Comparative genetic analysis among Moshtohor line rabbits and their parental lines using microsatellite markers

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Abstract

Sixteen microsatellite markers were used to investigate the genetic diversity and phylogenetic relationship among Moshtohor line rabbits (M-line) and their parental lines of Spanish V-line and Sinai Gabali rabbits using the French Giant Papillon (FGP) as a reference population. A total of 100 animals viz. 25 rabbits from each population were used. Microsatellite markers of INRACCDDV0003, SAT2, SAT3, SAT4, SAT5, SAT7, SAT8, SAT12, SAT13, SAT16, SOL30, SOL33, SOL44, D3Utr2, D6Utr4 and D7Utr5 were chosen. All microsatellite loci typed were polymorphic. The average number of alleles per locus was 6.75 and the average polymorphic information content (PIC) was 0.76; and ranged from 0.60 at locus SAT2 to 0.86 at SAT16 and SOL33 loci. The observed heterozygosity averaged 0.65±0.03, and ranged from 0.37 in SAT4 locus to 0.79 in SOL44 and D6Utr4 loci, while the expected heterozygosity averaged 0.71±0.01, and ranged from 0.66 in SAT2 locus to 0.88 in SAT16 and SOL33 loci. All loci, except SAT7, showed deviations from Hardy-Weinberg equilibrium with highly significant level. The highest reduction in heterozygosity (F_{IS}) was 0.437 in locus SAT4, and the lowest reduction was -0.135 in locus D3Utr2. The estimates of F_{IS} across the loci and populations were positive and averaged 0.083± 0.043. The value of F_{ST} over the studied populations averaged 0.107±0.017 with the range from 0.042 for SAT13 and SOL44 loci to 0.311 for SAT2 locus. The differences among the populations in allele frequencies and their sizes were non-significant. The lowest pairwise F_{ST} value (0.08) and the closest pairwise Nei's genetic distance (0.18) were recorded between M-line and V-line rabbits. Neigbour-Joining phylogenetic tree showed that M-line and V-line were clustered together in one clade. Moshtohor line rabbits recorded the highest value of observed (0.758) and expected (0.742) heterozygosity, while the Gabali breed recorded the lowest value of observed heterozygosity (0.533) and the highest value of inbreeding coefficient (F_{IS}=0.266). The highest value for Nei`s genetic distance was recorded between V-line and FGP rabbits (0.35), while the closest pairwise Nei`s distance was recorded between V-line and M-line (0.18). The most probable structure clustering the four populations studied was detected between V-line and M-line rabbits.

Key words: Moshtohor line rabbits, genetic diversity, microsatellite markers, phylogenetic tree, structure clustering.

Introduction

Diversity of rabbit breeds offers the opportunity to increase the efficiency of commercial meat production through crossing **(Piles *et al.*, 2004).** Synthetic populations have been formed to combine desirable genes for commercially important traits **(Brun and Baselga, 2005)**. As a result of many years of domestication and crossbreeding, a wide variety of rabbit breeds exist today. Accordingly, a new Egyptian line of rabbits called Moshtohor (M- line) was synthesized, which considered as a synthetic multi-purpose line **(Iraqi *et al.*, 2009).** This line resulted from crossing Egyptian Sinai Gabali bucks with Spanish V-line does, where selection was practiced on the crossbreds for litter weight at weaning and live weight at 56 days **(Iraqi *et al.*, 2007)**. These studies were based on evidence stating that SpanishV-line rabbits and their crosses could produce and reproduce efficiently under hot climatic conditions **(Khalil and Baselga, 2002; Iraqi *et al.*, 2009).**

Genetic diversity among the native breeds provides us with valuable information to understand the domestication and evolution history **(Khalil and Baselga, 2002; Zenger *et al.*, 2003; Hailu and Getu, 2015)**. This will lead us to suggest the conservation or breeding plans to improve the native breeds of rabbits **(Joly *et al.*, 2012).** Therefore, genetic diversity within breeds is essential firstly for the sustained ability of a population to respond to selection for increasing productivity, fitness, disease resistance and environmental adaptation. Secondly, genetic diversity among the breeds is important, because rare and local breeds may fulfill specific requirements that might be necessary in the future **(Groeneveld *et al.*, 2010; Markert *et al.*, 2010).** In practice, microsatellites are able to generate the information necessary for planning of crossing and the selection of genotypes in genetic breeding programs in rabbits **(Grimal *et al.*, 2012).**

Molecular markers are powerful tools to assess genetic diversity within and between the populations in breeding programs and to identify the genetic loci linked to litter, lactation and growth traits in rabbits along with the conservation programs of rabbits genetic resources **(Bolet *et al.*, 2000; 2002; Berthouly *et al.*, 2008; Osman *et al.*, 2010; Khalil *et al.*, 2008).**

According to the FAO recommendations, the highly polymorphic microsatellite markers are currently the powerful method of choice for investigating the genetic relationships **(FAO, 2004).** Microsatellite DNA is currently one of the most useful markers of choice for a wide range of molecular genetic studies such as establishing population structure in rabbits **(Bolet *et al.*, 2000).** Also, microsatellites are the markers of choice for biodiversity evaluation owing to their unique characteristics and ease of applications in rabbits **(Korstanje *et al.*, 2001 and 2003; Grimal *et al.*, 2012).** This study aimed to investigate the genetic diversity and genetic relationship among the synthetic Moshtohor rabbits and their parental lines of Egyptian Sinai Gabali and Spanish V-line rabbits using microsatellite markers.

Materials and Methods

Experimental animal populations

Three rabbit populations namely Moshtohor line (M-line) as a synthetic line and their parental lines of V-line and Gabali (G) rabbits were used in this study. Also, a French Giant Papillon breed (FGP) was used as a reference breed for normalization of the results. A total of 100 pedigreed rabbits (25 rabbits from each population) were selected from the rabbitry of the Animal Production Department, Faculty of Agriculture, Benha University, Egypt and the Inshas rabbitry, Animal Production Research Institute (APRI), Agriculture Research Center (ARC), Ministry of Agriculture, Egypt. Moshtohor line is an Egyptian synthetic line developed by crossing does of Spanish V-line with bucks of Sinai Gabali **(Iraqi *et al.*, 2008 and 2009).** Spanish V-line is a synthetic maternal line originated in 1983 at the Department of Animal Science, Valencia, Spain, by crossing animals that were progeny of four specialized maternal lines and after three generations without selection, the line has been selected for litter size at weaning **(Estany *et al.*, 1989).** The Gabali rabbits are raised by the Egyptian Bedouins in Sinai and the north coast of western desert **(Khalil, 1999; Afifi, 2002).** On the other hand, the French Giant Papillon is a foreign breed developed in the Lorraine region of north eastern France in the 19th Century where the breeders at that time were raising the Flemish Giant rabbits, the spotted wild rabbits and the French Lop rabbits all together **(Bunnyhugga, 2010)**.

Sampling and genomic DNA extraction

The blood samples were taken randomly from 75 animal belonging to three rabbit populations (25 animal from each population; V-line, Gabali and M-line) along with fur samples from 25 animal of FGP breed. The samples were selected from the pedigreed animals with the least relationship to decrease the genetic similarity between the genotyped animals. To insure that there is no error in their original pedigrees, these relationships were checked by analyzing the polymorphism after genotyping.

Genomic DNA was extracted from blood samples belong to V-line, Gabali and M-line. Approximately 3-5 ml venous blood sample per animal was collected from the rabbit ear vein by 2-gauge 1.5 injection needle into tubes containing EDTA as anticoagulant. Genomic DNA was extracted using GeneJet Whole Blood Genomic DNA Purification Mini Kit (Cat no. #K0781,Thermo Scientific). For the reference population (FGP), DNA was extracted from the fur according to the manufacture of GeneJet Genomic DNA Purification Mini Kit (cat no. #K0721,Thermo Scientific). The quantity and quality of DNA were determined by spectrophotometer and agarose gel electrophoresis, respectively to be used directly in a variety of downstream applications.

Microsatellites genotyping

A list of 16 microsatellite markers was chosen for the molecular genotyping (Table 1). The selection of markers were designed based on their uniformly distribution throughout the rabbits genome as reported by **Mougel *et al.* (1997), Xin-Sheng *et al.* (2008) and Tian-Wen *et al.* (2010).** The primers were ordered from Invitrogen^{TM}, Germany. As a confirmation step, individual test was performed for each marker in a small sample from each breed to make sure of marker amplification in the breeds.The non-amplifying markers or homozygote markers in the population were culled.

PCR amplification

PCR amplification was carried out in 25 µl reaction mixture composed of 2 µl DNA (50 ng/µl), 5 µl of 5x 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 Mastercycler (Eppendorf) were as follows: initial denaturation step at 94°C for 4 min, 35 cycles of amplification (40s of denaturation at 94°C, 60s of annealing at 55°C,58°C or 60°C based on the optimal annealing temperature for the used primer, 60s 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 125v for 6 hour using 50 bp promega DNA step ladder (G.152A), and then stained with ethidium bromide solution and visualized under gel documentation model.

Table 1. List of the microsatellite markers and their primer sequences and annealing temperatures.

