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THE GENETIC VARIATION AND POLYMORPHISM AT MICROSATELLITE LOCI IN CHICKENS OF WARM REGIONS SELECTED FOR MEAT PRODUCTION

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ABSTRACT

In a research project aiming at the genetic improvement of warm-region originated chickens, a breeding program was practiced on naturally heat-resistant local population in Egypt and four lines have been derived. The lines were the homozygous normally-feathered selected (CE1) and control (CE2) lines, and the homozygous naked-neck selected (CE3) and control (CE4) lines. Lines CE1 and CE3 have been selected for 6-wk body weight for eight generations. The objective of this study was to assess the genetic features of the selected lines at the microsatellite loci recognized by 27 microsatellite primers in generations 6, 7 and 8.

The number of alleles detected by each primer varied from one to seven. The number of alleles per primer and generation averaged 5.72, 2.35, 5.98 and 2.57 in lines CE1, CE2, CE3 and CE4, respectively. The genetic variability was in general low among lines and ranged 0.17–0.20. Polymorphic information content (PIC) averaged 0.50, .39, 0.55 and 0.50 in lines CE1, CE2, CE3 and CE4 respectively, and line-specific alleles (LSA) formed 4.3, 0.7, 4.6 and 1.2% of total alleles in corresponding lines. The differences between each selected line and its control line in PIC and LSA were significant, except between line CE3 and line CE4 in PIC. The results denoted to the possible linkages between the detected microsatellite loci and QTL for body weight. By generation 8, the genetic distance indices between line CE1 and line CE2 averaged 0.740, and between line CE3 and line CE4 averaged 0.815. The phylogenetic dendograms revealed the genetic progress of the selected lines over subsequent generations.

Keywords: Microsatellite Alleles, Polymorphism, Selection, Variability, Warm-Region Chickens

INTRODUCTION

Despite local chicken breeds have the genetic compositions that enable them to adapt the local environments and have further significance for sustainable

development **[1-4]**, they have not been genetically improved in commercial sense. Several genes in different local breeds contribute to the adaptation process to certain environmental conditions. The naked-neck (Na) gene reduces the feather mass over the body surface and total loss of feathers in the neck region, and accordingly heat dissipation increases when ambient temperature is elevated **[5-7]**. The naked-neck birds may therefore be able to withstand the high ambient temperatures **[8-9]**.

During the 20th century, tremendous changes occurred in poultry breeding, much of which were focused on understanding poultry genetics and the applications of quantitative genetics **[10]**. The applications of molecular genetics are expected to dominate the theory and practice of poultry breeding during the 21st century, because it enhances the accuracy of genotype identification **[11-12]**. So, the molecular techniques are becoming important components to improve the efficiency of breeding programs, and classical breeding may remain the cornerstone for the development of breeding strategies in poultry **[13-14]**. The use of techniques of molecular genetics in selection programs rests on the ability to determine the genotypes and breeding values of individuals from the genomic information **[15]**. It is well documented that accurate determination of the genetic variations within and between breeds is a fundamental step toward animal breeding **[3, 16]**. Molecular techniques have been applied to a wide variety of poultry species to assess the genetic variation. The DNA fingerprints were used to assess the genetic variation between several chicken breeds in comparison with a population of red jungle fowl **[17]**. The genomes of two chicken lines, selected for susceptibility and resistance to heat stress conditions, were scanned by restriction fragment length polymorphism markers, where specific bands for each line were detected **[18]**. The genetic variability in Egyptian and Iranian chicken breeds were also evaluated by DNA fingerprinting **[19-21]**. The random amplified polymorphic DNA (RAPD) was applied to assess the genetic specificity of the native Egyptian and exotic chicken and duck breeds in relation to the subtropical conditions **[2, 3]**. Four RAPD markers to characterize the genetic similarity among Malaysian chickens **[22]**. High level of genetic variation was observed among the village chickens of different locations. Also, the genetic diversity of red jungle fowl in India was studied, using 15 microsatellite markers **[23]**. The total number of detected alleles was 173 and the mean allelic number per locus was 11.53. **[24]** Scanning the

genomes of two Egyptian chicken lines, normally feathered and naked-necks, revealed a genetic distance of 1.03 between both lines after two generations of selection for 6-week body weight **[24]**. Also, the chromosomal regions associated with growth traits have been identified in a cross of chicken lines divergently selected for body weight **[25]**.

