 2128, 1486, 2112, 2113, 2077, 1507, 1398, and 1404 in E2 were placed in the completely ideal and ideal quarters. In addition to having high yield, these hybrids had other favorable traits such as fruit length, fruit diameter, flesh thickness, brix, fruit count, and fruit weight. Which-won-where polygon plot showed that hybrids 1507, 1782, 1404, and 2109 for E1, and hybrids 1486, 1491, 1323, and 2131 for E2 were ideal. Based on the ranking of the hybrids compared to the ideal hybrid, hybrids 1623, 2077, 1323, 1491, 1486, 2109, 1507, 1404, 1782, and 2131 were close to the ideal hybrid, respectively, and they were ideal hybrids in terms of higher fruit yield ability and stability compared to others.

According to the results of the TOPSIS index, among the mentioned hybrids, 6 hybrids including 1623, 1323, 1507, 2077, 1404, and 1491 were common between the two fields. Based on the GGE biplot results, these hybrids were close to the ideal hybrid in terms of higher fruit yield ability and stability, these hybrids also had genes for resistance to *Fom-1* or *Fom-2*, and as promising hybrids in terms of simultaneous selection of yield and desirable traits, yield stability and resistance to *Fom-1* or *Fom-2* genes can be introduced.

Hybrids 2113, 2128, 1398, 1948, 2081, 2080, and 1363, in E1, and hybrids 2092, 2046, 1948, 1363, and 1782 in E2 were placed in the completely non-ideal quarter. They showed a lower mean than the mean total fruit yield. Based on the GGE biplot results, these hybrids, except 1782, did not show interaction in response to environmental changes.

Hybrid 1782 showed a good response to the E1 environment, but based on the simultaneous selection of several traits along with fruit yield in the TOPSIS index, this hybrid was placed in the completely non-ideal quarter. Most of these hybrids had *Fom-1* or *-2* resistance genes, which can be used in breeding programs to transfer resistance genes. Among the mentioned hybrids, two hybrids including 1948 and 1363 were common between the two fields. Hybrid 1948 was sensitive to *Fom-1* and *-2* genes, but hybrid 1363 showed resistance to both of them.

In the previous studies, the TOPSIS index was used to identify superior genotypes and cultivars of jujube (Kou et al., 2015), forage grasses (de Oliveira et al., 2024), foxtail millet (Zhang et al., 2024), maize (Liu et al., 2024), and alfalfa (Zhi-guang et al., 2024).

In the current study, the first two principal components accounted for a cumulative total of 100% of the observed variation in the GGE biplot. This high level of explanation is consistent with findings reported by previous researchers (Bishwas et al., 2021; Greveniotis et al., 2023). The GGE biplot methodology has been extensively applied in melon research. For instance, Dehghani et al. (2012) utilized this approach to evaluate seven melon populations. They reported that the two principal components of the biplot explained 88, 86, 70, and 58% of the total observed variation for fruit count, the average fruit weight, yield, and acceptable yield, respectively. Dia et al. (2016) used the GGE-biplot to select parents for the improvement of watermelon cultivars. In another report, by GGE-biplot method the stability of yield and quality traits in nine melon genotypes in nine environments in south-central Texas was investigated over 3 years (Sharma et al., 2020). In previous research, the GGE-biplot method was used to identify fruit weight stability in 36 muskmelon genotypes under three different drought stress conditions (Naroui Rad and Bakhshi, 2021).

Hybrid genotypic investigations through resistance gene markers for *Fom-1* and *Fom*-2, determined 2 resistance genes in 8 hybrids from 23 total hybrids. The dominance of the Fom-1 resistance gene, as reported by Oumouloud et al. (2015), suggested that the presence of this band signified homozygosity or heterozygosity for the resistance allele, conferring genetic resistance to *Fusarium* wilt. Because these two genes (*Fom-1* and *Fom-2*) show good resistance against *Fusarium* wilt, the current research result is important and proves the existence of genetic resistance to these races in Negin Bazr Danesh company hybrids. In conclusion, breeders can use these genes to improve their lines and hybrids via transferring resistance genes by the classical approach.

Conclusion

The current research showed that selection based on several traits is one of the basic characteristics of vegetable crop research. The TOPSIS index is a suitable method for the simultaneous selection of several traits and for determining superior genotypes by considering the appropriate weight for each trait according to the selection objective. In general, the use of the TOPSIS 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. Therefore, the efforts of breeders and researchers in choosing a logical and correct method for determining the desired genotypes can encourage farmers to choose the appropriate cultivars according to the cultivation environment. Using resistant cultivars is an effective way to control *Fusarium* fungus. Clear insight into the interaction between pathogenicity and resistance genes can provide practical knowledge for designing breeding programs. Based on the TOPSIS index results, hybrids 1507, 1623, 1782, 1323, 2077, 1404, 2109, 2046, and 1491, in E1 and hybrids 1623, 1323, 2131, 1491, 2128, 1486, 2112, 2113, 2077, 1507, 1398, and 1404 in E2 were placed in the completely ideal and ideal quarters. Among the mentioned hybrids, six hybrids (1623, 1323, 1507, 2077, 1404, and 1491) were common between the two fields. Based on the GGE biplot results, these hybrids were close to the ideal hybrid in terms of higher fruit yield ability and stability. These hybrids also had genes for resistance to *Fom-1* or *Fom-2*, and can be introduced as promising hybrids in terms of simultaneous selection of yield and desirable traits, yield stability, and resistance to *Fom-1* or *Fom-2* genes.

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Author Contributions

Conceived and designed the research, data analysis, and write-u, MG; Investigation, conducted the field experiments, plant evaluation, data collection, molecular experiments and PCR, AAAF. All authors read and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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