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Horticultural and Genetical Evaluation of Le-Conte Pear and Seven Induced Mutants Through RAPD, ISSR and Microsatellite

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Abstract: Horticultural characteristics and molecular genetic markers were used to evaluate Le-Conte pear and seven induced mutants during seasons from 2014 to 2016. The highest yield and the best fruit quality were obtained from mutants LM16, LM18 and LM21, while the lowest fruit yield and quality parameters were resulted from LM17 when compared with Le-Conte pear during all seasons. RAPD, ISSR and microsatellite (SSR) molecular markers were used to determine genetic relationship among Le-Conte pear and seven induced mutants. PCR products of ten RAPD primers produced 218 amplified fragments. Polymorphism percent ranged from 75% to 100%. Ten specific fragments; based on RAPD, discriminated Le-Conte and its mutants, *i.e.* LM16, LM17, LM18, LM19, LM20 and LM22. Analysis of five ISSR primers showed 102 amplified fragments. Polymorphism percent ranged from 43% to 100%. LM17 and LM22 were distinguished by three specific fragments. Fourteen microsatellite markers gave 39 amplified alleles. Polymorphic information content (PIC) values ranged from 0 to 0.86 and genetic diversity (GD) ranged from 0 to 0.98. Nine specific alleles differentiated Le-Conte, LM16, LM17 and LM22. Based on combined analysis of RAPD, ISSR and microsatellite markers, similarity percent and phylogenetic tree revealed that all induced mutants can be considered new lines of Le-Conte pear. Finally, radiation can be used as an effective method for producing new lines of pear.

Key words: Induced Mutants · Pear · Fruit Characteristics · Fingerprinting · Microsatellite · SSR · ISSR · RAPD · PIC · GD

INTRODUCTION

Among the deciduous fruits, pear fruits are considered the third important fruit all over the world and the fourth among all fruits [1]. Le-Conte pear is one of the most important deciduous fruits in Egypt [2].

Selection of cultivars as parents in breeding programs mainly depends on fruit quality which can be determined according to some considerable parameters such as fruit juice soluble solids content (SSC), firmness and pH, these parameters are high weight indicators not only for breeding for fruit quality of pear [3] but also its valuable factors from the consumer's point of view [4]. Many studies carried out for improvement of fruit set, yield and fruit characters of Le-Conte pear used mutation breeding programs [5].

Morphological and phenological characterization gave basic data about the ecotypes, but still not sufficient to assess genetic diversity in pear genotypes. This means that assessment is not possible using morphological traits only [6]. So, molecular markers became an extension and an integral part of classical breeding, donating successfully to reduce breeding and selection processing [7].

Polymerase chain reaction (PCR) based molecular markers such as random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP) technique and simple sequence repeat (SSR) or microsatellite markers, have already been implemented for identification genetic relationship, genetic diversity and taxonomic relationship studies in pears. Molecular technique markers also used

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for breeding programs among different fruit species and establishment credible gene bank to create a resource for breeding, plant nurseries and quality control [8-14]. Molecular markers techniques differe in their ability discrimination between varieties, it can be concluded that SSR markers are more efficient than ISSR markers for the assessment of genetic diversity [15]. In a study to evaluate genotypes of *Pyrus syriaca* Boiss and three local Syrian pears cultivars, in addition to Egyptian Le-Conte cultivar, it could be determined that the levels of polymorphism for all genotypes as revealed by RAPD and AFLP were 81.47% and 92.5%, respectively. RAPD and AFLP techniques were useful methods to identify the different closely related pear genotypes [16]. Liu *et al.* [17] evaluated polymorphism of 385 pear resources belonging to five cultivated species or interspecies of *Pyrus*, based on SSR markers, high-quality and comprehensive evaluation of a wide range of pear cultivars by core SSR. Also, Šisko and Javornik [18] reported that both molecular marker techniques proved their reliability to assess genetic relationships among pear genotypes. Wang *et al.* [19] estimated that ISSR molecular marker can well identify the genetic variability among genotypes and cultivars and ISSR is suitable for grouping them.

This study aimed to evaluate horticulture characteristics of Le-Conte pear and seven radiation-induced mutants and estimate their genetic characterization and genetic relationship to determine the degree of similarity among all evaluated samples.

MATERIALS AND METHODS

Plant Materials: Seven years old radiation-induced mutants of Le-Conte pear trees were evaluated during 2014 to 2016 seasons in Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC) and El-Nubaria Horticultural Research Station, Horticulture Research Institute, Agricultural Research Center, Ministry of Agriculture and Reclamation Land, Egypt. These mutants were obtained from radiation breeding program, where, Le-Conte pear buds were irradiated with various doses of Gamma rays (0, 20, 35 and 50Gy). Then treated buds were grafted on one-year-old *Pyrus. Communis* rootstock and were cultivated on the farm of El-Nubaria Horticultural Research Station. After seven years, horticultural characteristics and fruit quality (Physical and chemical characteristics) were evaluated. Genetic characterizations were estimated with three molecular markers; RAPD, ISSR and microsatellite (simple sequence repeat; SSR), to study the genetic relationship among Le-Conte pear cultivar and induced-mutants.