In Egypt, selection of two local lines of chickens for 6-week body weight have been practiced for 8 generations in natural warm environmental conditions **[26]**. The objective of this study was to assess the genetic features of the selected lines at the microsatellite loci in generations 6, 7 and 8.

MATERIALS AND METHODS

Breeding Stocks

The data set presented in this study represent generations 6, 7 and 8 of a selection scheme for 6-week body weight in naturally heat resistant native chickens in Egypt **[26]**. The prenatal stock was established in 2000 by crossing between the grandparents of a commercial broiler male line and a heterozygous naked-neck local chicken population, and then four lines have been derived from the crossbred population. Lines CE1 and CE2 are the normally-feathered selected and random-bred control lines. Lines CE3 and CE4 are the homozygous naked-neck selected and random-bred control lines, respectively.

Generation Sampling

In generation 6, blood samples were collected from 10 males and 10 females in each of the selected lines. In the generations 7 and 8, blood samples were collected from all individuals of 6 sire families in each of the selected lines. The sire families were assigned based on their average 6-week body weights and the average number of offspring per family was 20 individuals. For the control lines, blood samples were drawn from 10 males and 10 females randomly assigned in each generation. All blood samples were drown from the wing veins and collected in 5-ml tubes containing EDTA. Blood samples were immediately stored at -20ºC until use.

Genome Analysis

DNA Extraction

Upon use, the blood samples were thawed and used to extract genomic DNA according to Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI 53711-5399, USA). For this, 300 µl of each blood sample were put in a sterile 1.5-ml micro-centrifuge tube and 900 µl of cell lysis solution were added to the blood, and the contents were mixed thoroughly. Then, the mixture was incubated for 10 minutes at room temperature during which it was shacked thoroughly, and then centrifuged at 13,000 –16,000 rpm for 20 seconds at room temperature. The supernatant was then

removed without disturbing the visible white pellet. The pellet was shacked by a vortex for 10–15 seconds. Three hundred µl of nuclei lysis solution were added to the resuspended cells and mixed by inversion. Then, 1.5 µl of RNase solution were added to the nuclear lysate, and mixed thoroughly 2–5 times. The mixture was incubated at 37°C for 15 minutes, and then cooled to room temperature. One hundred µl of the protein precipitation solution was added to the nuclear lysate, vigorously shacked by vortex for 10–20 seconds, and centrifuged at 13,000 –16,000 rpm for 3 minutes at room temperature, where a dark brown protein pellet became visible. The supernatant was transferred to a sterile 1.5-ml micro-centrifuge tube containing 300 µl of room-temperature isopropanol. The solution was mixed gently by inversion until the white thread-like strands of DNA formed visible mass. The supernatant was centrifuged at 13,000–16,000 rpm for one minute at room temperature, and DNA was seen as a small white pellet. The supernatant was removed and one sample volume of ethanol (70%) was added to DNA, at room temperature. The mixture was gently inverted several times to wash the DNA pellet, and then centrifuged at 13,000–16,000 rpm for one minute at room temperature. The ethanol was then aspirated using a sequencing pipette tip. The DNA pellet was left to air-dry for 10 –15 minutes. One hundred µl of DNA rehydration solution was added to the tube and incubated at 65°C for one hour. The DNA concentration was determined according to the protocol of Sambrook and colleagues **[27]**, using a spectrophotometer (FLUOstar OPTIMA F-Microplate Fluorimeter, Offenburg, Germany).

Microsatellite-PCR Procedures and Electrophoresis

In generation 6, the individual DNA samples of each selected line were randomly used to form two pooled samples based on their DNA concentrations. In generations 7 and 8, the individual DNA samples of each family in each selected line were mixed together, according to the DNA concentrations, to form one pooled sample per family and six pooled samples per line. The individual DNA samples of the control lines were randomly used to form two pooled samples per line and generation. Twenty seven microsatellite primers that are documented to detect loci on different chromosomes of the chicken genome (Table 1, Metabion international AG, D-82152 Martinsried, Deutschland) were used. PCR was carried out on a total volume of 25 µl mixture (2.5 µl of DNA sample, 2.5 µl of forward primer 30 pmol, 2.5 µl of reverse primer 30 pmol, 12.5 µl of master mix solution (Bio Basic Inc., Canada), and 5 µl of sdH_2O). The PCR program started with

initial denaturation at 95ºC/5 min, denaturation at 94ºC/45 sec, annealing at 53 to 56ºC/45 sec based on the micrsatellite, and extension at 72ºC/1 min. The denaturation, annealing and extension have been repeated for 35 cycles and the final extension was at 72ºC/10 min and the final hold was for 4 sec.

