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Genetic Survey of the Most Distributed Commercial Rabbits Along The Nile in



Egypt: A Case Study by Microsatellite Markers

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Abstract

THE investigation of genetic diversity is vital for managing farm animals. Hence, the current study examined the genetic status of the most rabbit lines distributed commercially along 804 km of the surrounding villages on the Nile River. The results of survey showed that the percentages of the Verde line (VL), Hyplus line (HL), Alexandria line (AL), and New Zealand (NWZ) were 79, 70, 64, and 53%, respectively. According to previous result, 338 biological samples from previous lines (86 for VL, 84 for LA, 83 for HL, and 85 for NWZ) were collected from three populations for each rabbit line in Delta (D), Middle Egypt (M), and Upper Egypt (U) to determine the genetic variability using 26 microsatellite markers. According to results of microsatellite loci, the total number of recorded alleles was 166. Among rabbit lines, the mean number of alleles (MNa) was the lowest in NWZ (5.772) while it was highest in AL (7.154). Furthermore, the south populations (U) expressed high values of private alleles (Pa) and all populations (7, 5, 4, and 3 for AL, VL, HL, and NWZ, respectively). It was noticed that observed heterozygosity (H_0) expected (H_e) among all lines and populations. The inbreeding coefficient (F_{IS}) recorded the highest value in NWZ (0.178) with bottleneck observation. In contrast, other lines were in negative values. Our findings show that the generated information is relevant for rabbit breeding and could be used as a guide in genetic improvement programs in Egypt.

Keywords: Commercial Rabbits, Egypt, Nile, Microsatellite, Genetic, rural areas.

Introduction

Rabbit husbandry projects meet the sustainable and alternative classifications [1]. It would likely succeed compared to other animal activity projects [2]. For farmers with little resources, it might promote food security, particularly in less developed nations (LDCs) like Cameroon, Egypt, Ghana, and Mexico [3]. The rabbit industry plays an important role in the agriculture sector in most Mediterranean countries [4]. Egypt is the third country in rabbit production by 75000 tyr-1 in 2021 [5]. It is considered the ideal project for improving the livelihood in rural areas for fast-growing and high productivity in rural areas [6].

The genetic variables of most farm animal species are suffering from a drastic decrease [7, 8]. According to FAO [9], 30-40% of farm animal genetic resources are necessary economically in the agriculture sector (FAGRs). The maintenance and documentation of FAGR's assistant decision-makers

in sustainable strategy design in developing countries [10]. In addition, it is considered a key to survival that could occur based on overall information regarding genetic variability and population structure [11]. Biodiversity plays an important role in the ecosystem by providing several services nutrition, water cycling, soil formation, and regulation of abiotic stress resistance [12]. Global warming affects genetically and physiologically animals, plants, and [13]. microorganisms Genetic markers are considered a wide method to identify species and to assess relationships among different populations which helps to estimate genetic status in a specific geographic area [12, 14].

Microsatellite markers are an important method for estimating genetic variability (novel mutation, genetic drift, bottleneck position, gene flow, genetic structure, and genetic distance) [15]. It contributes to enhance selection, particularly for the maintenance of

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animal breeds/ populations which ensures long-term food security[14]. This reflects on maximizing the productivity under several challenges such as changes in environmental hazards, marketing fluctuating, management, and vulnerability to certain diseases [16, 17].

Thus, the current study aims to evaluate the genetic variability, structure, and relationship among the commercial rabbit lines widely used in rabbit production along the Nile River in Egypt using 26 microsatellite markers.

Material and Methods

Rabbit survey

In this study, about 12.1 thousand bucks and does belonging to 89 commercial rabbit farms survived. These farms were in 47 rural centers along the Nile River as representative samples to determine rabbit lines distribution along the Nile in Egypt. The survey covered 3 main provinces that demonstrated 11 Egyptian governorates. They were Delta (Beheira, Monufia, Gharbia, Qalyubia, Dakahlia, Damietta, and Kafr El sheikh), Middle Egypt (Giza, Bani Suef, and Minya) and Upper Egypt (Asyut, Sohag, and Qena). Three governorates (Alexandria, Luxor, and Aswan) were excluded from the current study because the recording system was not available.

