Basateen MKM" a new early pear cultivar

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Abstract: The early pear cultivar "Basateen MKM" was evaluated and compared with common pear cultivar Le-Conte in Egypt based on blooming date, harvest date and fruit character. This cultivar is the result of selections during five years from several orchards in Northern Governorates of Egypt. It was very early of blooming date around five weeks than Le-Cont cultivar, so it escapes infection of fire blight disease. It was found to be significant over Le-Conte in having yield per tree (20.193 to 30.153Kg), fruit weight (176.100 to 205.233 gm). Fruit height of "Basateen MKM" cultivar was significant (up to 9.00cm), comparing with Le-Conte cultivar (around to 7.00cm).DNA fingerprint was also determined through RAPD technique using six primers to identify unique molecular markers characterizing the early pear cultivar Basateen MKM., which budded on two rootstocks (P.communis and P.betulifolia), and compared with Le-Conte pear on the same rootstocks. The result of molecular analysis in genomic DNA of pear showed that the total number of fragments were 50 with an average number of 8.3 fragments / primer. The polymorphism ranged from 25% to 100% with 25 unique bands. High variation was observed when two cultivars budded on different two rootstocks. This high polymorphism makes these markers useful for genetic studies, in pear cultivars.

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1-Introduction

The genus Pyrus, the pears, includes a wide range of species used partially as rootstocks but not or very rarely as human food. The genus Pyrus is a part of the family Rosaceae with 34 chromosomes (2n) Cao et al,2002. Kikuchi (1946), classified it into three groups, small fruited (with two carpels), large fruited (with five carpels) and their hybrids (with 3-4 carpels). Large fruited species with five carpels are three major species P.communis (European Pear), P.ussuriensis (Chinese pear) and P.purifolia (Japanese pear), which are commercially cultivated in temperate zone (Mohan Jain and Priyadarshan,2009 and Chittaranjan,2007).In Egypt, and according to the Ministry of Agriculture and reclamation statistic, the cultivated area was recorded 18286 feddan in 1988, decreased to 15400 feddan in 1994 and to 5800 feddan in 2000 which represented 5% of deciduous plants. Pear production ranged from 5-6 tones per feddan with a farm gate currently supplies only 30 – 40% of market requirements. Fire blight is the major constraint to increased pear production. This disease is a highly destructive caused by the bacterium Erwinia amylovara. (Deckers and Schoofs, 2002). It can be especially 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. (Van der - Zweet and Beer,1995. The insufficient chilling requirements of Le-Conte pear in Egyptian climate caused delayed and prolonged period of blooming in warm climate and high humidity. Under this conditions a great infection of fire blight occurred, and that caused in its crop losses of up to 95 percent were reported for 1985

Le-Conte pear cultivar, a hybrid between P.communis × P.serotina, is the only cultivated one in Egypt. It is susceptible to some diseases especially fire blight. The present risks to production are significant with the potential to cause yearly loss of marketable fruit, long-term problems on yield or the loss of trees. It is unlikely that major changes in production and diversity will occur within the next decades; therefore, it is growing interest in the selection and evaluation of new cultivars. (Lee,1948; Mehanna et al.,1992 a,b ;Overcash and Loomis,1959)

Thomas, 1979 stated that pear cv. Le-Conte is still in need for further studies to improve the quality of the fruit. Farther more, there is a great need for early blooming trees in order to escape the favorite environmental conditions for Erwinia amylovara bacteria (Marguery and Sangwan, 1993).

Productivity of pear varies in Egypt from year to year and location to another. This might be attributed to limited ovules viability and stigma receptivity, poor pollen germination ability ovule abortion, excessive flower abscission and low fruit set (Yehia and Hassan 2005).

Singh and Sharma, (1994) conducted experiments on Le-Conte pear trees to improve fruit set and yield. Singh and Sharma, (2005) examined some different chemicals to improve the fruit characters. Also, (Hegazi,2011) studied the effects of spraying some

chemical compounds on fruit set and fruit characteristics of Le-Conte pear. Holwah and El-Sheikh (2000) postulated that it enter into dormancy in December while its bud break was in March. Bud opening percent corresponded positively with increasing the chilling units under Kalubia governorate condition in Egypt.

In pear breeding programs improvement of fruit quality and chilling requirements are a main objective. A few reports on the inheritance of fruit characters have dealt with pears (Abe et al.,1995; Machida and Kozaki 1975& 1976;Bell,et al.,1996).

