Bull. Fac. Agric., Cairo Univ.,65: 303-317 (2014).

SELECTION AND EVALUATION OF GENETIC DIVERSITY IN POMEGRANATE
I- Some criteria for studying the progenies of selfing, open-pollination and hybrid between Nab-El Gamal and El- Tahrir Cultivars

(Received: 3.6. 2014)

By
B. M. Khalil, N. A. Awd, A. O. Rayan and M. A. El-Hamady*

Horticulture Research Institute, Agriculture Research Center
** Faculty of Agriculture Kafr elsheikh University, Giza, Egypt*

ABSTRACT

Pomegranate breeding program was initiated in 2004 in the Horticulture Research Institute, Agriculture Research Center, Egypt. Breeding objectives are dedicated predominantly by the demands of the European markets and exploit the principal advantages of the Egyptian cultivars. It was initiated by crossing between the main cultivars (Manfaloty, Nab-El Gamal, El-Tahrir and Bader), selfing and open pollination for each cultivar. The aim of this study was to develop very early ripening genotypes of high productivity, dwarfing habit and good quality of fruits. About 200 progenies resulting from open, self-pollination and crossing among these cultivars were planted in Shandawel, Sohag Governorate in 2008. The progenies were studied to examine the inheritance of important traits. Some trees of these progenies flowered and gave fruits in 2012 and 2013 seasons; from which, nine trees were selected on the basis of the yield. The present investigation studied the polymorphism variance based on vegetative growth, flowering, fruit characters and yield of these progenies. DNA fingerprinting was determined through ISSR technique, using five primers to identify unique molecular markers characterizing the progenies under study. Some progenies, such as the hybrid between El-Tahrir x Nab-El Gamal (tree No 9) exhibited a yield of 22.5Kg/tree. Trees (No7 and No9) of the hybrid between El-Tahrir♀ × Nab El-Gamal♂ gave the best ratio of T.S.S/Acidity of 31.96 and 29.86 respectively, as compared to the other progenies. The hybrid tree (No7) between the same two cultivars was the smallest tree compared with all the progenies under study. Open pollination progeny of Nab-El Gamal gave the highest number of pomegranate grains in 100g, while the self pollination progeny of El-Tahrir gave the highest juice percentage. A dark color of the grains was detected for the hybrid (El-Tahrir♀ × Nab El-Gamal♂) in the two seasons. The results of molecular analysis of genomic DNA of the nine trees (progenies) of pomegranate showed that the total number of amplicons amplified by the five primers was 57 with an average of 11.4/primer. The polymorphism ranged from 50% to 93.3%. The size of the amplified band varied between the used primers, ranging from 115 to 128 bp. Genetic similarity value was very low among the three tested hybrids ranging between 0.0 to 0.06. The variations observed in the genetic similarity could be attributed to the effect of pollen grains on the genetic structure of the resulting genotypes. On the other hand, a high value of genetic similarity was observed in the self and open pollination progenies of Nab-El Gamal, while the self and open pollination progenies of El-Tahrir cultivar exhibited intermediate values of genetic similarity. This polymorphism renders these markers useful for further genetic studies in pomegranate progenies.

Key words: *pomegranate, Punica granatum, selfing, hybrids, open-pollination, fingerprinting, ISSR.*

1. INTRODUCTION

Pomegranate *(Punica granatum* L.*)* is one of the oldest known fruit species, originated in south west Asia and probably in Iran (De-Candolle 1967). According to Smith (1976), pomegranate behaves as deciduous under temperate climate, but behaves as an evergreen or partially deciduous in subtropical and tropical climate.

Pomegranate is considered as a monoecious species developing male and perfect flowers. three types of flowers are present on the same plant, *i.e.,* male, hermaphrodite and intermediate. The ovary of the male flower is rudimentary, whereas that of the intermediate flowers is degenerate type (Nath and Randhawa, 1959a and b and Josan *et al.* 1979). Both self and cross pollinations are reported in pomegranate. Singh (1977) reported that it is an often cross – pollinated crop whereas Nalawadi, *et al.* (1973) reported that it is a cross pollinated crop. The greater percentage of fruit set was observed by hand pollination and pollination under natural conditions, *i.e.*, open pollination (Nath and Randhawa, 1959 b).

Pomegranate plants can be trained on single stem or in multi-stem system. Flowering of pomegranate depends on the conditions of the growing place and also the fruiting season. (Nalawadi *et al.*, 1973). Singh *et al.* (1978) stated that the inflorescence developed from mixed buds is situated terminally on the previous season's growth as well as on old spurs. Mir *et al.* (2007b) showed that significant variation in all growth yield parameters existed due to various cultivars. Recently, a number of pomegranate cultivars was studied in temperate region and indicated a high range of variability in vegetative growth behavior.

Khodade *et al.* (1990) studied the seedling selections of P-23 cultivar during the early, middle and late stages of fruit development. Fruit size, weight and volume increased whereas, the specific gravity decreased gradually throughout fruit development. Morphology and fruit characteristics of wild genotypes were studied by Bist *et al*. (1994). Also Jalikop and Kumar (1998) found significant variation among 18 genotypes representing soft, semi-soft and hard-seeded pomegranate. Pasad and Banker (1999) evaluated pomegranate trees in different regions in the world to determine the degree of similarities among genotypes, while the flower behavior was studied by El-Kasses *et al.* (1998). Fruit quality characteristics were studied by Feng *et al.* (1998). Abou-El-Khashab *et al.* (2005) evaluated six pomegranate cultivars and stated that Nab-El Gamal gave the best fruit characteristics and yield, under Ali Mubarak farm conditions at South Tahrir Research Station.

Some parts of the Mediterranean area are considered as native lands of pomegranate. Almost all the varieties in the region (local type) are selected by unknown persons and propagated by vegetative propagation. The grown local material may be considered as the pomegranate primary gene pool. The hybridization between cultivated and wild forms is probably, still taking place (Zukovskiy, 1950). Thus wild forms would be the secondary gene pool. Genetic studies are rare, but some studies based on morphometric criteria have recently been performed to determine the degree of polymorphism within local material (Mars and Marrakchi, 1999).

