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Molecular analysis of a new synthetic rabbit line and their parental populations using microsatellite and SNP markers

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ABSTRACT

Genetic diversity, clustering pattern and phylogenetic relationship between Moshtohor rabbits (M-line) as a new synthetic line and their founder line of Spanish V-line and Sinai Gabali were assessed using sixteen microsatellite markers. Moreover, polymorphism based on SNP G/A_{2464} located in the promoter region of progesterone receptor gene (*PGR*) was evaluated based on PCR-RFLP method. The French Giant Papillon (FGP) was used as an out-group breed. All the investigated microsatellite loci were polymorphic and the average N_O, H_O, *He*, F_{IS} were 6.75, 0.65, 0.80 and 0,083 respectively. M-line rabbits recorded the highest values of N_O (5.50), H_o (0.758) and H_e (0.742). Three genotypes (GG, AA and GA) were detected by genotyping G/A_{2464} SNP located in the promoter region of progesterone receptor (*PGR*) gene. M-line recorded the highest values of *Ne* (1.987), *He* (0.497) and Polymorphic information content (PIC) (0.373) and showed high potentiality for litter traits improvement by possessing the highest GG genotype frequency (0.120) among the studied populations. A close relationship between M-line and V-line (0.18) was supported by their clustering in one phylogenetic clade and their appearance as one admixed mosaic cluster in population structuring.

1. Introduction

Molecular markers are a powerful tool to assess genetic diversity within and between the rabbit populations in any breeding programs and to identify the genetic loci linked to different traits along with the conservation programs of rabbit genetic resources (Bolet et al., 2000; Markert et al., 2010; Hailu and Getu, 2015). There are many studies that have been conducted to evaluate the genetic diversity within and between rabbit populations including domestic and commercial types (Estes-Zumpf et al., 2008; Tian-Wen et al., 2010; Grimal et al., 2012). Microsatellite DNA is considered a marker of choice for a wide range of molecular genetic studies such as establishing rabbit population structure (Bolet et al., 2000), biodiversity evaluation owing to their unique characteristics and ease of applications in rabbits (Korstanje et al., 2003; Grimal et al., 2012). Single nucleotide polymorphism (SNPs) seem to be an appealing marker in molecular analysis of population structure and genetic diversity studies in rabbits (Peiró et al., 2008; Abdel-Kafy et al., 2015; Shevchenko, 2015).

There are few studies depend on SNP marker in evaluating the rabbit genetic diversity and the association with the important traits (Yang et al., 2013; Othman et al., 2015;Rafayová et al., 2009). A SNP located in the *MSTN* gene (Fontanesi et al., 2008; Rafayová et al., 2009; Shevchenko, 2015), a SNP located in the intron 5 of *POU1F1* gene (Wang et al., 2015), another SNP located in *GHR* gene (Sahwan et al., 2014; Abdel-Kafy et al., 2015). The SNP G > A_{2464} located in the promoter region of *PGR* gene showed that there are some differences in early embryo survival and development at day 3 of gestation between lines selected by uterine capacity (Peiró et al., 2008; Argente et al., 2010; Shevchenko, 2015). Progesterone receptor gene (*PGR*) encodes for a protein acts as a specific intracellular progesterone receptors (*PRs*) interacted with the progesterone hormone to mediate the reproductive events associated with pregnancy establishment and maintenance (Conneely et al., 2002). A new Egyptian rabbit line called Moshtohor line was developed and this line is considered as an important multi-purpose synthetic rabbit line (Iraqi et al., 2007, 2010) and there is only one study available about the genetic diversity of Moshtohor line based

Abbreviations: SNPs, single nucleotide polymorphisms; *PGR*, progesterone receptor gene; N_o, observed number of alleles; N_e, effective number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; PCR-RFLP, PCR-Restriction fragment length polymorphism; FGP, French Giant Papillon; M-line, Moshtohor rabbits; *PIC*, polymorphic information content; PRs, progesterone receptors; APRI, Animal Production Research Institute; *HWE*, Hardy-Weinberg equilibrium; F_{ST}, pairwise genetic differentiation among populations; F_{IT}, reduction in heterozygosity due to inbreeding for each locus; F_{IS}, reduction in heterozygosity due to inbreeding within each breed; MAS, marker assisted selection

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on RAPD marker (Osman et al., 2010).