Marker	Primer sequence 5'→ 3'	Ann.. Tem (°C)
INRA	GATCAGCGAGCGCCTCTC TCCATCTGAATGAGGCACAA	60
SAT2	GCTCTCCTTTGGCATACTCC GCTTTGGATAGGCCCAGATC	55
SAT3	GGAGAGTGAATCAGTGGGTG GAGGGAAAGAGAGAGACAGG	60
SAT4	GGCCAGTGTCCTTACATTTGG TGTTGCAGCGAATTGGGG	60
SAT5	GCTTCTGGCTTCAACCTGAC CTTAGGGTGCAGAATTATAAGAG	60
SAT7	GTAACCACCCATGCACACTC GCACAATACCTGGGATGTAG	60
SAT8	CAGACCCGGCAGTTGCAGAG GGGAGAGAGGGATGGAGGTATG	60
SAT12	CTTGAGTTTTAAATTCGGGC GTTTGGATGCTATCTCAGTCC	55
SAT13	CAGTTTTGAAGGACACCTGC GCCTCTACCTTTGTGGGG	55
SAT16	AATCAGCCTCTATGAATTCCC AATGCTACATGGTAACCAGGC	55
SOL30	CCCGAGCCCCAGATATTGTTACA TGCAGCACTTCATAGTCTCAGGC	60
SOL33	GAAGGCTCTGAGATCTAGAT GGGCCAATAGGTACTGATCCATT	55
SOL44	GGCCCTAGTCTGACTCTGATTG GGTGGGGCGGCGGGTCTGAAAC	58
D3Utr2	AGGAAGTGAGGGGAGGTGTT ATAATGTGCTGCCAAAATAGAAAT	55
D6Utr4	CAGAAGGGCATTTGTTTTG GGTGATTCTTTCTTCTGCCTCTTA	55
D7Utr5	ACACCTGGGGAATAAACAACAAG GAGGGAGGCAGAGGGATAAGA	58

Statistical analysis

The statistical analysis was performed by **SAS (2002)** to evaluate Duncan multiple range tests in the testing of the significance levels **(Duncan, 1955).** Genetic diversity was assessed by calculating the observed (*No*) and effective *(Ne*) number of alleles, the observed (*Ho*) and the expected (*He*) heterozygosity using GENALEX version 6.0 **(Peakall and Smouse, 2006)**. Hardy-Weinberg equilibrium (HWE) over the loci within each population was tested using GENEPOP program **(Raymond and Rousset, 1995;** http://genepop.curtin.edu.au/**)**. To determine the deviations from Hardy–Weinberg equilibrium,exact tests were applied using the Markov Chain Monte Carlo simulation (100 batches, 5000 iterations and a dememorization number of 10 000) as implemented in GENEPOP program **(Guo and Thompson, 1992)**. Polymorphism information content (PIC) was calculated using CERVUS version 3 software **(Kalinowski *et al.*, 2007)**.

$$Ne = \frac{1}{1 - HE}$$

$$Ho = \frac{No.\ of\ \text{heterozygosity}}{N}$$

$$He = 1 - \sum_{i=1}^{n} p2_i$$

$$PIC = 1 - \sum_{i=1}^{n} p2_i - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p2_i\ p2_j$$

Where Pi is the frequency of the i[th] allele, Pj the frequency of the j [th] allele and n is the number of alleles.

The F-statistics of pairwise genetic differentiation among the populations (F_{ST}**),** reduction in heterozygosity due to inbreeding for each locus (F_{IT}) and the reduction in heterozygosity due to inbreeding within each population (F_{IS}) were calculated using GENEPOP version 3.4 **(Raymond and Rousset, 1995**; http://genepop.curtin.edu.au/**).** The alleles fragment sizes were analyzed using software TotalLab™ Quant v13.2 supplied by Nonlinear Dynamics Company (**TotalLab Ltd, 2014;** http://totallab-quant.software.informer.com/).

$$F_{IS} = \frac{He - Ho}{He}$$

$$F_{IT} = \frac{HT - Ho}{HT}$$

$$F_{ST} = \frac{HT - HE}{HT}$$

The genetic distances among the four populations were evaluated by Nei's genetic distance **(Nei *et al.*, 1983)**. A phylogenetic tree was constructed based on the Nei's genetic distance by using the neighbor-joining (NJ) method **(Saitou and Nei, 1987)**.The robustness of the tree topologies was evaluated with a bootstrap test of 1,000 resampling across loci. These processes were conducted using POPULATIONS version 1.2.30 software **(Langella, 2008**;http://bioinformaticsorg/~tryphon/populations).

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 60,000 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;** http://bioinformatics.oxfordjournals.org/content/23/1/4/1801). Finally, the clustering pattern was graphically displayed for the selected K value using DISTRUCT software **(Evanno *et al.*, 2005).**

Results and Discussion

In the present study, the genetic differentiation measured between the populations and obtained from the molecular variance (AMOVA) was recorded to be 19 % of the total genetic variance. However, 81 % of the genetic variation was attributed to within-population genetic diversity. These results can be

interpreted according to walnut principle for hierarchical population structure.

Among loci genetic diversity across the populations

The observed and effective number of alleles for each locusacross the populations studied

Across the populations studied, the observed (*No*) and effective (*Ne*) number of alleles and their ranges in alleles sizes for each locus are shown in Table 2. The *Ne* is an index used to reveal the genetic diversity of the populations. In all the populations and across the markers studied, the *No* was higher than the *Ne*.

All the microsatellites showed significant polymorphism across the four populations (Table 2). Therefore, these loci will be useful in the determination of the population genetic structure and assessing the patterns of gene flow in the populations.

Table 2. The observed (*No*) and effective (*Ne*) numbers of alleles and their ranges in alleles' sizes per microsatellite marker across the populations studied.

Microsatellite marker (Locus)	*No* ± *SE*	*Ne* ± *SE*	Ranges in alleles' sizes (bp)
INRA	6±0.57^{de}	3.83±0.06^{gf}	212-252
SAT2	4±0.57^{f}	2.13±0.06^{j}	231-251
SAT3	7±0.57^{cd}	4.10±0.06^{e}	146-166
SAT4	7±0.57^{cd}	3.35±0.06^{h}	215-245
SAT5	7±0.57^{cd}	2.93±0.06^{i}	221-263
SAT7	4±0.57^{f}	3.46±0.06^{h}	195-211
SAT8	6±0.57^{de}	3.35±0.06 ^{h}	134-162
SAT12	6±0.57^{de}	3.48±0.06 ^{h}	118-142
SAT13	9±0.57^{ab}	5.73±0.06^{a}	132-176
SAT16	10±0.57^{a}	5.13±0.06^{b}	103-147
SOL30	7±0.57^{cd}	4.22±0.06^{e}	143-183
SOL33	10±0.57^{a}	4.79±0.06^{c}	215-267
SOL44	8±0.57^{bc}	4.64±0.06^{d}	206-258
D3Utr2	6±0.57^{de}	3.55±0.06^{h}	376-426
D6Utr4	5±0.57^{ef}	3.67±0.06^{g}	192-230
D7Utr5	6±0.57^{de}	3.86±0.06^{f}	131-167
Overall mean ± SE	6.75±0.45	3.88±0.22	

The estimate with the same letters in each column are not significantly different (p≤0.05); SE= standard error.

Ben Larbi *et al.* (2014) used 36 microsatellite loci to provide a comprehensive insight into the genetic status and the genetic relationship among 12 Tunisian indigenous rabbit populations, and found that all microsatellite loci were polymorphic. A total of 108 alleles were observed across the populations. The average number of alleles per locus was 6.75±0.45 and ranged from 4 to 10 alleles. The highest observed number was 10 alleles and was detected in markers SAT16 and SOL33 and the lowest number was four alleles and was detected in markers SAT2 and SAT7. **Ben Larbi *et al.* (2014)** found that a total of 119 alleles were observed for 36 loci surveyed across 12 populations and the number of alleles per locus per population ranged from 2 (INRA0105, INRA0143 and INRA0274) to 18 (INRA0172) with an average of 3.30.

The average *Ne* was 3.89±0.22, and ranged from 2.13 at SAT2 locus to 5.73 at SAT13 locus (Table 2). These results are relatively higher than those of **Xin-Sheng *et al.* (2008**) who reported that the average number of alleles in Wan line Angora rabbit was 4.5 and ranging from 3 to 6 alleles. **Estes-Zumpf *et al.* (2008)** used 10 polymorphic microsatellite loci for the pygmy rabbit (*Brachylagus idahoensis*) and reported that nine of the 10 loci were reliable and had low frequencies of alleles and the number of alleles per locus ranged from four to 12. **Tian-Wen *et al.* (2010)** used 15 microsatellite loci and found that, the number of alleles averaged 6.63±0.49 in seven Chinese rabbit populations and they were varied from 2.86 at SAT8 to 9.92 at SOL44. **Ben Larbi *et al.* (2014)** stated that the highest mean *Ne* was 4.55, while the lowest locus variability was 2.86.

