



Field Performance and DNA Fingerprint Identification of New Egyptian Eggplant F₁ Hybrids



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THIS study was undertaken to evaluate the field performance of the two newly promising bred eggplant F₁ hybrids (H1 and H2) relative to a commercial eggplant hybrid (Alabaster, C4) as a reference, as well as apply a dependable DNA fingerprinting technique (PCR-based, Inter Simple Sequence Repeat, ISSR markers) for identification and documentation of locally produced eggplant F₁ hybrids. Vegetative growth and yield parameters for H1, H2, and C4 eggplant hybrids were evaluated under four ecological conditions and in different geographic governorate locations (Giza “L-1”, Menofia “L-2”, Qalyubia “L-3” and El-Behaira “L-4”) during the two growing seasons of 2020 and 2021. The means of locally produced eggplant F₁ hybrids (H1 and H2) exceeded or equaled the means of the reference hybrid (C4), especially under the conditions of Qalyubia (L3) and Giza (L1) for the characteristics of plant height, number of branches/plant, number of leaves/plant, early flowering, early fruit yield, total fruit yield, number of fruits/plant and the average fruit weight. Furthermore, nineteen ISSR primers were used to identify the genetic fingerprint of the above-mentioned hybrids (H1, H2 and C4) in addition to two local parental lines (P1 and P2). ISSR analysis revealed 243 distinct DNA amplified bands with polymorphic bands of 50.21%. The percentage of polymorphism ranged between 9% and 88% with an average level of 50%. The PIC value varied from 0.09 to 0.49 with an average of 0.36. Seven ISSR primers (HB12, 17899-A, UBC-844, UBC-892, UBC-895, ISSR-59 and ISSR-DAT) produced distinctive fingerprints associated with H1 hybrid. In the same context, eight primers (HB10, HB12, UBC-892, UBC-808, ISSR-55, ISSR-01, ISSR-59 and ISSR-DAT) were distinctive for H2 hybrid. While the commercial hybrid “C4” was distinguished by only three primers (UBC-850, UBC-881 and ISSR-9). The highest similarity value of 0.89 was found between P1 and H1, while the lowest value of 0.78 between P1 and H2. It may be concluded that local eggplant hybrids (H1 and H2) performed superiorly in most studied traits and displayed more polymorphisms than the commercial eggplant hybrid “C4”.

Keywords: *Solanum melongena* L., Ecological conditions, ISSR markers, Genotype fingerprinting, New released hybrid, Vegetative growth parameters, Fruit yield and Quality traits, Breeder rights.

Introduction

Recently, the expansion of the agricultural sector in Egypt has faced many challenges for producing more food to meet the current and future food demand for the increasing population, such as climate change, water scarcity, and an insufficient local supply of high-quality seeds. These challenges prompted Egyptian plant breeders to accelerate their plans to produce newly promising cultivars as well as F_1 hybrids suitable for the preferences of Egyptian consumers and appropriate to Egyptian conditions, whereas the imported seeds are often not well adapted to growers' and consumers' preferences. Moreover, imported seed costs are going to be a limited factor for growers, especially after the Egyptian economic constraints and the global economic crisis. The vegetable crop seeds in the Egyptian market are fully occupied by imported seeds, which accounted for about 98% of all consumed vegetable crops. The vegetable crop seeds in the Egyptian market are fully occupied by imported seeds, which accounted for about 98% of all consumed vegetable crops equivalent to 2 billion pounds each year. Eggplant (*Solanum melongena* L.) is a warm-weather crop and one of the most important vegetable crops worldwide, especially in Africa, subtropical regions, Southeast Asia and the Middle East (FAO, 2017, Chapman, 2019). Eggplant fruit is a rich source of vitamins, essential minerals and some bioactive compounds that have beneficial properties for human health as well as a low caloric value (Kowalski et al., 2003, Oladosu et al., 2021). It is also one of Egypt's most economically important vegetable crops, ranking fourth in the Solanaceae family after tomato, potato, and pepper. Egypt is one of the top five producing countries, coming after China and India with 1.28 million tons per year (Taher et al., 2017, FAO 2020). The total cultivated area in Egypt reached 103344 feddans (43422 ha) in 2019/2020 producing 1277492 tons with an average of 12.366 tons/feddan (29.431 ton/ha) as indicated by the Economic Affairs Sector, Ministry of Agriculture and Land Reclamation (2020). Eggplant shows a wide variation in morphological characteristics (Iwuagwu et al., 2019 and Abu Al-Azm et al., 2021). Although eggplant fruits have a wide diversity of shapes and colors worldwide, Egyptian growers used to produce only 5 fruit types (ellipsoid white, ellipsoid very dark violet "black", ellipsoid stripes, globular very dark violet and obovate very dark violet) (UPOV, 2002, Sultana et al., 2018, Susilo, 2018, Konan et

al., 2020). Only the ellipsoidal white fruit type is considered in this investigation.

Newly bred cultivars or F_1 hybrids performance should be evaluated under a wide range of environmental conditions, before being released to the market in order to confirm that they have genetic stability as well as define the interaction between the genotypes and the environmental effects (Abd EL-Ghany et al., 2021). These interaction effects give the evaluated cultivars or hybrids their phenotypic values, which are used to select the high-yielding and more stable cultivars under different environmental conditions. Genotype by environment interaction is defined as the variation of the environmental conditions, such as soil types, soil or irrigation water salinity, irrigation water quality, drought, temperature, biotic and abiotic stresses, etc. which results in different genes expression within the same genotype but not necessarily, of different rank orders of genotypes through the tested environments, crop improvement programs include examining many genotypes in a wide range of environments, including stress and non-stress conditions. Genotype, that producing higher and stable yields under stress conditions, e.g., water scarcity, can be recommended for such environments (Dencis et al., 2000, Yan and Hunt, 2001 and Abd EL-Ghany et al., 2021).