The main objective of this work was to evaluate the genetic diversity of Moshtohor rabbits (M-line) as a new synthetic line relative to their founder breeds (Spanish V-line and Egyptian Sinai Gabali) using two molecular makers, the neutral molecular markers of 16 microsatellites and the functional molecular markers of G/A_{2464} SNP located in *PGR* gene.

2. Material and methods

2.1. Blood sampling and DNA extraction

Blood samples were obtained from a total of 100 individuals belonging to three Egyptian rabbit populations named Moshtohor line (M-line) as a synthetic line and their parents of V-line and Gabali (G) rabbits. Also, a French Giant Papillon breed (FGP) was used as an outgroup population, 25 samples from each breed. The animals used in the present study were chosen from three farms; the rabbitry of the Department of Animal Production, Faculty of Agriculture, Benha University, the rabbitry of Inshas, Animal Production Research Institute (APRI) and Agriculture Research Center, Ministry of Agriculture, Egypt. The samples were taken randomly from pedigreed animals with the least coancestry (avoiding full-sib and half-sibs). Approximately 3–5 ml venous blood sample per animal was collected from the rabbit ear vein by 2-gauge injection needle into tubes containing EDTA as anticoagulant. Genomic DNA was extracted from leukocytes using the Promega Wizard Genomic DNA Purification Kit (Cat No. A 1120).

2.2. Microsatellite genotyping

Molecular genotyping of the samples was carried out with a set of 16 microsatellite markers (Table 1). PCR amplification was carried out in 25 μl reaction mixture composed of 2 μl DNA (50 ng/μl), 5 μl of 5 × PCR Buffer, 2.5 μl dNTP's (20 m mol L), 2 μl of each primer (10 pmol/μl), and 0.2 μl Taq DNA polymerase and then the final volume was adjusted using dd.H_2O. The amplification conditions on a thermal cycler were as follows: initial denaturation step at 94 °C for 4 min, 35 cycles of amplification (40 s of denaturation at 94 °C, 60 s of annealing from 55 °C to 60 °C based on the optimal annealing temperature for the used primer, 60 s of extension at 72 °C), and followed by final extension at 72 °C for 10 min. Amplified products were visualized by polyacrylamide gel (8%) to separate PCR products with different sizes at 125 V for 6 h using 50 bp Promega DNA ladder and then they were stained with ethidium bromide stain and visualized under gel documentation device.

2.3. SNP G/A_{2464} genotyping using PCR-RFLP

Amplification of 558 bp DNA fragment located in promoter region of *PGR* gene was performed using these two primers, PGRP-F 5′ GAAGCAGGTCATGTCGA-TTGGAG 3′ and PGR-UTR 5′ CGCCTCTGGTGCCAAGTCTC 3′ designed by Peiró et al. (2008). The amplicons were digested with *Eco31I* restriction enzyme by incubation for 8 h at 37 °C. RFLP was carried out in reaction volume 30 μl consisted of: 10 μl of PCR product, 17.5 μl of dH2O, 2 μl of 10 × G buffer and 0.5 μl of *Eco31I* restriction enzyme (Fermentas). The restriction fragments were subjected to electrophoresis in 2% Agarose gel stained with ethidium bromide and visualized under UV trans-illuminator.

2.4. Statistical analysis

2.4.1. Microsatellite data

Genetic diversity was assessed by calculating the observed (N_o) and effective (N_e) number of alleles and the observed heterozygosity (H_o) and expected heterozygosity (H_e) using GENALEX version 6.0 (Peakall

Table 1
List of selected Microsatellite markers and nucleotide sequences.