The plant breeding methods have little impact on the development of pears because long Juvenility period, high heterozygosity and sexual compatibility in Pyrus spp. Traditional methods to identify cultivars are based on phenotypic observations, which a slow process. The incorporation of new methodologies into fruit certification process, by allowing the fingerprinting of each genotype at any stage of development and independently of environmental factors that may influence the phenotype (Ibrahim, et al.,2007)

Random amplified polymorphic DNA (RAPD) has been widely used on pear genetic studies because it has the advantages of being readily employed, requiring small amounts of genomic DNA. RAPD markers have been successfully used for identification and genetic relationships of pear (Itai,2009; Oliveira et al., 1999) investigated molecular characterization and phenetic similarities between several cultivars of P.communis and P.pyrifolia and several wild species by RAPD markers. (Teng et al., 2001-2002) evaluated genetic variation between 118 Pyrus spp. also Banno et al.,1999 used RAPD markers to identify parentage. RAPD analysis was carried out to evaluate polymorphism and genetic similarity between three apricot cultivars and its rootstocks. Seventy five amplification products were identified using four random primers, with an average of 18.8 bands per primer (Khalil and Abd-Alla,2002).

The present study was aimed at evaluating the new pear cultivar (Basateen MKM), resulted by selection from many orchards of pear and the adapted common Le-Conte cultivar in Egypt, based on dates of flowering, yield performance, physical and chemical characteristics of fruits. At the molecular level, RAPD markers were used for estimating genetic variability to determine the genetic variability in genomic of pear cultivars among two different rootstocks of pears.

2-Material and Methods:

2.1.Materials:

2.1.1. Sample:

The present investigation was carried out on Basateen MKM and Le-Conte pear cultivars grown in North Governorates (Behara and Nubaria), during seasons 2006, 2007 and 2008. Eighteen trees ,as uniform as, possible were selected for achieving this study, budded on Pyrus communis, grow in a loamy soil in private farm. Nine trees of early pear (Basateen MKM) were compared with nine trees of other cultivar Le-Conte growing in the same area and the study was based on the performance of the individual tree. The trees were six years old at the start of experiment and treated with normal agricultural practices.

Dates of bud break, full bloom, fruit set and beginning of harvest. Yield per tree and per feddan were calculated according the equations:

$$\text{Fruit set percent} = \frac{\text{Number of fruits after set}}{\text{Number of flowers}} \times 100$$

$$\text{Fruit retention percent} = \frac{\text{Number of fruits before harvest}}{\text{Number of flowers}} \times 100$$

Fruit setting (%) was taken into consideration, the number of flowers and fruit setting were observed in 30 spurs randomly on the tree.

2.1.2.Primers:

Six random primers (10-mer) were purchased from Kit A (Operon Technologies, Inc., Alameda, CA, USA) and screened for its ability to polymorphisms. The primers sequences were as follows:

Primer Code	Primer Sequences
OPA-07	5'-GAAACGGGTG-3'
OPA-18	5'-AGGTGACCGT-3'
OPG-12	5'-CAGCTCACGA-3'
OPK-04	5'-CCGCCCAAAC-3'
OPQ-12	5'-AGTAGGGCAC-3'
OPK-12	5'-TGGCCCTCAC-3'

2.2.Methods:

2.2.1. Horticultural studies:-

a-Fruit quality:

Sample of ten fruits per tree from each replicate were collected randomly, when they were yellow colored, then transported to the laboratory to determine physical and chemical fruit characteristics.

b-Physical fruit characteristics:

Fruit samples were weighted and the average fruit weight for each replicate was calculated. Fruit size was calculated as (cm^3). Diameter and height of fruits

were measured using a hand caliper. Flesh firmness was recorded in two opposite sides of fruits, using pressure tester and was expressed as (pound/Inch^2), according to (Magness and taylor, 1982). The color of the fruit was determined following Color Chart (Robert,1938).

c-Chemical fruit characteristics:

Fruit juice was used to determine the percentage of soluble solids content (S.S.C%), by hand refractometer according to the official methods of Analysis (A.O.A.C.1990). The percentage of total acidity was determined as in fruit juice measured as malice acid according to the official Methods of analysis (A.O.A.C.1990).

2.2.2. Random amplified polymorphic DNA (RAPD) markers:

Plant materials:

Total genomic DNA was extracted from young and fresh leaves of (Basateen MKM) Cultivar and other Le-Conte pear, which they budded on two rootstocks P.communis and P.betulifolia during growing time using the CTAB method which described in Rogers and Bendich (1985).