Eggplant cultivation required high-priced imported hybrid seeds, thus national breeding programs aimed to develop competitive and high-yielding local F_1 hybrids that are resistant to both prevailing biotic and abiotic stresses (Kaushik et al., 2018, Kaushik, 2019 and Rakha et al., 2020 & 2021). The capacity and efficiency of breeding programs are primarily determined by the combination of traditional phenotypic and molecular marker screening. Molecular marker techniques are useful to identify economically significant traits, genetic diversity and DNA fingerprinting in eggplant (Sultana et al., 2018, Shrestha et al., 2020 and Dubey & Saini, 2021). Molecular markers reflect the difference among the analyzed materials on the DNA level and have great advantages in seed purity identification (Korir et al., 2012). Utilizing DNA fingerprinting to characterize new genetic resources is important to ensure the purity of the new genetic materials and protect the breeder's rights. Moreover, this information could be useful for future breeding programs (Sultana et al., 2018). There are numerous molecular marker

techniques that have been developed and adapted for DNA fingerprinting among them the inter-simple sequence repeats (ISSR) technique. ISSR markers are PCR based, dominant markers that are reliable, easy, cost-effective, rapid, and highly informative for DNA fingerprinting (Gemmill and Grierson, 2021). Genetic diversity and genetic variation in plant species are well detected by morphological traits coupled with ISSR markers (Yuan et al., 2015 and Chen et al., 2020). ISSR markers have been extensively used in eggplant for genetic diversity, DNA fingerprinting and cultivar identification (Isshiki et al., 2008, Ali et al., 2011, Mahmoud & El-Mansy, 2012, Konan et al., 2020 and Al-Rowaily et al., 2021).

The purpose of this study was to compare the field performance of two new local F_1 hybrids to the most common commercial hybrid as a reference under four ecological conditions, for the vegetative growth and yield traits as well as establish DNA fingerprinting for the studied genotypes using ISSR markers to estimate the

genetic variation and produce a distinct fingerprint for each genotype.

Materials and Methods

Five eggplant genotypes with ellipsoidal white fruit types were used in this investigation, 1 (parent 1 “P1”), 2 (parent 2 “P2”), 3 (local F_1 hybrid “H1”), 4 (commercial hybrid C4 “Alabaster”) and 5 (local F_1 hybrid “H2”). Genotypes 1, 2, 3 and 5 were obtained from the national breeding program “Towards Breeding of Promising Hybrids and Cultivars of Some Strategic Vegetable Crops”, which is funded by the Academy of Scientific Research and Technology “ASRT” and carried out at the National Research Centre. Meanwhile, Genotype 4, a reference hybrid (commercial hybrid, Alabaster), was obtained from the Syngenta Company (<https://www.syngenta.com>). The three genotypes (H1, H2 and C4) were sown in the field experiments. In addition, they used the two parents (P1 and P2) in the DNA fingerprinting part of this study.

TABLE 1. The main ecological differences among the 4 experimental locations.

Locations	Abu Ghaleb area, Giza Governorate	El-Sadat city, Menofia Governorate	Damlo village, Qalyubia Governorate	Imam Malik village, El-Behaira Governorate
Practices	‘L1’	‘L2’	‘L3’	‘L4’
Water source	ground water	ground water	ground water	ground water
EC of irrigation water	1200 ppm	1000 ppm	700 ppm	2500 ppm
Soil texture	Sandy soil	Sandy soil	Clay soil	Sandy Loam soil
Irrigation system	Fertigation program	Fertigation program	Fertigation program	Fertigation program

TABLE 2. Weather data at the 4 experimental locations during 2020 and 2021 seasons

Location	Giza (L1)		Monofiya (L2)		Qalyubiyah (L3)		Behera (L4)	
Months	Average Temperature (°C)		Average Temperature (°C)		Average Temperature (°C)		Average Temperature (°C)	
	2020	2021	2020	2021	2020	2021	2020	2021
April	19.58	20.55	19.39	20.50	19.05	19.84	18.79	19.20
May	25.09	27.78	23.71	27.56	23.58	26.99	23.09	25.95
June	28.30	28.58	28.08	28.57	27.55	28.17	26.87	27.31
July	30.21	31.09	30.33	31.12	29.92	30.65	28.97	29.94
August	30.55	31.35	30.62	31.43	30.22	30.95	29.51	30.48
September	29.91	28.04	29.94	28.52	29.47	27.88	29.01	27.98
Average	27.27	27.90	27.01	27.95	26.63	27.42	26.04	26.81

Field Experiments

Two field experiments were carried out during the summer growing seasons of 2020 and 2021 at different geographic locations, Abu Ghaleb area, Giza Governorate (L1), El-Sadat city, Mnofia Governorate (L2), Damlo village, Qalyubia Governorate (L3) and Imam Malik village, El-Behaira Governorate (L4), aiming to evaluate the field performance of H1 and H2 with C4. The main ecological differences among the 4 experimental locations are summarized in Table 1. Weather data at the 4 experimental locations during 2020 and 2021 seasons are shown in Table 2. The seeds of all studied genotypes were sown in 209 cell Styrofoam seedling trays filled with a mixture of peat moss and vermiculite (1:1 v/v) medium with a capacity of one seed per cell on Feb. 15th and transplanted on April 1st in all locations during both growing seasons.

The experimental design was a randomized complete block design with three replicates in all locations. Each block consists of 1 ridge with 1 m width and 25 m long, the net block area was 25 m². Uniform 45 day-old eggplant seedlings with 4–5 true leaves, were transplanted into the four different experimental fields on both sides of the drip irrigated ridge and 50 cm apart. All agriculture management practices for eggplant crop production were carried out during the two growing seasons in all locations as recommended by the Ministry of Agriculture and Land Reclamation (MOALR).

After 90 days from the transplanting date, a representative five plants from each experimental block from each experimental location were randomly chosen and gently uprooted to determine the following vegetative growth parameters, plant height, number of branches/plant, number of leaves/plant and the number of elapsed days to 50% flowering (earliness). Moreover, early yield/plant was calculated as the first three harvestings, total yield/plant, average fruit weight and number of fruits/plant.

The data collected over the two seasons of the study were calculated and subjected to statistical analysis of variance procedures using two-way-ANOVA. Duncan's multiple range test was employed to separate and compare the significant differences among treatment means at the 5% level of probability as outlined by Gomes and Gomes (1984).