No.	Microsatellite marker	Primer sequence 5′ → 3′	Annealing temp. (°C)	Reference
1	INRACCDDV0003	GATCAGCGAGCGCCTCTC TCCATCTGAATGAGGCACAA	60	Grimal et al., 2012
2	SAT2	GCTCTCCTTTGGCATACTCC GCTTTGGATAGGCCCAGATC	55	Mougel et al., 1997
3	SAT3	GGAGAGTGAATCAGTGGGTG GAGGGAAAGAGAGAGACAGG	60	Mougel et al., 1997
4	SAT4	GGCCAGTGTCCTTACATTTGG TGTTGCAGCGAATTGGGG	60	Xin-Sheng et al., 2008
5	SAT5	GCTTCTGGCTTCAACCTGAC CTTAGGGTGCAGAATTATAAGAG	60	Mougel et al., 1997
6	SAT7	GTAACCACCCATGCACACTC GCACAATACCTGGGATGTAG	60	Mougel et al., 1997
7	SAT8	CAGACCCGGCAGTTGCAGAG GGGAGAGAGGGATGGAGGTATG	60	Mougel et al., 1997
8	SAT12	CTTGAGTTTTAAATTCGGGC GTTTGGATGCTATCTCAGTCC	55	Mougel et al., 1997
9	SAT13	CAGTTTTGAAGGACACCTGC GCCTCTACCTTTGTGGGG	55	Xin-Sheng et al., 2008
10	SAT16	AATCAGCCTCTATGAATTCCC AATGCTACATGGTAACCAGGC	55	Mougel et al., 1997
11	SOL30	CCCGAGCCCCAGATATTGTTACA TGCAGCACTTCATAGTCTCAGGC	60	Tian-Wen et al., 2010
12	SOL33	GAAGGCTCTGAGATCTAGAT GGGCCAATAGGTACTGATCCATT	55	Tian-Wen et al., 2010
13	SOL44	GGCCCTAGTCTGACTCTGATTG GGTGGGGCGGCGGGTCTGAAAC	58	Tian-Wen et al., 2010
14	D3Utr2	AGGAAGTGAGGGGAGGTGTT ATAATGTGCTGCCAAAATAGAAAT	55	Tian-Wen et al., 2010
15	D6Utr4	CAGAAGGGCATTTGTTTTG GGTGATTCTTTCTTCTGCCTCTTA	55	Tian-Wen et al., 2010
16	D7Utr5	ACACCTGGGGAATAAACAACAAG GAGGGAGGCAGAGGGATAAGA	58	Tian-Wen et al., 2010

and Smouse, 2006). Hardy-Weinberg equilibrium (*HWE*) among loci within each breed was tested using GENEPOP program (Raymond and Rousset, 1995). Polymorphism information content (*PIC*) was calculated by using CERVUS version 3 software (Kalinowski et al., 2007). The F-statistics of pairwise genetic differentiation among populations (F_{ST}), reduction in heterozygosity due to inbreeding for each locus (F_{IT}) and the reduction in heterozygosity due to inbreeding within each breed (F_{IS}) across the studied populations were calculated using GENEPOP version 3.4 (Raymond and Rousset, 1995).

The Nei's genetic distance and the pairwise F_{ST} estimates were estimated among the four rabbit populations across the 16 microsatellite studied loci (Nei et al., 1983). A phylogenetic tree was constructed based on the Nei's genetic distance by using the neighbor-joining method (Saitou and Nei, 1987). The robustness of tree topologies was evaluated with a bootstrap test of 1000 resampling across loci. These processes were conducted using POPULATIONS version 1.2.30 software (Langella, 2008). The genetic structure of the sampled populations was investigated using a Bayesian clustering procedure implemented in STRUCTURE software with the admixture method (Pritchard et al., 2000). A 50 runs were used for each value of K ($2 \leq K \leq 4$) with 50,000 iterations following a burn-in period of 100,000. Pairwise comparisons of the 50 solutions of each K value were run along with 50 permutations using CLUMPP software (Jakobsson and Rosenberg, 2007). Finally, the clustering pattern was graphically displayed for the selected K value using DISTRUCT software (Rosenberg, 2004; Evanno et al., 2005).

2.4.2. G/A_{2464} SNP data

Allelic and genotypic frequencies were calculated by the standard procedure cited by Falconer and Mackay (1996). The statistical analysis was performed by SAS (2002). Genetic diversity of SNP G > A2464 located in the promoter region of PGR gene was assessed by calculating the effective number of alleles (*Ne*), the observed (*Ho*) and the expected (*He*) heterozygosity using GENALEX version 6.0 (Peakall and Smouse, 2006). Hardy-Weinberg equilibrium (*HWE*) within each population was estimated using Chi-Square test. The polymorphism information content (*PIC*) was calculated using CERVUS version 3 software (Kalinowski et al., 2007).

3. Results and discussion

3.1. Genetic diversity and polymorphism based on microsatellite markers

3.1.1. Among microsatellite loci markers

A total of 108 alleles were observed across the four studied rabbit populations (Table 2). The average number of alleles per locus was 6.75 and the highest number of observed alleles was recorded in markers *SAT16* and *SOL33* (10 alleles) and the lowest number was recorded in markers *SAT2* and *SAT7* (4 alleles). In Wan line Angora rabbits, Xin-Sheng et al. (2008) found that the average number of alleles per locus was 4.5 and ranging from 3 to 6 alleles. Tian-Wen et al. (2010) using 15 microsatellite loci found that the genetic diversity among seven Chinese rabbit populations were varied from 2.860 for marker *SAT8* to 9.920 for marker *SAT4* and the mean number of alleles was 6.625.