PCR products were then separated by the electrophoresis on 2% agarose gel (Bio Basic Inc., Canada). The agarose gels were prepared by adding 2 g agarose to 100 ml 1X TAE buffer, and then heating the mixture using microwave oven for 1.5 minutes. The gel solution was then poured in the gel apparatus and was left to cool to 60°C. Ethidium bromide was added and mixed thoroughly. PCR products were loaded to the gel. The 100-bp DNA ladder was used to determine the molecular weight of the amplified bands. The DNA bands on gels were visualized on an ultraviolet trans-illuminator and photographed by a gel documentation system, by which the genomic data were generated.

Genomic Measurements

The molecular data set was used to estimate the genomic variability within generation and line according to Kuhnlein and colleagues **[28]**, and polymorphic information content (PIC) was calculated in each line. The percentage of line-specific allele (LSA) was also estimated as the percentage of microsatellite alleles detected by the primers in a line and were entirely absent in all generations in the other lines. The PIC and LSA per line were estimated for the primers as averages overall generations. The indices of genetic distance between lines and generations were also estimated **[28, 29]**. The PhyloDraw software was applied to the genetic distance indices to construct phylo-genetic dendograms between lines and between generations within lines **[30]**.

Statistical Analysis

The statistical pair-wise comparison between each selected line and its genetic control line was assessed for PIC and LSA using the t-test procedures of the statistical analysis system **[31]**.

RESULTS

The microsatellite markers recognized 1345 microsatellite alleles in all lines and generations, with averages of 5.72, 2.35, 5.98 and 2.57 alleles per primer and generation in lines CE1, CE2, CE3 and CE4, respectively **(Table 2)**. The number of alleles detected by each primer in all lines varied from one to seven, and the allele lengths were 30-346 bp **(Table 3)**.

The genetic variability estimates within line in the subsequent generations are presented **(Table 4)**. The variability was in general low in line CE1 and averaged, overall primers, 0.11, 0.25 and 0.23 in generations 6, 7 and 8 respectively, versus 0.14, 0.22 and 0.24 for its control line CE2. The

variability estimates averaged 0.10, 0.20 and 0.20 in the corresponding generations of line CE3 versus 0.19, 0.16 and 0.17 for its control line CE4.

Table 5 presents the average polymorphic information content (PIC) and the average percentage of line-specific alleles (LSA) for each line overall generations. Several microsatellite loci showed polymorphism of 100% in different lines. The pair-wise statistical comparison revealed significant differences between the selected line CE1 and its genetic control line CE2 in average PIC and LSA, overall generations and primers. The average PIC was 0.50 in the selected line CE1 and was 0.39 in the control line CE2. Average LSA formed 4.3 and 0.7% in both lines CE1 and CE2, respectively. The average PIC was 0.55 in the selected line CE3 versus 0.50 for the control line CE4, and the difference between them was insignificant. The average LSA was 4.6% in line CE3 and 1.2% in line CE4, and the difference was significant.

By generation 8, the genetic distance indices between the selected line CE1 and its control line CE2 averaged 0.740 **(Table 6)**, and the genetic distance indices between line CE3 and its control line CE4 was slightly larger and averaged 0.815. The phylogenetic dendogram of the lines in the 8^{th} selected generations **(Figure 1)** showed that lines CE1 and CE2 formed two close branches differing from those of lines CE3 and CE4. The farthest distances were between line CE1 and each of lines CE3 and CE4. Also, the phylogenetic dendograms reveal that all lines have been derived from the same origin. The phylogenetic dendogram of line CE1 **(Figure 2)** clarified the discrimination between subsequent generations which was apparently due to selection, versus narrower distances obtained in the control line CE2 over subsequent generations. Lines CE3 and CE4 formed similar patterns of phylogenetic dendograms **(Figures 3)**.

DISCUSSION

The average microsatellite alleles was 3.15 per primer, revealing that the loci were multi-allelic and polymorphic. The distinct difference between each selected line and its control line in number of recognized alleles may be accounted for the gene accumulation resulted from eight generations of selection, which in turn influences the frequencies of different alleles. **[32]** used 20 microsatellite primers to scan an Iranian chicken breed. The number of alleles varied from one to six with lengths of 86-310 bp. Also, **[33]** scanned the genomes of two F_1 generations of Brazilian broiler and layer populations, by 34 microsatellite primers targeting chromosomes 1, 3 and 4. A total of 163 different alleles were recognized, of which

31 and 44 alleles were unique to layer and broiler lines, respectively. Also, the genetic changes in selected chicken populations have been successfully monitored by microsatellite and RAPD genome scanning **[18, 34, 35]**.