Samples Collection and DNA Extraction

A total of 338 biological samples (tissue specimens and hair bulbs) were collected from widely rabbit lines distributed in commercial farms along the Nile (Fig. 1). Rabbit samples in current study were not related, and if they were, the only relationship from one parent with only one individual was considered from the offspring. The diversity for each rabbit line was examined in 3 different populations: Delta (D), Middle Egypt (M), and Upper Egypt (U). The commercial farms that use a recording system were included in the study to guarantee the purity of breeding. The extraction of DNA from rabbit samples was carried out using the NaOH method [18].

Microsatellites DNA Markers Selection and Polymerase Chain Reaction (PCR)

Twenty-sex microsatellite markers (Invitrogen, France) were classified into five multiplexes, were used in this study. The conditions of PCR reaction conditions for each multiplex were shown in Table S.1. The presence of PCR products was analyzed by a horizontal gel electrophoresis system (mini gel, Biometra[®]EU), using 2% agarose gel stained with ethidium bromide. The fragments size was determined using a genetic analyzer (ABI PRISM 3730 XL; Applied Biosystems).

Data Analysis

The estimated number of alleles per microsatellite marker (Na), observed at each microsatellite marker and the mean numbers of alleles (MNa), number of private alleles (Pa), observed and expected heterozygosity (H_o and H_e) were calculated by GENAIEX 6.4.1 program [19]. The calculation of allelic richness (Ar) and estimation of the inbreeding co-efficient within populations (F_{IS}) was carried out by FSTAT 2.9.3.2 software [20]. The situation of bottlenecks was detected by BOTTLENECK software v.1.2.02 [21] by1000 simulations as the probability distribution (Wilcoxon-P> 0.05) through the two-phase model (TPM) and stepwise mutation model (SMM) methods. The mode shift indicator based on the shape of the allele frequency distribution was also performed. The neighborjoining (NJ) tree was visualized in Mega tree explorer according to the Reynolds matrix [22]. The significance of the Hardy-Weinberg equilibrium (HWE) and polymorphism information content (PIC) calculation was carried out by Cervus 3.0.6 software [23]. The population structure evaluation was based on a Bayesian clustering analysis by the STRUCTURE 2.3.4 program [24] upon independent runs using 100000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 30000 steps and was also performed for 2≤K≤12 (K=number of assumed clusters). Evanno methods were used to compute the statistic ΔK was computed by graphic explanation [25].

Results

Data Survey

The percentages of rabbit lines distribution at commercial levels (Fig. 2). The survey results showed that the Verde Line (VL), Hyplus Line (HL), Alexandria Line (AL), and New Zealand (NWZ) were widely distributed commercially in survived rabbit farms (79, 70, 64, and 53%, respectively). Other commercial rabbits such as Flemish Giant (FG), California (CL), and Papillon (PAP) were found in lower percentages (36, 32, and 30%, respectively). We observed that other local Egyptian lines APRI, Moshtohour (ML), Red and black Baladi was limited distribution at commercial levels with 21%.

General population statistic

The summary of genetic variability for rabbit lines along the Nile is shown in Table 1. The mean values of MNa, Pa, and Ar were 6.379, 3.33, and 4.426, respectively. Moreover, the mean value of H_e is lesser than that of H_o (0.398 and 0.557, respectively). In this regard, AL showed the highest variability, while NWZ was the lowest. The value of MNa ranged from 5.772 in the NWZ to 7.313 in the AL. The mean values of Pa and Ar ranged from 2.000 and 3.999 in the NWZ and 4.333 and 5.068 in the AL, respectively. The mean values of H_e and H_o were 0.398 and 0.557, respectively. In all rabbit

lines, U populations expressed the highest genetic variability than others (D and M).

Inbreeding coefficient and bottleneck test

As shown in Fig. 3 a, the F_{IS} values for rabbit lines appeared in the significant negative (*0.190, *0.192, and *0.211 for AL, VL, and HL, respectively) except NWZ was in the positive values (0.178). In the same context, the F_{IS} was higher in Delta populations on all lines and reduced towards the south (Fig. 3 b). In Fig. 4 (a and b) the absence of bottleneck was found in VL, AL, and HL according to Wilcoxon signed-rank tests (P > 0.05), while it was observed in NWZ. The mean values of TMP SMM, and IAM varied from 0.462, 0.298, and 0.222 to 0.323, 0.254, and 0.179(NWZ and AL, respectively).