The reaction conditions were optimized and mixtures (20m/total volume) were composed of DNA (50ng), I × reaction buffer, I unit Taq DNA polymerase (M 1861, promega), 3mM Mg Cl_2, 0.2mM of each dNTP, and 20pM primer.

The mixture was covered with two drops of mineral oil. For DNA amplification, a coy Temp cycler Π (model 110p) programmed as follow: Denaturing (one cycle) 94˚c for 5min and then Annealing (35 cycles) of 1min at 94˚c; 1min at 35˚c and 1.5min at 72˚c. The extension (one cycle) was 5min at 72˚c.

-Detection of the PCR products:

The amplification products were separated in 1% (w/v) agarose gel in I × TAE buffer and visualized by staining with ethidum bromide(William, et al,1990) (The experiment reported was conducted in the Genetic Engineering Research Center. Faculty of Agriculture. Cairo University).

Pear were observed and analyzed statistically following Steel and Torrie (1980).

- Amplification reactions:RAPD- PCR was carried out according to the procedure given by (Vos et., 1995).

3-Results and Discussion

3.1.Horticultural studies:-

Differences between dates of bud burst, full bloom and beginning of harvest of the (Basateen MKM) and Le-Conte Cultivars budded on P.communis in the three successive seasons under study 2006, 2007 and 2008 were observed. Starting of bud burst and full bloom dates of Basateen MKM cultivar were very earlier than Le-Conte (around five weeks), while beginning of harvest was in the third week of July in all the tree seasons (Table 1).

Table1: Date of bud break, full bloom, fruit set and beginning of harvest for (Basateen MKM) and Le-Conte cultivars in 2006, 2007 and 2008 seasons

Cultivars	bud burst			full bloom			fruit set			beginning of harvest		
	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
Basateen MKM	Feb.5	Feb.5	Feb.6	Feb.20	Feb.22	Feb.22	1.5	1.9	1.9	Jul.20	Jul.20	Jul.20
Le-Conte	Mar.15	Mar.20	Mar.18	Apr.2	Apr.9	Apr.7	1.7	2.9	2.0	Aug.15	Aug.15	Aug.15
T. test							N.S.	N.S.	N.S.			

N.S. (non-significant)

Le-Conte pear cultivar began its harvest at 15 August in the three seasons. Holwah and el-Sheikh (2000) postulated that accumulated chill units at bud break of common Le-Conte and bud opening percent when the accumulated chill units was ranged between 350 to 400 under Kalubia governorate in Egypt.

Previous evaluations of two pear cultivars (Basateen MKM and Le-Conte) have focused primarily on blooming and harvest date. The first cultivar had shallow deep dormancy and needed less chilling hours comparing with another cultivar. So, Basateen MKM don't need applied protocol to escape of infection of fire blight. Concerning harvest date, also its yield matured earlier three weeks than Le-Conte.

These variations in dates of blooming and beginning of harvest for two cultivars under study may be due to a different genotype. These findings were in agreement with those of Khalil and El-Sheik (2000) in apricot and Khalil et al., (2009) in Orange.

According the results in the same table Basateen MKM cultivar shows insignificant differences for the fruit setting percent ranges between 1.5 to 1.9%, however in Le-Conte cultivar, it ranges between 1.7 to 2.9%. Hegazi (2011) found that the final fruit set percent for Le-Conte cultivar was 1.28%.

The yield per tree for Basateen MKM cultivar ranges between 20.193 to 30.153Kg, while in Le-Conte cultivar, it ranges between 3.873 to 7.610 Kg in the three seasons under study. On the other hand, Basateen MKM cultivar gave yield per feddan ranges between 3.753 to 5.180 ton while ranges between 0.567 to 1.227 ton for Le-Conte cultivar (Table 2). Faissal and Abdel All (2007), Fayek et al., (2011) improved the yield of Le-Conte pear by some applied treatments (ringing or amino acids applications). Singh and Sharma (1994) stated the improvement in fruit set and yield with GA_3 applications.

Table2: Yield per tree and per feddan, of Basateen MKM and Le-Conte Cultivars in 2006, 2007 and 2008 seasons

Cultivars	Yield / tree (Kg.)		
	2006	2007	2008
Basateen MKM	20.193	28.630	30.153
Le-Conte	7.610	5.500	3.873
T.test	S.	S.	S.

N.S.(non-significant) S. (significant)

Cultivars	Yield / feddan (Kg.)		
	2006	2007	2008
Basateen MKM	3.753	4.723	5.180
Le-Conte	1.227	0.950	0.567
T.test	N.S	S.	S.