Across the populations studied, the numbers of alleles per locus were 6, 4, 7, 7, 7, 4, 6, 6, 9, 10, 7, 10, 8, 6, 5 and 6 alleles with the range in allele size from 212-252, 231-251, 146-166, 215-245, 221-263, 195-211, 134-162, 118-142, 132-176, 103-147, 143-183, 215-267, 206-258, 376-426, 192-230 and 131-167 bp for the marker INRA, SAT2, SAT3, SAT4, SAT5, SAT7, SAT8, SAT12, SAT13, SAT16, SOL30, SOL33, SOL44, D3Utr2, D6Utr4 and D7Utr5, respectively (Table 2). In accordance and taking into account the corresponding loci**, Rico *et al.* (1994)** found that the number of alleles in locus SoL33; ranged in allele size from 153 to 172 bp. **Mougel *et al.* (1997)** reported that the numbers of alleles were 4, 5, 6, 5, 7, 4, 3 and 5 in loci SAT8 (with 136 to 158 bp), SAT7 (with 184 to 195 bp), SAT5 (with 206 to 234 bp), SAT4 (with 195 to 240 bp), SAT3 (with 146 to 162), SAT2 (with 241 to 253 bp)**,** SAT12 (with 122 to

138 bp), SAT16 (with 109 to 115 bp) and SAT13 (with 114 to 128 bp), respectively.**Andersson *et al.* (1999)** found that the number of obvious alleles were 9, 8, 3, 9 and 13 in loci SAT2 (with 227 to 255 bp), SAT5 (with 209 to 221 bp), SAT8 (with 94 to 102 bp), SAT12 (with 106 to 138 bp) and SOL30 (with 155 to 209 bp). **Surridge *et al.* (1999)** found that the number of detectable alleles were 9, 8, 15 and 16 in loci SAT12, SAT8, SOL30 and SOL33 with the range in allele size from 114 to 146 bp, 134 to 182 bp, 137 to 171 bp and 189 to 219 bp, respectively. **Korstanje *et al.* (2003**) detected that the number of alleles were 5, 5 and 5 in loci D3Utr2, D6Utr4 and D7Utr5 with the range in size from 233–355, 179-193 and 141–162 bp, respectively. **Xin-Sheng *et al.* (2008)** detected that the number of alleles in locus SOL44 was four alleles with the range in size from 210 to 234 bp and six alleles in locus SAT13 with the range in size from 124 to 158 bp.**Tian-Wen *et al.* (2010)** reported 6, 8, 8, 10 and 15 alleles in loci SOL30 (with 161 to 181 bp), SAT5 (with 231 to 263 bp), SAT8 (with 191 to 269 bp), SOL33 (with 214 to 248 bp) and SOL44 (with 194 to 262 bp), respectively. **Badr (2015)** reported that the numbers of alleles were 6**,** 4, 9, 6, 5, 6, 5, 8, 9, 7, 9 and 6 in loci INRA, SAT2, SAT4, SAT5, SAT7, SAT8, SAT12, SAT13, SAT16, SOL30, SOL33 and SOL44. The range in sizes of these loci were from 200 to 250, 233 to 251, 181 to 240, 199 to 263, 182 to 199, 134 to 158, 130 to 146, 96 to 158, 101 to 145, 151 to 187, 101 to 145 and 194 to 252 bp, respectively.

As presented in Table 2, the microsatellite markers used in this study showed significant differences in the observed and effective numbers of alleles across the populations. These results can be used in interpreting the good and successful choice for detecting genetic variation within and among the rabbit breeds.

Hardy-Weinberg Equilibrium and the observed allelic frequencies and sizes across the populations studied

Among the 16 loci studied, the Hardy-Weinberg equilibrium (HWE) was significantly differentiated in 15 loci (Table 3). These results indicated that the polymorphism and genetic diversity were high. **Zenger *et al.* (2003)** used seven SAT microsatellite lociin 252 wild rabbits from five populations across Australia (the populations were compared to each other and to the data from Europe) found that deviations ($P < 0.05$) from HWE were non-significant(with F_{IS}~0) in any of the Australian data.

Allelic frequencies across all the microsatellite loci were mostly polymorphic (Table 3), and this is due to the differences in distribution of the allele frequency for each allele size among the populations. Also, allelic sizes showed highly significant differences among loci due to participation of common alleles between populations, and this meaned absence of this allele. Thus, these results have been pointed out the potential use of microsatellites in genetic studies of populations and detecting a significant level of differentiation across the loci within each population.

Table 3. Hardy-Weinberg equilibrium (HWE), the observed allelic frequencies and sizes per microsatellite marker across the populations studied.

Locus	HWE across the populations		Allelic frequencies		Allelic sizes	
	P-value	HWE	Mean	SE	Mean	SE
INRA	0.0000	***	0.216^{abc}	0.03	231.7^{c}	3.38
SAT2	0.0074	**	0.313^{a}	0.04	242.5^{b}	4.14
SAT3	0.0000	***	0.204^{bc}	0.03	155.4^{ef}	3.1
SAT4	0.0000	***	0.238^{abc}	0.03	231.3^{c}	3.13
SAT5	0.0000	***	0.275^{abc}	0.04	240.0^{bc}	3.13
SAT7	0.0585	NS	0.271^{ab}	0.04	204.8^{d}	4.14
SAT8	0.0000	***	0.192^{bc}	0.03	146.7^{f}	3.38
SAT12	0.0000	***	0.238^{abc}	0.03	130.7^{g}	3.38
SAT13	0.0000	***	0.138^{c}	0.03	152.7^{ef}	2.76
SAT16	0.0000	***	0.141^{c}	0.03	123.2^{g}	2.62
SOL30	0.0000	***	0.204^{bc}	0.03	161.0^{e}	3.13
SOL33	0.0000	***	0.182^{bc}	0.03	233.2^{bc}	2.62
SOL44	0.0000	***	0.169^{bc}	0.03	231.5^{c}	2.93
D3Utr2	0.0000	***	0.238^{abc}	0.03	401.7^{a}	3.38
D6Utr4	0.0000	***	0.225^{abc}	0.03	210.8^{d}	3.71
D7Utr5	0.0000	***	0.217^{abc}	0.03	147.3^{f}	3.38

NS= Non-significant, *= p≤0.05, **= p≤0.01 and ***= p≤0.001.
P-values tested by the Markov chain method for each locus; SE= standard error.

The observed and expected heterozygosity across the populations used

The observed (*Ho*) and expected (*He)* heterozygosity and the polymorphic information content (PIC) for each locus across the populations are presented in Table 4. The most widely parameters used to measure the genetic diversity across and within the populations are *He* or the gene diversity as defined by **Nei (1973**). The *Ho* in all microsatellite markers (except D3Utr2 and D6Utr4) were lower than *He*. The *Ho* for different markers averaged 0.65±0.03, and ranged from 0.37 at marker SAT4 to 0.79 at markers SOL44 and D6Utr4. While, the overall mean of *He* was 0.80±0.01,and ranged from 0.66 at SAT2 to 0.88 at SAT16 and SOL33. **Surridge *et al.* (1999)** found that *Ho* ranging from 0.241 at locus SOL44 to 0.718 at locus SAT12, while *He* ranging from 0.694 at SAT5 to 0.891 at SOL03. **Xin-Sheng *et al.* (2008)** found that the heterozygosity ranged from 0.630 to 0.721, and **Estes-Zumpf *et al.* (2008)** reported that the *Ho* and *He* ranged from 0.26 to 0.89 and from 0.63 to 0.88, respectively. **Tian-Wen *et al.* (2010)** found that the microsatellite D6Utr4 used in the Rex rabbits showed the highest *He* (0.889), while the locus SAT5 in Fujian Yellow rabbits had the lowest *He* (0.161). The average *He* in all loci and populations was high and ranging from 0.675±0.031 in Fujian Black rabbits to 0.820±0.012 in American Rex rabbits. **Ben Larbi *et al.* (2014)** found that *Ho* ranged from 0.3 to 0.53 across 36 microsatellite markers used in 12 rabbit populations.

The polymorphic information content (PIC) could be used to determine the heterozygosity and the number of alleles in the population. The values of PIC for the microsatellite markers were highly informative (PIC>0.50) across the populations (Table 4), and this could suggest their usefulness in genetic polymorphism studies and linkage mapping programs in rabbits (**Schwartz *et al.*, 2007; Xin-Sheng *et al.*, 2008; Hongmei *et al.*, 2008; Tian-Wen *et al.*, 2010)**. For a given locus, when PIC > 0.5, the locus is highly polymorphic; when 0.25< PIC < 0.5, the locus is moderately polymorphic; when PIC < 0.25, the locus is lowly polymorphic.

Table 4. The observed (*Ho)* and expected (*He)* heterozygosity and the polymorphic information content (PIC) for each locus across the populations studied.

Microsatellite marker (Locus)	*Ho*± *SE*	*He*± *SE*	*PIC*± *SE*
INRA	0.62±0.06^{b}	0.81±0.06^{ab}	0.78±0.06^{ab}
SAT2	0.44±0.06^{c}	0.66±0.06^{b}	0.60±0.06^{c}
SAT3	0.44±0.06^{c}	0.83±0.06^{ab}	0.80±0.06^{ab}
SAT4	0.37±0.06^{c}	0.80±0.06^{ab}	0.77±0.06^{ab}
SAT5	0.67±0.06^{ab}	0.74±0.06^{b}	0.70±0.06^{bc}
SAT7	0.74±0.06^{ab}	0.74±0.06^{b}	0.69±0.06^{bc}
SAT8	0.62±0.06^{b}	0.81±0.06^{ab}	0.77±0.06^{ab}
SAT12	0.57±0.06^{b}	0.77±0.06^{ab}	0.73±0.06^{bc}
SAT13	0.78±0.06^{a}	0.86±0.06^{a}	0.84±0.06^{ab}
SAT16	0.77±0.06^{a}	0.88±0.06^{a}	0.86±0.06^{a}
SOL30	0.57±0.06^{b}	0.83±0.06^{ab}	0.80±0.06^{ab}
SOL33	0.73±0.06^{ab}	0.88±0.06^{a}	0.86 ±0.06^{a}
SOL44	0.79±0.06^{a}	0.82±0.06^{ab}	0.79±0.06^{ab}
D3Utr2	0.78±0.06^{a}	0.77±0.06^{ab}	0.74±0.06^{bc}
D6Utr4	0.79±0.06^{a}	0.77±0.06^{ab}	0.73±0.06^{bc}
D7Utr5	0.74±0.06^{ab}	0.80±0.06^{ab}	0.77±0.06^{ab}
Overall mean ± SE	0.65±0.03	0.80±0.01	0.76±0.02

The estimate with the same letters in each column are not significantly different (p≤0.05); SE= standard error.