Genetic parameters of Microsatellite markers

A total of 166 alleles were observed in the total samples at 26 microsatellite markers (Table 2). All microsatellites showed a polymorphism across the tested lines. The values of Na and *PIC* varied from 0 to 14 and 0.274 to 0.865 for INRA140 and INRA205, respectively. About 73% of microsatellite markers were highly informative (*PIC*> 0.5), while 27% represented moderately informative (0.25<*PIC*<0.5). Moreover, 69.2% of microsatellite markers showed non significance of HWE, whilst. 30.8% showed differences at the 3 levels (*P*<0.05; *P*<0.01 and *P*<0.001).

Relationships among rabbit lines and structure

As shown in Fig. 5, a neighbour-joining tree is visualized the genetic relationships based on genetic distances among lines. The results showed two main clusters: the first cluster included AL, VL, and NWZ. Figure 6 illustrated that the structure analysis results depending on Bayesian approach and the number of clusters (K). The highest values of ΔK were obtained when k=12 (41.82) shows the clustering pattern arising from the analysis. The number of predefined clusters varied from K2 to K12.

Discussion

The wide distribution of exotic rabbit lines commercially is due to the higher productive and reproductive performance than Egyptian lines [26]. Moreover, the current survey showed that the Alexandria line (AL) was distributed by 64%, this may be due to the high body gain in 28-63 days and adapted under the Egyptian climate [27]. Although high residence of abiotic stresses, other Egyptian rabbit lines were limited distributed for low production and reproduction traits [28].

The highest genetic variability values were recorded in the AL line. The same observation was stated in the ML was synthesized recently as AL lines (29). However, the lowest was recorded in NWZ for underwent to several selection programs that were carried out since 80's in Egypt (30, 31). The selective intense breeding programs decreased the genetic variability in commercial breeds (32). Among all populations, we observed that H_o> H_e, which might be attributed to an absence of inbreeding status in most populations is an indicator of the isolate-breaking effect inside each line population [33]. Furthermore, the current study showed that the highest genetic variability in all lines expressed in the southward direction was characterized by warm weather. The warm weather motivates mutations that cause genetic variability [34-37]. In the same context, it could be explained the increase of Ar values in Upper Egypt populations could be due to mutation richness.

The F_{IS} values were found in negative values in three lines (AL, VL, and HL) which is an indication of inbreeding avoidance [38]. The values of bottleneck nearby agree with European commercial rabbits [39]. The negative values of F_{IS} with the absence of a bottleneck position concur [40].

In Table 2, the high percentage of non-significant differences in HWE confirms the large size of populations and random mating [41]. Most microsatellite markers showed high informative polymorphism (73%) which is evidence of a genetic polymorphism and linkage mapping [42].

Figure 5 shows the relationship among 4 Lines. In the first cluster, 3 Lines (AL, VL, and NWZ) were occupied. According to previous literature, AL contained 87.5% of VL and 12.5% of Black Baladi [26, 27, 43]. In addition, VL resulted after four specialized selections for the Spanish maternal A line that were synched from NWZ rabbits [44]. The second cluster included the HL line only. Because that HL line was bred from several other meat-type rabbits [45].

According to structure results (Fig.6), the stability of Upper Egypt populations was noticed. Also, the classification according to geographical areas was reported in the Algeri .We conclude that commercial rabbit farms are dependent on AL, VL, HL, and NWZ. Commercial rabbit lines along the Nile River are characterized by high genetic variability and stability with low inbreeding and the absence of genetic bottleneck in three lines (AL, VL, and HL) in contrast, NWZ is suffering from inbreeding and genetic bottleneck. In addition, these findings could be used as an initial guide to design further investigations for the improvement of rabbit lines' genetic improvement programs for commercial rabbit lines. The findings from this study can assist researchers in drawing a sustainable agriculture strategy that could contribute to the rabbit industry development in rural areas in Egypt. Also, it will meet the first and second goals of the United Nations' sustainable development agenda for 2030

(no poverty and zero hunger) which could achieve food security and improved nutrition of rabbits [36].

Conclusion

We conclude that commercial rabbit farms are dependent on AL, VL, HL, and NWZ. Commercial rabbit lines along the Nile River are characterized by high genetic variability and stability with low inbreeding and the absence of genetic bottleneck in three lines (AL, VL, and HL) in contrast, NWZ is suffering from inbreeding and genetic bottleneck. In addition, these findings could be used as an initial guide to design further investigations for the improvement of rabbit lines' genetic improvement programs for commercial rabbit lines. The findings from this study can assist researchers in drawing a sustainable agriculture strategy that could contribute to the rabbit industry development in rural areas in Egypt. Also, it will meet the first and second goals of the United Nations' sustainable development agenda

for 2030 (no poverty and zero hunger) which could achieve food security.

