ISAG-recommended Microsatellite Marker Analysis Among Five Korean Native Chicken Lines

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ABSTRACT

The objective of this study was to determine genetic variation of five Korean native chicken lines using 30 microsatellite (MS) markers, which were previously recommended by ISAG (International Society for Animal Genetics). The initial study indicated that two microsatellite markers, MCW0284 and LEI0192, were not amplified in these lines and excluded for further analysis. Twenty eight microsatellite markers were investigated in 83 birds from five Korean native chicken lines. The identified mean number of alleles was 4.57. Also, the expected, observed heterozygosity (He, Ho) and polymorphism information content (PIC) values were estimated in these markers and they ranged from 0.31~0.868, 0.145~0.699, and 0.268~0.847, respectively. The results were used for the discrimination of five chicken lines using genetic distance values and also neighbor-joining phylogenetic tree was constructed. Based on the He and PIC values, eighteen markers are enough for the discrimination of these Korean native chicken lines for the expected probability of identity values among genotypes of random individuals (PI), random half sibs (PI_{half-sibs}) and random sibs (PI_{sibs}). Taken together, these results will help the decision of conservation strategies and establishment of traceability system in this native chicken breed. Also, the use of ISAG-recommended microsatellite markers may indicate that the global comparison with other chicken breeds is possible.

(Key words : Discrimination, Genetic diversity, Microsatellite, Korean native chicken)

INTRODUCTION

Consumption of native chicken meat is dramatically increased in Korea even though two to three times high price than that of broiler. In Korea, chicken meat is covering 30% of the total meat consumption where poultry industries have been providing 90% of chicken meat from imported breeds (MIFAFF, 2011). In this point of view, the Korean government has launched the conservation program for native chicken breed since 1994. Basically, five Korean native chicken lines were selected based on the feather colors and the selection program has been performed by the government institution, NIAS (National Institute of Animal Science). Recently, genetic markers which can differentiate these five lines were developed and these can be used for making the conservation strategies for the native chicken

lines.

Molecular markers are essential for the genetic management to ensure high accuracy in breeding program. The combined analysis of molecular markers and phenotypic traits in microsatellite DNA, MC1R gene, LEI0258 marker in MHC region and mitochondrial DNA (mtDNA) markers have been successfully investigated in Taiwanese indigenous chickens (Chang et al., 2011). The red jungle fowl is the ancient progenitor of the domesticated chicken, based on the investigation using 22 MS markers in 52 chicken populations (Hillel et al., 2003). Previously, native chicken conservation studies have been documented using microsatellite markers to assessing genetic diversity and breed structures in the world (Berthouly et al., 2008; Bodzsar et al., 2009; Ding et al., 2010; Kong et al., 2006; Muchadeyi et al., 2007; Mwacharo et al., 2007; Tadano et al., 2007a, b). Also, high accuracy

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of genetic discrimination has been documented in Japanese chicken lines (Tadano et al., 2008). Also, enormous progress with MC1R gene polymorphisms has been achieved in relation to coat color variations in the chicken breeds (Guo et al., 2010; Kerje et al., 2003). However, the MC1R marker was not enough for the differentiation of these Korean native chicken lines based on the plumage colors (data not shown). Phylogenetic relationships between Korean native chickens and other breeds have been revealed by the D-loop sequence variations in mtDNA (Hoque et al., 2009). Also, the combined assessment of mtDNA and LEI0258 marker has been well discriminated in the Korean commercial chicken lines (Hoque et al., 2011). However, MS markers are more suitable for analysis of genetic diversity with their high tandem repeat polymorphisms throughout the genome (Cheng and Crittenden, 1994; Muhammet et al., 2008). The Food and Agricultural Organization (FAO) is an international group, which has launched the native animal conservation project for the Maintenance of Domestic Animal Genetic Diversity (MoDAD) (FAO, 1997). Initially, MoDAD suggested the genetic diversity using analysis of 25 MS markers in 14 main species including four poultry species of domestic animals in the world (FAO, 1997). In 2004, FAO and ISAG (International Society for Animal Genetics) recommended a new group of 30 MS markers for study of genetic diversity and analysis of genetic distance (FAO, 2004).