Vechetel and Ruppel (1992) stated that carotene pigments were the most important photosynthetic pigments, and they prevented chlorophyll from the damage of absorbed energy by photoxidation. There are very few reports on molecular genetic studies with pomegranate. Only a handful of genes were isolated from *P. granatum* and deposited in Genebank. Most of the genes deposited are those involved in production of unsaturated fatty acids; genes that encode for parts of ribosomal RNA. A comparative work is now being conducted in several studies to study the level of their expression and the structural differences of these genes among pomegranate cultivars that display prominent differences in skin and aril colors. Molecular markers, such as AFLP, RAPD and ISSR, were reported by several groups. Although Jbir *et al.* (2006) and Zamani *et al.* (2007) concluded that pomegranates are highly polymorphic. Others concluded that the degree of polymorphism was surprisingly low (Aradhya, 2006 and Yilmaz *et al.*, 2006). Some studies observed that the apparent phenotypical differences among pomegranate cultivars were not reflected in the polymorphism of the molecular markers. Obviously, many more markers should be isolated from pomegranates to enhance breeding and evolutionary studies.

The main objectives of this investigation are to (1) study the effect of open, self and cross pollination on some local pomegranate cultivars which are highly adapted to the Egyptian environmental conditions, (2) to develop new genotypes characterized by high productivity and dwarfing habit trees and fruits of good quality, soft-seediness or absence of seeds, skin of red coloration, resistance to fruit cracking and good post harvest quality.

2. MATERIALS AND METHODS

This study was initiated out in 2004 through a breeding program in the Horticulture Research Institute, Agriculture Research Center in Egypt, using some local pomegranate cultivars to develop new Egyptian cultivars, having high yield, good color, seed softness, early and late ripening and high percentage of juice.

2.1. The first step of the program: was to produce the first generation progenies (open and self pollination and hand crossing between the four cultivars Manfaloty, Nab El-Gamal, El-Tahrir and Bader).

Five mature uniform and productive trees of each cultivar were selected as a parent in all combinations of pollinations (open, selfing and crossing). These trees were grown in Aly-Mubarak farm at South Tahrir, Research Station, at a distance of 5×5m, in a sand soil with drip irrigation. Flowers in all treatments (selfing and crossing), except the open pollination were protected from pollen contamination by using glyssine paper bags. Pollen grains of the four cultivars were collected from flowers at the balloon stage, spread on a paper at the laboratory for 24 hours to dry. Then, they were stored in a container and placed in desiccators at 4ºC. The flowers at balloon stage were emasculated (for applying crossing) by removing the anthers carefully. Emasculated flowers were hand pollinated with specific pollen to receptive stigmas using the rubber tip of a pencil. The pollinated flowers (except the open pollinated flowers) were bagged by using glyssine paper bags in all treatments to prevent any undesirable pollination. All possible cross combinations were made during 2004 season to produce the F_1 progeny. Fruits were picked (F_1 hybrid) at the end of the season, and the seeds of all combinations were extracted.

2.2. The second step of the program started in 2005 season. The extracted seeds were cold stratified in the refrigerator at 5-7ºC. After two-three weeks, stratified seeds were planted in boxes (filled with a mixture of sand: beatmoss at a ratio 1:1) in the greenhouse for one year in the Horticultural Research Institute in Giza. In the second year of seed germination (2006), the seedlings (progenies) were transferred into polyethylene bags, filled with the same mixture of soil. The remaining progenies of seedling belonged to Manfaloty (open, selfed and the cross Manfaloty x Nab –El Gamal); Nab –El Gamal (open, selfed and the cross Nab El Gamal x Manfaloty); El-Tahrir (open, selfed and the corss El-Tahrir x Nab–El Gamal); and Bader (only selfed).

2.3. The third step of the program: About 200 seedlings of these treatments were planted in Shandawel farm, Sohag Governorate in 2008 year. Seedlings were planted at a distance of 5×5m. Nine trees of the progenies of open, selfing and crossing of El-Tarir xNab-El Gamal, which flowered and gave fruits, were selected on the basis of the yield. They were studied at 2012 and 2013 seasons, as follows:

2.3.1. Morphological characteristics: The data of the vegetative and flowering traits were measured. Ten shoots were labeled on each the progeny (tree) in different directions to measure the vegetative growth and flowering traits. Plant height, shoot length, shoot diameter, number of leaves, number of internodes and leaf area were recorded.

2.3.2. Fruit characteristics: Ten fruits from the previously tagged flowers were collected randomly at maturity stage. Fruit weight, fruit size, fruit shape, fruit room number and color of fruit were recorded. Also total soluble sugar, acidity, percentage of grains, number of grains in 100g and percentage of juice were also recorded. Fruit set and yield for each progeny (tree) were studied in the two seasons 2012 and 2013.

2.3.3. Spectrophotometric determination of chlorophyll A and B and the total carotenoid contents of the small leaves of all the progenies (trees) under study were determined according to the method of Sukran *et al.* (1998) with some modifications.

2.3.4. Statistical analysis: all data were subjected to statistical analysis of variance (F test) "ANOVA". Moreover, Duncan's multiple range test was used for testing the significant differences among the two means of treatments at the level of significant of ($P < 0.05$ and $P < 0.01$). Gomez and Gomez (1984).

2.4. DNA Fingerprint

2.4.1. Inter simple sequence repeat (ISSR-PCR) procedure. Total DNA was extracted from young leaves as described by Porebski *et al.* (1997). Five ISSR primers were used for PCR amplification (Table 1). Each 25µl of the reaction mix contained 2.5 µl 10x Taq buffer (Promega), 1.0 µl dNTPs (20 mM), 2.0 µl of MgCl2 (25 mM), 1.0 µl primer (0.11 nmol/µl), 2.5 µl DNA template (10 ng/µl), 0.2 µl of Taq polymerase (Promega, 5u/µl) and sterile H_2O to 25 µL.

Amplification was performed under the following conditions: 4 min at 94 ºC for one cycle followed by 30s at 94 ºC, 45 s at 60 ºC, 2 min at 72 ºC for 35 cycles and 7min at 72 ºC for a final extension. The amplification products were separated by electrophoresis on 1.5% a garose gels with IX TBE buffer and detected with Ultraviolet light after ethidium bromide staining.

2.4.2. Data analysis

A similarity matrix using the similarity coefficients of Nei and Li (1979) was constructed for ISSR data based on the presence (coded as 1) or absence (coded as 0) of the resulted fragments for each primer. Moreover, the relationships among the different progenies as revealed by " dendogram" were done using SPSS. Windows program (V.10)

Table (1): List of the primers and their nucleotide sequences used for ISSR procedure.

No	Name	Sequence
1.	HB-08	5′ GAG AGA GAG AGA GG 3′
2.	HB-11	5′ GTG TGT GTG TGT CC 3′
3.	HB-12	5′ CAC CAC CAC GC 3′
4.	HB-13	5′ GAG GAG GAG GC 3′
5.	HB-15	5′ GTG GTG GTG GC 3′

3. RESULTS AND DISCUSSION

The study included nine progenies (trees) as follows: one progeny (tree) for each open pollination of Nab El Gamal (T1) and El-Tarir (T2), two progenies for selfing of each of Nab-El Gamal (T3 and T4) and El-Tarir (T5 and T6) and three progenies for the hybrids between El-Tahrir♀x Nab El-Gamal♂(T7,T8 and T9). Vegetative growth, fruit characteristics and molecular markers were investigated.