Acknowledgment

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Declaration of Conflict of Interest:

The authors declare that there is no conflict of interest.

Ethical Considerations

The present study has been conducted by the guidelines of the Ethics Committee of The International Animal Care and use Committee (IACUC) Faculty of science, Menoufia University, Egypt, Approval №: MUFAG/F/AP/2/21.



Fig. 1. Geographical sampling locations along the Nile River in Egypt.

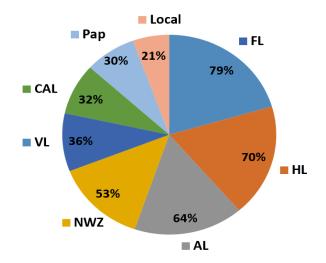


Fig. 2. The Percentages of Commercial Rabbits Distributions in Farms

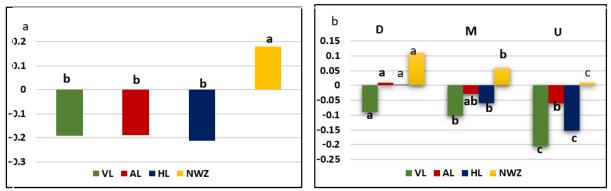


Fig. 3. The values of inbreeding coefficient (F_{IS}). a. The mean values of F_{IS} in rabbit lines. b. The comparative of F_{IS} in each geographical area. Verde Line (VL), Alexandria line (AL), Hyplus Line (HL), New Zeland (NWZ), Delta (D), Middle Egypt (M), and Upper Egypt (U).

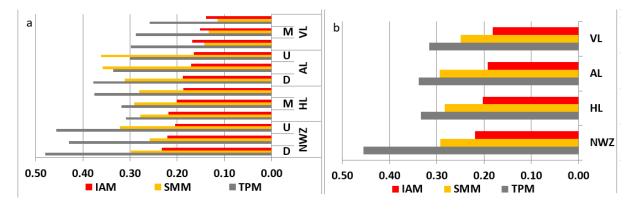


Fig. 4. a. Bottleneck test among lines, b. Bottleneck test among populations. VLine (VL), Alexandria line (AL), Hyplus Line(HL), New Zeland (NWZ), Delta (D), Middle Egypt (M), Upper Egypt (U), Infinite Allele Model (IAM)Two phase model (TMP), Step wise mutation model (SMM).

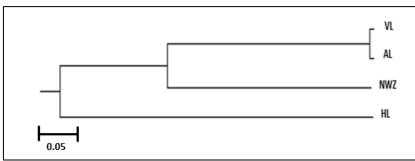


Fig. 5. Neighbor-joining tree for the rabbit populations. Verdi Line (VL), Alexandria line (AL), Hyplus Line(HL), New Zeland (NWZ).

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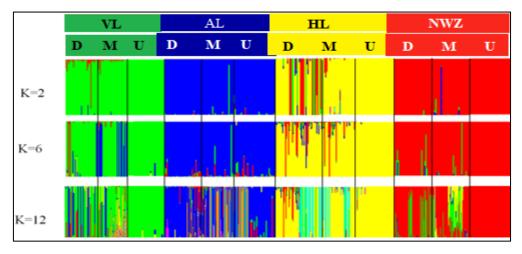


Fig. 6. Individual assignment of domestic rabbits inferred with Bayesian cluster analysis (STRUCTURE). Verde Line (V L), Alexandria line (AL), Hyplus Line (HL), New Zeland (NWZ), Delta (D), Middle Egypt (M), and Upper Egypt (U).

TABLE 1. Genetic variability for the most rabbit lines commercially distribution along the Nile River in Egypt.