In our study, ISAG-recommended 28 of 30 MS markers were investigated for identifying the relationships and discrimination among the five Korean native lines. Our results from ISAG-recommended markers will give valuable information for further breeding plans and strategies in relation to Korean native chicken conservations.

MATERIALS AND METHODS

1. Sample and DNA extraction

A total of 83 individuals in five different Korean native chicken lines were used in this study. The number of animals used in this study were gray brown (G, n=18), black (L, n=18), red brown (R, n=16), white (W, n=16), and yellow brown (Y, n=15) which were obtained from the National Institute of Animal Science (NIAS). The blood samples were collected from wing vein and transferred to EDTA containing tubes. Genomic DNAs were extracted from whole blood using modified manual methods (Miller et al., 1988).

2. Microsatellite (MS) marker and PCR amplification

The 30 MS markers used in this study are the recommended markers from FAO/ISAG as a part of the global management of farm animal genetic resources (FAO, 2004). The PCR reaction was performed in a 20 μ L volume including 50 ng of genomic DNA, 10 pmol fluorescent dye (FAM, VIC, NED, PET) labeled in the 5' forward primer (Applied Biosystems, USA), 10 pmol reverse primer (Cosmo gene tech, Korea), 2.5 mM of each dNTPs (Genet Bio, Korea), 10X reaction buffer (Genet Bio, Korea), 2.5 unit HS prime *Taq* DNA Polymerase (Genet Bio, Korea). The PCR was performed in an initial denaturation at 95°C for 10 min followed by 35 cycles of 30 sec at 95°C, 30 sec at 60°C, 30 sec at 72°C and final extension at 72°C for 10 min using My-Genie 96 Thermal Cycler (Bioneer, Korea). The PCR products for MS markers were electrophoresed on 4% agarose gels with ethidium bromide and DNA bands were visualized under ultra violet light.

3. Marker genotyping

A total of 28 MS markers were well genotyped (Table 1). The PCR products were diluted approximately 20 times for genotyping. The genotyping reaction contained 1 μ L of diluted PCR products, 10 μ L of Hi-DiTM Formamide (Applied Biosystems, USA) and 0.1 μ L of GeneScanTM-500 LIZTM size standard marker (Applied Biosystems, USA). After dilution, genotyping reaction mixture was denatured at 95°C for 2 min and genotyped using capillary array Genetic analyzer 3130 xl (Applied Biosystems, USA). The MS genotyping results were confirmed using GeneMapper ver. 3.0 (Applied Biosystems, USA).

4. Statistical analysis

The genotyping data were used to calculate mean number of allele (Na), expected heterozygosity (He), observed heterozygosity (Ho), and polymorphic information content (PIC) using Cervus 3.0 program (Marshall et al., 1998). Also, the construction of phylogenetic neighbor-joining tree and genetic diversity were estimated using PowerMarker v3.25 program (Liu and Muse, 2005). The expected probability of identity values among genotypes of random individuals (PI), random half sibs (PI_{half-sibs}) and random sibs (PI_{sibs}) were calculated using API-CALC ver1.0 (Ayres and Overall, 2004).