3.1. Horticultural studies

The differences between dates for blooming and fruit set of the nine progenies in the two successive seasons under study at 2012 and 2013 are presented in Table (2). Data revealed that the period of fruit set was the shortest in the progeny of selfing of Nab-El Gamal (T4) in the two seasons (46 days), while it was the longest for the selfed progeny of Nab-El Gamal (T3) for both seasons (57days) in the two seasons. The data showed that, although the ratio of male flowers to perfect flowers (hermaphrodite) was 1/5 in the tree progeny of hybrid between El-Tahrir ♀ and Nab-El Gamal ♂ (T7), the period of fruit set was 50 and 51 days in the two seasons, respectively. For the open pollination progeny of El-Tahrir, the ratio was 3/2 for two successive seasons, while the fruit set period ranged between 47 and 43 days for two successive seasons. Singh *et al.* (1978) studied the behavior of flowering and sex expression in the some pomegranate cultivars. They reported that inflorescence developed from mixed buds situated terminally on the previous seasons growth as well as on old spurs.

Table (2) shows the initial date of vegetative growth in the nine different progenies of pomegranate during 2012 and 2013 seasons. The earliest was the hybrid between El-Tahrir♀ and Nab El-Gamal ♂ (T9) (10 and 27 Jan) for both seasons, respectively. While, the latest one was Nab-El Gamal selefed (T4) (2 Feb), for both seasons.

Shoot growth of the nine progenies of pomegranate during 2012-2013 growing seasons is presented in Table (3). Data revealed that the selfed progeny of Nab-El Gamal (T3) exhibited significant superiority shoot length for both seasons (21.30 and 22.00 cm, respectively). On the other hand, the selfed progeny of El-Tahrir (T6) exhibited the shortest shoot length (19.00 and 19.7cm,) for 2012 and 2013 seasons, respectively. Statistical analysis revealed that insignificant differences occurred in shoot diameter for all the studied progenies in the first season, while there were slight differences in the second one.

Statistical analysis revealed significant differences in the number of leaves per shoot for all progenies of different origins. The two selfed progenies of El-Tahrir (T5 and T6) gave the highest number of leaves ranging between 400.0 and 430.3 in the two seasons, while the lowest number was detected for the selfed progeny of Nab-El Gamal (T3) ranging between 337.0 and 355.3, in the two seasons, respectively. In respect to the number of the internodes per shoot, the highest value was recorded by the selfed progeny of Nab-El Gamal (T4) 8.65 and 9.00 in the two seasons, respectively, the lowest (6.10 and 6.20) was recorded by the trees (T7 and T8) progenies of the hybrid between El-Tahrir♀ and Nab El-Gamal♂ in the growing year 2013.

Concerning the leaf area, tree (T8) progeny of the hybrid El-Tahrir X Nab- El Gamal exhibited the highest values (6.033 and 5.767 cm^2) for the two seasons, respectively, while the lowest value was recorded by the open-pollinated progeny Nab-El Gamal (T1) of, (3.490 and 3.410 cm^2 for both seasons, respectively.

Table (4), shows the percentage of fruit set, yield per tree and the number of fruits per tree

Table (2): Dates of flowering and fruit set mean, fruit set period initial date of the vegetative growth and the ratio of male/ perfect flowers (hermaphrodite)of different progenies (open, self and hybrid) of pomegranate in 2012 and 2013 seasons.

Tree No.	Progeny	Blooming date				Fruit set				Initial date of vegetative growth		Fruit set period (day)		Male flowers / Perfect Flowers (Ratio)	
		Initial		End		Initial		End							
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
T1.	Nab-El Gamal(O)	Mar.28	Mar.30	May17	May22	Mar.30	Apr. 2	May22	May12	Jan. 27	Jan. 30	52	40	1 / 4	2 / 3
T2.	El-Tahrir (O)	~ ~ 29	~ ~ 27	~ ~ 20	~ ~ 20	Apr.1	~ ~ 4	~ ~ 28	~ ~ 17	~ ~ 29	Feb. 5	47	43	3 / 2	3 / 2
T3.	Nab-El Gamal(S)	~ ~ 29	~ ~ 29	~ ~ 20	~ ~ 20	~ ~ 1	~ ~ 1	~ ~ 28	~ ~ 28	~ ~ 29	Jan. 29	57	57	3 / 2	3 / 2
T4.	Nab-El Gamal (S)	~ ~ 30	~ ~ 30	~ ~ 17	~ ~ 17	~ ~ 3	~ ~ 3	~ ~ 19	~ ~ 19	Feb. 2	Feb. 2	46	46	2 / 4	2 / 4
T5.	El-Tahrir (S)	~ ~ 28	~ ~ 25	~ ~ 17	~ ~ 10	Mar.30	Mar.27	~ ~ 22	~ ~ 20	Jan. 27	Jan. 20	53	54	1 / 4	2 / 5
T6.	El-Tahrir (S)	~ ~ 28	~ ~ 25	~ ~ 17	~ ~ 10	~ ~ 30	~ ~ 27	~ ~ 22	~ ~ 20	~ ~ 27	~ ~ 20	53	54	1 / 4	2 / 5
T7.	El-Tahrir♀ × Nab-El Gamal♂	~ ~ 27	~ ~ 22	~ ~ 10	~ ~ 5	~ ~ 28	~ ~ 25	~ ~ 17	~ ~ 15	~ ~ 28	~ ~ 25	50	51	1 / 5	1 / 5
T8.	El-Tahrir♀ × Nab-El Gamal♂	~ ~ 29	~ ~ 25	~ ~ 17	~ ~ 12	~ ~ 29	~ ~ 28	~ ~ 15	~ ~ 11	~ ~ 29	~ ~ 27	47	44	1 / 4	2 / 4
T9.	El-Tahrir♀ × Nab-El Gamal♂	~ ~ 15	~ ~ 25	~ ~ 23	~ ~ 12	Apr.7	~ ~ 29	~ ~ 30	~ ~ 11	~ ~ 10	~ ~ 27	53	43	2 / 3	2 / 4

(O) Progeny resulted from open pollination (trees No. 1 and 2)
(S) Progeny resulted from self pollination (trees No. 3 to 6)
Hybrid between El-Tahrir♀ × Nab El-Gamal ♂(trees No. 7 to 9)

Table (3): Means of vegetative growth traits of different progenies of pomegranate in 2012 and 2013 seasons.