Horticultural Studies: During Spring, flowers number, fruits set number per tree and the remained fruits number at harvest time were counted, then the fruit set percent (%) and fruit drop percent (%) were calculated according to the following equations:

$$\text{Fruit set \%} = \frac{\text{Number of fruit set}}{\text{Flowers number}} \text{x} 100$$

$$\text{Fruit drop\%} = \left(1 - \frac{\text{Number of retention fruits at maturity time}}{\text{Number fruit set}}\right) \text{x } 100$$

Fruit Yield: At harvest time (maturity), the yield of evaluated trees was determined as number of retention fruits/tree and fruits weight (Kg).

Fruit Quality: A sample of four replicates per tree; each replicate contained ten fruits, were collected randomly to determine physical and chemical parameters of fruit in both seasons.

Physical Fruit Characteristics: Each fruit was weighted and the average fruit weight (g) for each replicate was calculated. Fruit diameter (cm), fruit length (cm) and fruit shape (length/diameter ratio) were measured. In addition, five fruits of each experimental tree were used to determine the fruit firmness (pound/inch^2) using penetrometer [20]. Two readings were taken at two distinct positions on the flesh fruit after peeling.

Chemical Fruit Characteristics: According to the official methods of analysis [21], the percentage of total soluble solids of fruit juice (TSS %) was measured by hand refractometer. The percentage of total acidity in fruit juice was determined as malic acid according to the official methods of analysis [21].

Data Analysis: Samples were completely randomized collected, each treatment (eight treatments) was represented as four replicates per tree. Data were statically analyzed by using SAS package [22]. Differences among various treatments were compared using the least significant differences (LSD) at 5% level according to Steel and Torrie [23].

Genetic Characterization

Plant Materials: Young leaves samples were collected for DNA extraction from Le-Conte pear and seven induced mutants cultivated in farm of El-Nubaria Horticultural Research Station.

DNA Extraction: From each evaluated sample, young leaves were collected, grounded in liquid nitrogen and 200 mg of grounded leaves were used for DNA extraction by i-genomic Plant DNA Extraction Mini Kit (protocol A for Lyophilized leaf), (iNtRON Biotechnology Co.). Extracted DNAs were charge depend separated by electrophoresis (5 Vcm^{-1}) in 1% agarose to sure that DNA did not injure during extraction. DNA concentration was measured spectrophotometrically (Nano Drop 1000, USA) and DNA templates were diluted to 50 ng/µl.

PCR Amplification

RAPD and ISSR Markers: Ten RAPD primers (OP-A01, OP-A12, OP-A17, OP-A18, OP-B12, OP-N16, OP-S147, OP-S227, OP-S238 and OP-S253) and five ISSR primers (OP-A08, OP-Amic2, OP-Amic3, OP-Amic-5 and OP-Mic08) were obtained from Bio Basic Inc. (Table 4) and used in PCR reactions. Each PCR reaction contained 100ng of DNA template, 12.5µl master mix solution (i-Taq™, iNtRON Biotechnology), 1 µl of primer and 5 µl of PCR buffer with 1.5mM of $MgCl_2$ in a final volume of 25µl. PCR program conditions consisted of 94°C for 5 min as one step for an initial denaturation, then 35 cycles of 94°C for 1 min (denaturation) followed by 37°C for 1 min (annealing) and 72°C for 3 min (extension) and one cycle at 72°C for 7 min (final extension).

Microsatellite Markers Using Shutdown PCR: Fourteen microsatellites (SSR) primers obtained from Bio Basic Inc (OP- ARO120, OP- ARO123, OP- ARO177, OP- ARO196, OP- ARO780, OP-ATC09, OP-CAC15, OP-CAT01, OP TAA27, OP-Org 23, OP-AMB3, OP-CT19, OP-AG14, CIR016) were used (Table 5).

The PCR amplifications were conducted in a total volume of 25µl solution containing 75ng of genomic DNA, 12.5µl Master mix solution (i-Taq™, iNtRON Biotechnology), 1µl of each forward and reverse primers and 5µl of PCR buffer with 1.5mM of $MgCl_2$. Shutdown PCR program was used for DNA amplification. Program consisted of a cycle of 95°C for 5 min for an initial denaturation, 45 cycles of 95°C for 45s followed by annealing step at 65°C for 30s with -0.7°C/cycle for 15 cycles, then at 50°C for 30cycles, then at 72°C for 3min. for DNA fragment extension and one cycle at 72°C for 10min for final DNA fragments extension and held at 4°C until used.