Table 1. Primer information for 28 ISAG-recommended microsatellite markers

Marker	Chromosome	Sequence (5' → 3')	Dye	Allele size (bp)
ADL0268	1	F : CTCCACCCCTCTCAGAACTA R : CAACTTCCCATCTACCTACT	NED	105 - 117
MCW0020	1	F : TCTTCTTTGACATGAATTGGCA R : GCAAGGAAGATTTTGTACAAAATC	VIC	183 - 189
MCW0111	1	F : GCTCCATGTGAAGTGGTTTA R : ATGTCCACTTGTCAATGATG	FAM	98 - 106
MCW0248	1	F : TTGCATTAACTGGGCACCTTC R : GTTGTTCAAAAGAAGATGCATG	PET	215 - 223
LEI0234	2	F : ATGCATCAGATTGGTATTCAA R : CGTGGCTGTGAACAAATATG	PET	217 - 315
MCW0034	2	F : TGTCTTCCAATTACATTCATGGG R : TGCACGCACTTACATACTTAGAGA	VIC	217 - 237
MCW0206	2	F : CTTGACAGTGATGCATTAAAT R : ACATCTAGAATTGACTGTTC	PET	226 - 240
LEI0166	3	F : CTCCTGCCCTTAGCTACGCA R : TATCCCCTGGCTGGGAGTTT	PET	346 - 356
MCW0016	3	F : TGGCTTCTGAAGCAGTTGCTATGG R : ATGGCGCAGAAGGCAAAGCGATAT	FAM	134 - 146
MCW0037	3	F : GAAAGCTCACATGACACTGCGAAA R : ACCGGTGCCATCAATTACCTATTA	FAM	152 - 156
MCW0103	3	F : TTTCTAACTGGATGCTTCTG R : AACTGCGTTGAGAGTGAATGC	PET	267 - 271
MCW0222	3	F : GCAGTTACATTGAAATGATTCC R : TTCTCAAAACACCTAGAAGAC	VIC	217 - 223
LEI0094	4	F : GATCTCACCAGTATGAGCTGC R : TCTCACACTGTAACACAGTGC	VIC	246 - 280
MCW0098	4	F : CGATGGTCGTAATTCTCACGT R : GGCTGCTTTGTGCTCTTCTCG	VIC	260 - 262
MCW0295	4	F : ATCACTACAGAACACCCTCTC R : TATGTATGCACGCAGATATCC	NED	88 - 100
MCW0078	5	F : CCACACGGAGAGGAGAAGGTCT R : TAGCATATGAGTGACTGAGCTTC	FAM	135 - 143
MCW0081	5	F : GTTGCTGAGAGCCTGGTGCAG R : CCTGTATGTGGAATTACTTCTC	FAM	113 - 131
MCW0014	6	F : AAAATATTGGCTCTAGGAAGTGC R : ACCGGAATGAAGGTAAGACTAGC	NED	173 - 187
MCW0183	7	F : TGAGATTTACTGGAGCCTGCC R : ATCCAGTGTGAGTATCCGA	VIC	291 - 319
ADL0278	8	F : CCAGCAGTCTACCTTCCTAT R : TGTCATCCAAGAACAGTGTG	FAM	111 - 119
ADL0112	10	F : ATCTCAAATGTAATGCGTGC R : GGCTTAAGCTGACCCATTAT	NED	127 - 133
MCW0067	10	F : GAGATGTAGTTGCCACATTCCGAC R : GCACTACTGTGTGCTGCAGTTT	FAM	176 - 182
MCW0104	13	F : TAGCACAACCTAAGCTGTGAG R : AGACTTGCACAGCTGTGTACC	VIC	188 - 226
MCW0216	13	F : GGGTTTTACAGGATGGGACG R : AGTTTCACTCCCAGGGCTCG	NED	136 - 146
MCW0123	14	F : CCACTAGAAAAGAACATCCTC R : GGCTGATGTAAGAAGGGATGA	FAM	80 - 90
MCW0330	17	F : TGGACCTCATCAGTCTGACAG R : AATGTTCTCATAGAGTTCTCTGC	PET	254 - 286
MCW0165	23	F : CAGACATGCATGCCAGATGA R : GATCCAGTCTGCAGGCTGC	NED	114 - 118
MCW0069	26	F : ATTGCTTCAGCAAGCATGGGAGGA R : GCACTCGAGAAAACCTCCTGCG	NED	155 - 173

RESULTS AND DISCUSSION

1. Genetic diversity and differentiation

A total of 128 alleles were detected from 28 ISAG-recommended MS markers in the five Korean native chicken lines, ranged from 2 to 11 alleles with 4.57 mean number of alleles distributed on 14 autosomes. The expected heterozygosity (He), observed heterozygosity (Ho), and polymorphic information content (PIC) were obtained in these chicken lines (Table 2). The mean value of He was 0.615, ranged from 0.868 (LEI0234) to 0.31 (MCW0078). Also, the mean value of Ho was 0.499, ranged from 0.699 (MCW0104) to 0.145 (MCW0014). Besides, the mean value of PIC was