The low estimates of variability in both selected lines are possibly due to practicing selection for eight successive generations. However the low variability estimates in both control lines may be due to maintaining them randomly bred in small size populations over subsequent generations. In an earlier study using seven microsatellite markers **[36]**, the genetic variability was 0.54 in line CE3 after six generations of selection versus 0.46 in the genetic control line CE4. **[37]** reported that the within-breed genetic variability is a reflection of the population structure. **[38]** evaluated the genomes of nine Tanzanian local chicken ecotypes and a White Leghorn population using 20 microsatellite markers. The genetic variability within indigenous chicken ecotypes was lower than that between different ecotypes. Also, **[2, 3]** recognized moderate to high variability among the indigenous random-bred chicken and duck breeds in Egypt.

The high PIC reveals that the loci that have been detected are multi-allelic. The slightly higher PIC and the noticeably higher LSA percentages in both selected lines than in their corresponding control lines points out the possible linkages between the detected microsatellite loci and QTL for body weight. Selection for body weight has resulted in the accumulation of many genes over eight generations in lines CE1 and CE3, and accordingly the frequencies of linked microsatellite alleles have increased over subsequent generations. **[36]** reported PIC averages of 0.81 and 0.57 at the microsatellite loci recognized by only seven microsatellite markers after 6 generations in lines CE3 and line CE4, respectively. [39, 40] reported that the information on polymorphic loci can be employed to detect population-specific alleles, to measure the amount of genetic diversity and to evaluate the change in population variability over generations. **[33]** found many alleles unique for each of the Brazilian layers and broilers. **[41]** reported a high level of microsatellite polymorphism between two broiler lines and suggested the crossing between both lines to generate populations in which microsatellites will be very efficacious for mapping. **[3]** scanned the genomes of three native Egyptian breeds (White Baladi, Fayoumi and Sinai Bedouin) and a commercial broiler strain. Polymorphism represented 65.19% of the total alleles. **[42]** evaluated polymorphism in a native Isfahan

chicken population using 10 microsatellite markers. Nine microsatellite loci were found to be polymorphic and one locus was monomorphic, and PIC ranged between 0.38 and 0.70 per locus with an average of 0.49.

The results of genetic distances signified the breeding history of different lines. The discrimination between normally-feathered and naked-neck lines was obvious. The phylogenetic dendograms revealed the genetic progress of the selected lines over subsequent generations. The results indicated that the natural heat resistance of the lines was significant in achieving the selection target of heavier body weights under a natural warm environment. The narrow distances obtained in each control line over subsequent generations are expected since both lines are randomly bred. **[18]** obtained a genetic distance of 0.29 between two lines of chickens selected for heat resistance and susceptibility for 5 generations.

The genomes of 23 highly inbred chicken lines derived from White Leghorn, jungle fowl, Fayoumi and Spanish breeds were scanned by 42 microsatellite markers **[43]**. The largest distances were between the jungle fowl and the other lines and ranged 1.12-5.38. **[3]** scanned the genomes of three Egyptian breeds (White Baladi, Fayoumi and Sinai Bedouin) and a commercial broiler strain. An average genetic distance of 0.42 was found between White Baladi and each of Fayoumi and Sinai Bedouin. The genetic distance between Fayoumi and Sinai Bedouin was 0.53 and between White Baladi and commercial broilers was 0.45. The genetic distances reflected the origin of the native Egyptian breeds and indicated that these breeds have the genetic potential of fast growth.

CONCLUSION

The screening of the genomes of both selected lines by the microsatellite primers pointed out the possible linkages between the detected microsatellite loci and QTL for body weight. Selection for body weight has resulted in the accumulation of many genes over eight generations in the selected lines, and accordingly the frequencies of linked microsatellite alleles have increased. The genetic distance indices and phylogenetic dendograms revealed the genetic progress of the selected lines over subsequent generations. The results indicated that the natural heat resistance of the lines was significant in achieving the selection target of heavier body weights under a natural warm environment.

