INTRODUCTION

Chicken is one of the major livestock, especially for supplying proteins to human and the Korean native chicken (KNC) has been documented since approximately 2,000 years ago (Seo et al., 2013; Seo et al., 2015). But, due to their poor commercial performance, Korean native chicken breeds almost became extinct and the breeds that existed before the Korean War (1950-1953), are almost all extinct (Seo et al., 2013; Choi et al., 2015). After the Korean War, commercial native chicken companies maintained various independent breeds while continuing production and market distribution (Seo et al., 2018). Since 1992, a KNC conservation project was launched by the National Institute of Animal Science (NIAS) in an attempt to restore local chicken breeds (Choi et al., 2015; Roh et al., 2019). So, KNC breeds and other imported and adapted breeds in the 1960s have been restored (Heo et
NIAS has preserved two types of purebred chicken breeds: purebred KNCs, which include five breeds with different feather colors (red-brown (NR), yellow-brown (NY), gray-brown (NG), black (NL) and white (NW)) and the "imported and adapted chickens", which includes two Rhode Island Red breeds, two Cornish breeds and two Leghorn breeds (Seo et al., 2018; Choi et al., 2019). Also, NIAS developed the Woorimatdag version 1 (WM1) and 2 (WMT). WM1 breeds were commercial, KNC breeds generated from crossbreeding fast growing native male and good tasting female with increased egg production, and WM2 breeds were modified version of WM1 breeds with increased growth rates (Park et al., 2010; Choi et al., 2015). The private native chicken breeding-stock company (Hanhyup) is responsible for more than 80% of the native chicken distribution in Korea and has maintained purebred chicken breeds (Hanhyup breeds) for commercial use for the past 60 years (Seo et al., 2018; Choi et al., 2019). Hanhyup breeds produced by mating the KNC and economically superior and naturalized breeds (Seo et al., 2017). A number of Korean chicken breeds were registered in Domestic Animal Diversity Information System (DAD-IS, http://dad.fao.org/) of the Food and Agriculture Organization (FAO). But, at present, sufficient detailed information about these Korean chicken breeds is not available. Evaluating the genetic diversity and genetic structure of these breeds is very important step towards identifying and conserving valuable genetic resources (Suh et al., 2014).

Genetic marker polymorphisms provide a reliable method to assess the biodiversity within and among chicken breeds. Microsatellite markers or simple-sequence repeat (SSR) markers, are highly polymorphic, one to six base pair repeats, widely used since they are numerous, randomly distributed in the genome, and show co-dominant inheritance (Cheng et al., 1994; Crooijmans et al., 1996; Choi et al., 2015). Thus, microsatellites have been identified as reliable markers in chickens (Hillel et al., 2003; Tadano et al., 2007; Suh et al., 2014). The identification of these specific markers could aid the selection process for the development of native chickens that are more suitable for the chicken industry in Korea. Therefore, the aim of this study was to characterize the genetic diversity Korean chicken breeds available in Korea based on 12 microsatellite markers.

### MATERIALS AND METHODS

#### Animal and DNA isolation

A total of 782 individual samples from 22 Korean chicken breeds: 5 breeds of broilers (Arbor Acres (AB), Black Cornish (NH), Brown Cornish (NS), Cobb, Ross), 4 breeds of laying hens (Hy-line Brown (HL), Lohmann (LO), Leghorn F (NF), Leghorn K (NK)) and 13 breeds of Dual-purpose (Ogye (NO), Hanhyup A (HA), Hanhyup 3 (HCC), Hanhyup Z (HZ), WM, WMT, Rhode Island Red C (NC), Rhode Island Red D (ND), NR, NY, NW, NL) were collected from NIAS and Hanhyup. Genomic DNA was extracted from blood samples collected from the wing veins into ethylene diamine tetra acetic acid (EDTA) - coated tunes. Genomic DNA extraction from blood samples the using the methods described for AccuPrep® Blood DNA Extraction Kit (Bioneer, Korea). The concentration of DNA samples was measured using NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA) and stored at -20℃.

#### Microsatellite (MS) marker and polymerase chain reaction (PCR) amplification

Previously, 27 Microsatellite markers were investigated for the discrimination of KNC and commercial KNC (Seo et al., 2015; Seo et al., 2017; Choi et al., 2019). From these results, a total of 12 MS markers were initially selected, which have high expected heterozygosity (HEExp) and polymorphic information content (PIC) values (Supplementary Table 1).

All 782 DNA samples were amplified using a T100™ Thermal Cycler (Bio-Rad, USA). The amplifications were carried out using 15 µL reaction mixtures containing genomic DNA (5-20 ng), 10 pmol primer mix, 2.5 mM of each dNTPs (GeNet Bio, Korea) and 1.5 U Hot Start Taq polymerase (GeNet Bio, Korea) which were then subjected to 30 cycles of 30 s at 95℃, 30 s at 58℃, and 1 min at 72℃.

