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Evaluation of El-Sadat (M2K) Pear Clone Grown in El-Sadat City

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ABSTRACT

This study was conducted during three successive seasons of 2013 up to 2015 on Sadat pear (selected clone from (Le-Conte) pear (*Pyrus communise*, Rehd). Trees grafted on *Pyrus betulaefolia* [*P. betulifolia*] rootstock and grown in sandy soil at private orchard in Sadat city, Menofya Governorate, Egypt, to evaluated parameters [(date of: vegetative bud break, flower bud break, full bloom and petal fall, vegetative growth parameters (No. of leaves per shoot, shoot length and leaf area), fruiting (fruit set, maturity and yield) and fruit quality (physical and chemical analysis)] at harvest or cold storage at 5±1 ºC and 90% relative humidity for 60 days.The results showed that, bud break time was late at first week of April, and maturated at the end of September compare to 15/8 for "Le-Conte" and fruitful with a consistent bearing of regular shape, large size fruits (385g) and produced higher yield per tree 123.4 kg (average of three seasons) Sadat pear trees yield late fruits and has good keeping quality during cold storage so, it offer fresh fruits for long period in markets, where as was known that the most production of pear fruits persists just through two months.With respect to molecular genetic studies, the data obtained from RAPD-PCR showed nine bands as total bands with molecular weight ranging from 180-1100bp. On the other hand, ISSR-PCR analysis generated ten bands with molecular weight ranging from 120-1360bp.

Keywords: Pear, Evaluation, Morphological characterizations, physical and chemical analysis, yield, Cold storage.

INTRODUCTION

Pear is considered one of the most important pome fruits produced in Egypt. The planted area is about 20000 feddans[1] in 2015 which represented 5 % of deciduous fruits. Most of this area is concentrated in Behara, Alexandria, Monofia and Kafr El-Sheikh Governorates, according to Ministry of Agriculture. Average production ranged from 6-7 tones per feddan. Pear production decreased in last decades because (*Pyrus communis*) rootstock is low resistant to fire blight. Deckers and Schoofs (2002) found that the bacterial disease caused by *Erwinia amylovora*, which spreads from cankers in the bark throught the flowers very rapidly in worm humid weather. It can be consider problematic in regions where environmental conditions for disease development are favorable, specifically where spring time weather is warm and wet. It is responsible for serious production losses in Egypt. *Pyrus betulaefolia* is a vigorous rootstock, a deep rooted system; it is resistant to fire blight, pear decline, crown rot, root aphids and produce a large vigorous tree that grows under very wet or poor soil conditions and produce large round fruits (Strand 1999). Sadat pear trees (selected colon grafted on *Pyrus betulaefolia*) is one of the genotype that selected from a private orchard (one hundred feddans cultivated Le-Conte pear trees) at Sadat city. This colon is late in flowering and maturity, so it offers fresh fruits for long periods in markets.

Molecular markers are interest to plant geneticists and breeders as a source of new genetic information on plant genomes and for use in trait selection. Randomly Amplified Polymorphic DNA analysis (RAPD) can be used to identify many useful polymorphisms quickly and efficiently, and as such, it has tremendous potential for use in cultivar identification. RAPD analysis has been used to study genetic relationships in a number of fruit trees including almond (Bartolozzi *et al.,* 1998), plum varieties (Ortize *et al.,* 1997), peach varieties (Chaparro *et al.,* 1994 and Warburton and Bliss, 1996), peach rootstocks (Lu *et al.*, 1996) and RAPD markers have been used in peach genetics and breeding programs (Rajapakse *et al*., 1995). The ISSR strategy was therefore performed to access the DNA diversity among crop genotypes (Zehdi *et al*., 2004). On the other hand, Kim and Ko-Kwang (2004) used RAPD technique to identify 33 Asian pears (*Pyrus spp*.). Nine primers out of 18 primers were produced distinct and reproducible bands. Most of the Asian pears could be identified. The obtained results and Neis genetic distance were used to construct the dendrogram. The Asian pears were differentiated to four clusters. Therefore, this investigation has been initiated to evaluate a new selected colon of pear as for flowering, fruiting, and good quality of fruits and storability under cold storage at 5 ±1 ºC and 90% relative humidity for 60 days.