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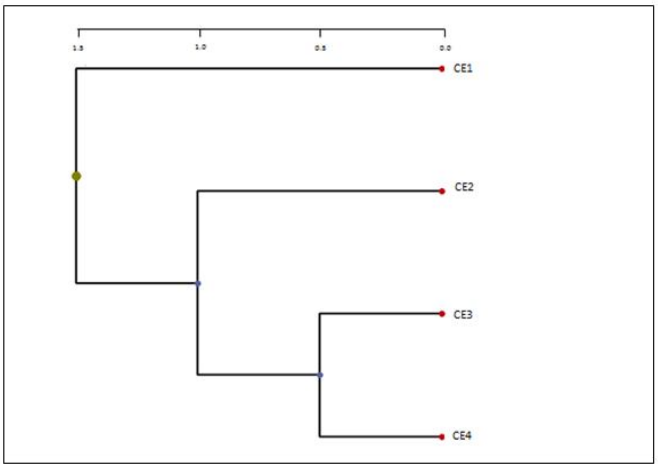

Figure 1: The phylogenetic dendogram among normally-feathered selected line ce1, normally-feathered control Line CE2, naked-neck selected line CE3, and naked-neck control line CE4, in the 8th selected generation

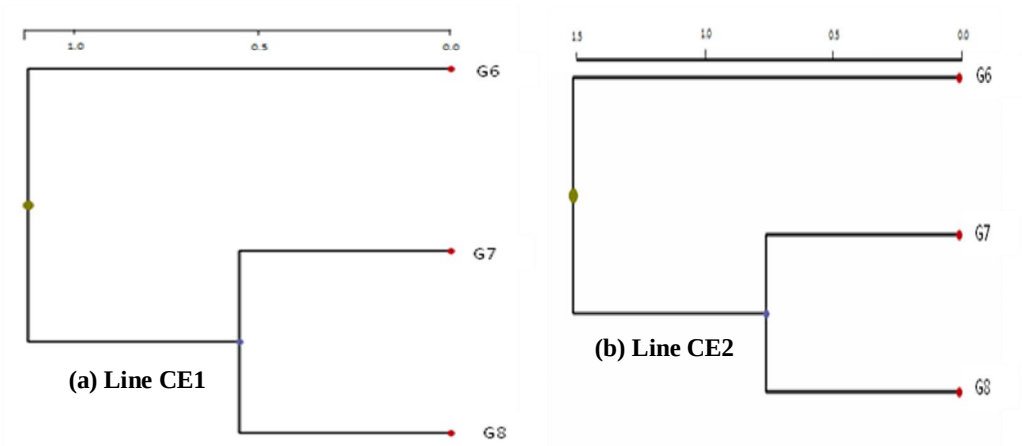

Figure 2: The phylogenetic dendogram for the 6th, 7th and 8th generations in the normally-feathered selected line CE1 (a) and its genetic control line CE2 (b)

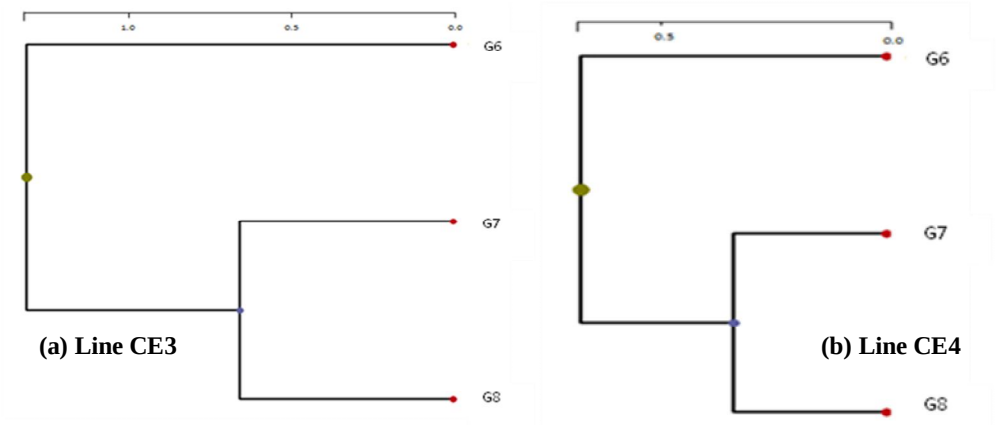

Figure 3: The phylogenetic dendogram for the 6th, 7th and 8th generations in naked-neck selected line CE3 (a) and its genetic control line CE4 (b)