Tree No.	Progeny	Mean of shoot length (cm)		Mean of shoot diameter (cm)		No. of leaves per shoot		No. of internodes per shoot		Leaf Area (cm^2)	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
T1.	Nab-El Gamal(O)	20.00 ab	21.00 a-c	3.30 a	3.23 b	410.0 ab	432.0 a	8.63 a	8.20 c	3.490 c	3.410 e
T2.	El-Tahrir (O)	20.60 ab	20.00 cd	3.27 a	3.30 ab	390.0 b	360.3 c	8.13 bc	8.50 b	4.400 b	4.950 b
T3.	Nab-El Gamal (S)	21.30 a	22.00 a	3.27 a	3.30 ab	337.0 c	355.3 c	8.53 ab	9.13 a	4.597 b	5.123 b
T4.	Nab-El Gamal (S)	21.70 a	21.33 ab	3.30 a	3.27 b	413.0 ab	336.0 c	8.65 a	9.00 a	4.523 b	3.723 d
T5.	El-Tahrir (S)	18.70 b	20.60b-d	3.23 a	3.23 b	418.0 a	400.0 b	7.42 d	8.50 b	4.100 b	4.377 c
T6.	El-Tahrir (S_2	19.00 b	19.7 d	3.30 a	3.23 b	425.3 a	430.3 a	7.80 cd	8.23 c	4.203 b	3.987 d
T7.	El-Tahrir♀ × ab-El Gamal♂1	20.00 ab	20.00 cd	3.30 a	3.30 ab	388.7 b	335.0 c	8.70 a	6.10 e	4.500 b	4.307 c
T8.	El-Tahrir♀ × Nab-El Gamal♂	20.00 ab	21.27 ab	3.37 a	3.27 b	389.0 b	334.7 c	8.00 c	6.20 e	6.033 a	5.767 a
T9.	El-Tahrir♀ × Nab-El Gamal♂	20.70 ab	20.33 b-d	3.30 a	3.40 a	359.0 c	364.3 c	6.83 e	7.80 d	6.267 a	2.527 f

(O) Progeny resulted from open pollination (trees No. 1 and 2)
(S) Progeny resulted from self pollination (trees No. to 6)
Hybrid between El-Tahrir♀ × Nab El-Gamal ♂(trees No. 7 to 9)
Means followed by the same letter within the same column are not significantly different P=0.05

for all progenies under investigation. The highest percentage of fruit set was recorded by selfed progeny of El-Tarir (T5) and the hybrid progeny (T8). On the other hand, open-pollination progeny of Nab El-Gamal (T1) exhibited the lowest percentage which reached to 17.15%. As regards yield/tree, the progenies resulted from self pollination of Nab-El Gamal tree (T3) and the hybrid between El-Tarir X Nab-El Gamal gave the highest yield/tree ranging between 21.75 and 22.50 Kg/tree. In contrast, the open-pollination progeny of Nab El-Gamal (T1) gave the lowest yield/tree (11.25 Kg).

Concerning the number of fruits/tree, the highest value was recorded by selfed progeny of El–Tarir (T6), of 70.940, while the lowest value of 38.825 was achived by the open pollination progeny of El-Tarir (T2). Nath and Randhawa (1959b) studied the fruit set and seed formation of some pomegranate cultivars and reported that the greater percentage of fruit set was observed by hand pollination and pollination under natural conditions (open-pollination). Bist *et al.* (1994) reported a great variation in fruit set of some promising selections of wild pomegranate. Moreover, Khalil *et al.* (1985) and Abou El-Khashab *et al.* (2005) studied the vegetative growth of some selections of some cultivars of pomegranate under a new reclaimed region and stated that Nab El Gamal cultivar gave the best fruit characteristics and yield.

Table (4): Percentage of fruit set, yield per tree (Kg) and the number of fruits per tree of different progenies of pomegranate during the two seasons.

Tree No.	Progeny	Fruit set %	Yield / tree (Kg)	No. of fruits /tree
T1.	Nab-El Gamal(O)	17.15 d	11.25 d	56.000 ab
T2.	El-Tahrir (O)	18.70 cd	13.50 cd	38.825 b
T3.	Nab-El Gamal (S)	19.30 b-d	22.50 a	51.090 ab
T4.	Nab-El Gamal (S)	20.25 b-d	21.75 a	56.420 ab
T5.	El-Tahrir (S)	25.65 a	12.75 ab	61.900 ab
T6.	El-Tahrir (S)	23.90 ab	17.50 ab	70.940 a
T7.	El-Tahrir♀ × Nab-El Gamal♂	23.05 a-c	15.25 ab	63.690 ab
T8.	El-Tahrir♀ × Nab-El Gamal♂	25.15 a	16.00 ab	52.335 ab
T9.	El-Tahrir♀ × Nab-El Gamal♂	23.25 a-c	22.50 a	63.420 ab

(O) Progeny resulted from open pollination (trees No. 1 and 2)
(S) Progeny resulted from self pollination (trees No. 3 to 6)
Hybrid between El-Tahrir♀ × Nab El-Gamal ♂(trees No. 7 to 9)
Means followed by the same letter within the same column are not significantly different P=0.05

Table (5) showed significant differences in the vegetative growth of nine progenies resulted from different origins of pomegranate. Statistical analysis revealed that significant differences occurred in tree height. El-Tarir selfed progenies (trees No) and the hybrid progeny of El-Tarir X Nab-El Gamal (T8) gave the highest value ranging from 3.4 to 3.60 m respectively, while the lowest value was 2.33m for the open progeny

Table (5): Tree height, trunk circumference and chlorophyll A, B and Caroten in the leaves of different progenies of pomegranate during two seasons

Tree No.	Progeny	Tree height (m)	Trunk circumference (cm)	Chlorophyll A (mg/g)	Chlorophyll B(mg/g)	Caroten (mg/g)
T1.	Nab-El Gamal(O)	2.3250 e	13.2500 b	1.1400 ab	0.3895 c	0.7430 f
T2.	El-Tahrir (O)	2.6000 de	17.7500 ab	1.2130 ab	0.4565 bc	0.9120 c
T3.	Nab-El Gamal (S)	2.6250 de	19.5000 a	1.3370 a	0.5870 b	1.0360 b
T4.	Nab-El Gamal (S)	2.9000 cd	16.5000 ab	1.1805 ab	0.4035 bc	0.8405 d
T5.	El-Tahrir (S)	3.0750 bc	20.5000 a	1.0900 ab	0.3765 c	0.7380 f
T6.	El-Tahrir (S)	3.4000 ab	20.0000 a	1.1030 ab	0.3900 c	0.7705 e
T7.	El-Tahrir♀ × Nab-El Gamal♂	2.8750 cd	17.5000 ab	1.5925 a	0.5350 bc	1.0170 b
T8.	El-Tahrir♀ × Nab-El Gamal♂	3.6000 a	17.5000 ab	0.6210 b	1.3100 a	1.2940 a
T9.	El-Tahrir♀ × Nab-El Gamal♂	3.4000 ab	20.5000 a	1.1985 ab	0.4645 bc	0.8520 d