The PIC average is 0.76 with the values ranging from 0.60 at locus SAT2 to 0.86 at loci SAT16 and SOL33. These values are relatively higher than those of **Xin-Sheng*et al.* (2008),** who found that the average PIC was 0.642 with the range from 0.559 at locus SAT4 to 0.705 at locus SOL33. The PIC reported here was also higher than that reported by **Hongmei *et al.* (2008)** across 10 microsatellite markers, with arange in PIC from 0.499 to 0.700. **Schwartz *et al.* (2007)** reported that the PIC value was the highest at locus SAT16 (0.70), while the lowest PIC was at locus SOL33 (0.27). In contrast, **Tian-Wen *et al.* (2010)** found that the estimate of PIC averaged 0.625 to 0.796, that indicating high polymorphic across the 15 microsatellites.

Across the populations, the microsatellite markers showed significant differences in *Ho*, *He* and PIC for each locus (Tble 4), and suggesting that the microsatelite can be used as a tool to understand and detect the genetic variability within the populations.

The reduction in heterozygosity due to inbreeding

TheF-statistics presented in Table 5 showed the reduction in heterozygosity across the populations used due to inbreeding (F_{ST}, F_{IT} and F_{IS}) for each locus across the four populations investigated.

The highest F_{IS} was observed for locus SAT4 (0.437) and the lowest value was observed for locus D3Utr2 (-0.135). The estimate of F_{IS} across all loci and populations ws moderately positive (0.083± 0.043), indicating that there is a moderate inbreeding. The low positive F_{IS} values are very close to zero for loci SAT2, SAT13, SAT16 and SOL33; indicating low inbreeding within the populations. While, the high positive values of F_{IS} for loci INRA, SAT3, SAT4, SAT8, SAT12 and SOL30 showed that there is a high inbreeding. The negative F_{IS} for loci SAT5, SAT7, SOL44, D3Utr2, D7Utr4 and D6Utr5 indicated that there is a homozygosity deficiency and/or an excess in the heterozygosity. However, the high inbreeding values can be attributed to non-random mating and some loci might be linked to some economical traits. **Tian-Wen *et al.* (2010)** reported that F_{IS} was negative (-0.114±0.050), indicating an excess in heterozygosity.The average F_{IS} among the four rabbit populations (0.081) was relatively lower than that of 0.172 reprted by **Grimal *et al*. (2012)** among the four Egyptian populations of Baladi Black, Baladi Red, Gabali and Giza White rabbits.

The F_{ST} is the inbreeding coefficient of an individual related to the subpopulation and it was calculatd from the subpopulation observed and expected heterozygosity **(Falconer and Mackay, 1996).** The value of F_{ST} over the studied populations was high (0.107±0.017) indicating that there are genetic differentiations among the populations. The F_{ST} valuesranged from 0.311 for the marker SAT2 to 0.042 for the markers SAT13 and SOL44. **Tian-Wen *et al.* (2010)** reported F_{ST} of 0.099, and **Grimal *et al.* (2012)** reported F_{ST} of 0.0137.

Table 5. The F-statistics of reduction in heterozygosity due to inbreeding (F_{ST}, F_{IT} and F_{IS}) in each locus across the four populations investigated.

Locus	F_{IS}± SE	F_{ST}± SE	F_{IT}± SE
INRA	0.153±0.005^{e}	0.091±0.005^{e}	0.231±0.005^{f}
SAT2	0.027±0.005^{h}	0.311±0.005^{a}	0.330±0.005^{c}
SAT3	0.413±0.005^{b}	0.090±0.005^{e}	0.466±0.005^{b}
SAT4	0.437±0.005^{a}	0.177±0.005^{b}	0.536±0.005^{a}
SAT5	-0.115±0.005^{l}	0.179±0.005^{b}	0.085±0.005^{i}
SAT7	-0.049±0.005^{j}	0.047±0.005^{fg}	0.001±0.005^{l}
SAT8	0.111±0.005^{f}	0.131±0.005^{c}	0.227±0.005^{f}
SAT12	0.194±0.005^{d}	0.080±0.005^{e}	0.259±0.005^{e}
SAT13	0.049±0.005^{g}	0.042±0.005^{g}	0.090±0.005^{i}
SAT16	0.039±0.005^{gh}	0.081±0.005^{e}	0.117±0.005^{h}
SOL30	0.248±0.005^{c}	0.078±0.005^{e}	0.306±0.005^{d}
SOL33	0.045±0.005^{g}	0.125±0.005^{c}	0.165±0.005^{g}
SOL44	-0.010±0.005^{i}	0.042±0.005^{g}	0.032±0.005^{k}
D3Utr2	-0.135±0.005^{m}	0.107±0.005^{d}	-0.013±0.005^{l}
D6Utr4	-0.086±0.005^{k}	0.056±0.005^{f}	-0.026±0.005^{m}
D7Utr5	-0.002±0.005^{i}	0.076±0.005^{e}	0.074±0.005^{j}
Overall mean± SE	0.083±0.043	0.107± 0.017	0.180± 0.042

The estimate with the same letters in each column are not significantly different (p≤0.05); SE= standard error.

The F_{IT} is an estimate of the total inbreeding coefficient of an individual related to the whole population, calculated using the expected and observed heterozygosity for the whole population. The value of the inbreeding coefficient of the individual relative to the total population (F_{IT}) was high and averaged 0.180±0.042. Most of these values are higher than that of -0.004 reported by **Tian-Wen *et al.* (2010)**, but they are close to 0.279 reported by **Grimal *et al.* (2012)**. The highest value of 0.536 for F_{IT} was recorded for SAT4 locus, while the lowest value of -0.026 was recorded for D6Utr4 locus. In general, these values showed that there were significant differences in the level of reduction in heterozygosity due to inbreeding for each locus across the populations. Hence, these results are confirmed from the results of all microsatellites genotyped and they are showing the high polymorphism across the four populations.

Among populations genetic diversity across the loci

The observed and effective number of alleles in each population

Within each population, the observed (*No*) and effective (*Ne*) number of alleles are presented in Table 6. The highest allelic numbers of *No* (5.50) and *Ne* (4.43) were obtained for M-line, followed by V-line with *No*=5.00 and *Ne*=3.91. While the lowest values for the same were obtained for FGP breed (*No* = 4.69 and *Ne* = 3.55). The highest value of *No* agreed with that recorded by **Grimal *et al.* (2012),** who showed high value of *No* (3.94) for Gabali breed among the four Egyptian rabbit breeds. In Table 6, the microsatellite markers showed significant differences in *No* among the populations, except Gabali and FGP in which was not significant, while M-line showed significant differences in *Ne* compared to the other populations. This trend may be related to the breeding history of each population.

Table 6. The observed (*No*) and effective (*Ne*) numbers of alleles per population of rabbits.

Population	N	*No*± SE	*Ne* ± SE
V-line	25	5.00±0.274[b]	3.91±0.200[b]
Gabali	25	4.75±0.359[c]	3.67±0.299[b]
M-line	25	5.50±0.398[a]	4.43±0.363[a]
FGP	25	4.69±0.362[c]	3.55±0.305[b]
Overall mean ± SE	100	4.98±0.176	3.89±0.151

The estimate with the same letters in each column are not significantly different (p≤0.05); SE= standard error.

Hardy-Weinberg Equilibriumin each population

Out of the 16 microsatellite loci, the Hardy-Weinberg Equilibrium (HWE) was significantly differentiated in 14 loci in V-line, 11 loci in Gabali, 13 loci in M-line and 13 loci in FGP rabbits (Table 7). These results might be attributed to disequilibrium resulted from genetic drift or occurred by selection which has been conducted in M-line to improve the reproductive traits specially litter size traits. On contrast, in the pure breeds (Gabali and FGP), the disequilibrium may be attributed to the inbreeding practiced in these breeds and/or the samples were selected from the same rabbitry.

Table 7. Hardy-Weinberg Equilibrium (HWE) per microsatellite marker in each population.

Locus	V-line		Gabali		M-line		Papillon	
	P-value	HWE	P-value	HWE	P-value	HWE	P-value	HWE
INRA	0.0035	**	0.0020	**	0.0000	***	0.0000	***
SAT2	0.0005	***	0.5861	NS	1.0000	NS	0.1021	NS
SAT3	0.0000	***	0.0000	***	0.0000	***	0.0000	***
SAT4	0.0007	***	0.0024	**	0.0000	***	0.0052	**
SAT5	0.4225	NS	0.1558	NS	0.0000	***	1.0000	NS
SAT7	0.5169	NS	0.1622	NS	0.0998	NS	0.0651	NS
SAT8	0.0000	***	0.0005	***	0.0044	**	0.0000	***
SAT12	0.0036	**	0.0007	***	0.0002	***	0.0020	**
SAT13	0.0000	***	0.0000	***	0.0000	***	0.0000	***
SAT16	0.0002	***	0.0000	***	0.0000	***	0.0000	***
SOL30	0.0000	***	0.0000	***	0.0001	***	0.0026	***
SOL33	0.0406	*	0.0006	***	0.0000	***	0.0001	***
SOL44	0.0010	***	0.0011	***	0.0011	***	0.0002	***
D3Utr2	0.0000	***	0.1337	NS	0.0003	***	0.0329	*
D6Utr4	0.0005	***	0.0697	NS	0.0111	**	0.0005	***
D7Utr5	0.0027	**	0.0032	**	0.0610	NS	0.0139	**

NS= Non-significant, *= p≤0.05, **= p≤0.01 and ***= p≤0.001.
P-values tested by the Markov chain method for each locus.

The observed allelic frequencies and sizes in each population

The observed allele frequencies and their sizes in each population (Table 8), showed non-significant differences among the populations.

As shown in Figure 1, the observed allele sizes and their frequencies for the marker INRA in each population showed that the highest frequency was 0.460 for the allele with size of 252 bp in V-line rabbits, while the lowest frequency was 0.0.60 for the allele with size of 212 bp in M-line rabbits.