In all studied microsatellite markers (except *SAT7*, *D3Utr2* and *D6Utr4*), the values of H_o were lower than the H_e across the studied populations (Table 2), indicating heterozygosity deficiency and inbreeding which might be occurred due to non-random mating. The H_o ranged from 0.0.34 in *SAT4* to 0.79 in *SOL44 and* D6Utr4, while the H_e ranged from 0.66 in *SAT2* to 0.88 in *SAT16 and SOL33* loci. Grimal et al. (2012) reported that the mean H_o was lower than the H_e in four Egyptian rabbit breeds (Baladi Black, Gabali, Baladi Red, White Giza) and New Zealand White (NZW) where H_o ranged from 0.477 in *NZW* to 0.581 in Giza White. The values of polymorphic information content (*PIC*) of the selected markers were high (Table 2). *PIC* values in all studied markers ranged from 0.60 at locus *SAT2* to 0.86 at *SAT16* and *SOL33* loci. These values could suggest their usefulness for genetic polymorphism studies and linkage mapping programs in rabbits. In this concept, Xin-Sheng et al. (2008) found that the average *PIC* was 0.642 and ranged from 0.559 in locus *SAT4* to 0.705 in locus *SOL33*. All the loci studied except *SAT7* showed deviations from the Hardy-Weinberg equilibrium with highly significant level (Table 2). These results might be attributed to disequilibrium created by selection which conducted on Moshtohor rabbit's line to improve litter traits and could be attributed to non-random mating practiced on other three populations (V-line, Gabali and Papillon).

As shown in Table 2, the highest F_{IS} was observed for *locus SAT4* (0.437) and the lowest value was found for locus *D3Utr2* (-0.135). The mean F_{IS} value across all loci and populations was moderately positive (0.083), indicating that there is a moderate level of inbreeding. However, the high inbreeding values can be attributed to non-random mating and some loci might be linked to some economic traits. Tian-Wen et al. (2010) reported that the negative value of F_{IS} (-0.114)

Table 2
The observed (N_o) and effective (*Ne*) numbers of alleles, observed (H_o) and expected (H_e) heterozygosity, polymorphic information content (*PIC*) and Hardy-Weinberg equilibrium (*HWE*), F-statistics (F_{ST}, F_{IT} and F_{IS}) per microsatellite marker across the studied populations.

Marker (Locus)	N_o	N_e	H_O	H_E	*PIC*	*HWE*	F_{IS}	F_{ST}	F_{IT}
INRA	6^{de}	3.83^{gf}	0.62^{b}	0.81^{ab}	0.78^{ab}	***	0.153^{e}	0.091^{e}	0.231^{f}
SAT2	4^{f}	2.13^{j}	0.44^{c}	0.66^{b}	0.60^{c}	**	0.027^{h}	0.311^{a}	0.330^{c}
SAT3	7^{cd}	4.10^{e}	0.44^{c}	0.83^{ab}	0.80^{ab}	***	0.413^{b}	0.090^{e}	0.466^{b}
SAT4	7^{cd}	3.35^{h}	0.37^{c}	0.80^{ab}	0.77^{ab}	***	0.437^{a}	0.177^{b}	0.536^{a}
SAT5	7^{cd}	2.93^{i}	0.67^{ab}	0.74^{b}	0.70^{bc}	***	-0.115^{l}	0.179^{b}	0.085^{i}
SAT7	4^{f}	3.46^{h}	0.74^{ab}	0.74^{b}	0.69^{bc}	NS	-0.049^{j}	0.047^{fg}	0.001^{l}
SAT8	6^{de}	3.35^{h}	0.62^{b}	0.81^{ab}	0.77^{ab}	***	0.111^{f}	0.131^{c}	0.227^{f}
SAT12	6^{de}	3.48^{h}	0.57^{b}	0.77^{ab}	0.73^{bc}	***	0.194^{d}	0.080^{e}	0.259^{e}
SAT13	9^{ab}	5.73^{a}	0.78^{a}	0.86^{a}	0.84^{ab}	***	0.049^{g}	0.042^{g}	0.090^{i}
SAT16	10^{a}	5.13^{b}	0.77^{a}	0.88^{a}	0.86^{a}	***	0.039^{gh}	0.081^{e}	0.117^{h}
SOL30	7^{cd}	4.22^{e}	0.57^{b}	0.83^{ab}	0.80^{ab}	***	0.248^{c}	0.078^{e}	0.306^{d}
SOL33	10^{a}	4.79^{c}	0.73^{ab}	0.88^{a}	0.86^{a}	***	0.045^{g}	0.125^{c}	0.165^{g}
SOL44	8^{bc}	4.64^{d}	0.79^{a}	0.82^{ab}	0.79^{ab}	***	-0.010^{i}	0.042^{g}	0.032^{k}
D3Utr2	6^{de}	3.55^{h}	0.78^{a}	0.77^{ab}	0.74^{bc}	***	-0.135^{m}	0.107^{d}	-0.013^{l}
D6Utr4	5^{ef}	3.67^{g}	0.79^{a}	0.77^{ab}	0.73^{bc}	***	-0.086^{k}	0.056^{f}	-0.026^{m}
D7Utr5	6^{de}	3.86^{f}	0.74^{ab}	0.80^{ab}	0.77^{ab}	***	-0.002^{i}	0.076^{e}	0.074^{j}
Overall mean ± SE	6.75 ± 0.45	3.88 ± 0.22	0.65 ± 0.03	0.80 ± 0.01	0.76 ± 0.02		0.083 ± 0.043	0.107 ± 0.017	0.180 ± 0.042