DNA Electrophoresis: Amplified PCR products were charge depend separated by electrophoresis (5Vcm^{-1}) in 1.5% agarose for RAPD and ISSR primers and 3.0% Agarose for SSR primers and stained with ethidium bromide as described by Sambrook and Russel [24]. DNA ladder (1-Kb plus blue DNA Ladder, GeneOne.Co.) was used to determine the molecular weight of amplified DNA. Agarose gel photographic record was taken under UV transilluminator.

DNA Data Analysis: For RAPD, ISSR and SSR, each DNA amplified fragment was scored as present (1) or absent (0) fragment or allele. Data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package version 2.02 [25]. Cluster analysis was carried out by unweighted pair group's method arithmetic average (UPGMA) with Jaccard similar coefficient. Polymorphic information content (PIC) provides an estimate of the discriminatory power of a locus by considering, not only the number of alleles that are expressed but also the relative frequencies of those alleles. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies) [26]. PIC values were calculated according to Smith *et al.* [26] using the following algorithm for all primers:

$$PIC = 1-Pij2$$

where P is the frequency of the ij^2 *ith* allele

Genetic diversity (GD) was calculated by using the following formula of Nei [27].

$$GD= n (1- p)/ (n-1)$$

where (*n*) is the samples number and (*p*) is the frequency of one allele.

RESULTS

Horticultural Studies: Flowering number, fruit set, fruit drop percent, fruit retention, fruit yield (kg/tree) and seed number/fruit as an average of three seasons 2014, 2015 and 2016 are presented in Table (1).

Table 1: Evaluation of flowering, fruit set and yield of Le-Conte cultivar and seven induced mutants as average of three seasons (2014, 2015 and 2016)

Parameters		Flowers NO./Tree	Fruit set NO./Tree	Fruit set %	Fruit drop (%)	Fruit retention NO./Tree	Yield(kg)/tree	Seeds NO./Fruit
Le-Conte		910	49.0	5.2	20.40	39	6.46	2.55
Induced mutants	LM16	4395	295.0	11.5	6.63	261	50.04	5.00
	LM17	1535	100.0	14.0	6.48	86	7.86	6.00
	LM18	1835	192.0	23.4	10.78	147	26.64	9.00
	LM19	2925	132.0	18.1	4.48	108	13.04	1.00
	LM20	2860	125.0	12.8	4.28	109	22.16	2.00
	LM21	3900	187.0	15.5	6.16	158	31.73	1.50
	LM22	990	86.0	8.1	8.78	79	16.75	1.00
L.S.D(0.05)		78	36.7			36	3.76	2.20

Table 2: Evaluation of physical fruit characteristics of Le Conte cultivar and seven induced mutants during two seasons (2015 and 2016)

Parameters		Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)		Fruit shape (L/D ratio)		Fruit firmness (pound/inch^2)	
Season		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Le-Conte		165.85	165.50	8.25	8.60	10.27	9.91	0.802	0.867	14.700	14.76
Induced mutants	LM16	191.50	193.00	9.15	9.16	9.02	9.02	1.014	1.015	12.475	12.70
	LM17	91.50	91.00	5.92	5.88	6.05	6.08	0.978	0.967	13.450	13.62
	LM18	181.25	181.50	7.85	7.88	8.05	8.07	0.975	0.976	11.375	11.62
	LM19	120.75	120.25	8.45	8.41	9.16	9.16	0.922	0.918	12.575	12.65
	LM20	203.35	208.50	11.27	11.19	11.47	11.38	0.982	0.983	13.125	13.24
	LM21	200.85	208.50	9.35	9.32	9.95	10.01	0.939	0.931	13.425	13.56
	LM22	212.11	211.75	10.02	10.01	11.40	11.41	0.878	0.877	10.400	10.43
L.S.D (0.05)		13.97	11.81	0.33	0.29	0.24	0.49	0.074	0.077	0.582	1.20

Data showed that mutants LM16, LM21, LM19 and LM20 maximized the number of flowers/tree (4395, 3900, 2925 and 2860 flowers/tree, respectively) compared with Le-Conte pear cultivar and LM22 which gave 910 and 990 flowers/ tree, respectively. Mutants LM16, LM18, LM21 and LM20 significantly enhanced fruit set number to 295, 192, 187 and 125 fruit/tree, respectively, while Le-Conte pear cultivar minimized the number of fruits set (49 fruit/tree). The same mutants exploited fruit retention number in a significant way to 261, 158, 147 and 109 fruit/tree, respectively compared with Le-Conte pear cultivar (39fruit/tree). Although fruit drop percentage showed the highest value in LM18 (23.4%) compared with Le-Conte pear cultivar (20.40%), LM18 possessed high number of fruit retention (147 fruit/tree). The highest average yield per tree was obtained from mutants LM16, LM21, LM18 and LM20 (50.04, 31.73, 26.64 and 22.16 Kg/tree, respectively), compared with Le-Conte pear cultivar (6.46 Kg/tree).