Table 8. The observed allele frequencies and their sizesin each population

Population	*No* of alleles	Allele frequencies		Allele sizes	
		Mean	SE	Mean	SE
V-line	80	0.209[a]	0.015	202.7[a]	1.65
Gabali	78	0.221[a]	0.016	202.7[a]	1.65
M-line	88	0.197[a]	0.014	202.7[a]	1.65
FGP	75	0.237[a]	0.016	202.7[a]	1.65

The estimate with the same letters in each column are not significantly different (p≤0.05); SE= standard error.

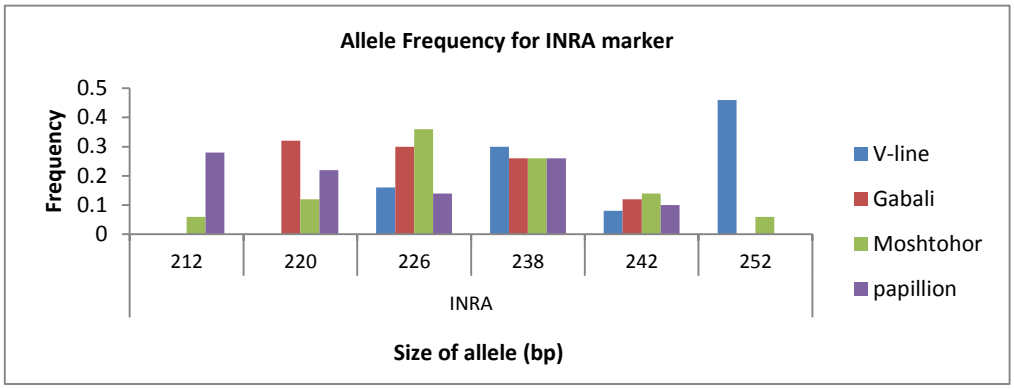
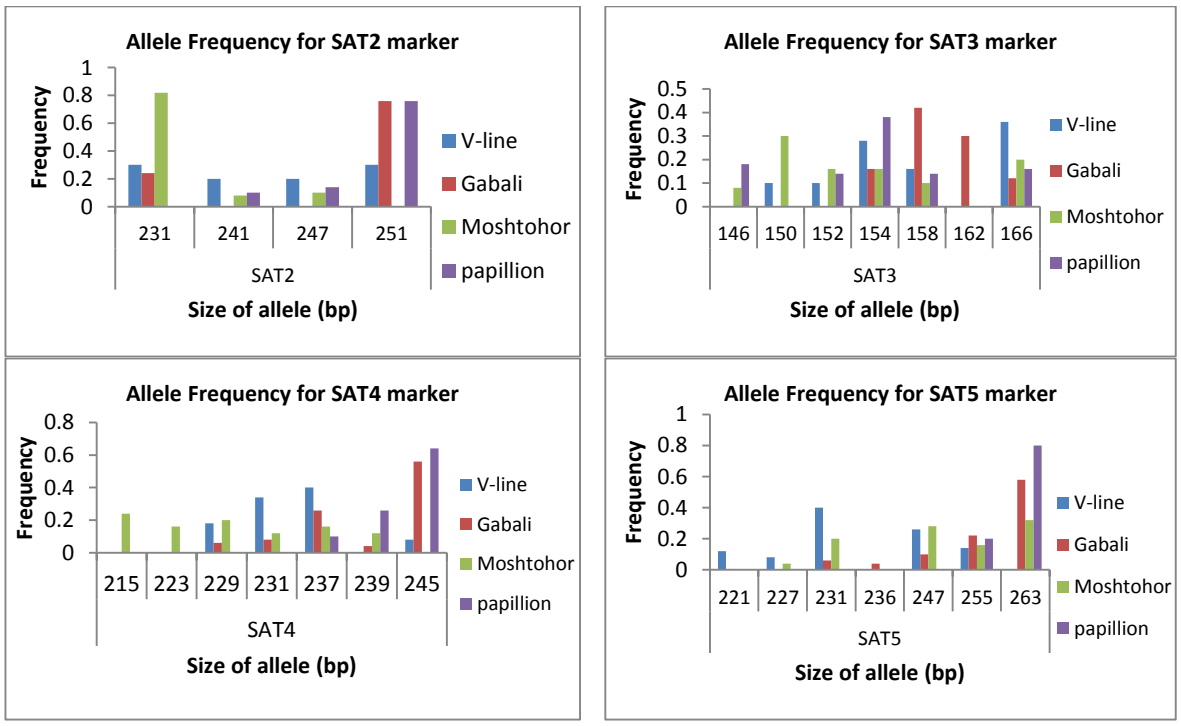


Figure1. The observed allelic frequency and size in each population of rabbits for the locus INRA.

For the SAT loci, the highest observed allele frequencies were 0.820, 0.420, 0.560, 0.800, 0.460, 0.420, 0.480, 0.280 and 0.400 were recorded for the markers SAT2, SAT3, SAT4, SAT5, SAT7, SAT8, SAT12, SAT13 and SAT16 with the allele sizes of 231 bp in M-line rabbits, 158 bp in Gabali rabbits, 245 bp in FGP rabbits, 263 bp in FGP rabbits, 211 bp in V-line rabbits, 134 bp in M-line, 130 bp in M-line, 148 bp in V-line and 133 bp in V-line, respectively (Figure 2). On the other hand, the lowest allele frequencies were 0.100, 0.100, 0.040, 0.040, 0.160, 0.020, 0.060, 0.040 and 0.020 for the markers SAT2, SAT3, SAT4, SAT5, SAT7, SAT8, SAT12, SAT16 and SAT16 with the allele sizes of 241 bp in FGP rabbits and 247 bp in M-line rabbits, 152 bp in V-line rabbits, 158 bp in M-line, 239 bp in Gabali rabbits, 227 bp in M-line, 236 bp in Gabali, 195 bp in FGP, 136 bp in Gabali and FGP rabbits, 142 bp in FGP, 132 bp in V-line, 132 bp in FGP, 164 bp in M-line and 117 bp in Gabali, respectively. **Mougel *et al.* (1997)** in Europe and **Zenger *et al.* (2003)** in Australia used the microsatellite loci SAT2, SAT3, SAT4, SAT5, SAT7, SAT8 and SAT13 to detect the genetic diversity in *Oryctolagus cuniculus* and reported that the mean number of unique alleles was significantly higher ($P<$ 0.05) in the Spanish source populations when compared with French and Australian rabbits.



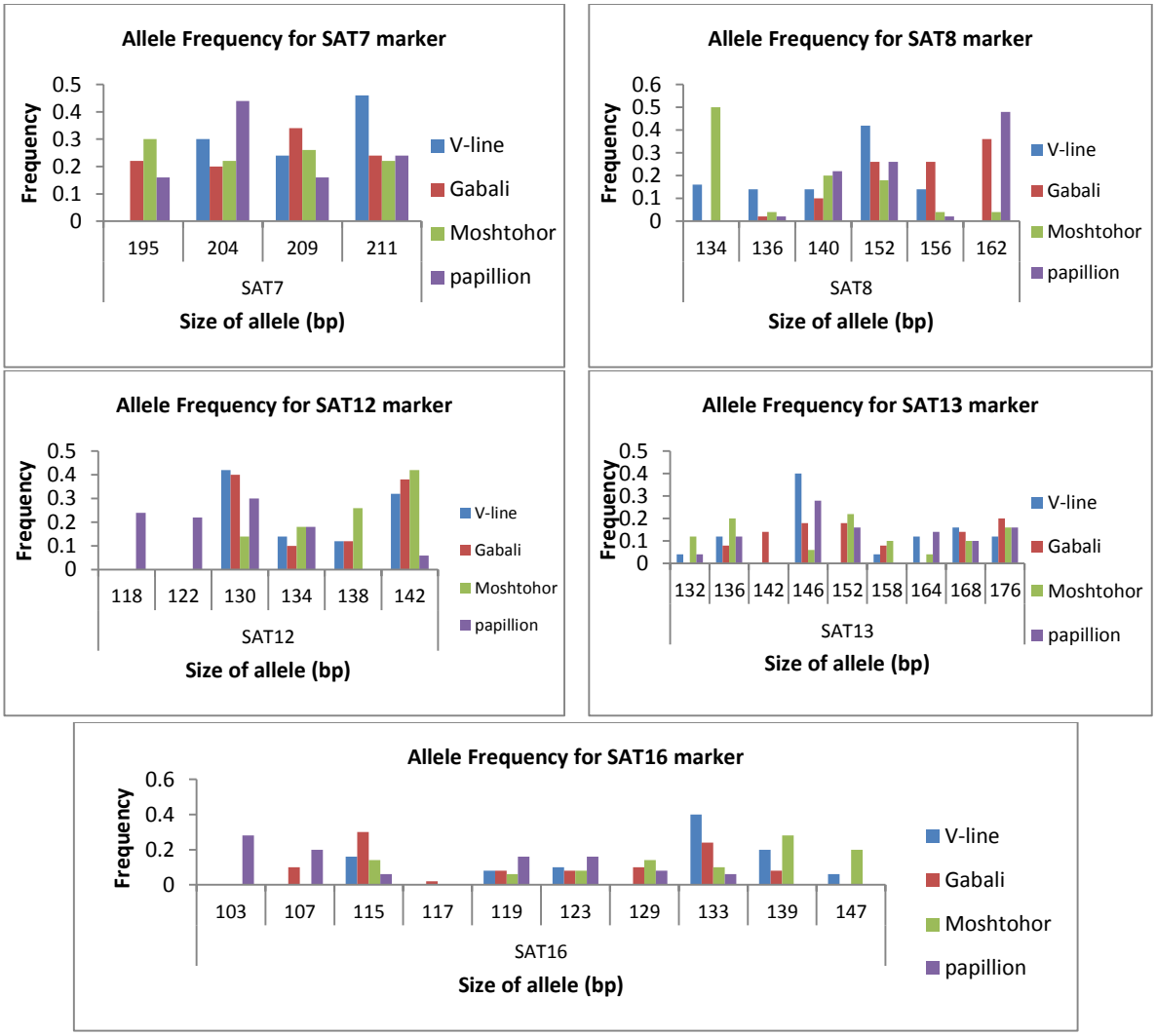
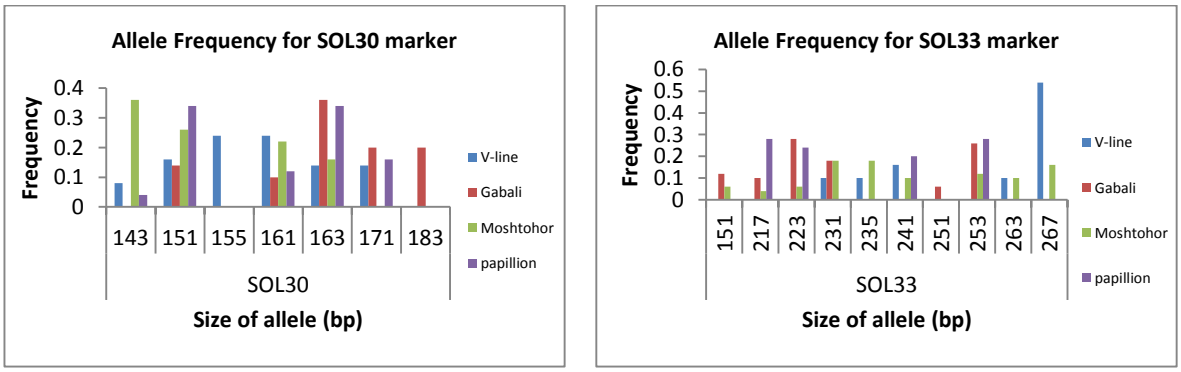