DNA fingerprinting

Plant materials

The genetic materials used in this study consisted of the five eggplant genotypes. It included two parents (P1 and P2), two local F₁ hybrids (H1 and H2) and a reference hybrid (C4). Five seedlings of each genotype were grown at the laboratory in earthen tubs containing soil collected from the nursery and then fresh leaf samples from 2-week-old young seedlings were collected for genomic DNA isolation. Leaf samples were immediately immersed in liquid nitrogen and ground to a fine powder with a mortar and pestle. The DNA extraction was carried out using the "i-genomic DNA" Mini Kit for Plant DNA Extraction (iNtRON Biotechnology, Inc., Korea) according to the manufacturer's protocol. Nineteen ISSR primers were used (Table 3). These primers were selected from the previous studies conducted by Mahmoud and El-Mansy (2012), Husnudin et al. (2019), Konan et al. (2020) on the basis of their high discriminating capacity. These primers were synthesized by (Willowfort, UK). PCR amplifications were performed in a final volume of 12.5 µL, containing 1 µL of diluted DNA, 1 µL of ISSR primer, 6.3 µL of PCR Master-Mix (Cosmo, Master Mix Sigma-Aldrich) and sterile ddH₂O. A PCR reaction was performed on a lab thermal cycler (Sensoquest, Germany). The DNA amplification program consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at a primer-specific annealing temperature for 45 s, and extension at 72°C for 2 min. After the last cycle, a final step of 10 min at 72°C was included to allow complete extension of all amplified fragments, and then the samples were held at 4°C.

The final amplified products were subjected to 1 hr of electrophoresis at 100 V on a 1.5% agarose gel containing 1 µL ethidium bromide (EtBr 10 mg/L) in 1 X TBE buffer. After electrophoresis, the amplified product profiles were visualized on a UV transilluminator in a gel documentation system and photographed for analysis. The DNA molecular weight marker 100 bp DNA ladder (Willowfort, UK) was used in this study against the amplified fragments. The gel images were analyzed using the Gel Analyzer 3 software to determine the molecular weights of the amplified fragments. The amplified fragments were scored as present (1) or absent (0). PIC values were determined using the online program for calculating polymorphic information content and heterozygosity (<https://gene-calc.pl/pic>). The

TABLE 3. List of ISSR used primers, sequence and annealing temperature.

No.	Primer name	Primer sequence (5' - 3')	Annealing temp. (°C)
1	HB10	GAGAGAGAGAGACC	40
2	HB12	CACCACCACGC	45
3	17899-A	CACACACACACAAG	44
4	UBC 808	AGAGAGAGAGAGAGAGC	52
5	UBC-809	AGAGAGAGAGAGAGAGG	53
6	UBC-844	CTCTCTCTCTCTCTRC	49
7	UBC-850	GTGTGTGTGTGTGTGYC	48
8	UBC-864	ATGATGATGATGATGATG	50
9	UBC-880	GGAGAGGAGAGGAGA	48
10	UBC-881	GGGTGGGGTGGGGTG	45
11	UBC-892	TAGATCTGATATCTGAATCCC	58
12	UBC- 895	AGAGTTGGTAGCTCTTGATC	58
13	ISSR-1	CACTCCTCCTCCTCCTCC	56
14	ISSR-09	CTCCTCCTCCTCCTCCTC	56
15	ISSR-34	AGAGAGAGAGAGAGAGAA	53
16	ISSR-40	ACCACCACCACCACCACC	53
17	ISSR-55	TGTGTGTGTGTGTGTGGG	48
18	ISSR-59	AGAGAGAGAGAGAGAGGC	50
19	ISSR DAT	GAGAGAGAGAGAGAAC	44

similarity coefficient was estimated by Jaccard's coefficient. Cluster analysis was carried out with NTSYS-PC software, to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) according to Rohlf (2000).

Results

Vegetative growth parameters of eggplant hybrids

Plant height

The results in Table 4 revealed that the mean performances of studied genotypes or locations for the plant height parameter showed nonsignificant differences during both seasons. Moreover, local F_1 hybrid (H2) gave the highest insignificant values for genotype means (110.7 and 120.7 cm). The mean of location was 109.7 cm under Qalyubia (L3) in the first season and 121.6 cm under El-Behaira (L4) in the second season.

Under Giza (L1) H1 gave the lowest values of plant height (82.8, 90.8 cm), whereas H2 recorded the highest values (122.6 and 130.6 cm), respectively, in both seasons. In addition, local F_1 hybrids (H1) illustrated the highest values (119.7 and 130.7 cm) in both seasons when grown in Qalyubia (L3).

Number of branches/plant

Regarding the number of branches/plant (Table 5) illustrated that the mean performance for local F_1 hybrid (H2) exceeded the mean recorded by commercial F_1 hybrid (C4) during both seasons of the study. The highest number of branches/plant (8.7 and 10.3) were detected with local F_1 hybrid (H2) and (9.8 and 11.6) with Qalyubia (L3) in the first and second seasons, respectively.

Local F_1 hybrid (H2) eggplant recorded the highest values (10.7 and 12.5) when grown in Qalyubia (L3), while the lowest values (6.8 and 7.8) were recorded when grown in El-Behaira (L4) in both seasons. However, all studied genotypes gave the lowest values for the aforementioned parameter when grown in El-Behaira (L4) during both seasons.

Number of leaves/plant

Concerning the number of leaves/plant (Table 6) showed that the commercial F_1 hybrid (C4) significantly exceeded the means of the two local F_1 hybrid (H1 and H2). The highest significant values of number of leaves/plant (260 and 253) were recorded by a commercial F_1 hybrid (C4), followed significantly by (247 and 240) local F_1 hybrid (H2) in the first and second seasons,

TABLE 4. The mean performance of two local F₁ eggplant hybrids compared with a commercial F₁ hybrid for the plant height (cm) in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F ₁ hybrid (H1)	82.8 b	115.1 ab	119.7 a	104.6 ab	105.6 A
Local F ₁ hybrid (H2)	122.6 a	103.2 ab	100.6 ab	116.5 ab	110.7 A
Commercial F ₁ hybrid (C4)	108.0 ab	107.4 ab	108.7 ab	107.6 ab	107.9 A
Locations mean	104.5 a	108.6 a	109.7 a	109.6 a	
Season 2021					
Local F ₁ hybrid (H1)	90.8 b	124.1 ab	130.7 a	116.6 ab	115.6 A
Local F ₁ hybrid (H2)	130.6 a	112.2 ab	111.6 ab	128.5 a	120.7 A
Commercial F ₁ hybrid (C4)	116.0 ab	116.4 ab	119.7 ab	119.6 ab	117.9 A
Locations mean	112.5 a	117.6 a	120.7 a	121.6 a	

TABLE 5. The mean performance of two local F₁ eggplant hybrids compared with a commercial F₁ hybrid for the number of branches/plant in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F ₁ hybrid (H1)	8.1 ce	7.0 e	8.6 bd	6.9 e	7.7 B
Local F ₁ hybrid (H2)	9.5 ac	7.9 de	10.7 a	6.8 e	8.7 A
Commercial F ₁ hybrid (C4)	8.7 bd	7.3d e	10.0 ab	7.2 de	8.3 AB
Locations mean	8.8 b	7.4 c	9.8 a	7.0 c	
Season 2021					
Local F ₁ hybrid (H1)	10.1 ce	8.5 fg	10.4 bd	7.9 g	9.2 B
Local F ₁ hybrid (H2)	11.5 ac	9.4 df	12.5 a	7.8 g	10.3 A
Commercial F ₁ hybrid (C4)	10.7 bc	8.8 eg	11.8 ab	8.2 fg	9.9 AB
Locations mean	10.8 b	8.9 c	11.6 a	8.0 d	

respectively. In the same regard, the highest significant values of the number of leaves/plant (288 and 283) were attained under Qalyubia (L3) in the two seasons.