The estimate with the same letters in each column is not significantly different ($P \leq 0.05$).

Table 3
The observed (N_o) and effective (N_e) numbers of alleles, the observed (H_O) and expected (H_E) heterozygosity, and the fixation coefficient of an individual within a subpopulation (F_{IS}) per population of rabbits.

Breed	N	N_O ± SE	N_e ± SE	H_O ± SE	H_e ± SE	F_{IS} ± SE
V-line	25	5.00 ± 0.274^{b}	3.91 ± 0.200^{b}	0.698 ± 0.032^{a}	0.734 ± 0.014^{a}	0.047 ± 0.044^{a}
Gabali	25	4.75 ± 0.359^{c}	3.67 ± 0.299^{b}	0.533 ± 0.040^{b}	0.695 ± 0.029^{a}	0.266 ± 0.055^{b}
M-line	25	5.50 ± 0.398^{a}	4.43 ± 0.363^{a}	0.758 ± 0.055^{a}	0.742 ± 0.032^{a}	-0.032 ± 0.064^{c}
FGP	25	4.69 ± 0.362^{c}	3.55 ± 0.305^{b}	0.618 ± 0.046^{a}	0.675 ± 0.037^{a}	0.083 ± 0.050^{b}
Mean ± SE	100	4.98 ± 0.176	3.89 ± 0.151	0.651 ± 0.024	0.711 ± 0.015	0.081 ± 0.029

The estimate with the same letters in each column isn't significantly different ($P \leq 0.05$).
SE = standard error.

indicating an excess in heterozygosity and the low F_{IS} value (very close to zero) indicating low level of inbreeding within the population, while the high positive value indicating a high level of inbreeding. The mean value of F_{ST} was high (0.107) and ranging from 0.042 for locus *SAT13* and locus *SOL44* to 0.311 for locus *SAT2* (Table 2). This value is lower than 0.137 recorded by Grimal et al. (2012). However, these values indicating that there is genetic differentiation among the studied four populations.

3.1.2. Among rabbit populations

Among the populations, the mean observed (N_O) and effective (N_e) numbers of alleles, observed (H_o) and expected (H_e) heterozygosity and the fixation coefficient of an individual within a subpopulation (F_{IS}) are presented in Table 3. The highest value of N_O (5.50), N_e (4.43), H_O (0.758) and H_e (0.742) were recorded for M-line, while the lowest value for the N_O (4.69), N_e (3.55) and H_e (0.675) were recorded for *FGP*. The highest value of N_O, N_e, H_O, H_e and F_{IS} which were recorded by M-line might be attributed to that this line was recently synthesized from crossing G buck with V-line doe compared with the other populations studied (V-line, G and *FGP*). Grimal et al. (2012) found that the average number of alleles per locus per population was 3.6, ranging from 2.7 in *NZW* to 3.9 in *BR* and *G* rabbits; the observed heterozygosity averaged 0.527, ranging from 0.477 in *NZW* to 0.581 in Giza White rabbits.

The highest value of F_{IS} was recorded for G population (0.266), while the lowest value of F_{IS} was recorded for M-line (Table 3). The value of F_{IS} among the studied four rabbit populations averaged 0.081 that was relatively lower than that of 0.172 reported by Grimal et al. (2012) for four Egyptian rabbit breeds.