Table 1: Microsatellite Primers, Chromosome on Which Alleles are Recognized and the Forwarded and Reverse Sequences

Primer	Chromosome	Base sequence	
		Forward	Reverse
ADL0183	1	ACAGAAATGGAAAGCGAGAC	TTGTGAAGTGGATAAGATGA
ADL0188	1	GTGGACACAATGAGTTCCTC	CACTTCCAGTATTAACGTGA
LEI0079	1	TCATTATCCTTGTGTGAAACTG	AGGCTCCTGAATGAATGCATC
LEI0106	1	GTCAGCATGACAGCAGCTGAG	AAACCTTCAAATGGTTAAAATGC
MCW0010	1	TAGTACAAGAATCTAGTGTTAAAA	CTGTAGAATTACAGAAATACA
ADL0266	4	AATGCATTGCAGGATGTATG	GTG GCATTCAGGCAGAGCAG
LEI0094	4	TCTCACACTGTAACACAGTGC	GATCTCACCAGTATGAGCTGC
ADL0292	5	AAATGGCCTAAGGATGAGGA	CCAAATCAGGCAAAACTTCT
LEI0082	5	CCTTAGCTGGCTCAGTGGATG	TATCCATACAGTACCCTCCTG
MCW0193	5	ATTACGTCTGCACCAGTACAG	TATTCAATAGAGTTACGCTGTC
MCW0064	8	TCTCAGCACTACAAAATACACAGG	CTTCAAGAGCCATAGGTGGTCT
ADL0158	10	TAGGTGCTGCACTGGAAATC	TGGCATGGTTGAGGAATACA
LEI0112	10	TATCATACCAGCGCAGCTCTG	GGGAACATACAGGGTGCTGCT
ADL0240	12	CGTCCCGTCCTGANTGTTTG	ACCTGGGAGATTGGATTCAA
ADL0372	12	GGCGCCGTTCAAGGAAGCAC	CGCCCCCGTTTACTGATTTG
LEI0120	15	TTAGAATGAAAAGGCTGTTCC	CGTAACACATGCAACTCAATG
ADL0304	18	CCTCATGCTTCGTGCTTTTT	GGGGAGGAACTC TGGAAATG
MCW0217	18	CTGCACTTGGTTCAGGTTCTG	GATCTTTCTGGAACAGATTTC
MCW0300	27	TGTGCACATTTCTCTGCTGAC	CAGAGAAACGTGCATGTGGAC
MCW0328	27	CTCCAATCCCAGGCTCCAAC	ATGGAAACAGATGGAGCTGGC
ADL0299	28	CCACCCCCATGT TCAGGTCA	GTCTAGGCCCCTTGCCAAAC
ROS0095	28	AGCTGCTTTGAAGGAGAAACC	CCCTCCCTCTGTGCTCTG
DL0022	Z	GGTCAAGGAAATCATAGAAA	GCATCAGAGGAAGAAGGAAA
ADL0273	Z	TGGTAGATGCTGAGAGGTGT	GCCATACATGACAATAGAGG
LEI0075	Z	ATCCAGTGCGTGTCTGGTCAG	CTATGCTATCATTGAAACACAGC
LEI0111	Z	CCTGTTTGCCGTACACTTGGC	CCCACAAAAGAGACACCGTGG
MCW0055	Z	GTTTGCATTGTCTACAGCTCCTTG	TTTGTAGTTACCTGGTACTGA

Table 2: Total Number of Alleles Recognized by the Microsatellite Primers in the Subsequent Generations in Each Line

Parameter	CE1			CE2			CE3			CE4		
	F_6	F_7	F_8	F_6	F_7	F_8	F_6	F_7	F_8	F_6	F_7	F_8
∑ bands/F	71	198	194	65	65	60	74	221	189	65	72	71
Average/primer	2.63	7.33	7.19	2.41	2.41	2.22	2.74	8.19	7.0	2.41	2.67	2.63
∑bands/line	463			190			484				208	
Average	5.72			2.35			5.98				2.57	

NOTE: CE1, CE2, CE3 and CE4 indicate normally-feathered selected, normally-feathered control, naked-neck selected and naked-neck control lines, respectively; F_6, F_7 and F_8 indicate 6^{th}, 7^{th} and 8^{th} generations, respectively

Table 3: Number and Length of Different Alleles Recognized by The Primers in Each Line, Overall Generations