Fruit Physical Characteristics: Fruit weight, length, diameter, shape (length/diameter ratio) and firmness of Le- Conte and seven induced mutants are recorded in Table (2) and Fig. (1). All physical characteristics of fruits refer to the superiority of mutants LM20, LM21, LM22, LM16 and LM18 when compared with Le-Conte in both seasons and all the mutants varied in fruit shape. LM22, LM20, LM21, LM16 and LM18 possessed the highest fruit weight in both seasons (212.11 and 211.75, 203.35 and 208.50, 200.85 and 208.50, 191.50 and 193.00 g, respectively). Also, the best fruit firmness was obtained from LM22, LM18 and LM16 which ranged from 10.40 to 12.70 pounds/inch^2). compared with fruit firmness of Le- Conte (14.76 pounds/inch^2).

Fruit Chemical Characteristics: Data in table (3) concerning total soluble solids of fruit (TSS), fruit acidity percent and the ratio between TSS and acidity % of Le-Conte pear and seven induced mutants during two seasons 2015 and 2016. Le-Conte pear possessed the highest significant value of TSS compared with all mutants during season 2015 (12.8), while, TSS of mutants ranged from 11.1 to 12.3 for LM21 and LM20, respectively in the same season. On the contrary, during season 2016, there was no significant difference among TSS of Le-Conte, LM20 and LM16 (12.2, 12.3 and 12.6, respectively). On the other side, fruit acidity percent of Le-Conte pear showed low value (0.29%) compared with most induced mutants, LM20, LM17 and LM16 which cleared the highest acidity % significantly (0.41, 0.39 and 0.35%, respectively) during season 2015. While acidity % of Le-Conte pear possessed high percent (0.40%) with no significant differences with LM16, LM20, LM19, LM18 (0.43, 0.42, 0.41 and 0.39%, respectively) during season 2016.

Fig. 1: Fruit shape, fruit color, fruit size of Le-Conte and some induced mutants

Table 3: Differences of chemical fruit characteristics among mutated clones and Le Conte cultivars (control) at seasons (2015 and 2016)

Parameters		TSS (%)	Acidity (%)	TSS/Acidity ratio	TSS (%)	Acidity (%)	TSS/Acidity ratio
Season		2015			2016		
Le-Conte		12.8	0.29	44.3	12.2	0.40	30.1
Induced mutants	LM16	12.1	0.35	35.0	12.6	0.43	29.5
	LM17	12.2	0.39	31.2	13.2	0.34	34.2
	LM18	11.7	0.33	35.2	11.7	0.39	29.8
	LM19	12.2	0.32	39.2	12.5	0.41	30.4
	LM20	12.3	0.41	30.5	12.3	0.42	29.2
	LM21	11.1	0.34	33.1	11.3	0.34	33.4
	LM22	12.2	0.28	43.1	12.3	0.36	34.4
L.S.D: at 0.05				4.3			3.2

Regarding TSS/acidity ratio, Le-Conte pear gave the maximum value (44.3) with no significant difference with LM22 and LM19 (43.1 and 39.0, respectively). The lowest fruit TSS/acidity ratio was obtained from LM20 (30.50) during season 2015. Fruit TSS/acidity ratio generally decreased during season 2016, but LM20 remained the lowest ratio (29.2). These results refer that fruit TSS and acidity % may be affected by environmental conditions which lead to enhance or inhibit these parameters through affecting gene expression.

DNA Fingerprint

RAPD Markers Analysis of Le-conte Pear and Seven Induced Mutants: Data in table (4) and Fig. (2) cleared that amplification DNA of Le-Conte pear and seven induced mutants using ten operon RAPD primers resulted in 218 amplified fragments. The highest total number of amplified fragments resulted from both OP-A01 and OP-B12 (29 AF with 87.5% polymorphism). One monomorphic fragment was observed in five operons (OP-A01, OP-B12, N-16, S147 and S227). The maximum number of specific fragments (4SF) were produced by OP-A12; which discriminated Le-Conte, LM16, LM19 and LM20; one SF for each, followed by OP-B12 (3SF) discriminated for three induced mutants (LM16, LM20 and LM22). Also, two specific fragments discriminated mutant LM20 were observed in OP-A18, while one specific fragment for Le-Conte pear and LM18 were observed in OP-A01 and OPA-17, respectively. All operon primers cleared high polymorphic percent.