Figure2. The observed allelic frequency and size in each population of rabbits for the loci SAT.

For the SOL loci, the highest allele frequencies were 0.360, 0.540 and 0.380 for the markers SOL30, SOL33 and SOL44 with the allele sizes of 143 bp and 163 bp in M-line and Gabali rabbits, 267 bp in V-line rabbits, and 222 bp in Gabali and FGP rabbits, respectively (Figure 3). The lowest allele frequencies were 0.100, 0.040 and 0.020 for the allele with size of 161 bp in Gabali rabbits, 217 bp in M-line rabbits, and 220 bp in FGP rabbits.



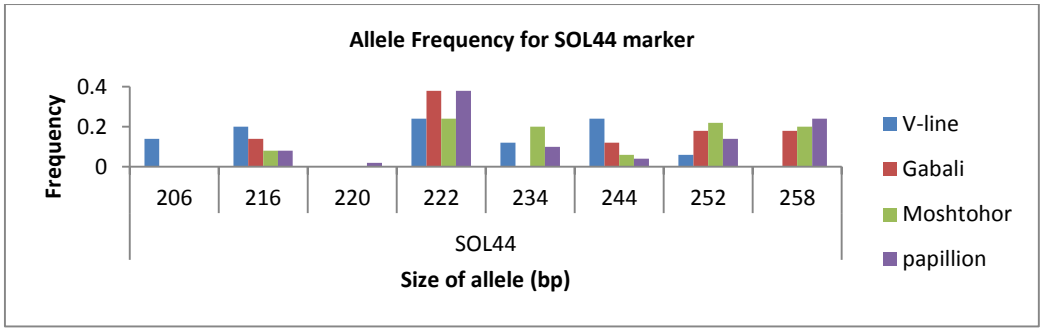


Figure 3. The observed allelic frequency and size in each population of rabbits for the loci SOL.

Regarding the D3Utr loci (Figure 4), the highest observed allele frequencies were 0.580, 0.340 and 0.420 for the markers D3Utr2, D6Utr4 and D7Utr5 with the allele sizes of 376 bp in Gabali rabbits, 192 bp in V-line and Gabali rabbits and 167 bp in FGP rabbits, while the lowest frequency was 0.060 for the markers D3Utr2, D6Utr4 and D7Utr5 with the allele sizes of 402 bp in FGP rabbits, 230 bp in Gabali rabbits and 143 bp in V-line rabbits.

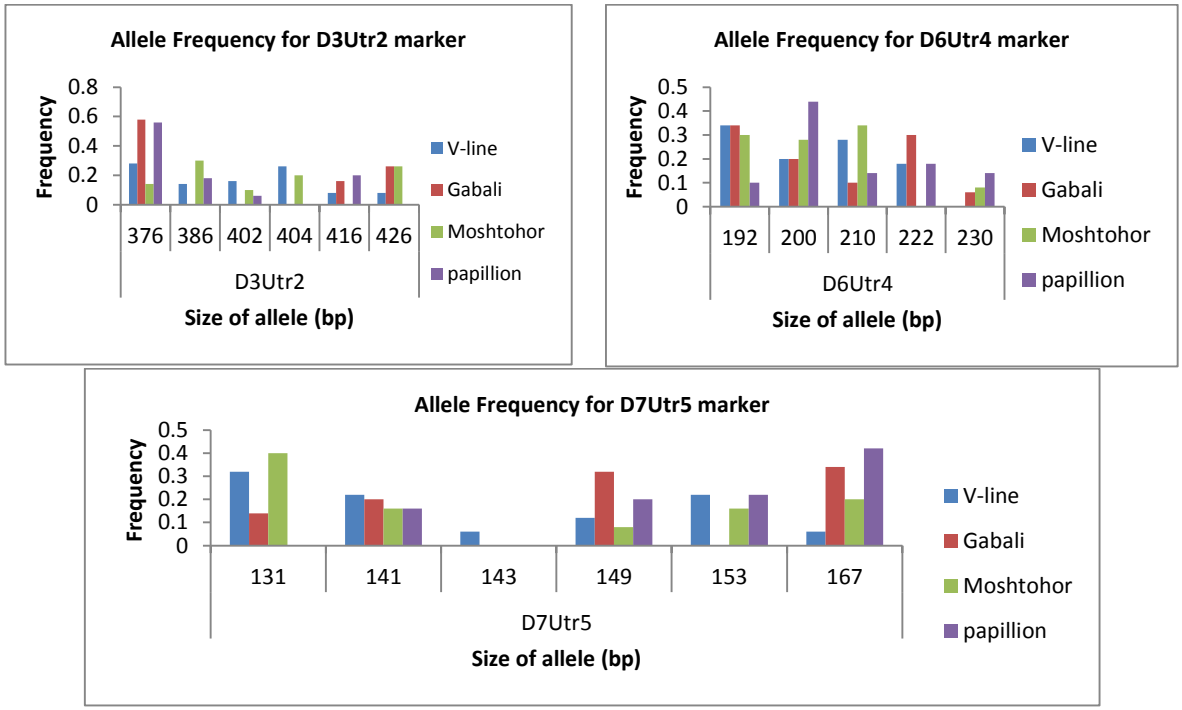


Figure4. The observed allelic frequency and size in each population of rabbits for the loci DUtr.

The observed and expected heterozygosity in each population

The observed (*Ho*) and expected (*He*) heterozygosities, and the fixation coefficient of an individual within a subpopulation (F_{IS}) are presented in Table 9. M-line had the highest value for *Ho* (0.742), and this might be attributed to its origin as a synthetic line compared with the other pure breeds. As a result of that, this line showed the lowest inbreeding coefficient (F_{IS}= -0.032). In contrast, Gabali breed had the lowest value of *Ho* (0.533) and the highest value of inbreeding coefficient (F_{IS}= 0.266).

The highest value of *He* was obtained for M-line (0.742), followed by V-line (0.734), while the lowest value was obtained for FGP breed (*He* = 0.675).The estimates of *Ho* was significantly less in Gabali breed compared with the other populations, however there were no significant differences in *He* among different populations.

As presented in Table 9, the estimates of *Ho* and *He* showed non-significant differences in rabbit populations studied except Gabali breed showed significant difference in *Ho* compared with the other populations.

Table 9. The observed (*Ho*) and expected (*He*) heterozygosities, and the fixation coefficient of an individual within the subpopulation (F_{IS}).

Population	N	*Ho* ±*SE*	*He* ± *SE*	F_{IS} ± *SE*
V-line	25	0.698 ±0.032[a]	0.734± 0. 014[a]	0.047±0.044[a]
Gabali	25	0.533 ±0.040[b]	0.695± 0. 029[a]	0.266±0.055[b]
M-line	25	0.758 ±0. 055[a]	0.742± 0.032[a]	-0.032±0.064[c]
FGP	25	0.618± 0.046[a]	0.675± 0.037[a]	0.083±0.050[b]
Overall mean ± SE	100	0.651 ±0.024	0.711 ±0.015	0.081±0.029

The estimate with the same letters in each column are not significantly different (p≤0.05); SE= standard error.

The fixation coefficient of an individual within a subpopulation (F_{IS}) illustrated significant differences within the populations. These results can be interpreted according to the walnut principle for hierarchical population structure.