The commercial F₁ hybrid (C4) gave the superiority of the number of leaves/plant (300 and 295) when grown under under Qalyubia (L3). During both experimental seasons, the worst results (175 and 168) were obtained by the local F₁ hybrid (H2) when grown under El-Behaira (L4).

Number of elapsed days to 50% flowering (earliness)

The data in Table 7 clearly indicated that there were no significant differences in the number

of elapsed days to 50% flowering found among the studied genotypes in both seasons. On the other hand, significant differences were observed among the different locations concerning the number of days from the transplanting date to 50% flowering. Eggplants grown under Menofia (L2) gave the highest value for the number of elapsed days to 50% flowering. In contrast, under El-Behaira (L4) the lowest value was achieved. In another way, eggplant plants flowered earlier when grown in El-Behaira (L4) than those grown under Menofia (L2). The highest and lowest significant values for the number of elapsed days to 50% flowering were attained by a commercial F₁ hybrid (C4) when grown under Menofia (L2) and Qalyubia (L3), respectively, in both seasons.

*Yield and its components**Early yield*

Data presented in Table 8 revealed the mean performance of genotypes and locations for the early yield in both seasons. The local F₁ hybrid (H2) gave the highest significant values for early yield during both seasons of the study. Also, significant differences were detected among genotypes studied in both seasons. Concerning the location means, the highest values for early yield in the two years were noticed under Menofia (L2) and Qalyubia (L3), without a significant difference between them. The local F₁ hybrid (H2) gave the highest significant values for early yield (2.360 and 3.430 kg/plant) when grown under Menofia (L2). Conversely, the lowest

values (1.593 and 2.093 kg/plant) were observed by commercial F₁ hybrid (C4) when grown under El-Behaira (L4) in the first and second seasons, respectively.

Total yield

Regarding the total yield trait (Table 9), the results summarized that there were significant differences realized among all studied genotypes in both experimental seasons. The highest significant values for total yield were found with the local F₁ hybrid (H2) during both seasons. Furthermore, the highest values for total yield were recorded under Menofia (L2) and Qalyubia (L3), with an insignificant difference between them, in the first season and under Qalyubia (L3) only in the second season.

TABLE 6. The mean performance of two local F₁ eggplant hybrids compared with a commercial F₁ hybrid for the number of leaves/plant in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F ₁ hybrid (H1)	255 ef	261 de	278 bc	194 g	247 B
Local F ₁ hybrid (H2)	243 f	200 g	286 b	175 h	226 C
Commercial F ₁ hybrid (C4)	266 ce	270 cd	300 a	205 g	260 A
Locations mean	255 b	244 c	288 a	191 d	
Season 2021					
Local F ₁ hybrid (H1)	247 de	252 d	273 bc	187 f	240 B
Local F ₁ hybrid (H2)	235 e	191 f	281 b	168 g	222 C
Commercial F ₁ hybrid (C4)	258 d	261 cd	295 a	198 f	253 A
Locations mean	247 b	235 c	283 a	184 d	

TABLE 7. The mean performance of two local F₁ eggplant hybrids compared with a commercial F₁ hybrid for the number of elapsed days to 50% flowering in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F ₁ hybrid (H1)	48 ad	54 ab	41 be	34 de	44 A
Local F ₁ hybrid (H2)	45 ae	51 ac	39 ce	34 de	42 A
Commercial F ₁ hybrid (C4)	51 ac	58 a	32 e	37 de	44 A
Locations mean	48 a	54 a	37 b	35 b	
Season 2021					
Local F ₁ hybrid (H1)	52 ad	59 ab	47 bd	40 d	50 A
Local F ₁ hybrid (H2)	49 bd	56 ac	45 cd	40 d	48 A
Commercial F ₁ hybrid (C4)	55 ac	63 a	38 d	43 cd	50 A
Locations mean	52 ab	59 a	43 bc	41 c	

TABLE 8. The mean performance of two local F₁ eggplant hybrids compared with a commercial F₁ hybrid for the early yield trait (kg/plant) in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F ₁ hybrid (H1)	1.932 ce	2.158 bd	2.206 bd	1.842 de	2.035 B
Local F ₁ hybrid (H2)	2.370 ab	2.630 a	2.325 ac	1.810 de	2.284 A
Commercial F ₁ hybrid (C4)	1.803 de	1.848 de	2.047 bd	1.593 e	1.823 C
Locations mean	2.035 a	2.212 a	2.193 a	1.748 b	
Season 2021					
Local F ₁ hybrid (H1)	2.632 cd	2.958 bc	3.106 ab	2.342 de	2.760 B
Local F ₁ hybrid (H2)	3.070 ab	3.430 a	3.225 ab	2.310 de	3.009 A
Commercial F ₁ hybrid (C4)	2.503 d	2.648 cd	2.947 bc	2.093 e	2.548 C
Locations mean	2.735 b	3.012 a	3.093 a	2.248 c	

TABLE 9. The mean performance of two local F1 eggplant hybrids compared with a commercial F1 hybrid for the total yield trait (ton/fed.) in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F ₁ hybrid (H1)	8.278 de	9.023 bc	9.287 bc	7.683 e	8.568 B
Local F ₁ hybrid (H2)	9.353 bc	10.663 a	9.526 b	8.367 de	9.477 A
Commercial F ₁ hybrid (C4)	7.771 e	7.748 e	8.680 cd	6.909 f	7.777 C
Locations mean	8.467 b	9.145 a	9.164 a	7.653 c	
Season 2021					
Local F ₁ hybrid (H1)	7.451 de	9.113 b	10.216 a	6.147 g	8.232 B
Local F ₁ hybrid (H2)	8.417 c	10.770 a	10.479 a	6.693 f	9.090 A
Commercial F ₁ hybrid (C4)	6.994 f	7.826 d	9.548 b	5.527 h	7.474 C
Locations mean	7.621 c	9.236 b	10.081 a	6.122 d	

The highest significant values for total yield were found with the local F₁ hybrid (H2) when grown under Menofia (L2). Conversely, the lowest values were observed by the commercial F₁ hybrid (C4) when grown under El-Behaira (L4) in the two seasons. It is of interest to note that all studied genotypes expressed the lowest values for the above-mentioned trait when grown under El-Behaira (L4) in both seasons.