3.2. Genetic diversity and polymorphism based on G/A_{2464} SNP

3.2.1. G/A_{2464} SNP polymorphism across the rabbit populations

Progesterone receptor gene (*PGR*) plays an important role in the reproductive traits in rabbits. The PCR–RFLP assay was carried out to genotype the four rabbit populations as a result of polymorphisms arose in promoter region of *PGR* gene due to SNP (G/A_{2464}). Three different genotypes were obtained across the studied populations; AA genotype for undigested 558 bp fragment, GG genotype for the digested 558 bp fragment yielding416 and 142 bp fragments and heterozygous GA genotype (558, 416 and 142 bp fragments).

The alleles and genotypes frequencies of the SNP $G > A_{2464}$ were estimated for the three Egyptian rabbit populations (V-line, Gabali, M-line) in addition to French Giant Papillon as an outgroup population. The distribution of AA, GG and GA genotypes for polymorphic variants were 28%, 4% and 68% in V-line rabbits and 24%, 4% and 72% in Gabali rabbits and 4%, 12% and 84% in M-line rabbits, respectively (Table 4). Across the three studied Egyptian rabbit populations, M-line recorded the highest genotypic frequency for both of GG genotypes (0.12; $P < 0.05$) and GA genotype (0.04; $P < 0.05$), while V-line recorded the lowest frequency for the same genotypes (0.04 and 0.68 respectively).

The allelic frequency showed the same trend as the genotypic frequency, where the M-line showed the highest frequency for the G allele (0.54; $P < 0.05$), while V-line showed the lowest frequency for the same allele (0.38). Our results are in agreement with Peiró et al., 2008 who reported that GG genotype showed the highest frequency compared to AA genotype (GG = 0.329, AA = 0.171). On the contrary, Shevchenko (2015) detected the polymorphism in the SNP G/A_{2464}, in New Zealand White rabbit females, and reported an opposite trend where the GG genotype showed lower frequency than the AA genotype (GG = 0.183 and AA = 0.317). The highest and significant frequency of GG genotype in M-line rabbits might be as a result of selection for maternal effect applied during the establishment program of the M-line, which might indicate the potentiality of M-line for increasing uterine capacity and improving litter size traits (Iraqi et al., 2010). Peiró et al. (2010) reported that GG genotype was the most frequent genotype in the line selected for increasing uterine capacity and the AA genotype was the most frequent genotype in the line selected for decreasing uterine capacity.

Table 4
Genotypic and allelic frequencies and their standard errors (SE) for the SNP (G/A_{2464}) of *PGR* gene in the four studied populations.

Genotype and allele	V-line	Gabali	M-line	FGP	All populations Mean ± SE
Genotypic frequency:					
AA	0.28^{a}	0.24^{b}	0.04^{a}	0.12^{a}	0.16 ± 0.06
GA	0.68^{b}	0.72^{a}	0.84^{b}	0.88^{b}	0.77 ± 0.05
GG	0.04^{c}	0.04^{b}	0.12^{c}	0.000	0.07 ± 0.03
SE	0.006	0.006	0.006	0.006	
Allelic frequency:					
A	0.62^{a}	0.60^{a}	0.46^{a}	0.56^{a}	0.56 ± 0.04
G	0.38^{b}	0.40^{b}	0.54^{b}	0.44^{b}	0.44 ± 0.04
SE	0.006	0.006	0.006	0.006	

The estimate with the same letters in each row are not significantly different ($P \leq 0.05$);
SE = standard error.

3.2.2. G/A_{2464} SNP polymorphism among the rabbit populations

Based on SNP of *PGR* gene, the values of effective number of alleles (*Ne*), observed (*Ho*) and expected (*He*) heterozygosity and the polymorphic information content (*PIC*) of populations were presented in Table 5. The value of *Ho* was higher than the value of *He* in all populations. Moreover, the Chi-Square tests showed that the genotype frequencies were not in Hardy-Weinberg equilibrium ($P < 0.05$ or $P < 0.001$) in all studied four populations. This might be attributed to the potential population dynamics, selection program and the nature of the sampling process. The four studied rabbit populations showed high levels of genetic diversity with average of *Ne* = 1.943, *He* = 0.485 and *PIC* = 0.367. These indices were higher than those values of *Ne* = 1.665, *He* = 0.395 and *PIC* = 0.320 reported by Yang et al. (2013) for SNP $G > A_{214}$ located in exon 1 of *TBC1D1* gene in two rabbit breeds. Wang et al. (2015) reported intermediate level of genetic polymorphism of *Ne* = 1.898, *He* = 0.472 and *PIC* = 0.361 for a SNP located at 536 bp in intron 5 of *POU1F1* gene in four rabbit breeds.