The genetic distances among the populations

The estimates given in Table 10 illustrated that the highest value for Nei's genetic distance was recorded between V-line and FGP (0.35) followed by V-line and Gabali (0.29) and Gabali and M-line (0.27), while the closest pairwise Nei's genetic distance was recorded between V-line and M-line (0.18), followed by Gabali and FGP (0.19). The highest pairwise F_{ST} value (0.16) was recorded between V-line and FGP and between M-line and FGP rabbits, while the lowest pairwise F_{ST} value was recorded between Gabali and FGP (0.06) and also between V-line and M-line (0.08). These low levels of differentiations, are within the range reported in some literature **(Bolet *et al.*, 2002; Grimal *et al.*, 2012)**, while they are higher than those reported by **Carneiro *et al.* (2011)** for the European populations.In Egypt, **Osman *et al.* (2010)** assessed the genetic similarity coefficients among six rabbit breeds to be 0.648 between NZW and Black Rex, 0.685 between NZW and Hyplus, 0.648 between NZW and V-line, 0.721 between NZW and M-line, 0.807 between NZW and Gabali, 0.807 between Black Rex and Hyplus, 0.855 between Black Rex and V -line, 0.873 between Black Rex and M-line, 0.855 between Black Rex and Gabali, 0.765 between Hyplus and V-line, 0.786 between Hyplus and M-line, 0.807 between Hyplus and Gabali, 0.924 between V-line and M-line, 0.709 between V-line and Gabali and 0.797 between M-line and Gabali. **Tian-Wen *et al.* (2010)** reported that the range in F_{ST} was from 0.041 for the marker 6L3F8 to -0.195 for the marker SAT, while **Grimal *et al.* (2012)** found that the overall variation among populations (F_{ST}) was 0.137, where the NZW breed was the most differentiated population (F_{ST} = 0.194) relative to the Egyptian populations of Baladi Black, Baladi Red, Gabali and Giza White rabbits. **Ben Larbi *et al.* (2014)** found that the F_{ST} values between pairs of 12 rabbit populations across 36 microsatellite markers indicated high level of genetic differentiation, ranging from 0.03 to 0.28 and the overall genetic differentiation among the populations (F_{ST}) was low (1.1%), and this implies that 98.9% of the total genetic variation was explained by the individual variability.

Table 10. The estimates of Nei's genetic distance (below the diagonals) and the pairwise F_{ST} (above the diagonals) among the four populations using 16 microsatellite loci

Population	V-line	Gabali	M-line	FGP
V-line	0	0.11	0.08	0.16
Gabali	0.29	0	0.12	0.06
M-line	0.18	0.27	0	0.16
FGP	0.35	0.19	0.32	0

Neigbour-Joining phylogenetic tree showed that M-line and V-line rabbits were clustered together in one clade (Figures 5 & 6), which reflects the high genetic similarity between V-line (as the maternal parent of M-line) and its progeny, while the genetic distance between M-line and Gabali (as the paternal parent of M-line) was 0.27. This close relation was supported by the clustering pattern in the neighbor joining phylogenetic tree. The tree topology showed a close relation between V-line and M-line which might be explained by selection processes which has been conducted in the direction of V-line to improve reproductive traits specially the litter traits such as litter size and weight and milk production in M-line **(Iraqi *et al.*, 2009).** These results are confirmed by **Iraqi *et al.* (2008),** who stated that M-line is a synthetic line formed from crossing the Egyptian Sinai Gabali bucks (50%) and the Spanish V-Line does (50%).

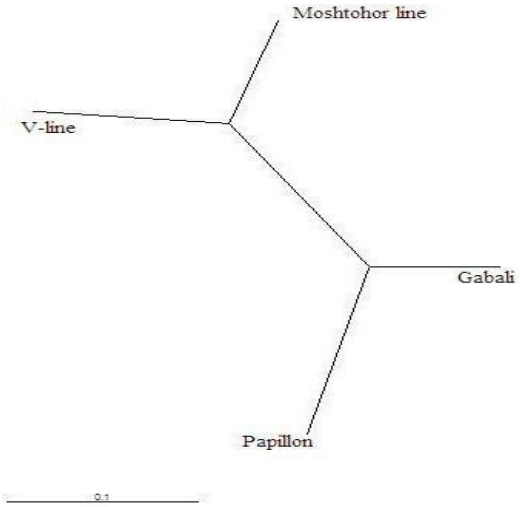


Figure 5. The Neigbour-Joining phylogenetic tree among the four rabbits populations based on 16 microsatellite loci. The numbers within the nodes are bootstrapping values from 1000 replicates across the set of loci.

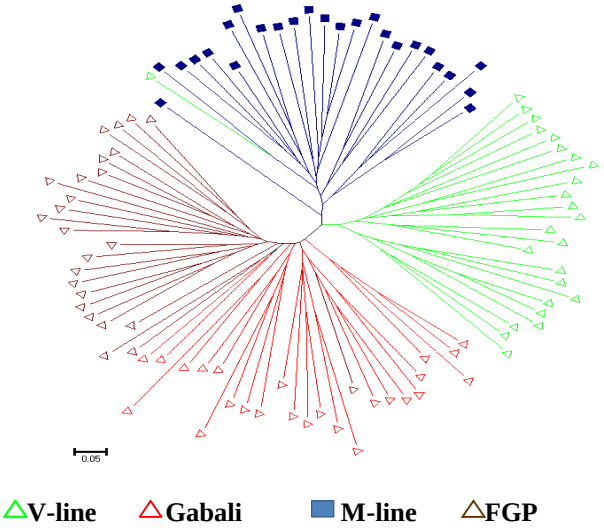


Figure 6. The Neigbour-Joining phylogenetic tree among 100 individuals using the allele shared distance based on 16 microsatellite loci.

In agreement with the present results, **Osman *et al.* (2010)** reported that the highest similarity coefficient was between V-line and M-line (0.924), followed by the similarity between Gabali and M-line (0.797), while the lowest similarity was between NZW and Black Rex, and NZW and V-line; and the dendrogram showed that V-line and M-line were close to each other while New Zealand White and Hyplus were more distant breeds.

Population structure clustering

The structure clustering of rabbit populations were clarified using STRUCTURE software and the most probable structure clustering the four rabbit populations studied was at $K = 2$ (Figure 7). The V-line and M-line rabbits brought together and showing robust relationship. This was because V-line is considered as one of the parents of M-line as stated before. However, selection processe was conducted in a direction of V-line to improve litter traits. So, V- line and M-line rabbits were clustered together forming admixed mosaic cluster.

A high degree of relationship was observed between Gabali and French Giant Papillon (Figure 7). Due to Gabali rabbits were raised in Sinai, north coast of western desert and western Giza Governorate and were considered as native Egyptian rabbits **(Mahmoud, 1938).** This could possibly explain,on the basis, that Gabali rabbits were developed by crossing with Flemish Giant rabbits. And, French Giant Papillon raised from crossing among the Flemish Giant, the spotted wild and the French Lop rabbits. This interprets, the relationship between Gabali and FGP, and this can be refer to the common ancestor (Flemish Giant rabbits).

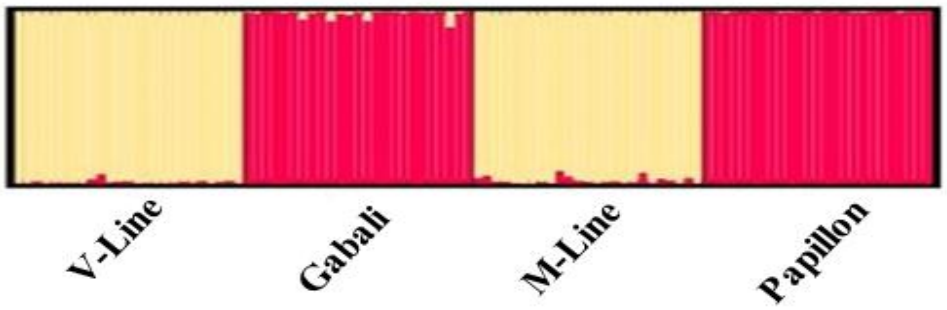


Figure 7.STRUCTURE clustering of the four rabbits populations obtained for K = 2.

Conclusions

1) All the microsatellite loci used were effective markers in detecting the genetic relationships among and within the rabbit populations investigated. Therefore, microsatellite markers can be used as an initial guide to detect the relationship between synthetic lines and their parents. For more efficacy, we can recommend that various sets of microsatellite primers could be used.
2) Among the four populations, M-line and V-line rabbits had the closest relationship which was confirmed with the estimates of the Neigbour-Joining phylogenetic tree.
3) This study provides the basic molecular information for the design of genetic improvement and conservation programs for the Egyptian breeds of rabbits investigated. In this concern, SNPs might be useful for future studies targeting the assessment of the genetic variation in rabbit population.

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References

Afifi, E.A. (2002). The Gabali rabbits. In Khalil MH, Baselga M (Eds). Rabbit Genetic Resources in Mediterranean Countries. Options Méditerranénnes, Series B, No. 38:51-64. http://www.iamz.ciheam.org/medrabbit/docs/gabali.pdf

Andersson, A.C., Thulin, C.G. and Tegelstrom, H. (1999). Applicability of rabbit microsatellite primers for studies of hybridization between an introduced and a native hare species. *Hereditas*. 130: 309-315.

Badr, Omnia A. (2015). Molecular analysis for some rabbit populations. *M.Sc*. Thesis, Faculty of Agriculture, Benha University, Egypt.

Ben Larbi, M., San-Cristobal, M., Chantry-Darmon, C. and Bolet, G. (2014). Population structure in Tunisian indigenous rabbit ascertained using molecular information. *World Rabbit Science*. 22: 223-230.

Berthouly, C., Bed'Hom, B., Tixier-Boichard, M., Chen, C.F., Lee, Y.P., Laloë, D., Legros, H., Verrier, E. and Rognon, X. (2008). Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. *Animal Genetic*s. 39: 121-129.