N.S.(non-significant) S. (significant)

Tables 3 and 4 show the characteristics of mature fruits (Physical and Chemical) of two pear cultivars, Basateen MKM and Le-Conte statistical analysis revealed that significant differences occurred in weight and size of fruits in three seasons under study. Highest fruit weight was obtained of (Basateen MKM) ranged between 176.100 to 205.233 gm, while ranged between 121.167 to 167.233 gm Le-Conte cultivar.

3.2.Major mature fruit characters:

Table3: Physical fruit character of (Basateen MKM) and Le-Conte Cultivars in 2006, 2007 and 2008 seasons

Cultivars	fruit weight (gm)			Fruit size (cm^3)			Fruit diameter (cm)			Fruit height (cm)		
	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
Basateen MKM	189.933	176.100	205.233	186.833	171.333	203.000	6.63	6.53	6.87	9.27	9.40	9.73
Le-Conte	167.233	121.167	140.733	162.067	119.833	138.767	6.77	6.63	6.07	6.97	7.87	7.00
T. test	S.	S.	S.	S.	S.	S.	N.S.	N.S.	S.	S.	S.	S.

N.S.(non-significant) S. (significant)

Cultivars	fruit circumference (cm)			Fruit firmness (pound/L)			Fruit color		
	2006	2007	2008	2006	2007	2008	2006	2007	2008
Basateen MKM	20.57	20.07	20.40	10.70	7.63	11.17	Indian yellow page 6 6/1	Indian yellow page 6 6/1	Indian yellow page 6 6/1
Le-Conte	19.63	20.67	20.03	11.60	11.60	12.03	Indian yellow page 6 6/3	Indian yellow page 6/3	Indian yellow page 6/3
T. test	N.S.	N.S.	N.S.	N.S.	S.	S.			

N.S.(non-significant) S. (significant)

The fruit size behaved similarly in three seasons. The first cultivar ranged between 171.333 to 203.000 (cm^3), and ranged between 119.833 to 162.067 (cm^3) for the second cultivar. Fruit diameter was not significantly in 2006 and 2007 ranged between 6.53 to 6.77 (cm^3) for two cultivars under study. In 2008, fruit diameter were 6.87 and 6.07(cm^3) for Basateen MKM and Le-Conte Cultivars, respectively. Marked differences existed between height of fruits produced by two different Cultivars. Basateen MKM had fruits with height (9.27, 9.40 and 9.73 cm in 2006, 2007 and 2008) Le-Conte produced the shorter (6.97, 7.87 and 7.00 cm), respectively. No significant differences in fruit circular for the both Cultivars under study. Significant variations were detected in fruit firmness for two Cultivars in 2007 and 2008 ranged between 7.63 to 12.03 pound/In. Table 3 and Fig I present the color of the fruit for two Cultivars (Basateen MKM and Le-Conte), in three seasons. (Basateen MKM) gave skin colors of Indian yellow.

Table4: Chemical fruit character of Basateen MKM and Le-Conte Cultivars in 2006, 2007 and 2008 seasons

Cultivars	S.S.C (%)			Acidity (%)			Seed per Fruit		
	2006	2007	2008	2006	2007	2008	2006	2007	2008
Basateen MKM	14.03	13.00	12.06	0.477	0.453	0.456	0.72	0.87	1.00
Le-Conte	12.37	12.70	12.17	0.433	0.440	0.421	0.55	0.73	0.62
T. test	S.	N.S.	N.S.	S.	N.S.	N.S.	S.	N.S.	S.

N.S.(non-significant) S. (significant)

Table 4 shows the Chemical fruit character of Basateen MKM and Le-Cont Cultivars in the three seasons under study. Significant differences on percentages of S.S.C and total acidity in 2006, while in 2007 and 2008 the same percentages were non significant for the two Cultivars.

The total number of developed seeds/fruit is presented in Table 4. There is lowest significant number of seeds per fruit, ranged between 0.55 to 1.00, for two Cultivars in the three seasons under study. Similar results were found by Fayek et al., (2004), Fayek et al., (2011), Bahlool et al., (2000) in Le-Cont pear Cultivar in the fruit characters.

3.3.The genetic variability

It is related to phenotypic characteristics such as fruit shape, tree vigour, susceptibility to diseases, level of acidity, etc. Isoenzyme analysis has found similar genetic variability at the biochemical level (Moore and Castle, 1988). Selection scheme or genetic analysis based on phenotype is a function of the heritability of the trait, factors such as the environment, multi-genic and quantitative inheritance often confound the expression of a genetic trait. For

this reason, DNA – based genetic markers are being integrated into several genetic system and are expected to play an important role of plant breeding.