0.554, ranged from 0.847 (LEI0234) to 0.268 (MCW0103). Previously, the identified mean values of He, Ho, and PIC were 0.452, 0.466, 0.401 for Asian (Taiwan, Japan, and China) breeds, 0.575, 0.488, 0.432 for Chinese breeds and 0.517, 0.488, 0.373 for Japanese native chicken breeds, respectively (Berthouly et al., 2008; Ding et al., 2010; Tadano et al., 2007). The Korean native chicken lines had higher mean values compared to other Asian chickens, having He, Ho, and PIC values of 0.615, 0.499 and 0.554, respectively.

In addition, total inbreeding (*Fit*), genetic distance (*Fst*), and within line inbreeding (*Fis*) among the Korean native chicken lines were calculated (Table 2). The mean inbreeding co-efficient of *Fit* and *Fis* were observed as 0.2177 and

Table 2. The calculated number of alleles (Na), heterozygosity values (He and Ho), polymorphism information content (PIC), and *F*-statistic values (*Fit*, *Fst* and *Fis*) using 28 microsatellite markers in the Korean native chickens

Loci	Na	He	Ho	PIC	<i>Fit</i>	<i>Fst</i>	<i>Fis</i>
ADL0268	5	0.7	0.542	0.65	0.2512	0.1635	0.1048
MCW0020	4	0.691	0.639	0.634	0.1013	0.1411	-0.0464
MCW0111	5	0.703	0.687	0.639	0.0528	0.1598	-0.1274
MCW0248	3	0.468	0.386	0.405	0.1846	0.0487	0.1428
LEI0234	10	0.868	0.313	0.847	0.6497	0.1367	0.5943
MCW0034	7	0.726	0.53	0.682	0.3068	0.2531	0.0718
MCW0206	5	0.641	0.57	0.573	0.1469	0.2040	-0.0718
LEI0166	3	0.468	0.422	0.411	0.1105	0.0671	0.0465
MCW0016	5	0.738	0.542	0.689	0.2947	0.2025	0.1156
MCW0037	3	0.628	0.554	0.552	0.1501	0.1878	-0.0465
MCW0103	2	0.32	0.349	0.268	-0.0651	0.1232	-0.2148
MCW0222	4	0.66	0.55	0.588	0.1959	0.1783	0.0215
LEI0094	9	0.83	0.699	0.803	0.1861	0.1666	0.0234
MCW0098	2	0.497	0.386	0.372	0.2740	0.3276	-0.0797
MCW0295	5	0.725	0.639	0.674	0.1463	0.1535	-0.0086
MCW0078	3	0.31	0.289	0.28	0.0841	0.0978	-0.0152
MCW0081	2	0.49	0.463	0.368	0.0941	0.2100	-0.1467
MCW0014	4	0.603	0.145	0.549	0.7655	0.0907	0.7421
MCW0183	3	0.586	0.566	0.497	0.0708	0.2040	-0.1673
ADL0278	4	0.562	0.518	0.508	0.1143	0.2060	-0.1156
ADL0112	3	0.417	0.418	0.347	0.0115	0.0651	-0.0573
MCW0067	3	0.613	0.53	0.536	0.1449	0.0553	0.0948
MCW0104	11	0.85	0.699	0.827	0.2111	0.2054	0.0072
MCW0216	4	0.635	0.518	0.561	0.2215	0.2370	-0.0203
MCW0123	5	0.699	0.482	0.638	0.3627	0.3865	-0.0387
MCW0330	4	0.58	0.602	0.525	-0.0264	0.0669	-0.0999
MCW0165	3	0.598	0.463	0.523	0.2522	0.1620	0.1076
MCW0069	7	0.624	0.47	0.574	0.2748	0.1861	0.1091
Mean	4.57	0.615	0.499	0.554	0.2177	0.1741	0.0528

Na = number of allele, He = expected heterozygosity, Ho = observed heterozygosity, PIC = polymorphism information content, *Fit* = total inbreeding, *Fst* = genetic distance, and *Fis* = within inbreeding.