Number of fruits/plant

Concerning the number of fruits/plant (Table 10), the highest number of fruits per plant (180 and 155 fruits/plant) was detected with local F₁ hybrid (H2) while the lowest (133 and 112 fruits/plant) was detected with commercial F₁ hybrid (C4) when grown under El-Behaira (L4) in both seasons.

plant) was obtained by commercial F₁ hybrid (C4) in the first and second seasons, respectively. Significant differences were noticed among all studied genotypes in both seasons. The highest significant values for the number of fruits/plant were attained under El-Behaira (L4) in the first season, while in the second season obtained under Menofia (L2).

The local F₁ hybrid (H2) recorded the highest values of number of fruits/plant when grown under El-Behaira (L4) in the first season and under Menofia (L2) in the second season. On the other side, the commercial F₁ hybrid (C4) gave the lowest values of the number of fruits/plant

when grown under Qalyubia (L3) in both seasons of 2020 and 2021.

Average of fruit weight

The data shown in Table 11 strongly reported that there were significant differences among all studied genotypes in both seasons. The commercial F_1 hybrid (C4) gave the highest significant values of average fruit weight, while the local F_1 hybrid (H2) recorded the lowest values in both seasons of the study. Under Qalyubia (L3) conditions, the highest significant values of average fruit weight were noticed during both experimental seasons.

During both seasons, the commercial F_1 hybrid (C4) showed the highest significant values when grown under Qalyubia (L3) conditions. In addition, the lowest values were obtained by the local F_1 hybrid (H2) when grown under El-Behaira (L4) conditions in the first season and under Menofia (L2) conditions in the second season.

Molecular characterization of eggplant hybrids Profiling of ISSR bands and their polymorphisms

Nineteen ISSR primers were selected to assess DNA fingerprints for the five eggplant genotypes. The amplification results of the ISSR primers used are presented in Table 12. All the ISSR primers showed polymorphism and exhibited good amplification. In addition, the ISSR banding pattern is shown in Figure 1.

A total of 243 reproducible and scorable bands were generated by the 19 primers, of which 122 were polymorphic (50.21%). The number of amplified DNA fragments per primer ranged from 9 to 16, with an average of 12.8 bands per primer. The primers UBC-892 and ISSR-59 yielded the maximum number of 16 fragments, while the minimum number of 9 fragments was amplified with primer ISSR-55. The number of polymorphic bands ranged from 1 (ISSR-01) to 14 (UBC-892),

TABLE 10. The mean performance of two local F_1 eggplant hybrids compared with a commercial F_1 hybrid for the number of fruits/plant trait in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F_1 hybrid (H1)	142 f	159 e	143 f	182 c	157 B
Local F_1 hybrid (H2)	166 d	196 b	156 e	203 a	180 A
Commercial F_1 hybrid (C4)	134 g	139 fg	113 h	145 f	133 C
Locations mean	147 c	165 b	137 d	177 a	
Season 2021					
Local F_1 hybrid (H1)	127 e	174 b	107 g	119 f	132 B
Local F_1 hybrid (H2)	150 c	218 a	117 f	134 d	155 A
Commercial F_1 hybrid (C4)	119 f	152 c	82 i	93 h	112 C
Locations mean	132 b	181 a	102 d	116 c	

TABLE 11. The mean performance of two local F_1 eggplant hybrids compared with a commercial F_1 hybrid for the average fruit weight (g) in different locations during the seasons of 2020 and 2021.

Genotypes	Locations				Genotypes mean
	Giza (L1)	Menofia (L2)	Qalyubia (L3)	El-Behaira (L4)	
Season 2020					
Local F_1 hybrid (H1)	58.1 cd	56.9 d	64.9 b	42.2 f	55.5 B
Local F_1 hybrid (H2)	56.4 d	54.4 d	61.0 c	41.2 f	53.2 C
Commercial F_1 hybrid (C4)	58.0 cd	55.8 d	76.8 a	47.7 e	59.6 A
Locations mean	57.5 b	55.7 b	67.6 a	43.7 c	
Season 2021					
Local F_1 hybrid (H1)	58.5 d	52.3 ef	95.9 b	51.5 ef	64.6 B
Local F_1 hybrid (H2)	56.2 de	49.5 f	89.6 c	49.9 f	61.3 C
Commercial F_1 hybrid (C4)	58.6 d	51.6 ef	115.9 a	59.1 f	71.3 A
Locations mean	57.8 b	51.1 c	100.5 a	53.5 c	

with an average of 6.4. ISSR primers produced 29 unique bands with an average of 1.5 bands per primer. The highest number of unique bands (3) was generated by primers HB10, UBC-892 and ISSR-59. The percentage of polymorphism ranged between 9% and 88% with the primers ISSR-1 and UBC-892, respectively, with an average level of 50%, which indicated a medium level of polymorphism among the studied genotypes. The polymorphic information content (PIC) values ranged from 0.09 (ISSR-40) to 0.49 (UBC-881 and UBC-892), with an average of 0.36. Moreover, the size of the amplified fragments varied with the different primers and ranged from 170 to 2500 bp.

Out of 19 ISSR primers, 17 generated unique bands. Among them, seven ISSR primers (HB12, 17899-A, UBC-844, UBC-892, UBC-895, ISSR-59, and ISSR-DAT) were uncovered and generated eight unique fingerprints for the F_1 hybrid (H1).

In addition, eight ISSR primers (HB10, HB12, UBC-892, UBC-808, ISSR-55, ISSR-01, ISSR-59 and ISSR-DAT) exhibited 11 unique bands for F_1 hybrid (H2). As a result, the DNA bands become specific band markers for the two local F_1 hybrids (H1 and H2). These bands enhanced the hybrids' distinctive fingerprinting, making it easier to set them apart from one another.