ISSR Markers Analysis of Le-conte Pear and Seven Induced Mutants: Analysis of DNA amplification of Le-Conte and seven induced mutants based on five ISSR operon primers presented in Table (4) and Fig. (2). Results showed that the total number of amplified fragments was

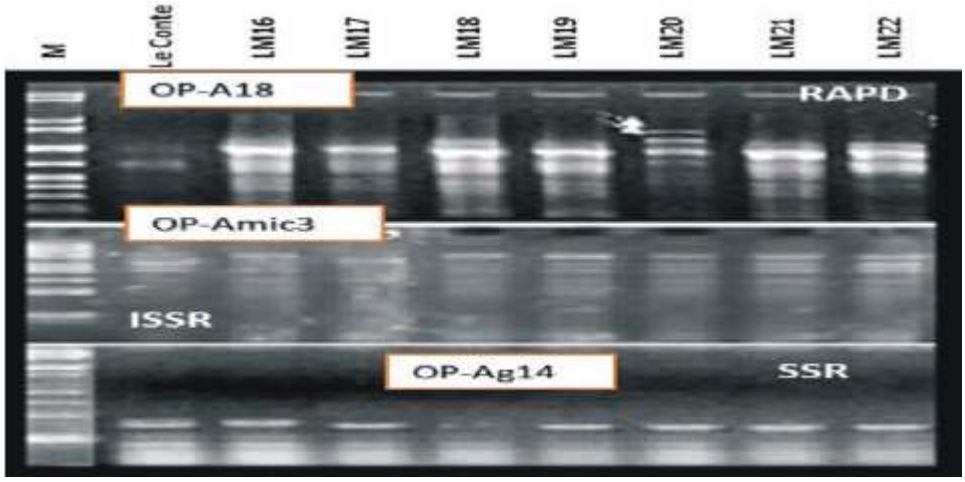

Fig. 2: Samples of electrophoreses of PCR amplified products of Le-Conte pear and seven induced mutants based on RAPD, ISSR and Microsatellites (SSR) markers

Table 4: Sequence of RAPD and ISSR markers and their genetic characterization among Le-Conte pear and seven induced mutants

	Primer	Sequences 5' to 3'	TAF NO.	M F NO.	P F NO.	S F NO. (Name of discriminated mutants)	P%
RAPD markers	OP-A01	CAGGCCCTTC	29	1	7	1 (Le-Conte)	87.5
	OP-A12	TCGGCGATAG	15	==	7	4(Le-Conte, LM16, LM19 and LM20)	100
	OP-A17	GACCGCTTGT	18	==	5	1 (LM18)	100
	OP-A18	AGGTGACCGT	22	==	6	2(LM20)	100
	OP-B12	CCTTGACGCA	29	1	7	3(LM16, LM20 and LM22)	85.7
	OP-N16	AAGCGACCTG	21	1	3	==	75.00
	OP-S147	AGCTGCAGCC	26	1	4	==	80.00
	OP-S227	GAAGCCCAGC	20	1	3	==	75.00
	OP--S238	TGGTGGCGTT	22	=	5	1 (LM17)	100
	OP-S253	GGCTGGTTCC	16	=	5	==	100
Total Number.			218	5	52	10	90.32
ISSR maker	OP-A08	AGC AGC AGC AGC GC	24	=	24	=	100
	OP-Amic02	GAC GAT AGA TAG ATA GATA	23	=	23	==	100
	OP-Amic03	AGA TAG ATA GAT AGA TA	16	8	8	1 (LM22)	50
	OP-Amic-05	CGG CAC ACA CAC ACA CA	25	=	25	2 (LM17& LM22)	100
	OP-Mic-08	CGA CGA CGA CGA CGA	14	8	6	=	43
Total Number.			102	16	86	3	

*TAF NO.: Number of total amplified fragments M F NO.: Number of monomorphic fragments P F NO.: Number of polymorphic fragments P%: polymorphism percent

102 fragments, OP- Amic05, OP-A02 and OP-Amic02 possessed the high number of amplified fragments (25, 24 and 23 AF, respectively) with no monomorphic fragment and high polymorphism appearance (100%). While OP-Amic03 and OP-Mic08 gave the lowest number of amplified fragments (16, 14 AF, respectively) with eight amplified fragments for each one and lowest polymorphism percent (50 and 43%, respectively). Specific fragments were detected for LM22 in OP-Amic03 and for LM17 and LM22 in OP-Amic05.

Microsatellite Markers Analysis of Le-Conte Pear and Seven Mutants: Data in Table (5) and Fig. (2) presented the analysis of the amplification of Le-Conte pear DNA and seven induced mutants using 14 Microsatellite operons. The total number of amplified alleles (AA) was 39 alleles resulted from all used microsatellite operons. The highest amplified alleles number detected for OP-ARO177 (7AA) followed by OP-RO123, OP-ARO120 and OP-CIR016 (4AA for each) and OP-CAT01 (3AA). While, OP-ATC09 and OP-CT9 produced only one allele for each

Table 5: Sequence of microsatellite markers (SSR) and their genetic characterizations among Le-Conte pear and seven induced mutants