(O) Progeny resulted from open pollination (trees No. 1 and 2)
(S) Progeny resulted from self pollination (trees No. 3 to 6)
Hybrid between El-Tahrir♀ × Nab El-Gamal ♂(trees No. 7 to 9)
Means followed by the same letter within the same column are not significantly different (P=0.05)

of Nab El-Gamal (T1). Significant differences were recorded in trunk circumference, ranging from 13.25cm for open pollination progeny of Nab-El Gamal (T1) to 20.50cm for El-Tarir selfed progeny (T5) and the studied hybrid (T9).

Considerable variation in chlorophyll A and B and carotene in the leaves was observed among progenies in Table (5). The highest value of chlorophyll A (1.5925mg/g) was recorded by the hybrid tree (T7). Meanwhile, the tree (T8) of the hybrid (El-Tarir xNab-El Gamal) gave the lowest value of chlorophyll A (0.6210 mg/g). With respect to chlorophyll B and carotene, the highest values of chlorophyll B and carotene (1.3100 and 1.2940 mg/g, respectively) were recorded by hybrid EL-Tarir X Nab- El Gamal tree (T8). It can be concluded that genetic changes in the progenies had an effect on the quantity of chlorophyll A and B and carotene. These results are in agreement with those reported by Sukran *et al.* (1998).

Table (6) shows the characteristics of mature fruits produced by the nine studied progenies. Statistical analysis revealed that highly significant differences were recorded in fruit weight and size. In both seasons, Nab-El Gamal selfed trees T3 and T4 (Fig.1) recorded the highest values, while El-Tahrir open-pollination progeny recorded the highest value in the first season only. In contrast, the open pollinated progeny of Nab-El Gamal, gave the lowest fruit weight in the first season (100.167g), while this tree had the highest value in the second season.

Regarding the fruit size, the tree (T3) of selfed progeny of Nab-El Gamal gave the highest value (387.0 and 466.7 cm^3 for the two seasons, respectively. The lowest value was recorded by open pollination progeny of Nab-El Gamal (T1) (95.00 cm^3), in 2012.

In respect to fruit diameter, significant differences were recorded between the different progenies; the highest values of fruit diameter, and fruit length were recorded by El-Tarir (T2) and selfed tree of Nab-El Gamal (T3), in the first season, while in the second season the same tree gave the highest value for fruit diameter and fruit length. On the other hand, the open-pollination progeny Nab-El Gamal (T1) gave the lowest fruit diameter (5.9 cm) and fruit length (5.2 cm) in first season, while El-Tarir selfed progeny (T6) gave the lowest fruit diameter (7.67 cm) and fruit length (6.97 cm) in second season. Regarding the fruit circumference, the highest value by El-Tahrir open-pollination progeny (T2) (30.07 cm), while the lowest value was recorded by Nab-El Gamal open-pollination progeny T1 (14.23 cm), in the first season. In the second season, Nab-El Gamal selfed progeny (T3) gave the highest value of (31.40 cm); while the lowest value (23.50cm) was achived by the tree T6 of El-Tarir (selfed). In respect to fruit room number, five progenies (T1, T2, T4, T7 and T9) exhibited the highest values of room number (6.00), in first season. While in the second season, six progenies (T1, T3, T4, T6, T7 and T8) showed high number of rooms ranging` from 6.00 to 6.67. Levin (1990) studied the mean performance of hybrids resulting from crossing of 53 maternal varieties and 13 pollen parents, in pomegranate. He reported that dwarfing habit is a desirable character of pomegranate tree for mechanical and easy harvesting in particular growing conditions.

Table (7) shows the fruit chemical characters of the nine progenies. Significant differences existed for percentage of T.S.S and total acidity in the two seasons 2012 and 2013. Regarding T.S.S./Acidity ratio, in the first season, El-Tahrir open-pollination (T2) progeny gave the highest ratio of T.S.S /Acidity (24.93), while the lowest was recorded for the tree (T8) of hybrid (El-Tahrir X Nab-El Gamal) (17.66).In the second season, all progenies gave high ratio comparing with the first season. The highest value was recorded by Nab El Gamal selfed progeny (T4) and El-Tahrir selfed tree (T5) and the hybrid tree (T7) of (El-Tahrir X Nab-El Gamal). For the percentage of grains, the selfed progenies of El-Tahrir (T5 and T6) showed the highest values, in both seasons, ranging between 75.50 and 59.70%. The lowest percentage was recorded by Nab-El Gamal open-pollination progeny (T1) and the hybrid progeny of El-Tarir x Nab-El Gamal (T9), in the first season. In the second season, the selfed progeny of Nab-El Gamal (T4) exhibited the lowest value (43.23%).

The number of grains in 100g for all progenies is presented in Table (7). Nab-El Gamal open pollination progeny (T1) showed the highest value; it ranged between 383.0 and 412.0 in the two seasons, respectively. The lowest value for the tree T3 (Nab-El Gamal selfed) was 206.0 and 265.8, in the two seasons, respectively. For percentage of juice, significant differences were recorded between the different progenies. El-Tahrir selfed progeny (tree T6) gave the highest value (38.10 and 38.5%) in the two seasons, while the lowest value was found for the hybrid (El-Tahrir X Nab-El Gamal) (tree T9 Table 7), which gave 21.00 and 18.03% in

Table (6): Fruit physical characteristics of different progenies(open, self pollination) and hybrid) of pomegranate, in 2012 and 2013 seasons.