Genetic similarity and cluster analysis within and among the studied genotypes based on the ISSR marker

The genetic similarity within and among five eggplant genotypes was estimated in terms of using Dice's similarity coefficients (DSC's) as shown in Table 13, to compute the similarity matrix based on the scored ISSR data matrix. This similarity matrix was used to construct a dendrogram using the unweighted pair group method arithmetic averages (UPGMA) method.

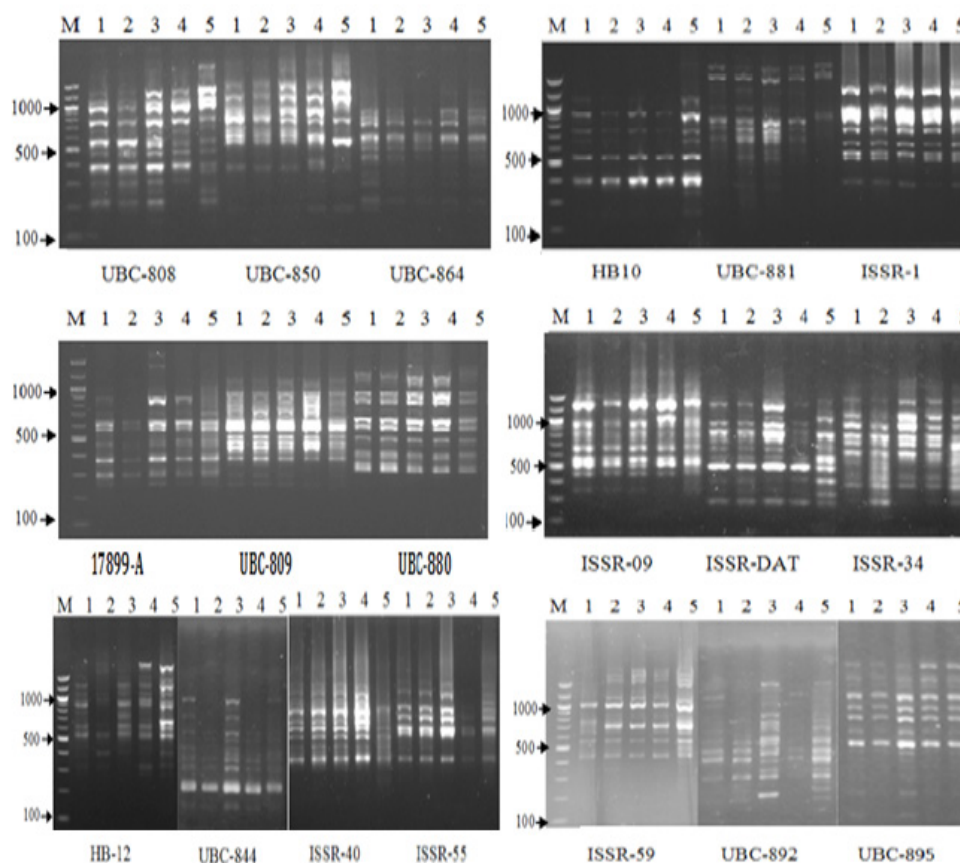


Fig. 1. ISSR profile of the five eggplant genotypes (1 – 5), as detected by nineteen ISSR primers. Lane 1: parent 1, lane 2: parent 2, lane 3: F_1 hybrid (H1), lane 4: reference hybrid (C4), lane 5: F_1 hybrid (H2) and M, DNA molecular weight marker 100 bp DNA ladder.

The genetic similarity was estimated between 0.78 and 0.89, revealing a high level of similarity. The closest relationship was scored between parent 1 and H1 (similarity of 0.89). On the other hand, the most distant relationship was found between parent 1 and H2 (similarity of 0.78).

The constructed dendrogram showed two major clusters, A and B. The first major cluster (A) contained an F₁ hybrid H2. This cluster exhibited highly significant genetic differences from the second cluster (B). The second major cluster (B) was recognized as two sub-clusters, one of which contained one sample of commercial F₁ hybrid C4. Thus, C4 showed genetic differences among the other genotypes within cluster (B), while the other sub-cluster was separated into two sub-sub-

clusters. Sub-sub-cluster I had only parent 2. On the other hand, sub-sub-cluster II consisted of parent 1 and the F₁ hybrid H1.

Discussion

In the present investigation, there were clear-cut variations in the effect of different geographic governorate locations (Giza "L1", Menofia "L2", Qalyubia "L3" and El-Behaira "L4") on the performance of evaluated eggplant F₁ hybrids. These might be due to the differences in microclimatic conditions among locations during the growing period in the summer of the 2020 and 2021 seasons. It may also be attributed to the difference in soil characterization and/or the EC of irrigation water as previously shown in Table 1. The favorable effects of Qalyubia (L3) location

TABLE 12. The amplification results revealed by ISSR markers among the 5 studied eggplant genotypes.

No.	ISSR primer	AB	PB	UB	P %	PIC	Product size (bp)
1	HB10	11	7	3	64	0.47	260-1200
2	HB12	12	10	2	83	0.43	450-2500
3	17899-A	14	9	2	64	0.32	250-1700
4	UBC 808	14	6	2	43	0.39	170-2200
5	UBC-809	14	3	0	21	0.45	240-1150
6	UBC-844	11	5	1	45	0.37	280-1200
7	UBC-850	15	8	2	53	0.40	190-1900
8	UBC-864	10	4	1	40	0.35	230-1200
9	UBC-880	14	4	2	29	0.15	300-1300
10	UBC-881	10	8	1	80	0.49	540-1900
11	UBC-892	16	14	3	88	0.49	220-1900
12	UBC-895	14	6	1	43	0.36	380-2100
13	ISSR-01	11	1	1	09	0.25	330-1200
14	ISSR-09	13	5	1	38	0.33	340-2300
15	ISSR-34	14	5	1	36	0.32	290-2000
16	ISSR-40	13	6	0	46	0.09	380-1400
17	ISSR-55	9	3	1	33	0.36	420-1500
18	ISSR-59	16	12	3	75	0.46	410-1850
19	ISSR-DAT	12	6	2	50	0.37	270-1300
Total		243	122	29			
Average		12.8	6.4	1.5	50	0.36	

AB= amplified bands, PB= polymorphic bands, UB= unique bands, P%= polymorphism percentage and PIC= polymorphic information content.