According to the classification of *PIC* values (*PIC* < 0.25 = low polymorphism; 0.25 < *PIC* < 0.50 = intermediate polymorphism;

Table 5
The effective numbers of alleles (*Ne*), the observed (*Ho*) and expected (*He*) heterozygosity, the Hardy-Weinberg equilibrium (*HWE*) and the polymorphic information content (PIC) of rabbit populations for the SNP of *PGR* gene (G/A_{2464}).

Population	N	*Ne*	*Ho*	*He*	χ^2 *HWE*	*PIC*
V-line	25	1.891^{d}	0.680	0.471^{b}	4.9*	0.360^{b}
Gabali	25	1.923^{c}	0.720^{c}	0.480^{b}	6.3*	0.365^{ab}
M-line	25	1.987^{a}	0.840^{b}	0.497^{a}	11.9***	0.373^{a}
FGP	25	1.972^{b}	0.880^{a}	0.493^{a}	15.4***	0.371^{a}
SE		0.001	0.006	0.003		0.003
Overall mean ± SE	100	1.943 ± 0.02	0.780 ± 0.05	0.485 ± 0.01		0.367 ± 0.002

The estimate with the same letters in each column are not significantly different ($P \leq 0.05$); SE = standard error, χ^2 = Hard-Weinberg Equilibrium χ^2 value; * = $P < 0.05$, ** = $P < 0.001$.

Table 6
The estimates of Nei's genetic distance (above the diagonals) and pairwise F_{ST} (below the diagonals) among the four populations based on 16 microsatellite loci studied.

Breed	V-line	Gabali	M-line	FGP
V-line		0.29	0.18	0.35
Gabali	0.11		0.27	0.19
M-line	0.08	0.12		0.31
FGP	0.15	0.06	0.16	

PIC > 0.50 = high polymorphism) the four studied populations showed moderate values of polymorphism of 0.360 for V-line, 0.373 for M-line, 0.365 for G and 0.371 for FGP population. Moreover, the *PIC* of this study was higher than that reported by Rafayová et al. (2009) for a SNP located at intron 2 of *MSTN* gene in two rabbit lines (*PIC* = 0.3447).

3.3. The genetic relationship and population structure

The closest pairwise Nei's genetic distance was recorded between V-line and M-line (0.18), followed by G and FGP (0.19), while the lowest pairwise F_{ST} value was recorded between G and FGP (0.06) and also between V-line and M-line (0.08) (Table 6). These levels of differentiations are within the range reported in some literature (Grimal et al., 2012). These close relationships between M-line and V-line were supported by the clustering pattern of the neighbor joining phylogenetic tree (Fig. 1) and population structure (Fig. 2). The tree topology showed that M-line and V-line were clustered in one clade. Moreover, the population structure at the most probable structure clustering ($K = 2$) showed that V-line and M-line appeared as one admixed mosaic cluster (Fig. 2).

The closer relationship between M-line and its maternal parent (V-line) than with its paternal parent (Gabali) might be attributed to the possibility of linkage of some loci with litter size, weight and milk productive traits characterizing V-line than Gabali breed and were used