#### Genotyping and statistical analysis

The amplified DNA was performed using an automated Genetic Analyzer 3730 (Applied Biosystems, USA). The genotyping reaction contained 1 µL of PCR products, 8.9 µL of Hi-Di formamide, and 0.1 µL of GeneScan500LIZ size standard in 10 µL total volume. The results were obtained using GeneMapper V 5.0 (Applied Biosystems, USA).
| Marker   | NA | $H_{Exp}$ | $H_{Obs}$ | PIC  | $F_{st}(\theta)$ | $F_{in}(F)$ | $F_{is}(f)$ |
|----------|----|-----------|-----------|------|-----------------|-------------|-----------|
| ADL0293  | 11 | 0.5704    | 0.5841    | 0.5205 | 0.224           | 0.172       | -0.066    |
| ADL0304  | 10 | 0.6365    | 0.5805    | 0.5755 | 0.137           | 0.175       | 0.044     |
| ADL0317  | 12 | 0.6778    | 0.6532    | 0.6292 | 0.187           | 0.364       | 0.218     |
| GCT0016  | 15 | 0.6508    | 0.3424    | 0.5781 | 0.232           | 0.57        | 0.441     |
| LEI0094  | 17 | 0.7063    | 0.6871    | 0.6548 | 0.136           | 0.104       | -0.037    |
| MCW0029  | 18 | 0.7034    | 0.7202    | 0.6536 | 0.187           | 0.149       | -0.046    |
| MCW0087  | 13 | 0.7149    | 0.6374    | 0.667  | 0.185           | 0.234       | 0.06      |
| MCW0104  | 22 | 0.6938    | 0.651     | 0.6446 | 0.222           | 0.268       | 0.06      |
| MCW0123  | 7  | 0.5123    | 0.5323    | 0.4452 | 0.25            | 0.202       | -0.065    |
| MCW0127  | 19 | 0.7416    | 0.6631    | 0.6905 | 0.096           | 0.148       | 0.057     |
| MCW0145  | 9  | 0.7184    | 0.7451    | 0.6595 | 0.118           | 0.064       | -0.062    |
| MCW0330  | 11 | 0.6607    | 0.5654    | 0.599  | 0.216           | 0.277       | 0.078     |
| Mean     | 13.667 | 0.666     | 0.606    | 0.61  | 0.183           | 0.231       | 0.058     |

Table 1. Statistical analysis result of 12 ms markers

NA, Number of Alleles; $H_{Exp}$, Expected heterozygosity; $H_{Obs}$, Observed heterozygosity; PIC, Polymorphism Information Content; $F_{st}$, Genetic distance; $F_{in}$, Total inbreeding; $F_{is}$, Within inbreeding.

| Pop | MNA | $H_{Exp}$ | $H_{Obs}$ | PIC  |
|-----|-----|-----------|-----------|------|
| AB  | 5.75| 0.6878    | 0.6859    | 0.6352|
| COBB| 6.33| 0.7266    | 0.6008    | 0.6716|
| HA  | 4.08| 0.6151    | 0.5980    | 0.5391|
| HCC | 6.25| 0.7541    | 0.7109    | 0.7113|
| HL  | 4.83| 0.6804    | 0.8389    | 0.6179|
| HZ  | 6.25| 0.7253    | 0.6708    | 0.6786|
| LO  | 4.92| 0.6782    | 0.8674    | 0.6171|
| NC  | 4.17| 0.5996    | 0.4138    | 0.5306|
| ND  | 4.67| 0.6446    | 0.5241    | 0.5775|
| NF  | 3.75| 0.4679    | 0.3924    | 0.4131|
| NG  | 5.83| 0.6703    | 0.5956    | 0.6182|
| NH  | 4.67| 0.5860    | 0.4083    | 0.5265|
| NK  | 3.92| 0.4706    | 0.3663    | 0.419 |
| NL  | 6.42| 0.7414    | 0.6312    | 0.689 |
| NO  | 5.25| 0.6919    | 0.6443    | 0.6341|
| NR  | 6.50| 0.7162    | 0.6054    | 0.6571|
| NS  | 5.00| 0.6237    | 0.4697    | 0.5624|
| NW  | 6.08| 0.6914    | 0.6480    | 0.6353|
| NY  | 6.75| 0.7260    | 0.6414    | 0.6713|
| ROSS| 7.00| 0.7100    | 0.6833    | 0.6659|
| WM  | 6.42| 0.7256    | 0.7092    | 0.6826|
| WMT | 6.58| 0.7097    | 0.6260    | 0.6619|
| Mean| 5.52| 0.666     | 0.606     | 0.61  |

Table 2. MNA, $H_{Exp}$, $H_{Obs}$ and PIC observed across 12 MS markers in 22 Korean chicken breeds