Primer	# different alleles	Length, bp	Line			
			CE1	CE2	CE3	CE4
ADL0183	5	38-331	4	2	2	2
LEI0106	4	94-273	3	1	2	1
LEI0079	4	48-419	4	3	3	2
MCW0010	1	307	0	1	0	0
ADL0188	3	77-263	2	2	3	3
LEI0094	6	88-292	3	2	4	3
ADL0266	2	76-234	2	1	1	1
ADL0240	1	79	1	1	1	1
MCW0193	4	85-417	2	2	3	2
LEI0082	4	66-346	2	1	4	2
ADL0292	4	97-267	3	2	4	2
MCW0064	2	70-165	2	2	1	2
ADL0158	2	30-118	2	2	2	2
LEI0112	1	76	1	1	1	1
LEI0120	7	58-313	4	3	6	3
MCW0217	4	95-344	2	2	2	1
ADL0304	1	178	1	1	1	1
MCW0300	4	84-223	3	2	3	4
MCW0328	3	78-238	2	2	2	3
ROS0095	1	86	1	1	1	1
ADL0299	4	96-318	3	2	3	4
ADL0022	3	223-384	2	1	2	2
LEI0111	2	87-142	1	1	1	1
LEI0075	6	65-386	2	2	2	3
MCW0055	3	67-241	2	2	3	3
ADL0273	2	84-165	1	1	2	2
ADL0372	2	54-112	2	1	1	1
Total	85	30-386	57	44	60	53
Average	3.15		2.11	1.63	2.22	1.96

NOTE: CE1, CE2, CE3 and CE4 indicate normally-feathered selected, normally-feathered control, naked-neck selected and Naked-neck control lines, respectively; F_6, F_7 and F_8 Indicate 6^{th}, 7^{th} and 8^{th} generations, respectively

Table 4: The Genetic Variability Within Lines in the Subsequent Generations

Primer	CE1			CE2			CE3			CE4		
	F_6	F_7	F_8	F_6	F_7	F_8	F_6	F_7	F_8	F_6	F_7	F_8
ADL0183	0.00	0.25	0.00	0.00	0.33	0.00	0.00	0.11	0.00	0.00	0.33	0.00
LEI0106	0.00	0.33	0.39	0.00	1.00	1.00	0.00	0.11	0.20	0.00	0.00	0.00
LEI0079	0.50	0.35	0.27	0.00	0.00	0.50	0.00	0.18	0.27	0.33	0.00	0.33
MCW0010	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ADL0188	0.00	0.09	0.11	0.33	0.00	0.00	0.00	0.11	0.13	0.00	0.00	0.00
LEI0094	0.00	0.33	0.56	0.00	0.00	1.00	0.00	0.11	0.33	0.00	0.33	0.33
ADL0266	0.00	0.60	0.26	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
ADL0240	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
MCW0193	0.33	0.15	0.30	0.00	0.33	0.00	0.00	0.09	0.27	0.00	0.00	0.00
LEI0082	0.00	0.11	0.44	1.00	0.00	0.00	0.00	0.43	0.53	0.00	0.00	0.50
ADL0292	0.20	0.26	0.26	0.00	0.33	0.33	0.00	0.37	0.27	0.00	0.33	1.00
MCW0064	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00
ADL0158	0.00	0.11	0.11	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.33
LEI0112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LEI0120	0.00	0.53	0.18	0.00	1.00	0.33	0.33	0.39	0.60	1.00	0.00	0.00
MCW0217	0.00	0.11	0.22	0.00	0.33	0.00	0.33	0.00	0.13	0.00	0.00	0.00
ADL0304	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MCW0300	0.00	0.32	0.23	0.00	0.33	0.00	0.00	0.55	0.16	0.50	0.00	0.32
MCW0328	0.33	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	1.00	0.00
ROS0095	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ADL0299	0.50	0.11	0.11	0.33	0.00	0.00	0.00	0.40	0.10	0.20	0.33	0.33
ADL0022	0.00	0.87	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00	1.00	0.00
LEI0111	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LEI0075	0.00	0.33	0.00	0.00	0.00	1.00	0.00	0.00	0.13	0.00	0.00	0.50
MCW0055	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.40	0.00	0.00	0.00
ADL0273	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ADL0372	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Average	0.11	0.25	0.23	0.14	0.22	0.24	0.10	0.20	0.20	0.19	0.16	0.17