0.0528, and ranged from 0.7655 to -0.0651 and 0.7421 to -0.2148 in MCW0014 and MCW0103 markers, respectively. Also, the observed mean *Fst* value was 0.1741, ranged from 0.3865 (MCW0123) to 0.0487 (MCW0248) in Korean native chicken lines. Previously, *F*-statistics mean values have been documented in Chinese (*Fit* = 0.056, *Fst* = 0.013, and *Fis* = 0.059) and Japanese (*Fit* = 0.45, *Fst* = 0.383 and *Fis* = 0.109) native chicken populations (Ding et al., 2010; Tadano et al., 2007). The Korean native chicken global deficit heterozygosity is higher than Chinese native chicken. Also, the calculated genetic distances in Korean native chicken lines were slightly higher than Chinese native chickens.

The number of allele (Na), He, Ho, and PIC values were

also calculated using 28 MS markers in five Korean native chicken lines (Table 3). In the gray brown (G) chicken line, the number of alleles was between 2 to 4. The MCW0216 marker in this line has highest value for He, Ho, and PIC of 0.76, 0.778 and 0.691, respectively. The MCW0103 marker showed lowest value for He, Ho, and PIC of 0.056, 0.056, and 0.053, respectively. In black (L) chicken line, Na ranged between 2 to 8 and Ho ranged from 0.222 (MCW0248, MCW0014) to 0.833 (LEI0094). Also, highest value of He (0.854) and PIC (0.809) were observed in LEI0234 marker, whereas lowest value of He (0.259) and PIC (0.241) were observed in ADL0278. In the red brown (R) chicken line, the MCW0078 marker was homozygous

Table 3. The distribution of number of alleles (Na), heterozygosity (He and Ho) values, and polymorphism information content (PIC) within 5 lines of Korean native chicken