Tree No.	Progeny	Fruit Weight (gm)		Fruit size (cm^2)		Fruit diameter (cm)		Fruit length (cm)		Fruit circumference (cm)		Fruit room No.		Color of fruit
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	Two seasons
T1.	Nab-El Gamal(O)	100.167f	386.100a	95.000 f	359.667c	5.90 e	8.57 c	5.20 e	8.33 a-c	14.23 f	29.00 b	6.00 a	6.67 a	Reddish Yellow
T2.	El-Tahrir (O)	440.100a	292.667c	363.000a	291.333d	9.60 a	8.47 cd	8.30 a	7.97 b-c	30.07 a	26.43 d	6.00 a	5.67 b-d	Reddish Yellow
T3.	Nab-El Gamal (S)	400.000a	479.000a	387.000a	466.667a	9.371 a	9.83 a	8.03 a	8.80 a	29.50 a	31.40 a	5.33 ab	6.00 a-c	Greenish Yellow
T4.	Nab-El Gamal (S)	356.633a	424.000a	327.000b	410.000b	8.77 b	9.50 ab	8.07 a	8.50 ab	24.73 b	31.00 a	6.00 a	6.33 ab	Greenish Yellow
T5.	El-Tahrir (S)	160.333e	258.000d	155.000e	243.000e	6.20 e	8.03 d-f	5.60 d	7.70 c	19.03 e	25.33 e	4.67 b	5.00 d	Red Yellow
T6.	El-Tahrir (S)	265.000b	234.333e	244.333c	226.000e	8.57 b	7.67 ef	7.67 a	6.97 d	27.03 a	23.50 f	4.67 b	6.00 a-c	Red Yellow
T7.	El-Tahrir♀ × Nab-El Gamal♂	210.000d	264.533d	194.333d	246.333e	7.00 d	8.00 f	6.23 c	6.90 d	23.03 d	26.17 d	6.00 a	6.00 a-c	Reddish Yellow
T8.	El-Tahrir♀ × Nab-El Gamal♂	240.700c	356.933b	222.333c	359.667c	7.30 d	9.17 b	7.00 b	8.03 a-c	24.17 c	29.47 b	5.33 ab	6.00 a-c	Reddish Yellow
T9.	El-Tahrir♀ × Nab-El Gamal♂	341.000a	366.000ab	314.667b	310.667d	8.20 c	8.37 c-e	7.03 b	8.27 a-c	27.47 a	27.57 c	6.00 a	5.33 cd	Reddish Yellow

(O) Progeny resulted from open pollination (trees No. 1 and 2)
(S) Progeny resulted from self pollination (trees No. 3 to 6)
Hybrid between El-Tahrir♀ × Nab El-Gamal ♂(trees No. 7 to 9)
Means followed by the same letter within the same column are not significantly different P=0.05

Table (7): Fruit chemical characteristics of different progenies (open, self pollination and hybrid) in 2012 and 2013 season.

Tree no.	Progeny	T.S.S %		Acidity %		T.S.S/ acidity Ratio		Percentage of grains %		No. of grains in 100g		Percentage of juice %		Color of grains
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	Two seasons
T1.	Nab-El Gamal(O)	9.17 c	12.33 cd	0.509 b	0.482 c	18.33 d	25.64 d	44.40 e	50.70 bc	383.0 a	412.0 a	29.10 bc	28.40 b	Light Pink
T2.	El-Tahrir (O)	12.00 a	14.00 b	0.482 b	0.525 b	24.93 a	26.66 cd	69.20 b	59.97 a	214.2 de	275.7 bc	32.00 bc	38.80 a	Light Pink
T3.	Nab-El Gamal (S)	9.67 bc	10.50 e	0.405 c	0.369 d	24.15 ab	26.98 cd	49.90 d	46.57 b-d	206.0 e	265.8 c	28.90 bc	27.30 b	Light Pink
T4.	Nab-El Gamal (S)	10.33 bc	12.93 c	0.488 b	0.405 d	20.60 b-d	31.93 a	51.00 d	43.23 d	240.2 cd	330.2 bc	30.67 bc	31.07 a	Pink
T5.	El-Tahrir (S)	10.17 bc	15.33 a	0.520 b	0.481 c	19.59 cd	31.90 a	79.27 a	51.30 b	291.0 b	342.7 b	32.97 b	35.20 a	Light Pink
T6.	El-Tahrir (S)	12.00 a	14.33 ab	0.509 b	0.492 bc	23.65 ab	29.18 a-c	75.50 a	59.70 a	297.7 b	332.7 bc	38.10 a	38.50 a	Light Pink
T7.	El-Tahrir♀ × Nab-El Gamal♂	12.00 a	12.00 d	0.533 b	0.376 d	22.57 a-c	31.96 a	57.07 c	51.42 b	250.0 c	310.8 bc	28.00 c	23.80 c	Dark Pink
T8.	El-Tahrir♀ × Nab-El Gamal♂	11.17 ab	16.83 a	0.632 a	0.600 a	17.66 d	28.05 b-d	58.30 c	60.03 a	214.1 de	320.0 bc	23.00 d	21.90 d	Light Pink
T9.	El-Tahrir♀ × Nab-El Gamal♂	9.17 c	15.00 a	0.482 b	0.502 bc	19.08 cd	29.86 ab	41.00 e	45.80 cd	214.1 de	287.4 bc	21.00 d	18.03 e	Light Pink

(O) Progeny resulted from open pollination (trees No. 1 and 2)
(S) Progeny resulted from self pollination (trees No. 3 to 6)
Hybrid between El-Tahrir♀ × Nab El-Gamal ♂(trees No. 7 to 9)
Means followed by the same letter within the same column are not significantly different P=0.05

the two seasons, respectively. The table (6 and 7) presents the color of the fruit and the color of grains for different progenies. The tree T9 of hybrid (El-Tahrir X Nab-El Gamal) gave a good color (dark pink) for grains in the two seasons (Fig. 2).

Mir *et al.* (2007a and b) observed a high range of variability in pomegranate for fruit weight, volume, number of seeds and fruit color. Also, Varasteh *et al.* (2009) found a great variation in the important fruit characteristics of five commercial cultivars in Iran. Shulman *et al.* (1984), studied the growth curve of fruits, especially juice and T.S.S content, which increased continuously during maturation while acidity decreased.

3.2. Genetic studies

3.2.1.Polymorphism detected by ISSR marker

Five ISSR primers were tested with the DNA of nine progenies resulting from open and self pollination of Nab-El Gamal and El-Tahrir cultivars and the resulted hybrids between them (Fig.3). These primers produced multiple band profile which ranged from 8 to 15 amplicon (Table 8). The total number of amplicons amplified by the five primers was 57 with an average of 11.4/primer. The number of polymorphic bands ranged from 4 (HB-13) to 14 (HB-08), representing a percentage of polymorphism ranging from 50% (HB-12 and HB-13) to 93.3% (HB-08). The size of the amplified bands varied according to the used primers, it ranged from 115 bp to 128 bp.

Table (8): Polymorphism and its percentage as detected by ISSR marker.