Bolet, G., Monnerot, M., Arnal, C., Arnold, J., Belle, D., Bergoglio, G., Besenfelder, U., Bosze, S., Boucher, S., Brun, J.M., Chanteloup, N., Ducourouble, M.C., Durand-Tardif, M., Esteves, P.J., Ferrand, N., Hewitt, G., Joly, T., Koehl, P.F., Laube, M., Lechevestrier, S., Lopez, M., Masoero, G., Piccinin, R., Queney, G., Saleil, G., Surridge, A., Van Der Loo, W., Vanhommerig, J., Vicente, J.S., Virag, G. and Zimmermann, J.M. (2000). Evaluation and conservation of european rabbit genetic resources: first results and inferences. *World rabbit Science*. 8: 281-315.

Bolet, G., Monnerot, M., Besenfelder, U., Bosze, S., Boucher, S., Ferrand, N., Hewitt, G., Joly, T., Lechevestrier, S., Lopez, M., Masoero, G., Van Der Loo, W., Vicente, J. and Virag, G. (2002). Inventory, characterisation and conservation of European rabbit genetic resources. *7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, Communication, 4-11*.

Brun, J.M. and Baselga, M. (2005). Analysis of reproductive performances during the formation of a synthetic rabbit strain. *World Rabbit Sci*., 13: 239-252.

Bunnyhugga 2010. Giant Papillon - Rabbit breeds library.http://www.bunnyhugga.com/a-to-z/breeds/giant-papillon.html

Carneiro, M., Afonso Sandra., Geraldes, A., Garreau, H., Bolet, G., Boucher, S., Tircazes, A., Queney, G., Nachman, W. and Ferrand, N. (2011). The genetic structure of domestic rabbits. *Molecular Biology Evaluation.* 28: 1801-1816. doi:10.1093/molbev/msr003

Duncan, David.B. (1955). Multiple range and multiple F tests. *Biometrics l l.* 1-42.

Estany, J., Baselga, M., Blasco A. and Camacho, J. (1989). Mixed model methodology for the estimation of genetic response to selection in litter size of rabbits. *Livestock. Prod. Sci.,* 21:67-76.

Estes-Zumpf, W., Rachlow, J. and Waits, L. (2008). Ten polymorphic microsatellite markers for the pygmyrabbit (*Brachylagus idahoensis). Molecular Ecology Resources*. 8: 360–362.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE, a simulation study. *Molecular Ecology*. 14, 2611-2620.

Falconer, D.S. and Mackay, T.F.C. (1996). Introduction to Quantitative Genetics. Longman, Essex, U.K., 4th Edition.

FAO (2004). Secondary guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD): Recommended microsatellite markers. http://www.fao.org/3/a-aq569e.pdf

Grimal, A., Safaa, H.M., Saenz-de-Juano, M.D., Viudes-de-Castro, M.P., Mehaisen, G.M.K.,Elsayed, D.A.A., Lavara, R., Marco-Jiménez, F., Vicente, J.S. (2012). Phylogenetic

relationship among four Egyptian andone Spanish rabbit populations based onmicrosatellite markers. *World Rabbit Congress, Sharm El-Sheikh, Egypt*, 177-181

Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Finlay, E.K., Jianlin, H., Groeneveld, E., Weigend, S. and the GLOBALDIV Consortium (2010). Genetic diversity in farm animals – A review. *Animal Genetics*. 41(s1):6-31.

Guo, S. and Thompson, E. (1992). Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, 48:361–372

Hailu, A. and Getu, A. (2015). Breed characterization tools and their applications. *International Journal of Genetics*. *5*(1):7-14.

Hongmei, S., Xiumei, X., Min, R. and Bo, C. (2008). Genetic diversity and phylogenetic relationship of rabbit breeds based on microsatellite DNA markers. *Biodiversity Science*. 16(5): 492-497. http://www.biodiversityscience.net/EN/abstract/abstract8488.shtml.

Iraqi, M.M., Shenana, M.E. and Baselga, M. (2007). Some factors affecting production and milk composition characters in a crossbreeding experiment involving Gabali and V- line rabbits in Egypt. *World Rabbit Sci.*, 15: 151 – 159.

Iraqi, M.M., Afifi, E.A., Baselga, M., Khalil, M. H. and García M. L. (2008). Additive and heterotic components for post-weaning growth traits in a crossing project of V-line with Gabali rabbits in Egypt. In Proc. *9th World Rabbit Congress*, Verona, Italy. 131-136.

Iraqi, M.M., Baselga, M., Khalil, M.H., Afifi, E.A. and García, M.L. (2009). Line Moshtohor (Egypt). In Khalil M.H., Baselga, M. (Eds). Rabbit Genetic Resources in Mediterranean Countries. *Options Méditerranénnes, Series B, Nº 38, Spain*; http://www.iamz.ciheam.org/medrabbit/docs/moshtohor.pdf

Jakobsson, M. and Rosenberg, N.A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics.* 23: 1801-1806.

Joly, T., Neto,V. and Salvetti, P. (2012). Cryopreservation of genetic diversity in rabbit species (*Oryctolagus cuniculus*). Current Frontiers in Cryopreservation, Prof. Igor Katkov (Ed.),179 – 186. http://www.intechopen.com/books/current-frontiers-incryopreservation/

Kalinowski, S.T., Taper, M.L. and Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*. 16: 1099-1106.

Khalil, M. H. (1999). Rabbit genetic resources of Egypt. *Animal Genetic Resources Information*. *26*: 95-111.

Khalil, M. and Baselga, M. (2002). Rabbit Genetic Resources in Mediterranean Countries. *Options Méditerranénnes, Series B, Nº 38*, pp 262, Spain,

Khalil, M.H., Motawei, M.I., Al-Saef, A.M., Al-Sobayil, K.A. and El-Zarei, M.F. (2008). RAPD markers linked to litter, lactation and growth traits in rabbits. *In proceedings of 9th World Rabbit Congress,Verona, Italy, paper No. G45.*

Korstanje, R., Gillissen, G.F., den Bieman, M.G., Versteeg, S.A., van Oost, B., Fox, R.R., van-Lith, H.A. and van-Zutphen, L.F.M. (2001). Mapping of rabbit chromosome 1 markers generated from a microsatellite-enriched chromosome- specific library. *Anim. Genet*., 32:308–312.

Korstanje, R., Gillissen, G.F., Versteeg, S.A., van Oost, B.A., Bosma, A.A., Rogel-Gaillard, C., van-Zutphen, L.F.M., van-Lith, H.A. (2003). Mapping of rabbit microsatellite markers using chromosome-specific libraries. *Journal of Heredity*. 94(2):161–169.

Langella, O. (2008). Populations, 1.2.30 Copyright (C), CNRS UPR9034.

Mahmoud, I.N. (1938). Bases of Veterinary Medicine, 2nd Edn. Cairo University, Egypt (In Arabic).

Markert, J. A., Champlin, D. M., Gutjahr-Gobell, R., Grear, J. S., Kuhn, A., McGreevy, T. J., Nacci, D. E., Roth Annette., Bagley, M.J. and Nacci Diane E. (2010). Population genetic diversity and fitness in multiple environments. *BMC Evolutionary Biology*. 10(1): 205.doi: 10.1186/1471-2148-10-205

Mougel, F., Mounolou, J.C. and Monnerot, M. (1997). Nine polymorphic microsatellite loci in the rabbit (*oryctolagus cuniculus*). *Animal Genetics*. 28: 58-71.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA, 70: 3321–3323.

Nei, M. Tajima, F. and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*. 19: 153–170.

Osman, M.M., Hemeda, S.A., Hassanin, Abeer A.I. and El-Aswad, A.H. (2010). Molecular genetic evaluation of six rabbit breeds by Random Amplified Polymorphic DNA (RAPD)-PCR. *SCVMJ*, XV., (1):1-11.

Peakall, R. and Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology*. Notes, 6: 288- 295.

Piles, M., Rafel, O., Ramón, J. and Gómez, E. A. (2004). Crossbreeding parameters of some

productive traits in meat rabbits. *World Rabbit Science*. 7: 59 – 64.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*. 155: 945-959.

Raymond, M. and Rousset, F. (1995). GENEPOP (version 1.2): population Genetics Software for Exact Tests and Ecumenicism. *J Hered*., 86 (3): 248-249.

Rico, C., Rico, I., Webb, N., Smith, S., Bell, D. and Hewitt, G. (1994). Four polymorphic microsatellite loci for the European wild rabbit, *Oryctolaguscuniculus*. *Animal Genetics*. 25: 367.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. 4: 406-425.

SAS (2002). SAS Version 9.0 (TS M0), SAS Institute Inc., Cary, NC, USA. http://ftp.sas.com/techsup/download/hotfix/90_updates.html

Schwartz, M.K., Pilgrim Kristine L., McKelvey, K.S., Rivera, P.T. and Ruggiero, L.F. (2007). DNA markers for identifying individual snowshoe hares using field collected pellets. *Northwest Science*. 81 (4): 316-322

Surridge, A. K., Bell, Diana J., Ibrahim, K. M. and Hewitt, G. M. (1999). Population structure and genetic variation of European wild rabbits (*Oryctolagus cuniculus*) in East Anglia. *Heredity*, 82: 479-487.

Tian-Wen, W., Gui-Jiang, X., Yu-Lai, P., Xi-Ping, X., Bi-Chun, L. and Xin-Sheng, W. (2010). Study on gennetic diversity of 7 rabbit populations evidenced by microsatellite makers. *Journal of Animal and Veterinary Advances. 9*: 359-365.

Xin-Sheng, W., Tian-Wen, W., Hui –ling, Z., long, C.G., Qi, X., Jin- Hua, C., Xiu-Bai, Z. and Guo-Hong, C. (2008). Correlation analysis of wool yield in Wan line Angora rabbits using microsatellite DNA markers. *Journal of Biological Sciences*. 8(3): 679-682.

Zenger, K.R., Richardson, B.J.andVachot-Griffin, A.M. (2003). A rapid population expansion retains genetic diversity within European rabbits in Australia. *Molecular Ecology*. 12: 789-794.