Locus	G				L				R				W				Y			
	Na	He	Ho	PIC	Na	He	Ho	PIC	Na	He	Ho	PIC	Na	He	Ho	PIC	Na	He	Ho	PIC
ADL0268	2	0.246	0.278	0.211	3	0.66	0.556	0.568	5	0.792	0.563	0.73	4	0.625	0.625	0.525	4	0.754	0.765	0.682
MCW0020	3	0.522	0.556	0.438	3	0.652	0.722	0.558	3	0.635	0.625	0.536	3	0.583	0.625	0.503	3	0.643	0.588	0.554
MCW0111	3	0.56	0.5	0.445	5	0.665	0.722	0.602	3	0.683	0.688	0.588	3	0.522	0.813	0.406	3	0.656	0.706	0.564
MCW0248	2	0.489	0.444	0.362	3	0.375	0.222	0.327	3	0.417	0.375	0.367	2	0.484	0.375	0.359	3	0.469	0.529	0.375
LEI0234	3	0.652	0.444	0.558	8	0.854	0.444	0.809	5	0.772	0.188	0.706	4	0.734	0.375	0.658	7	0.799	0.118	0.744
MCW0034	4	0.59	0.611	0.533	4	0.586	0.556	0.525	4	0.7	0.563	0.612	4	0.47	0.438	0.425	5	0.49	0.471	0.45
MCW0206	3	0.519	0.588	0.437	2	0.428	0.588	0.329	3	0.646	0.467	0.545	2	0.508	0.625	0.371	5	0.641	0.563	0.564
LEI0166	2	0.108	0.111	0.099	3	0.541	0.333	0.45	3	0.575	0.375	0.474	3	0.502	0.688	0.414	3	0.504	0.647	0.44
MCW0016	4	0.586	0.556	0.525	4	0.49	0.444	0.437	4	0.629	0.5	0.559	3	0.655	0.563	0.561	5	0.749	0.647	0.685
MCW0037	3	0.679	0.611	0.586	2	0.322	0.389	0.264	3	0.679	0.75	0.584	2	0.353	0.313	0.283	3	0.633	0.765	0.54
MCW0103	2	0.056	0.056	0.053	2	0.386	0.389	0.305	2	0.387	0.5	0.305	2	0.498	0.688	0.366	2	0.214	0.118	0.186
MCW0222	3	0.541	0.444	0.45	3	0.508	0.647	0.397	2	0.497	0.4	0.365	3	0.623	0.688	0.533	3	0.639	0.625	0.539
LEI0094	4	0.633	0.5	0.566	6	0.825	0.833	0.775	6	0.813	0.938	0.755	4	0.575	0.5	0.514	5	0.717	0.706	0.647
MCW0098	2	0.475	0.5	0.355	2	0.457	0.556	0.346	2	0.315	0.25	0.258	2	0.063	0.063	0.059	2	0.471	0.471	0.352
MCW0295	3	0.617	0.611	0.528	3	0.513	0.389	0.446	5	0.798	0.813	0.737	3	0.619	0.875	0.515	4	0.663	0.588	0.597
MCW0078	2	0.203	0.222	0.178	3	0.567	0.556	0.492	1	0	0	0	2	0.387	0.375	0.305	2	0.299	0.353	0.248
MCW0081	2	0.413	0.444	0.321	2	0.513	0.588	0.374	2	0.517	0.6	0.375	2	0.226	0.25	0.195	2	0.387	0.375	0.305
MCW0014	3	0.446	0.278	0.386	4	0.76	0.222	0.691	2	0.484	0	0.359	2	0.466	0.063	0.349	3	0.642	0.118	0.546
MCW0183	2	0.457	0.667	0.346	3	0.579	0.556	0.495	3	0.583	0.813	0.503	3	0.401	0.375	0.334	2	0.471	0.353	0.352
ADL0278	2	0.489	0.444	0.362	4	0.259	0.278	0.241	3	0.522	0.688	0.45	3	0.605	0.625	0.52	3	0.506	0.588	0.415
ADL0112	2	0.356	0.333	0.286	2	0.428	0.471	0.329	3	0.553	0.643	0.424	2	0.387	0.5	0.305	3	0.28	0.188	0.248
MCW0067	3	0.538	0.389	0.412	3	0.514	0.611	0.449	3	0.599	0.375	0.489	3	0.607	0.688	0.523	3	0.686	0.647	0.592
MCW0104	4	0.544	0.5	0.456	7	0.775	0.778	0.724	6	0.758	0.813	0.691	5	0.665	0.625	0.6	6	0.804	0.706	0.745
MCW0216	4	0.76	0.778	0.691	3	0.452	0.444	0.399	3	0.619	0.75	0.515	2	0.272	0.313	0.229	2	0.371	0.235	0.295
MCW0123	3	0.298	0.278	0.269	4	0.346	0.333	0.317	5	0.663	0.688	0.597	2	0.417	0.438	0.323	4	0.649	0.706	0.566
MCW0330	3	0.294	0.333	0.258	4	0.578	0.722	0.512	3	0.647	0.75	0.551	4	0.7	0.563	0.627	3	0.59	0.647	0.481
MCW0165	3	0.608	0.667	0.504	3	0.633	0.412	0.532	2	0.287	0.333	0.239	3	0.486	0.563	0.386	3	0.613	0.313	0.511
MCW0069	4	0.589	0.444	0.512	4	0.576	0.444	0.508	4	0.556	0.5	0.483	3	0.679	0.75	0.584	3	0.308	0.235	0.269
Mean	2.86	0.474	0.45	0.397	3.54	0.544	0.507	0.471	3.46	0.578	0.53	0.498	2.86	0.504	0.514	0.42	2.86	0.504	0.513	0.420

Na = Number of allele, He = Expected heterozygosity, Ho = Observed heterozygosity, PIC = Polymorphism information content. G = gray brown, L = black, R = red brown, W = white, and Y = yellow brown