MATERIALS AND METHODS

This study was carried out during three successive seasons from 2013 to 2015 on mature healthy trees of Sadat pear (selected colon from Le-Conte pear budded on *Pyrus betulifolia* rootstock in a private orchard at El-Sadat region, Menofyia Governorate). The experimented trees were grown on sandy soil, nearly uniform in vigor. All trees received the same cultural practices, under drip irrigation, spaced 5 x 5 m, age 12-year-old, nine fruiting trees were selected and the following parameters were determined.

Vegetative bud break, flower bud break, full bloom and petal fall, fruit set and maturity dates to are recorded as well as fruit age from full bloom till maturity was estimated as days.

Vegetative growth (No. of leaves per shoot, shoot length and leaf area) were calculated in mid October.

Fruiting (fruit set percentage was recorded and yield was counted as kg/tree and ton/fed.).

Fruit quality (Fruit physical and chemical characteristics) determinations at harvest or cold storage at 5 ±1 ºC and 90% relative humidity for 60 days as following:-

[1] **One feddan area = 4200 m^2**

1-Fruit physical characteristics determinations

a- Fruit age (day): days from full bloom to harvest date in all seasons were calculated and recorded.

b- Fruit weight (g), volume, length (cm) and diameter (cm): were recorded.

d- Fruit weight loss (%):

The loss in mass fruit weight during storage at cold storage (5 ± 1 °C) was calculated as the difference between fruit weight at the start of storage and fruit weight at the inspection date as the following equation: [(A-B)/A] ×100**.**

Where: A=the initial fruit weight, B= fruit weight at the inspection date

e- Fruit Texture (g / cm^2):

Fruit firmness were determined by Lfra texture analyzer using a penetrating needle of 3 mm of diameter, 3 mm in distance, speed of 2 mm per second and the peak of resistance was recorded as g / cm^2.

f- Fruit peel color (L& h◦ values): It was measured by averaging two measurements taken on two opposite points of each fruit equator with a Minolta colorimeter (Minolta Co. Ltd., Osaka, Japan) on the basis of the CIELAB color system. In this system values of (a & b) specify the green-red and blue-yellow axis, while Hue (h◦) determines the position of such vector. h° values are calculated based on (a & b) values according to the following equation: h° =180-tan^{-1} (b / a). h° values were determined, calculated and used as an indicator of loquat ripeness according to (Mc Guire, 1992). Data of hunter L (ranging from black=0 to white=100) were used as surface browning indicator with out further conversion.

2- Fruit chemical characteristic determinations:

a- Total soluble solids percentage (TSS %) were determined in fruit juice by hand A`bbe refractometer .

b- Total acidity percentage (TA %) as malic acid determined in fruit juice according to A.O.A.C. (2005).

Post harvest treatment study

At the last week of September, in all seasons, mature pear fruits were harvested at one maturity stage (yellow colour) and directly transported to the laboratory, nearly uniform pear fruits were washed, air dried and divided into three replicates and each replicate was packed in 3 carton boxes and stored at 5 ±1° C and 90% relative humidity for 2 months to determine physical and chemical properties at 10 days intervals.

Molecular Genetic studies:

RAPD-PCR Analysis DNA Extraction

Yong and freshly excised leaves were collected from Pear clone. Then DNA extraction was performed according to Dellaporta *et al.* (1983).

Random Amplified Polymorphic DNA RAPD -PCR Analysis

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, $MgCl_2$ and Taq polymerase. A total of twenty-one random DNA oligonucleotide primers were independently used according to Williams *et al*. (1990) in the PCR reaction. Only six primers succeeded to generate reproducible polymorphic DNA products. Table 1 lists the base sequences of these DNA primers that produced informative polymorphic bands.

PCR amplification was performed using six random 10 mer arbitrary primers synthesized by (Operon biotechnologies, Inc.Germany).

Inter Simple Sequence Repeat ISSR-PCR Analysis

ISSR-PCR reactions were conducted according to Williams *et al*. (1990) in the PCR reaction.using six primers. Only six primers succeeded to generate reproducible polymorphic DNA products. Table 2 lists the base sequences of these DNA primers that produced informative polymorphic bands.

Gel documentation:

Gels were photographed scanned, analyzed using Gel Doc Vilber Lourmat system to capture the image and to calculate band intensities.

Table 1. List of the used RAPD primers, names and their nucleotides sequences.