Primer	Repeat motif	Sequence (F&R)	Amplified Alleles No.	Specific Alleles NO.	PIC	GD
OP-AG14	AG	F: AAAGGGAAAGCCCTAATCTCA R: CTTCCTCTTGGAGTGTTG	2Alleles	==	0.50	0.57
OP-AMB-3	TC	F: AACACACACACTCGCCTCAC R: CAGCCAAATGTGGAGAGACC	2Alleles	==	0.50	0.57
OP-ARO120	AG	F: AAGGGAAAGTGGCTCAGCTC R: GTTGCTTCCCCACAGTTTCA	4Alleles	1 (LM17)	0.75	0.86
OP-ARO123	TC	F: TTAATCCTGCCCACCTCTCC R: AAGCAAAAGCATTTTCATGTTCA	4Alleles	1 (LM16)	0.75	0.86
OP-ARO177	CT	F: CCCTGCCCTGAACTACCTTC R: GCTGCAAGCAAATGAAAAGC	7Alleles	2 (Le-Conte &LM22)	0.86	0.98
OP-ARO196	GT	F: GATTGTGGCCTGGTCAAGTG R: TCCGTTTGTCTGCTGTGTGA	3Alleles	==	0.67	0.77
OP-ARO780	TG	F: TGTGGGGTTTTTGAAGCCTA R: GAAACCCCCTCTTCCTTGTG	4Alleles	2 (Le-Conte &LM22)	0.75	0.86
OP-ATC09	TG	F: TTCCTTATGTAATTGCTCTTTG R: TGTGAGTGTTTGTGCGTGTG	1Allele	==	0	0
OP-CAC15	CAC	F: TAAATCTCCACTCTGCAAAAGC R: GATAGGAAGCGTCGTAGACCC	2Allelle	==	0.50	0.57
OP-CAT01	CAT	F: GCTTTCGATCCCTCCACATA R: GATCCCTACAATCCTTGGTCC	3Alleles	1 (Le-Conte)	0.67	0.77
OP-CT19	CT	F: CGCCAAGCTTACCACTCACTAC R: GCCACGATTTGTAGGGGATA	1Allele	==	0	0
CIR016	ATG	F: AGC GGG AAA TGA AAA GGT AT R: ATG AAA ACG TGC CAA ATG TC	4Alleles	2(Le-Conte)	0.75	0.86
OP-Org 23	TG	F: AGGTCTACATTGGCATTGTC R: ACATGCAGZTGCTATAATGAATG	2Allele	==	0.50	0.57b
OP-TAA27	TAA	F: GGATGAAAAATGCTCAAAATG R:TAGTACCCACAGGGAAGAGAGC	2Allele	==	0.50	0.57
Total	==	==	39	9	==	==

PIC: Polymorphism information content GD: Genetic diversity value

to become monomorphic alleles. Data showed the total number of specific alleles was 9 specific alleles distributed as the following two specific alleles for OP-ARO177 and OP-CIR016 which distinguished Le-Conte pear. Also, two specific alleles discriminate Le-Conte and LM22 in OP-ARO780. One specific allele differentiated LM17, LM16 and Le-Conte pear resulted from OP-ARO120, OP-ARO123 and OP-CAT01. Polymorphic information content (PIC) gave an estimate of the discriminatory force of a locus by concerning not only account, but also the number of alleles that are expressed, but also the relative frequencies of those alleles. OP-ARO177 gave the heights PIC value (0.86) to be highly discriminative followed by OP-ARO120, OP-ARO123, OP-ARO780 and OP-CIR016(PIC=0.75 for each of them). On the other hand, the lowest PIC values (0) were observed for OP-ATC09 and OP-CT19 which were monomorphic. Genetic diversity varied from zero; for monomorphic operon primers, to 0.98. for high discriminative operon primer OP-ARO177. Also, OP-ARO120, OP-ARO123, OP-ARO780 and OP-CIR016 were highly discriminative operons with GD value 0.86. OPAG14, OP-AMB3, OP-CAC15, OP-Org23 and OPTAA24 gave low genetic diversity (0.50).

Genetic Characteristics of Le-Conte Pear and Seven Induced Mutants Based on RAPD, ISSR and Microsatellite (SSR) Markers: Results in Table (6) included total genetic characterization of evaluated DNA samples based on the three genetic markers, RAPD, ISSR and SSR. Regarding RAPD marker, the highest TAF was possessed by LM18, LM16, LM21 and Le-Conte (31, 29, 28 and 27 AF). Le-Conte pear and seven induced mutants gave high polymorphism percent, induced mutants LM20 possessed the highest polymorphism percent (84%). Specific fragments (SF) based on RAPD cleared 12 specific fragments for all examined samples. The diverse number of specific fragments discriminated Le-Conte pear, LM16, LM17, LM18, LM19, LM20 and LM22 (2, 2, 1, 1, 1, 3 and 2SF, respectively). Concerning ISSR analysis, induced mutants LM20 and LM22 in addition to Le-Conte pear maximized total amplified fragments number (15, 15 and 13 AF) also, LM20 and LM22, Le-Conte pear, LM17 and LM19 possessed the highest polymorphism percent (87, 87, 85, 85 and 85 %).