NOTE: CE1, CE2, CE3 and CE4 Indicate normally-feathered selected, normally-feathered control, naked-neck selected and naked-neck control lines, respectively

Table 5: Polymorphic Information Content (PIC) and the Percentage of Line-Specific Alleles (LSA) in Different Lines, Overall Generations

Primer	#alleles	PIC		LSA, %		PIC		LSA, %	
		CE1	CE2	CE1	CE2	CE3	CE4	CE3	CE4
ADL0183	5	0.20	0.00	40.0	20.0	0.20	0.20	0.0	0.0
LEI0106	4	0.50	0.25	25.0	0.0	0.50	0.25	0.0	0.0
LEI0079	4	0.25	0.50	0.0	0.0	0.50	0.25	0.0	0.0
MCW0010	1	0.00	0.00	0.0	0.0	0.00	0.00	100.0	0.0
ADL0188	3	0.67	0.67	0.0	0.0	1.00	1.00	0.0	0.0
LEI0094	6	0.50	0.33	0.0	0.0	0.67	0.50	0.0	0.0
ADL0266	2	1.00	0.50	0.0	0.0	0.50	0.50	0.0	0.0
ADL0240	1	1.00	1.00	0.0	0.0	1.00	1.00	0.0	0.0
MCW0193	4	0.50	0.50	0.0	0.0	0.75	0.50	0.0	0.0
LEI0082	4	0.50	0.25	0.0	0.0	0.75	0.50	25.0	0.0
ALD0292	4	0.75	0.50	0.0	0.0	1.00	0.50	0.0	0.0
MCW0064	2	1.00	0.50	0.0	0.0	0.50	1.00	0.0	0.0
ADL0158	2	0.50	0.50	0.0	0.0	0.50	0.50	0.0	0.0
LEI0112	1	0.00	0.00	0.0	0.0	0.00	0.00	0.0	0.0
LEI0120	7	0.57	0.43	0.0	0.0	0.86	0.43	0.0	0.0
MCW0217	4	0.50	0.50	0.0	0.0	0.50	0.25	0.0	0.0
ADL0304	1	0.00	0.00	0.0	0.0	0.00	0.00	0.0	0.0
MCW0300	4	1.00	0.50	0.0	0.0	0.75	100.0	0.0	0.0
MCW0328	3	0.67	0.67	0.0	0.0	0.67	0.67	0.0	0.0
ROS0095	1	0.00	0.00	0.0	0.0	0.00	0.00	0.0	0.0
ADL0299	4	0.75	0.50	0.0	0.0	0.75	1.00	0.0	0.0
ADL0022	3	0.67	0.33	0.0	0.0	0.67	0.67	0.0	0.0
LEI0111	2	0.50	0.50	0.0	0.0	0.50	0.50	0.0	0.0
LEI0075	6	0.33	0.33	0.0	0.0	0.33	0.17	0.0	33.3
MCW0055	3	0.67	0.67	0.0	0.0	1.00	1.00	0.0	0.0
ADL0273	2	0.50	0.50	0.0	0.0	1.00	1.00	0.0	0.0
ADL0372	2	0.00	0.00	50.0	0.0	0.00	0.00	0.0	0.0
Average	3.15	0.50 [a]	0.39 [b]	4.3 [a]	0.7 [b]	0.55 [a]	0.50 [a]	4.6 [a]	1.2 [b]

NOTE: CE1, CE2, CE3 and CE4 Indicate normally-feathered selected, normally-feathered control, naked-neck selected and naked-neck control lines, respectively;

[a, b] Different superscripts significantly differentiate means of each selected line and its control line ($P \leq 0.05$)

Table 6: Average Genetic Distance Indices, Overall Primers, Between Different Lines And Generations

Generation	Between lines within generation					
	CE1*CE2	CE1*CE3	CE1*CE4	CE2*CE3	CE2*CE4	CE3*CE4
F_6	0.820	0.820	0.825	0.795	0.800	0.805
F_7	0.790	0.815	0.835	0.785	0.805	0.830
F_8	0.740	0.800	0.785	0.770	0.755	0.815
	Between generations within line					
	CE1		CE2	CE3		CE4
G_6*G_7	1.253		0.529	1.097		0.383
G_6*G_8	0.984		0.425	0.866		0.389
G_7*G_8	0.554		0.322	0.894		0.438

NOTE: CE1, CE2, CE3 and CE4 indicate normally-feathered selected, normally-feathered control, naked-neck selected and naked-neck control lines, respectively