Table 4. The genetic distances among Korean native chicken lines

line	Black (L)	Gray brown (G)	Red brown (R)	White (W)
Gray brown (G)	0.2154	–	–	–
Red brown (R)	0.1004	0.1891	–	–
White (W)	0.2155	0.2095	0.1352	–
Yellow brown (Y)	0.1662	0.1547	0.1295	0.2073

and highest genetic diversity was observed in Na, He, Ho, and PIC for 6, 0.813, 0.938, and 0.755, respectively, in LEI0094 marker. In the white (W) chicken line, the identified allele numbers were between 2 to 5 and calculated Ho values were 0.063 to 0.875 in MCW0098 and MCW0295 markers, respectively. Besides, the highest values of He (0.734) and PIC (0.658), lowest values of He (0.063) and PIC (0.059) were observed in LEI0234 and MCW0098 markers, respectively. Finally, in the yellow brown (Y) chicken line, the identified allele numbers were between 2 to 7 and highest Ho value of 0.765 was observed in ADL0268 and MCW0037 markers and lowest value of 0.118 was observed in LEI0234, MCW0103, and MCW0014 markers. Also, highest He and PIC values of 0.804 and 0.745 were identified in MCW0104 marker, whereas lowest values of 0.214 and 0.186 were observed in MCW0103 marker. The identified He and PIC values were very effectively used for the breed discrimination (Bathouly et al., 2008). In our investigation of ISAG recommend markers, the Na, He, Ho, and PIC values varied among Korean native chicken lines.

2. Genetic distance and phylogenetic analysis

The genetic distance was calculated among the Korean native chicken lines using Pair-raise co-ancestry matrix (Table 4). Based on this analysis, the black chicken line differed from gray brown and white chicken lines with similar distance value of 21.5%. Also, 20.9% genetic distance was observed between white and gray brown chicken lines. Thus, the black and red brown chicken line had comparatively low genetic distance value of 10%. Also, red brown chicken line differed from gray brown, white and yellow brown chicken lines with the genetic distance of 18.9%, 13.5% and 12.9%, respectively. Additionally, yellow brown chicken line differentiated in genetic distance from black, gray brown, and white chicken line with the genetic distance of 16.6%, 15.4% and 20.7%, respectively.

Based on the Nei (1983) equations, the unrooted neighbor-joining (NJ) phylogenetic tree was constructed for the genetic

relationships among the Korean native chicken lines (Fig. 1). In our phylogenetic studies, five chicken lines were clearly separated by using 28 ISAG-recommended MS markers. On the other hand, the red brown chicken line was separated in two groups. Based on these results, the ISAG-recommended markers used in this study will be used for discrimination of the Korean native chicken lines.

3. Discrimination of the Korean native chicken lines

The estimated He and PIC values were used for calculating the expected probability of selection of MS markers to discrimination of Korean native chicken lines. In case of random individuals (PI), random half-sib ($PI_{half-sibs}$), and random sibs (PI_{sibs}), the marker numbers of sixteen and eighteen markers from 28 ISAG-recommended markers were sufficient for discrimination of Korean native chicken lines, respectively (Fig. 2). Based on the PI and $PI_{half-sibs}$, 16 markers were selected for the enough identification power, where lowest values of He and PIC of 0.614 and 0.558, respectively, were observed. Among the 18 MS markers for PI_{sibs} identification, the lowest values for He and PIC were 0.603 and 0.526, respectively. For the animal breed discrimination, He (>0.60) and PIC (>0.50) are indicating highly informative locus (Botstein et al., 1980). Also, our results suggested that the identified lowest values of 0.603 (He) and 0.526 (PIC) are appropriate for chicken discrimination. Moreover, the calculated PI, $PI_{half-sibs}$ and PI_{sibs} values using all 28 MS markers were 1.21×10^{-52} , 6.57×10^{-37} and 1.89×10^{-15} , respectively. These results indicated the high discrimination power in Korean native chicken line using these markers.

Based on this study, ISAG-recommended MS markers show very high discrimination power in Korean native chicken lines. Our estimated genetic diversity values, relationship and discrimination power will give very important information for the future breeding plan and conservation policy in Korean native chicken lines.

chicken lines using 28 MS

individuals (PI), random half-sibs
or discrimination of chicken

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