Discrimination of Le-Conte pear and seven induced mutants based on ISSR marker seemed week; only three specific fragments were detected one of the discriminated

Table 6: Genetic characteristics of Le-Conte pear and seven induced mutants based on RAPD, ISSR and microsatellite (SSR) markers

Markers		RAPD			ISSR			SSR	
Parameters		TAF	SF	P%	TAF	SP	P%	TAN	SA
Le-Conte		27	2	77.8	13	0	85	28	5
Induced mutants	LM16	29	2	79.3	11	0	82	24	1
	LM17	25	1	76	13	1	85	24	1
	LM18	31	1	81	10	0	80	25	0
	LM19	25	1	76	13	0	85	24	0
	LM20	25	3	84	15	0	87	23	0
	LM21	28	0	79	12	0	83	27	0
	LM22	24	2	75	15	2	87	29	2

TAF: Total number of amplified fragments SF: Specific fragments number P%: polymorphism percent TAN: total alleles number SA: specific alleles

Table 7: Assessment of similarity percent among Le-Conte pear and seven induced mutants based on RAPD, ISSR and Microsatellite

Combination	Le-Conte	LM16	LM17	LM18	LM19	LM20	LM21	LM22
Le-Conte	100							
LM16	76.1	100						
LM17	59.8	66.7	100					
LM18	70.1	70.1	64.1	100				
LM19	63.3	66.7	709	72.7	100			
LM20	62.4	65.8	56.4	59.8	68.4	100		
LM21	75.2	70.1	64.1	67.5	70.9	75.2	100	
LM22	64.1	64.1	56.4	56.4	63.2	69.2	78.6	100

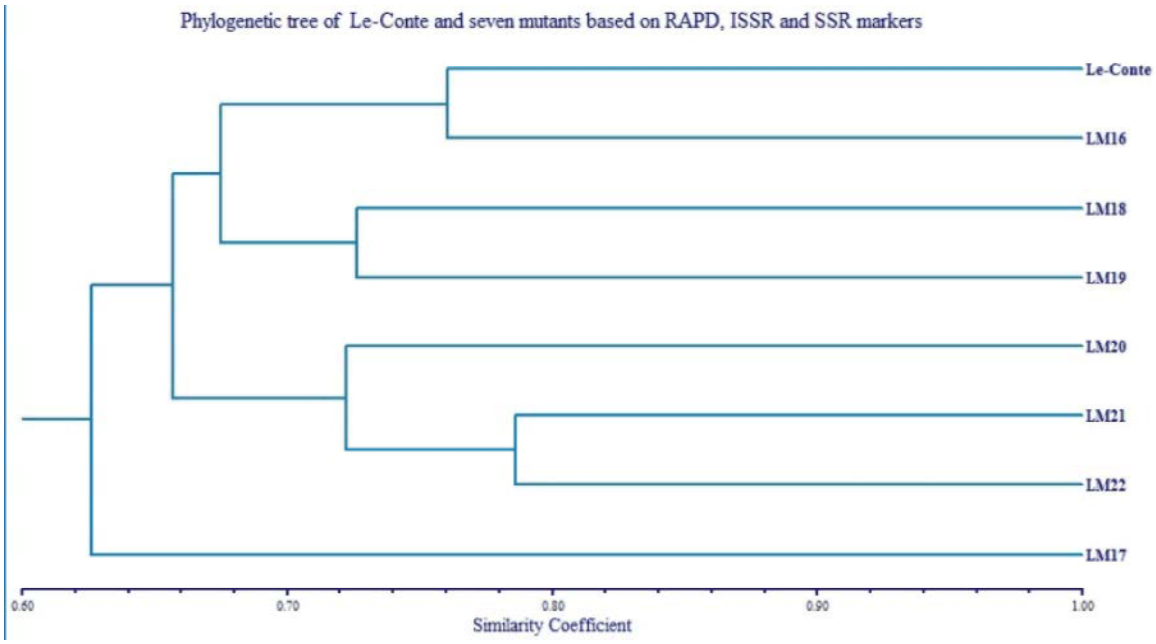

Fig. 3: Phylogenetic tree of Le-Conte pear and seven induced mutants based on RAPD, ISSR and Microsatellites (SSR) markers

LM17 and two discriminated LM22. The weakness of ISSR in discrimination may be due to use few number of operon primers. Using more number of ISSR operon primers may be maximized the benefit of this marker. Results of the microsatellites marker indicate that LM22 superior in the total alleles number (29alleles) followed by Le-Conte, LM21 and LM18 (28, 27and 25 alleles). Microsatellite marker resulted in nine specific alleles, five of them discriminated Le-Conte pear and two discriminate LM22 and one allele discriminate LM16 and LM17. RAPD, ISSR and microsatellite markers are useful in genetic discrimination of Le-Conte pear and its induced-mutants.