Line	Population	Ν	MNa± SD	Pa	Ar± SD	H _e ± SD	$H_o \pm SD$
VL	D	30	5.885 ± 0.274	2	3.711 ±0.220	0.350 ± 0.033	0.510 ± 0.025
	М	25	6.000 ± 0.200	4	3.982±0.194	0.380 ± 0.033	$0.529 \ \pm 0.027$
	U	31	6.231 ±0.339	5	4.193 ± 0.204	0.385 ± 0.030	0.582 ± 0.032
	Mean Valu	ies	6.038 ±0.271	3.667	3.962 ± 0.366	0.372 ± 0.032	0.540 ± 0.028
AL	D	32	7.253 ± 0.330	2	5.866 ± 0.361	0.435 ± 0.027	0.604 ± 0.030
	М	25	7.196 ±0.304	4	4.365±0.301	0.440 ± 0.035	0.604 ± 0.030
	U	27	7.492 ± 0.460	7	4.981 ±0.307	0.445 ± 0.042	0.578 ± 0.035
	Mean Valu	ies	7.154 ±0.364	4.333	$5.068{\pm}0.523$	0.442 ± 0.034	0.595 ± 0.035
HL	D	31	6.462 ±0.237	3	4.698±0.303	0.445 ± 0.042	0.588 ± 0.035
	М	24	6.557 ± 0.278	3	4.512 ± 0.333	0.363 ± 0.033	0.561 ± 0.033
	U	28	6.642 ± 0.304	4	4.82 2±0.393	0.386 ± 0.031	0.602 ± 0.029
	Mean Valu	ies	6.553 ±0.273	3.333	$4.677{\pm}0.443$	0.398 ± 0.035	0.583 ± 0.032
NWZ	D	28	5.145 ±0.190	1	3.391 ±0.239	0.350 ± 0.040	0.397 ± 0.034
	М	27	5.885 ± 0.224	2	3.696 ± 0.255	0.380 ± 0.037	0.595 ± 0.023
	U	30	6.308 ± 0.336	3	4.392 ± 0.301	0.355 ± 0.044	0.584 ± 0.033
	Mean Valu	ies	5.772 ±0.250	2.000	3.999 ± 0.265	0.379 ± 0.046	0.509 ± 0.033
	Overall mean		6.379±0.284	3.333	4.426±0.301	0.398 ± 0.033	0.557±0.038

Verde Line (VL), Alexandria line (AL), Hyplus Line (HL), New Zealand (NWZ), Delta (D), Middle Egypt (M), Upper Egypt (U), Number of samples (N), Mean number of observed alleles (MNa), Stander deviation (SD), number of private alleles (Pa), Allelic richness (Ar) mean observed and expected heterozygosity (H_e and H_o).

TABLE 2. The characteristics of 26 microsatellite markers used for rabbit lines along the Nile.

Multiplex	Microsatellite loci	Na	Allele range	H _e ± SD	$H_o \pm SD$	Mean <i>PIC</i>	HWE
1	INRA106	6	122-134	0.353 ±0.039	0.582 ± 0.028	0.645	Ns
	INRA176	6	227-240	0.118 ± 0.022	0.323 ± 0.064	0.388	***
	INRA203	6	204-216	0.357 ± 0.053	0.630 ± 0.036	0.650	Ns
2	INRA087	12	190-216	0.596 ±0.073	0.725 ±0.051	0.852	Ns
	INRA119	2	229-243	0.342 ± 0.054	0.486 ± 0.048	0.518	*
	INRA140	0	183-189	0.458 ± 0.036	0.490 ± 0.023	0.274	Ns
	INRA157	7	138-144	0.401 ± 0.033	0.585 ± 0.039	0.609	***
	INRA190	1	200-210	0.126 ± 0.034	0.365 ± 0.031	0.455	Ns
	INRA201	9	133-143	0.371 ± 0.032	0.512 ± 0.039	0.498	Ns
	SAT12	6	144-164	0.276 ± 0.037	0.584 ± 0.028	0.582	***