Primer	Total No. of amplicons	Monomorphic amplicons	Polymorphism amplicons	Percentage of polymorphism
HB-08	15	1	14	93.3
HB-11	8	1	7	87.5
HB-12	14	7	7	50.0
HB-13	8	4	4	50.0
HB-15	12	5	7	58.3
Total	**57**	**18**	**39**	
Average	**11.4**	**3.6**	**7.8**	**67.82**

3.2.2. Genetic similarity

The genetic similarity ranged from zero (between hybrid 1 and hybrid 2) to 1 (between the two genotypes of Nab-El Gamal self pollinated). A high value of genetic similarity was observed between Nab-El Gamal (open pollinated) and each of Nab-El Gamal (selfed T3 and T4) which reached 0.81 and 0.75, respectively (Table 9).

El-Tahrir cultivar revealed an intermediate value of genetic similarity between the open pollinated progeny and the two self pollinated progenies (0.55 and 0.7, respectively). However, the genetic similarity between the two self pollinated progenies of El-Tahrir was 0.92.

Regarding the three tested hybrid progenies, it was noted that hybrid (T7) was close to Nab-El Gamal and the farthest from El-Tahrir. Hybrid (T7) recorded genetic similarity with Nab-El Gamal ranging from 0.41 to 0.80; meanwhile, it ranged from 0.04 to 0.20 with El-Tahrir. On the other hand, hybrid T8 showed a lower genetic similarity with El-Tahrir progenies (open, selfed T5&T6) (0.05,0.30 and 0.11, respectively). The same observation was found with hybrid T9, it was closer to Nab-El Gamal of the values (0.69, 0.21 and 0.60) for (open, and self pollination T1, T3 and T4, respectively), compared to El-Tahrir progenies (open, selfed T2, T5 and T6) of the values (0.41, 0.16 and 0.27, respectively). Genetic similarity value was very low among the three tested hybrid progenies of (El-Tahrir X Nab-El Gamal), which was zero between hybrids 1&3 and 0.06 between hybrid T8 & T9. These variations observed in genetic similarity could be attributed to the effect of pollen grains source on the genetic structure of the resulted progenies.

3.2.3. Cluster analysis

The dendrogram obtained from UPGMA cluster analysis of the genetic distances (Fig 4) revealed that all of El-Tahrir progenies were separated in one cluster either for self-or open pollination progenies. Whereas, the second cluster consisted of the self and open pollination progenies of Nab-El GamaL. The second sub-cluster was also divided into two groups, where the hybrid T9 was separated in one of these groups.

The second group was divided in two sub-groups, one of them consisted of the two self

Fig. (1): pomegranate fruit of the resulting progenies under self of Nab-El Gamal.

Fig. (2): pomegranate fruit of the resulting progenies under the hybrid between El-Tahrir X Nab-El Gamal cultivars.

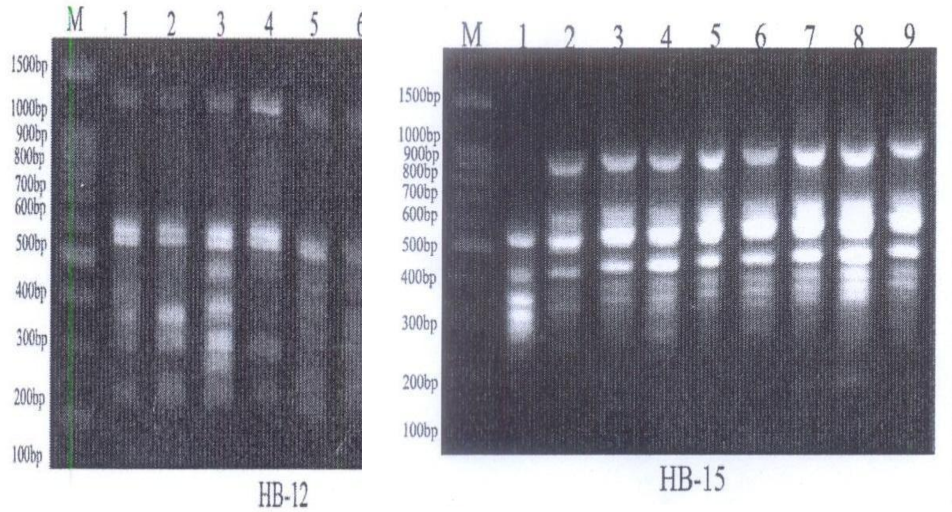

Fig. (3): DNA polymorphism of nine progenies of pomegranate amplified with ISSR primers. 1- Nab-El Gamal(O) (T1), 2- El-Tahrir(O) (T2), 3- Nab-El Gamal (S) (T3), 4- Nab-El Gamal (S) (T4), 5- El-Tahrir (S) (T5), 6- El-Tharir (S) (T6), 7- El-Tahrir x Nab-El Gamal (T7), 8- El-Tharir x Nab-El Gamal (T8) and El-Tahrir x Nab-El Gamal (T9).

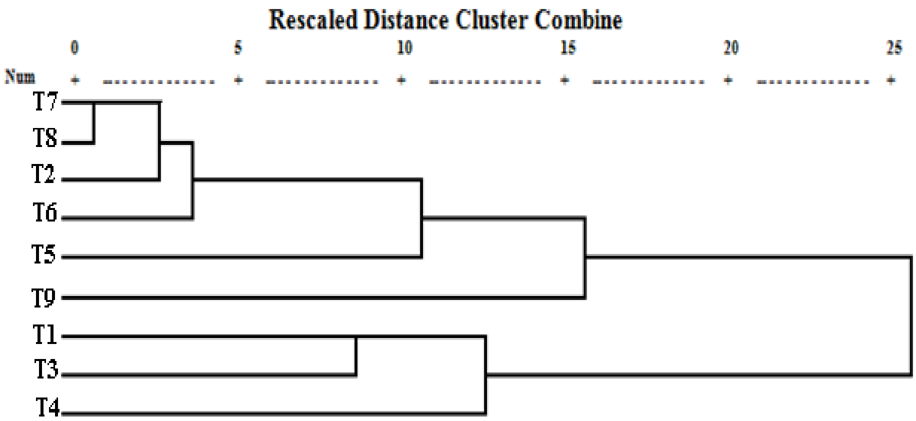

Fig.(4): Dendrogram of the nine progenies of pomegranate genotypes based on ISSR markers.

Table (9): Similarity indices among progenies of Nab El-Gamal (N), and El-Tahrir (T) measured by Nei coefficient methods for ISSR data.