TABLE 13. Genetic similarity matrix within and among the five eggplant genotypes as computed according to Dice's similarity coefficient (DSC's) from ISSR generated data.

Genotypes	P1	P2	Local F ₁ hybrid (H1)	Reference F ₁ hybrid (C4)	Local F ₁ hybrid (H2)
P1	1.00				
P2	0.87	1.00			
Local F₁ hybrid (H1)	0.89	0.86	1.00		
Reference F₁ hybrid (C4)	0.85	0.87	0.86	1.00	
Local F₁ hybrid (H2)	0.78	0.80	0.80	0.79	1.00

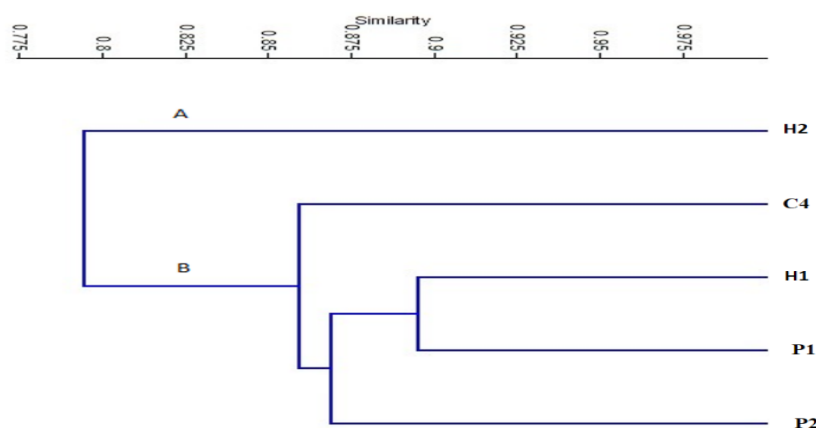


Fig. 2. A dendrogram for the five eggplant genotypes constructed from ISSR generated data using the UPGMA method and a similarity matrix computed according to DSC's.

conditions, on the vegetative growth and total fruit yield traits as compared with El-Behaira (L4) location during both seasons of study might be due to the favorable microclimate conditions such as higher maximum and minimum air temperatures and/or lower average radiation throughout the growing period of eggplant F₁ hybrids grown under the open field condition (Table 2). It may also be due to the texture of the soil in Qalyubia Governorate (L3), as it is a clay soil, which is characterized by high fertility, organic matter content and its retention of irrigation water. Also, the irrigation water in this location has a lower salinity compared to the El-Behaira Governorate "L4" as shown in Table (1). The obtained results are in good accordance with those of Elzbieta et al. (2016) who studied the effect of temperature and precipitation conditions on the growth yield and quality of processing tomato. These results are

also in agreement with those obtained by Radwan (2018) who studied the effects of different tomato varieties grown under the El-Behaira and El-Fayoum governorates. They found that there were significant differences in the number of leaves and fresh weight of plant foliage among the tested locations (El-Behaira and El-Fayoum) during the first season of the study, while in the second season there were significant differences in plant height as well as fresh and dry weight of plant foliage. Tomato plants grown under the El-Fayoum location recorded the highest values of plant height, number of leaves per plant, fresh and dry weight for plant foliage, as well as the number of branches per plant compared with those plants grown under the El-Behaira location during the two seasons of study. The highest values of vegetative growth parameters and fruit yield were mostly recorded by the cv. Super Strain B tomato

plants compared with the cv. Castlerock grown under El-Fayuom conditions. Moreover, Abou-Shleel and Saleh (2011) revealed that growing locations affected the tomato fruit yield per plant, the Ismailia location showed the largest yield compared to that obtained from the Qaluobiya location, with means of 651.2 and 462.8 g, respectively. The superiority of local hybrids (H1 and H2) may be due to the fact that these hybrids are the result of cross-breeding between Egyptian lines that are characterized by the suitability of Egyptian environmental conditions and their resistance to some common diseases.

The importance of DNA fingerprinting is due to its ability to determine the purity of hybrids and new varieties, thus protecting the rights of breeders. The ISSR technique is an effective DNA fingerprinting to characterize the new genotypes (Dubey and Saini, 2021). This marker was successfully applied to assess the phylogenetic relationship among eggplant genotypes (Isshiki et al., 2008, Tiwari et al., 2009). Therefore, the ISSR marker was used as a DNA marker for checking the purity of hybrids, protecting the rights of breeders and genetic fingerprinting to set them apart from one another. In general, the ISSR primers produced good reproducible and scorable patterns, and the amplification profiles were screened for the presence of polymorphism among the five eggplant genotypes.

Based on the results of this study, the percentage of polymorphism ranged from 9% to 88%, with an average of 50%, indicating a medium level of polymorphisms (genetic variations) among the five studied eggplant genotypes. This finding was less than that observed by Weihai et al. (2008) who found that the percentage of ISSR polymorphisms in southern Chinese long-eggplant cultivars was 71%. Isshiki et al. (2008) reported that the level of polymorphism was 99.1% using ISSR markers on eight Japanese eggplants. Moreover, Mahmoud and El-Mansy (2012) found higher levels of polymorphisms ranging from 70.0 to 100% with four ISSR primers. Also, Husnudin et al. (2019) found that the level of polymorphisms in Indonesian eggplant accessions using ISSR was 64.5%. Likewise, Konan et al. (2020) discovered that the percentage of polymorphism ranged from 57.14 to 100% when using an ISSR marker to compare nine local and exotic eggplant cultivars growing in Côte d'Ivoire. The current results showed relatively lower genetic variability among genotypes, this could be due to a narrower

genetic background from which the cultivated forms developed (Nunome et al., 2003, Ge et al., 2013, Augustinos et al., 2016). In contrast, the obtained level of polymorphism was higher than that observed by Ali *et al.* (2011) on cultivated Chinese eggplant revealed by ISSR primers, 13.5, 20.7 and 15.3% in landraces, hybrid cultivars and across, respectively. This might be explained by variations in the markers or in the genotypes (Lamare and Rao, 2015). The obtained results showed that these six ISSR primers (HB10, HB12, 17899-A, UBC881, UBC-892 and ISSR-59) exhibited a high level of polymorphism (64.00 - 88.00%), which indicates the ability of these primers to assess genetic variability among the five studied eggplant genotypes.