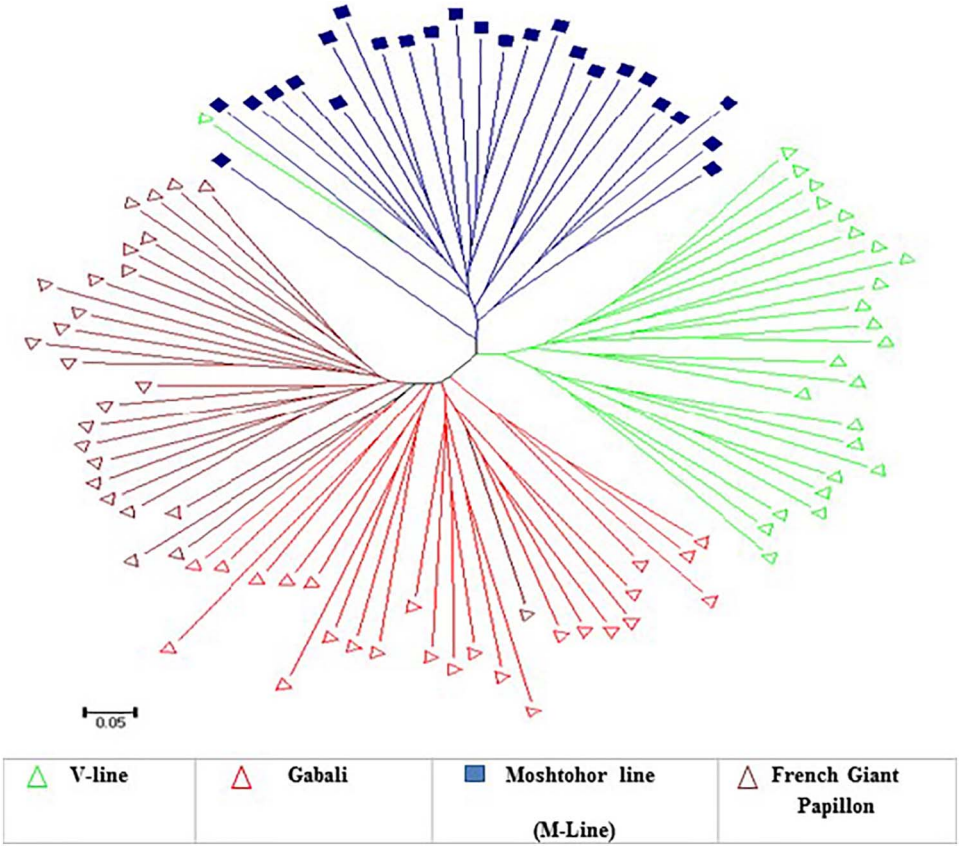

Fig. 1. The Neighbour-Joining phylogenetic tree among 100 individuals using the allele shared distance based on 16 microsatellite loci. The numbers within the nodes are bootstrapping values from 1000 replicates across the set of loci.

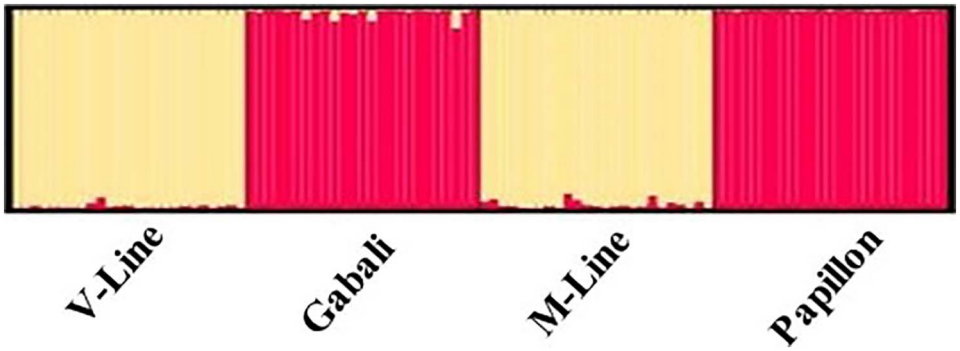

Fig. 2. Structure clustering of the four rabbits populations obtained for $K = 2$.

as a selection criteria during establishment of M-line (Iraqi et al., 2010).

Our results were in agreement with that of Osman et al. (2010) who reported that the highest similarity coefficient was recorded between M-line and V-line (0.924) followed by the similarity between M-line and Gabali (0.797).

High degree of relationship observed between G and FGP (Figs. 1 & 2) might be attributed to that G rabbits were raised by the Egyptian Bedouins in Sinai and in the north coast of western desert (Khalil and Baselga, 2002). On the other hand, the FGP is a foreign breed developed in the Lorraine region of north eastern France (Bunnyhugga, 2010). Gabali rabbits might suffer from gene introgression from the French rabbits during the French occupation of Egypt in eighteen century or due to recently unintended crosses between FGP and Gabali breed in the farm from where we collected our samples.

4. Conclusion

The microsatellites used in the present study were effective markers in detecting the genetic diversity and relationship among and within the investigated rabbit populations. Synthetic M-line rabbits showed high genetic diversity based on both microsatellites and SNP markers. M-line recorded the highest and significant frequency of GG genotype of G/A_{2464} SNP located in the promoter region of *PGR* gene indicating its potentiality for the improvement of litter traits. M-line showed closer relationship with its maternal founder (V-line) than with its paternal founder (Gabali) based on phylogenetic tree and structure clustering pattern.

Conflict of interest

There is no conflict of interest.

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