No.	Name	Sequence	No.	Name	Sequence
1	OP-A5	5´ CCTTGACGCA 3`	4	OP-B17	5` CTCACCGTCC 3`
2	OP-A7	5´ GAA AGG GGT G 3`	5	OP-C1	5` ACCGCGAAGC 3`
3	OP-B4	5´ GAT GAC CGC C 3`	6	OP-C12	5` GGACCCAACC 3`

Table 2. List of the used ISSR primers, names and their nucleotides sequences.

No.	Name	Sequence	No.	Name	Sequence
1	14A	5´ TCGGCCATAG 3`	5	HB-14	5´ CTC CTC CTC GC 3`
2	44B	5´ CCTTGACGCA 3`	6	HB-15	5` GTGTGTGTGTGTGC 3
3	HB-10	5` GAGAGAGAGAGACC 3`			
4	HB-13	5` GAGGAGGAGGC 3`			

Statistical analysis

All obtained data in three seasons were subjected to analysis of variance according to the method described by Snedecor and Cochran (1990). Differences among treatment means were compared using Duncan's Multiple Range test (Duncan, 1955) at $p \leq 0.5$ in of probably.

RESULTS AND DISCUSSION

1- Flowering parameters:-

Data presented in Table 3 clarify showed that vegetative bud break begin 25-27 March, flower break from 19 to 28 March. Also full bloom, petal fall, fruit set and maturity are shown in Table 3 for 2013, 2014 and 2015 seasons. The delay of maturity than "Le-Conte" cultivar (15/8) has helped to the pear marketing period.

These results generally agree with the findings of Angelini (1986), Trachen (1984) and Deckers and Schoofs (2002).

Table 3. Time of vegetative, buds break, flower bud break, blooming, fruit set and maturity stage of pear fruits during 2013, 2014 and 2015 seasons.

Season	Vegetative bud break	Flower bud break	Full bloom	Petal fall	Fruit set	Maturity
2013	3 / 26	3 / 28	4 / 1	4 / 10	4 / 14	9 / 28
2014	3 / 25	3 / 19	3 / 29	4 / 8	4 / 12	9 / 22
2015	3 / 27	3 / 21	4 / 5	4 / 12	4 / 3	10 / 2

2- Vegetative growth parameters:-

Table 4 showed vegetative growth of Sadat pear. It is clear that, average number of leaves/ shoot were 42.4, 38.9 and 39.2 through 2013, 2014 and 2015 seasons. Also, shoot length recorded 87.3, 76.1 and 79.6 cm respectively. In addition, leaf area recorded 30.4, 32.1 and 31.7 cm^2 respectively with insignificant differences. These results about the same which reflect that, Sadat "M2K" clone has fixed parameters and are in agreement with the work of Angelini (1986).

Table 4. Some vegetative growth parameters of Sadat pear trees during 2013, 2014 and 2015 seasons.

Season	Number of leaves / shoot	Shoot length (cm)	Leaf area (cm^2)
2013	42.4A	87.3A	30.4AB
2014	38.9AB	76.1A|B	32.1A
2015	39.2AB	79.6AB	31.7A

3- Fruit set % and Yield (kg/tree and ton/feddan):

According to the results in the Table 5, Sadat pear progeny shows insignificant differences between the three years in fruit set (%), yield (kg/tree) and (ton/feddan). The tree yield through the three years average were 119.9 to 130 (kg per tree) and the total yield per feddan were 21.4, 19.7 and 18.8 ton/feddan in 2013, 2014 and 2015 seasons, respectively. These results are in line with the findings of Fayek *et al*. (2004) and Faissal & Abdel All (2007) that they improved yield of "Le-Conte" pear by some applied treatments (ringing or amino acids application). Singh and Sharma (2005) stated the improvement in fruit and yield with GA_3 application.

Table 5. Fruiting parameters fruit set (%); yield per tree and per faddan of Sadat pear trees during 2013, 2014 and 2015.

Season	Fruit set (%)	Yield (Kg /tree)	Yield (ton/feddan)
2013	27.1AB	130.1A	21.4A
2014	30.4A	119.9A	19.7A
2015	28.6AB	122.3A|B	18.8AB

Table 6. Some fruit parameters of Sadat pear at harvest in 2013, 2014 and 2015.