Multiplex	Microsatellite loci	Na	Allele range	H _e ± SD	$H_o \pm SD$	Mean PIC	HWE
3	SAT 03	10	146-162	0.508 ± 0.037	0.595 ± 0.027	0.687	***
	SAT 04	8	195-241	0.426 ± 0.054	0.642 ± 0.051	0.701	Ns
	SAT 05	7	206-232	0.338 ± 0.044	0.505 ± 0.047	0.501	Ns
	SAT 07	7	184-194	0.388 ± 0.044	0.573 ± 0.041	0.675	Ns
	SAT 08	3	136-156	0.309 ± 0.024	0.470 ± 0.036	0.471	**
4	INRA102	6	128-136	0.542 ±0.033	0.669 ± 0.037	0.610	Ns
	INRA104	7	117-127	0.410 ± 0.028	0.606 ± 0.036	0.701	Ns
	INRA169	8	174-180	0.494 ± 0.049	0.623 ± 0.048	0.699	Ns
	INRA185	5	166-175	0.366 ± 0.045	0.420 ± 0.094	0.438	Ns
	INRA192	8	114-132	0.607 ± 0.029	0.643 ± 0.026	0.699	***
	INRA205	14	178-190	0.566 ± 0.044	0.719 ± 0.037	0.865	***
	INRA228	5	228-232	0.356 ± 0.063	0.415 ± 0.052	0.423	Ns
	SAT13	4	114-126	0.411 ±0.029	0.530 ± 0.036	0.575	Ns
5	INRA259	6	173-195	0.518 ±0.054	0.609 ± 0.039	0.666	Ns
	INRA313	7	239-275	0.450 ± 0.026	0.619 ± 0.020	0.695	Ns
	INRA342	6	170-196	0.458 ± 0.046	0.581 ± 0.043	0.594	Ns

Number of observed alleles (Na), mean observed and expected heterozygosity (H_o and H_e), Stander deviation (SD), mean *PIC*, mean polymorphism information content per microsatellite marker and Hardy-Weinberg Equilibrium (HWE). **P*<0.05; ***P*<0.01,*** *P*<0.001, NS: non-significant.

TABLE S1. Basic information of microsatellite markers and PC	R reaction
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Multi- plex	Locus	Accession №	Repeat Pattern	Chromo- some	Temperature	Time	Cycles
1	INRA106	AJ874448	(CA)14	13	95	15 m	
	INRA176	AJ874514	(TC)11(TG)13AG(TG)3	12	95	20 s	7X
	INRA203	AJ874540	(GT)16	3	63 - 56 (↓10)	1 min	
					72	45 s	
					95	30 s	35X
					55	30 s	
					72	45 s	
					60	15 m	
2	INRA087	AJ874430	(TG) ₁₄	8	95	15 m	
	INRA119	AJ874461	(GT) ₁₆	18	95	30 s	7X
	INRA140	AJ874480	(TG) ₁₄	14	61 - 55 (↓10)	45 s	
	INRA157	AJ874497	(GT) ₁₂	8	72	45 s	
	INRA190	AJ874528	(TG) ₁₂	18	95	30 s	37X
	INRA201	AJ874538	(TG) ₁₄ (AG) ₁₀	12	55	45 s	
	SAT12	X99891	(CTAT) ₁₀	7	72	1 min	
					60	20 m	
					10	x	
3	SAT 03	J03744	(TC) ₂₂	3	95	15 min	
	SAT 04	M33582	(TC) ₁₃ (N) ₂ (TC) ₂ TG(TC) ₇	15	95	30 sec	32x
	SAT 05	X99887	(TC) ₂₃ TTT(CT) ₅	3	60	30 sec	
	SAT 07	X99889	(CT)14(GT)8TT(GT)5	17	72	45 sec	8x
	SAT 08	X99888	(TG) ₁₄	10	95	30 sec	
					53	30 sec	
					72	45 sec	
					60	20 min	
					12	œ	

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Multi- plex	Locus	Accession №	Repeat Pattern	Chromo- some	Temperature	Time	Cycles
4	INRA102	AJ874444	(AC) ₁₈	19	95	15 m	
	INRA104	AJ874448	(GT) ₁₆	108	54	45 s	
	INRA169	AJ874508	(CA) ₁₇	1	95	30 s	8x
	INRA185	AJ874523	(AC) ₁₃	4	72	45 s	
	INRA192	AJ874530	(TG ₎₁₁	96	95	30 s	31x
	INRA205	AJ874542	(TG) ₁₇	4	60	15 m	60
	INRA228	AJ874561	(TG) ₁₂	-			
	SAT13	X99892	(GT) ₁₃	1			
5	INRA259	AJ874589	(GT) ₁₄ (GA) ₉	1	95	15 m	
	INRA313	AJ874634	(TG)7(AC)10GC(AC)6GC(14	95	30 s	8X
	INRA342	AJ874659	(GA)22	13	63 - 56 (↓10)	45 s	
					72	45 s	
					95	30 s	28X
					56	30 s	
					72	45 s	
					60	20 m	
					10	x	

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الملخص

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