Genetic identification for a Basateen MKM and other known cultivar Le-Cont in Egypt was performed using six random amplified polymorphic DNA (RAPD) primers.

All primers used in the present study showed the appearance of PCR products with a variable number of bands. A total of 50 DNA markers was detected among the four pear cultivars (Basateen MKM and Le-Cont budded on two rootstocks P. communis and P. betulifolia) of which 27 bands were polymorphic (54%) and can be considered as useful RAPD markers for the pear cultivars.

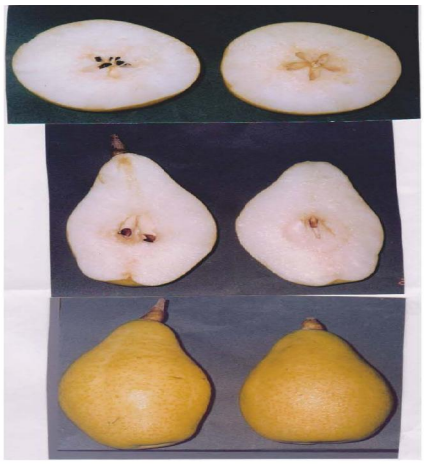

Fig (1): Basateen MKM Cultivars

Table5: Primers used in RAPD analysis and the number of bands generated.

Primer	Polymorphic No.	Polymorphic %	Monomorphic No.	Monomorphic %	Unique band	Total No. Of bands	Band size range
OPA-07	6	75.0%	2	25.0%	6+	8	160-1310
OPA-18	3	42.9%	4	57.1%	3-	7	300-1000
OPG-12	2	25.0%	6	75.0%	1+& 1-	8	150-900
OPK-04	3	33.3%	6	66.6%	1-	9	300-1050
OPQ-12	2	28.6%	5	71.4%	1+& 1-	7	150-1050
OPK-12	11	100.0%	0	00.0%	8+& 3-	11	180-2000
Total	**27**	**54%**	**23**	**46%**	**25**	**50**	

(Fig(1)and (2) and Table 5). The highest number of RAPD bands was detected for primers OPK-12 (11 bands), their size ranged between 180-2000. While the lowest was recorded for the two primers OPG-12 and OPQ-12 (2 bands), and its percent were 25.0% and 28.6%, and. its size ranged between (150-900) and (150-1050), respectively. Moreover the primer OPA-07 gave 6 bands polymorphic (75.0%) and its size ranged between 160-1310. Both of primers OPA-18 and OPK-04 gave 3 bands polymorphic its percent were 42.9% and 33.3% and their size ranged between (300-1000) and (300-1050), respectively.

Finally the use of RAPD-PCR is aming to show fast and reliable discrimination of any variations. The purified genomic of the pear on different rootstocks were produced when they are used as templates for RAPD-PCR, characteristic reproducible multiple band profiles of amplified fragments.

In conclusion, the present data revealed that the studies to identify pear cultivars with different molecular DNA markers have succeeded in distinguishing among them variability even if they were budded on different rootstocks. DNA fingerprinting proved to be very important in the case of any rootstocks.

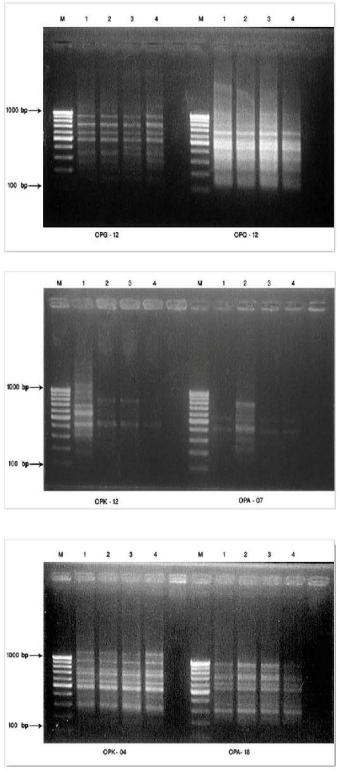

Fig (2): RAPD banding patterns of the four pear genotypes using six selected random primers, M: molecular weight marker (1Kbp ladder marker), Lane 1, Basateen MKM on P. communis, Lane 2, Le-Conte on P. communis, Lane 3, Basateen MKM on P. betulifolia, Lane 4, Le-Conte on P.betulifolia.

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