Years	Fruit age (days) (full bloom maturity)	Fruit weight(g.)	Volume (cm^3)	Diameter (cm)	Length (cm)
2013	185	388.67A	386.67A	8.78A	9.46C
2014	178	384.00B	387.5A	8.50B	10.90A
2015	180	386.33AB	387.17A	8.62AB	10.18B

4- Fruit age and properties:-

The present data in Table 6 showed that, fruit age average (from full bloom till maturity) were 178-185 days through 2013, 2014 and 2015 seasons compare to 130 days with "Le-Conte" cultivar (Makarem *et al*., 2012). Also, this Sadat pear progeny recorded heavy and large fruits with 384-388.67 g weight, 386.67-387.5 cm^3 volume, 9.46-10.9 cm length as well as 8.5-8.78 cm diameter in the three years of study.

5- Post harvest study:

Data in Table 7 showed the effect of cold storage periods on weight loss percentage of pear fruits during 2013, 2014 and 2015 seasons. Weight loss percentage was gradually increased with prolong the storage periods with significant differences among all storage periods in the three seasons of study.

These results agreed with those reported by El-Shiekh *et al*. (2002) and El-Shemy *et al*. (2007) on "Le Conte" pears.

The weight loss attributed mainly to water loss from the fruit tissues and partially for the respiration.

Table 7. Effect of cold storage periods at 5 °C and 90 % RH on weight loss percentage of pear fruits during 2013, 2014 and 2015 seasons.

Periods (days)	Weight loss (%)		
	1st season	2nd season	3rd season
0	0.00G	0.00G	0.00G
10	0.74F	0.66F	0.71F
20	1.99E	1.54E	1.80E
30	3.51D	3.12D	3.35D
40	4.62C	5.00C	4.87C
50	5.44B	6.25B	5.90B
60	6.34A	8.74A	7.61A

6- Texture (g/cm^2):

Data obtained regarding fruit texture for seasons of 2013, 2014 and 2015 are presented in Table 8 and clear that, texture decreased with progress of storage periods in the three seasons of study may be as a result of much water loss (Table 7), Yaman and Bayoindirli (2002) noticed that, the retention of firmness which occurred during storage could be explained by retarded degradation of insoluble protopectins to the more soluble pectic acid and pectin. During fruit ripening depolymerization or shortening of chain length of pectin substances occurs with an increase in pectin esterase and polygalactronase activities. Also pectinesterase activity is expected to increase progressively during storage and as a result peel and pulp hardness decreased during storage as reported by Ponomarev (1968) and El-Shemy *et al*. (2007) on pears.

Table 8. Effect of cold storage periods at 5° C and 90 % RH on texture (g/cm^2) of pear fruits during 2013, 2014 and 2015 seasons.

Periods (days)	Texture (g/cm^2)		
	1st season	2nd season	3rd season
0	127.40A	122.50A	139.95A
10	110.85B	115.05B	112.95B
20	104.65C	107.6C	106.15C
30	99.10D	104.65C	101.9E
40	98.10D	96.6D	98.85E
50	89.50E	94.15D	91.80F
60	84.85E	87.75E	86.30G

7- Fruit color:

Lightness (L* value): Data in Table 9 showed that, lightness (L) gradually increased towards the end of the storage period (60 days).

As for Hue angle, data indicated that hue angle (h°) decreased (increase density of yellow color) with the advance of cold storage periods.

This result agree with those reported by Bower *et al*. (2003) they noted that "Bartlett" pears stored at 2°C and moved to ambient temperatures, color change continued more rapidly in pears that exposed to ethylene than in those kept in air.

Table 9. Effect of cold storage periods at 5 °C and 90 % RH on fruit color percentage of pear fruits during 2013, 2014 and 2015 seasons.

Periods	Lighteness (L)			Hue angle (H)		
(days)	1st season	2nd season	3rd season	1st season	2nd season	3rd season
0	69.84A	68.06A	68.95A	98.02A	97.41A	97.71A
10	68.6A	67.94A	68.27A	95.39A	94.96B	95.18AB
20	68.52A	67.04A	67.78A	95.26A	92.13C	93.70B
30	62.84B	64.42B	63.63B	88.42B	88.33D	88.38C
40	61.5BC	63.77B	62.63BC	81.19C	86.71D	83.95D
50	60.52BC	61.53C	61.03CD	79.79C	82.37E	81.09DE
60	60.01C	60.33C	60.17D	79.74C	80.13E	79.94E

8- Soluble solid content (SSC %) and acidity:

The data introduced in Table 10 showed that, there were significant differences in soluble solid content of fruits. SSC (%) gradually increased with the advance of cold storage periods.