Even though the five eggplant genotypes differed morphologically, the eggplant clusters shared a lot of similarities (0.78 - 0.89), implying a narrow genetic base between the genotypes but distinct from the local F_1 hybrid (H2). The local F_1 hybrid H2 was separated alone in group "A" because it has more genetic differences that are sufficient to distinguish it based on patterns of ISSR markers. So, this genotype must be introduced into the breeding program because it has a broad genetic background. Furthermore, the local F_1 hybrid H1 demonstrated a close relationship when compared to parent 1, but H1 was characterized by its own unique bands. Since these genotypes were closely related, it was possible that they shared alleles, which was demonstrated by McMurrin (2010). The achieved results highlighted the higher genetic diversity of the two local F_1 hybrids compared to the commercial F_1 hybrid and the two parents. Additionally, H1 and H2 were highly significant in most determined traits compared to the commercial F_1 hybrid (C4). This makes the local hybrids a promising material that can be useful for cultivation and breeding programs. An almost similar level of similarity coefficient was reported by Husnudin et al. (2019) who showed that the similarity coefficient based on ISSR generated data ranged from 0.66-0.88 among eggplant accessions. However, the current similarity was higher than that reported by Mahmoud and El-Mansy (2012) who found that the similarity among eggplant cultivars using ISSR data ranged from 0.04-0.77.

Conclusion

It could be concluded that the two local F_1 hybrids (H 1 and H2) of eggplant achieved better

results for vegetative growth and fruit yield than the commercial F₁ hybrid (C4), especially under Qalyubia Governorate conditions (L3). ISSR fingerprinting of the studied genotypes supports the morphological data to characterize the local F₁ hybrids produced as a promising alternative to the high prices of imported F₁ hybrid seeds in cultivation and breeding programs. ISSR used primers have the ability to discriminate among the five studied eggplant genotypes, estimate the genetic variation and produce a distinct fingerprint for each genotype.

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Conflicts of Interest

No conflicts of interest during this study.

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السلوك الحقلية وتحديد البصمة الوراثية لهجن مصرية جديدة من الباذنجان

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تعتبر تربية وإنتاج بذور الخضر محلياً واحدة من أهم مهام القطاع الزراعي في مصر في الوقت الحالي وذلك لارتفاع أسعار بذور هجن الخضر المستوردة وبالتالي زيادة الإحتياج للعمولات الأجنبية في ظل معوقات الإقتصاد المصري والأزمة الإقتصادية العالمية. لذلك أصبح من الضروري تربية وإنتاج هجن خضر محلية واعدة لتحقيق الإكتفاء الذاتي نسبياً. هذه الدراسة تهدف إلى التقييم الحقلية لإثنين من هجن الباذنجان المنتجة محلياً (H₁ ، H₂) مقارنة مع أحد الهجن التجارية وهو هجين الألبستر (C₄) بالإضافة إلى تحديد البصمة الوراثية لهذه الهجن. تم تقييم سلوك الهجن H₁ و H₂ مقارنة مع C₄ من حيث النمو الخضري والإنتاجية تحت أربعة ظروف بيئية مختلفة (محافظة الجيزة "L₁" ، محافظة المنوفية "L₂" ، محافظة القليوبية "L₃" ، محافظة البحيرة "L₄") والتي تختلف فيما بينها من حيث نوع التربة (طينية - رملية - رملية كلسية) وملوحة مياه الري (٧٥٠-٢٥٠٠ جزء في المليون) خلال الزراعة في الموسم الصيفي لعامي ٢٠٢٠ و ٢٠٢١. أوضحت النتائج المتحصل عليها تفوق هجين الباذنجان المنتجين محلياً (H₁ & H₂) وفي بعض الأحيان تساوت مع الهجن التجاري (C₄) من حيث ارتفاع النبات، عدد الأفرع والأوراق لكل نبات، والتبكير في الأزهار، المحصول المبكر والكلبي، عدد الثمار/نبات، متوسط وزن الثمرة خاصة تحت ظروف محافظة القليوبية والجيزة (L₁ & L₃). تم استخدام تسعة عشر من بادئات ISSR لعمل بصمة وراثية للهجن تحت الدراسة (H₁, H₂, C₄) بالإضافة إلى الأبويين الأصليين (P₁, P₂). وأظهر تحليل بادئات ISSR الحصول على ٢٤٣ معلم جزيئي متضاعف بنسبة تباين (٢١,٥٠٪). وتراوحت عدد المعلمات الجزيئية المتباينة من ٩٪ الي ٨٨٪ بمتوسط ٥٠٪. كما تراوحت قيمة PIC من ٠,٠٩ إلى ٠,٤٩ بمتوسط ٠,٣٦. وقد أظهرت سبعة بادئات وهي (HB₁₂, UBC-١٧٨٩٩, A-٨٤٤, UBC-٨٩٢, UBC-٨٩٥, ISSR-٥٩ and ISSR-DAT) بصمة وراثية مميزة للهجين H₁. وبالمثل أظهرت ثمانية بادئات وهي (HB₁₀, HB₁₂, UBC-٨٩٢, UBC-٨٠٨, ISSR-٥٥, ISSR-٥١, ISSR-DAT) بصمة وراثية مميزة للهجين H₂. بينما تم تميز الهجن التجاري C₄ بثلاثة بادئات فقط وهي (UBC-٨٥٠, UBC-٨٨١ and ISSR-٩). أظهر هجين الباذنجان المحليين (H₁ ، H₂) نتائج أفضل في معظم الصفات والتباين الوراثي أكثر من الهجن التجاري «C₄»، مما يجعل هذه الهجن مناسبة للزراعة تجارياً واستخدامها في برامج التربية. وجد ان أعلى تشابه وراثي كان بين (P₁ ، H₁) وقدره ٨٩,٠٠، بينما كانت أقل قيمة تشابه هي ٧٨,٠٠ بين (P₁ ، H₂). وإعتماداً على تحليل علاقات القرابة الوراثية باستخدام طريقة الـ UPGMA لإيجاد شجرة القرابة بين التراكيب الوراثية الخمسة المستخدمة في الدراسة فقد تم فصلهم إلى مجموعتين رئيسيتين. كان لبادئات ISSR المستخدمة القدرة على التمييز بين الأنماط الجينية الخمسة تحت الدراسة بالإضافة إلى تقدير التباين الوراثي ودرجات القرابة الوراثية وإنتاج بصمة وراثية مميزة لكل نمط وراثي.