	N-open T1	N-self T3	N-self T4	T-open T2	T-self T5	T-self T6	Hybrid T7	Hybrid T8	Hybrid T9
N-open(T1)									
N-self(T3)	0.81								
N-self (T4)	0.75	1.00							
T-open(T2)	0.43	0.53	0.06						
T-self (T5)	0.18	0.26	0.40	0.55					
T-self(T6)	0.29	0.05	0.33	0.74	0.92				
Hybrid(T7)	0.70	0.80	0.41	0.04	0.20	0.10			
Hybrid(T8)	0.50	0.60	0.22	0.05	0.30	0.11	0.00		
Hybrid(T9)	0.69	0.21	0.60	0.41	0.16	0.27	0.35	0.06	

pollinated genotypes of Nab-El Gamal (T3 and T4). The second sub-group contained hybrid T8, hybrid (T9) and the open pollinated genotype of Nab-El Gamal. Some investigations reported that the clustering of the cultivars is not related to the geographical distance (Jbir *et al*., 2006).

3.2.4. Unique markers identified by ISSR markers

Genotype specific ISSR unique markers could distinguish six out of the nine studied progenies (Table 10). Only, the hybrid progeny T9 (El-Tahrir X Nab-El Gamal) was characterized by both positive and negative unique markers, the five remaining progenies were identified by either positive or negative unique markers.

El-Tahrir self pollinated progenies (T5) was characterized by one unique positive marker by HB-08 primer at 935 bp. On the other hand, El-Tahrir self pollinated progeny (T6) was identified by five positive unique markers with approximately molecular weight ranging from 115 to 840 bp with for different primers (HB-08,HB-11,HB-12 and HB-13). The two self pollinated progenies of Nab-El Gamal (T3 and T4) were characterized by negative unique markers, the self pollinated progeny (T3) was identified by three markers, one of them by HB-12 (400 bp), were remaining two markers were identified by HB-15 (115 and 230 bp). Whereas, the open pollinated progeny of Nab El-Gamal (T1) was characterized by two negative markers (630 bp and 880 bp) with primer HB-15.

Hybrid (T7) was identified by two unique

Table (10): Unique positive and negative markers as detected by ISSR markers.

Genotype	Unique positive markers			Unique negative markers			Grand Total
	Size of marker (bp)	Primer	Total # of markers/ genotype	Size of marker (bp)	primer	Total # of marker/genotype	
El-Tahrir Self (T5)	935	HB-08	1	---	---	---	1
El-Tahrir Self (T6)	840-690	HB-08	5	---	---	---	5
	360	HB-11					
	115	HB-12					
	840	HB-13					
Nab-El Gamal Self (T3)	---	---	---	400	HB-12	3	3
				230-115	HB-15		
Nab El-Gamal Open (T1)	---	---	---	880-630	HB-15	2	2
Hybird (T7)	260	HB-08	2	---	---	---	2
	1260	HB-12					
Hybird (T9)	610	HB-08	1	480	HB-08	2	3
				710	HB-11		
6 Genotypes			9			7	16

positive markers at 260 bp and 1260 bp with HB-08 and HB-12, respectively. The molecular data represented in this study are in harmony with those reported by Awamleh *et al.* (2009); Ebrahim *et al.* (2010) and Hasnaoui *et al.* (2010).

Acknowledgement

This research is dedicated to the soul of the late. Prof. Dr. Mostafa Atef El-Hamady, Professor of Pomology, Faculty of Agriculture, Kafre lsheikh University, and Supervisor of the Breeding Research Department, Horticultural Research Institute of A. R.C.

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الانتخاب والتقييم للاختلافات الوراثية في الرمان
1- بعض معايير التعرف على انسال تلقيحات ذاتيه ومفتوحه وهجن بين صنفى الرمان ناب الجمل والتحرير

بهان محمود خليل – نهلة عبد الفتاح عوض – أحمد عثمان ريان – مصطفى عاطف الحمادى*

معهد بحوث البساتين – مركز البحوث الزراعية - * كلية الزراعة – جامعة كفر الشيخ - مصر

ملخص

يهتم برنامج التربية لمحصول الرمان فى معهد بحوث البساتين – مركز البحوث الزراعية – الذى بدأ فى عام 2004 – بالتهجين بين الأصناف المصرية الرئيسية (المنفلوطى – ناب الجمل – التحرير – بدر) واخصائها ذاتيا واجراء التلقيح المفتوح لدراسة أنسالها تم زراعة حوالى 200 شتلة من هذه الانسال فى مزرعة محطة بحوث جزيرة شندويل محافظة سوهاج سنة 2008، – حيث بدأ بالاثمار عام 2011. أختيرت 9 اشجار مثمرة لدراسة الاختلافات المورفولوجية والوراثية بها من نمو خضرى، تزهير، صفات ثمرية، كمية المحصول. ايضا تم عمل البصمة الوراثية لتلك الأشجار من خلال تكنيك ISSR باستخدام خمسة بادءات مختلفة. وأظهرت النتائج أن الشجرة الثالثة من (التحرير× ناب الجمل) أعطت احسن

محصول (22 كيلوجرام/ شجرة). والشجرة الأولي والثانية من نفس الهجين أعــطوا أحسن نسبة من المــواد الصلبة الكلية والحموضة (31.96 , 29.86) فى الموسم الثانى. الشجرة الأولي من نفس الهجين اعطت اقل نمو خضرى بالمقارنة بباقى الانسال تحت الدراسة. انتخبت الشجرة الناتجة من نـــاب الجـمل (بالتلقيح المفتــوح) اعـلى عدد من البذور /100جرام . انتخبت الشجرتان الناتجتان من صنف التحرير (بالتلقيح الذاتى) اعلى نسبة عصير. انتخبت الشجرة الأولي من الهجين (التحرير × ناب الجمل) أعطت حبوب داكنة اللون. أظهرت النتائج على المستوى الجزيئى للـ DNA باستخدام خمس بادئات لتحليل ISSR 57 حزمة بمتوسط 11.4 حزمة لكل بادئ ويتراوح الاختلاف بين 50% الى 93.3 % وكان حجم الحزمة يتراوح بين 115 الى 128 bp. التشابه الوراثى بين الثلاث شجرات الهجين تحت الدراسة يتراوح من (0.0 - 0.06). لذا ربما يكون هذا البعد الوراثى راجع الى الاختلاف فى التركيب الوراثى لحبوب اللقاح. وأظهرت شجرة نسل ناب الجمل الناتج من التلقيح الذاتى والمفتوح نسبة عالية من التشابه الوراثى بينما كانت النسبة متوسطة بين الشجرة نسل صنف التحرير من التلقيح الذاتى والشجرة من التلقيح المفتوح لنفس الصنف. ستكون هذه المعلمات مفيدة جدا فى دراسة الجيل الثانى لهذه الأنسال.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (65) العدد الثالث (يوليو 2014) 303-317.