The increment in the SSC (%) could be due to the degradation of complex insoluble compounds like starch to simple soluble compounds like sugars, which are the major component of SSC content in the fruits and that changes increased with the progress of storage time, where it allowed the accumulation of SSC in the fruits (El-Shemy *et al*. 2007) on pears.

Concerning titratable acidity (TA), data showed significant decreases in titratable acidity of pear fruits in the three seasons of study with the progress of the storage period.

There may be a connection between the enhanced respiration with progress of storage period and the reduction in TA. Acidity reflects the level of organic acids in fruit tissues, particularly malic acid. These acids are the substrate for respiratory cycle, and enhanced respiration would lead to decrease in their levels (El-Shemy *et al*. 2007).

This results are in agreement with those observed by Jan (2010) and Wawrzyńczak *et al*. (2008), they noticed that, TSS (%) increased by increasing storage periods due to starch hydrolysis in pears. However, data in Tables (8, 9 and 10) showed that, Sadat pear progeny quality get better with storage at 5°C and 90 % RH for 60 days, where fruit texture, color and TSS were improved while acidity decreased.

Table 10. Effect of cold storage periods at 5 °C and 90% RH on SSC and acidity percentages of pear fruits during 2013, 2014 and 2015 seasons.

Periods	SSC (%)			Acidity (%)		
(days)	1st season	2nd season	3rd season	1st season	2nd season	3rd season
0	12.00D	11.90C	11.95E	0.192A	0.192A	0.192A
10	12.50CD	12.30C	12.40DE	0.192A	0.180AB	0.184A
20	12.60CD	13.10B	12.82CD	0.160AB	0.170AB	0.165B
30	13.00C	13.70B	13.33C	0.160AB	0.160AB	0.160B
40	14.50B	14.50A	14.48B	0.128BC	0.149B	0.139C
50	15.00B	14.60A	14.78B	0.128BC	0.149B	0.139C
60	16.00A	15.20A	15.58A	0.096C	0.162AB	0.129C

9- Molecular Genetic studies:

Randomly amplified polymorphic DNA (RAPD) markers:

Data of the amplified fragments using those six 10-mer arbitrary primers for Pear prongy succeeded in amplifying DNA fragments (Table, 11 and Plate, 1).

Primer OP-A5 resulted in eight bands with molecular sizes from 180 to 760bp and Primer OP-A7 resulted in seven bands with molecular sizes from 180 to 1100bp. While, Primer OP-B4 indicated the amplification of eight bands with molecular size range from 180-1100bp and Primer OP-B17 indicated the amplification of five bands with molecular weight size range from 260-700bp. On the other hand, primer OP-C1 resulted in three DNA fragments with molecular weight ranging in 260-400bp and primer OP-C12 produced five bands with molecular weight size range from 260-700bp .

10- Inter Simple Sequence Repeats (ISSR):

Data of the amplified fragments using those six ISSR primers for the Pear prongy in amplifying DNA fragments (Table, 12 and Plate, 2).

Primer 14 A resulted in eight bands with molecular sizes from 230 to 1360bp and Primer 44 B resulted in seven bands with molecular sizes 120 and 1360bp. While, Primer HB-10 indicated the amplification of five bands with molecular size range from 230-1200bp and also Primer HB-13 indicated the amplification of two bands with molecular weight size range from 120 -570bp. On the other hand, primer HB-14 resulted in eight DNA fragments with molecular weight ranging in 120 to 1360bp and primer HB-15 produced eight bands with molecular weight size range from 120 -1360bp.

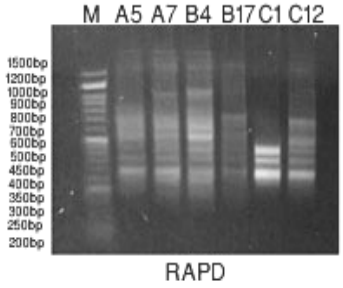

Table 11. Densitometric analysis for RAPD-PCR from Pear cultivar.

Band No.	A5	A7	B4	B17	C1	C12
1	-	1100	1100	-	-	-
2	760	-	-	-	-	
3	700	-	-	700	-	700
4	600	600	600	600	-	-
5	520	520	520	520	-	520
6	400	400	400	400	400	400
7	320	320	320	320	320	320
8	260	260	260	260	260	260
9	180	180	180	180	-	-

Plate 1. RAPD profiles of Pear cultivar amplified with six primers.