Assessment Similarity: Information extracted from RAPD, ISSR and microsatellite (SSR) markers were used for calculating the similarity percent between the Le-Conte pear and seven induced mutants. Similarity matrix based on RAPD and ISSR (not presented) indicated that similarity percent ranged from 47.4% to 84.2%, while microsatellite markers cleared similarity percent ranged from54.1 to 89.2. Data in Table (7) showed similarity percent matrix which calculated based on collected genetic information of RAPD, ISSR and Microsatellite markers. Similarity percent matrix cleared various similarity percent between each pair of samples. The highest similarity percent observed between LM21 and LM22 (78.6%) followed by Le-Conte and LM16 (76.1%). The lowest similarity percent was 56.4% which observed between LM17 and both LM20 and LM22. Anyway, most of evaluated induced mutants cleared low similarity percent with Le-Conte pear (less than 65%).

Phylogenetic Analysis: Similarity tree based on combination between RAPD, ISSR and Microsatellites (Fig. 3) showed two main clusters, the first cluster contain only LM17 and the other main cluster divided into two sub-clusters, the first sub-cluster contained two sub-sub clusters the first contained only one mutant (LM20) and the second contained LM21 and LM22. Also, the second sub cluster contained two sub sub-clusters, the first contained LM18 and LM19 and the second contained LM16 and Le-Conte pear. Phylogenetic tree revealed that all induced mutants considered new lines of Le-Conte pear and radiation is an effective method for pear improvement.

DISCUSSIONS

Evaluation of seven induced mutants compared with Le-Conte pear is a major step in the breeding program to select new and superior lines. The phenological and horticultural evaluation resulted in determination the valuable mutants which gave high yield with valuable fruit quality parameters, but not sufficient to detect new lines. So molecular markers play the significant role in determination similarity degree among all investigated plants and discrimination new lines. The highest average yield per tree was obtained from mutants LM16, LM21, LM18 and LM20 (50.04, 31.73, 26.64 and 22.16 Kg/ tree, respectively) when compared with Le-Conte pear cultivar (6.46 Kg/ tree). The high percentage of fruit set, fruit retention and the high yield of mutated trees may be resulted from their superiority in seed number per fruit when compared with Le-Conte or may refer to the ability of these mutants to avoid the unsuitable environmental conditions during flowering and fruit set. All physical characteristics of fruits refer to the superiority of mutants LM20, LM21, LM22, LM16 and LM18 when compared with Le-Conte. LM22, LM16 and LM18 maximized the fruit firmness value. TSS, acidity % and the TSS/acidity ratio differed among mutants and Le-Conte. These results refer that the fruit TSS and acidity % may be affected by environmental conditions which lead to enhance or inhibit these parameters through affecting gene expression. Results came in line with [6, 28, 29] who stated that selection of superior genotypes is an important task in pear breeding programs. Evaluation of pear cultivars and mutants basically focused on the evaluation of yield, morphological, physical and chemical fruits characterization; fruit shape and colour, total soluble solids, total sugar and acidity as well as to determine the firmness of fruit flesh. These fruit characters high weight indicators not only for breeding for fruit quality of pear [3] but also for consumer's point of view [4].

The genetic relationship based on PCR was the supported step in discrimination mutants. RAPD and ISSR molecular marker indicated high polymorphism of most induced-mutants and could distinguish some mutants by distinct 12 and 3 specific fragments for RAPD and ISSR primers, respectively. Also, microsatellite marker cleared relatively high polymorphism information content (PIC) and genetic diversity value (GD) and could discriminate some mutants through 9 specific alleles. similarity% and phylogenetic tree; which resulted from analysis of the combination of RAPD, ISSR and microsatellite markers, indicated that all induced mutants can be considered new lines of Le-Conte pear. Generally, it could be concluded that RAPD, ISSR and microsatellite markers able to genetical discriminate mutated plants and varieties and radiation is an effective method for pear improvement. Data agree with Lei *et al*. and Nishio *et al*. [30, 31] who used SSR (microsatellite) marker to differentiate pear cultivars and determine the genetic diversity. Also, results can be supported with Hamza, Ahmed *et al*. and Dhyani *et al*. [14, 32, 33] who stated that using RAPD, ISSR and SSR markers are efficient in discrimination partial genome in breeding programs.

CONCLUSION

The phenological and horticultural evaluation may be used as primary methods for evaluation and discrimination mutants in breeding programs. Genetic molecular markers like RAPD, ISSR and microsatellite markers able to genetical discriminate mutated plants

though specific fragments or alleles. Molecular markers can determine the genetical similarity percent, genetic diversity among examined samples and predict the efficiency of breeding program in producing new lines.

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