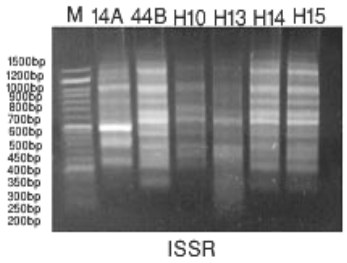

Table 12. Densitometric analysis for ISSR-PCR from Pear cultivar.

Band No.	14A	44B	HB-10	HB-13	HB-14	HB-15
1	1360	1360	-	-	1360	1360
2	1200	-	1200	-	-	-
3	1000	1000	1000	-	1000	1000
4	760	760	-	-	760	760
5	-	630	630	-	630	630
6	570	570	570	570	570	570
7	500	-	-	-	-	-
8	350	-	-	-	350	350
9	230	230	230	-	230	230
10	-	120	-	120	120	120

Plate 2. ISSR profiles of Pear cultivar amplified with five primers.

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تقييم سلالة من الكمثرى (السادات M2K) منزرعة بمدينة السادات

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أجريت هذه الدراسة على أشجار سلالة منتخبة من الكمثرى بالغة نامية بمزرعة خاصة بمدينة السادات بمحافظة المنوفيه – مصر. لتقييمها خلال ثلاثة مواسم (2013 حتى 2015). هذه الأشجار مطعومة على أصل *Pyrus betulaefolia* و نامية في تربة رملية لتقييم: 1- موعد تفتح البرعم الخضري و الزهري - اكتمال التزهير - تساقط البتلات والعقد. 2- قياسات النمو الخضري (عدد الاوراق على الفرع- طول الفرع- المساحة الورقية). 3- قياسات الإثمار (موعد: عقد الثمار – إكتمال نمو الثمار – المحصول للشجرة "كجم" والفدان بالطن). 4- كمية المحصول وصفات جودة الثمار عند الحصاد و أثناء التخزين. أوضحت نتائج هذا التقييم أن قياسات التزهير لهذه السلالة المنتخبة متأخرة (الاسبوع الاول من أبريل ومتوسط عدد الأوراق على الفرع 40 ورقة وطول الفرع 40 سم و مساحة الورقة 31.4 سم^2 وكذلك تأخر اكتمال نمو الثمار حتى نهاية سبتمبر مقارنة بأشجار الليكونت (منتصف أغسطس) وأن هذه السلالة منتظمة في الحمل وفي شكل الثمار وكبر حجمها (385 جرام) وتتميز بالإنتاجية العالية (123.4 كيلو جرام) للشجرة (متوسط ثلاثة مواسم). تم استخدام تقنية التضاعف العشوائي لأجزاء من مادة DNA في جهاز سلسلة تفاعل إنزيم البلمرة (RAPD-PCR) وتقنية البوادئ المتخصصة للقطع المتكررة على الجينوم (ISSR-PCR) لعمل التعريف الوراثي الجزيئي لصنف الكمثرى تحت ظروف الأراضي الصحراوية . وقد أظهرت النتائج التعرف على تسعة مواقع جينية على جينوم الكمثرى تحت الدراسة في مدى من الأوزان الجزيئية يتراوح من 1100 bp–180 وذلك باستخدام تقنية (RAPD-PCR) في حين أظهرت تقنية (ISSR-PCR) التعرف على عشرة مواقع جينية على جينوم الكمثرى تحت الدراسة في مدى من الأوزان الجزيئية يتراوح من 1360 bp – 120 تصل ثمار هذه السلالة لاكتمال النمو بعد 180 - 185 يوم من اكتمال التزهير مقارنة بـ 130 يوم فى صنف الليكونت عندما يكون متوسط وزن الثمرة 385 جم، طول الثمرة 10.18 سم ،قطر الثمرة 8.66 سم، والمواد الصلبة الذائبة الكلية 11.95%، والحموضة الكلية 192 .0 % وتحول لون الثمرة إلى الأصفر، وصلابة الثمار 259.9 جم/سم^2 على عمق 3 مم في سمك اللحم. و أوضحت النتائج أيضاً أن تأخر الإثمار وجودة الصفات الثمرية لأشجار هذه السلالة أدى إلى إطالة الفترة التسويقية لثمار الكمثرى بالاسواق وكذلك تحسن صفاتها الاكلية بعد تخزينها لمدة شهرين على درجة حرارة 5 مئوي ورطوبة نسبية 90 %.