MNA, Mean Number of Alleles; $H_{Exp}$, Expected heterozygosity; $H_{Obs}$, Observed heterozygosity; PIC, Polymorphism Information Content; Arbor Acres (AB), Cobb (COBB), Hanhyup A (HA), Hanhyup 3 (HCC), Hy-line Brown (HL), Hanhyup Z (HZ), Lohmann brown (LO), Rhode Island Red C (NC), Rhode Island Red D (ND), Leghorn F (NF), Gray Korea Native Chicken (NG), Black Cornish (NK), Black Korea Native Chicken (NL), Ogye (NO), Red Korea Native Chicken (NR), Brown Cornish (NS), White Korea Native Chicken (NW), Yellow Korea Native Chicken (NY), Ross (ROSS), Woorimatdag1 (WM), Woorimatdag2 (WMT).
The genotyped data were analyzed using MS toolkit software (Park, 2001) version 3.1 to calculate allele frequencies at each locus for each population, \( H_{\text{Exp}} \), observed heterozygosity \( (H_{\text{Obs}}) \), and PIC values. The amount of inbreeding-like effects within subpopulations (genetic distance: \( F_{st} \)), among subpopulations (within inbreeding, \( F_{is} \)), and within the entire population (total inbreeding, \( F_{it} \)) were analyzed by \( F \)-statistics (Wright, 1965). Wright’s \( F \)-statistics were computed according to Weir and Cockerham using FSTAT software (Weir and Cockerham, 1984). The Neighbor - Joining method was used to construct a phylogenetic tree (Saitou and Nei, 1987). The principal coordinates analysis (PCoA) was conducted for 22 chicken breeds using GenAlEx 6.4 program. The Factorial correspondence analysis (FCA), which is a weighted principal component analysis, was performed using the allele frequency data for the individuals of all 22 breeds and the 12 MS markers using GENETIX software (Belkhir, 2003; Tantia et al., 2006).

**RESULTS**

**Polymorphisms of microsatellite markers**

The number of alleles, \( H_{\text{Exp}} \), \( H_{\text{Obs}} \), and PIC values for the 12 markers used in this study summarized in Table 1. A total of 164 different alleles were detected, ranging from 7 (MCW0123) to 22 (MCW0104) and the mean number of alleles (MNA) was 13.667. \( H_{\text{Exp}} \) and \( H_{\text{Obs}} \) ranged from 0.512 (MCW0123) to 0.742 (MCW0127) and 0.342 (GCT0016) to 0.745 (MCW0145), with mean value of 0.666 and 0.606, respectively. PIC values ranged from 0.445 (MCW0123) to 0.691 (MCW0127), with a mean value of 0.610. Estimation of genotypic diversity in heterozygosity and PIC value of MS markers were previously used for determining animal breed selection (Berthouly et al., 2008; Choi et al., 2019). For the animal traceability, \( \text{PIC} > 0.5 \) and \( H_{\text{Exp}} > 0.6 \) are the most reasonable informative locus for application in genetics (Botstein et al., 1980; Jung et al., 2021).

\( F \)-statistic were estimated in a fixation index as genetic differentiation \( (F_{st}) \), the global heterozygote deficit among 22 chicken breeds \( (F_{is}) \), and the heterozygote deficit within the breed \( (F_{it}) \) among the 12 MS markers (Table 1). Among these markers, \( F_{st} \) values ranged from 0.096 (MCW0127) to 0.232 (GCT0016); \( F_{is} \) values ranged from 0.064 (MCW0145) to 0.364 (ADL0317) and the \( F_{it} \) ranged from -0.065 (MCW0123) to 0.218 (ADL0317). The estimated mean value of the \( F_{st} \), \( F_{is} \), and \( F_{it} \) were 0.183, 0.231 and 0.058, respectively (Table 1).

The breed statistics generated by the 12 microsatellite markers in 22 chicken breeds are shown in Table 2. The mean NA for each variety was 5.52, ranging from a 3.75 (NF) to a 7.0 (ROSS). The most diverse breed was the HCC, which had the highest \( H_{\text{Exp}} \) (0.754) and PIC (0.711). The NF was the least diverse population, having the lowest \( H_{\text{Exp}} \) (0.467) and PIC (0.413).

**Genetic distance among Korean Chicken breeds**

Fig. 1 illustrates the population relationships based on the PCoA using individual multilocus genotypes of 12 MS markers. The first and second components contributed 31.48% and 25.29%, respectively, and the third component contributed 15.8%. Clearly, by the first component, Leghorn (NF, NK) was confirmed to be separated from the other groups. Cornish (NS, NH) was confirmed near the KNC, HL and LO by the second component. And it showed that HL and LO are genetically very close by the variance of first and second components.

Also, we conducted FCA, using allele frequencies of the 12 MS markers, as an alternative approach to understand the genetic relationships among breeds (Fig. 2). Fig. 2 shows close relationship among individuals which belong to the KNC, Cornish, and NO, and it was the leghorn (NK, RIR).
NF) breeds that are clearly separated from other groups. Overall, it was confirmed that results similar to those of PCoA appeared.

The genetic divergences among the 22 chicken breeds based on allele frequencies were calculated according to DA genetic distance. The phylogenetic relationships among these 22 chicken breeds were determined using the neighbor-joining tree (Fig. 3). The genetic distances of 22 chicken breeds were in the range of 0.0515 (HL and LO) to 0.726 (HA and NK). The HL and LO were grouped into the same branch. Thus, the relationship between PCoA and FCA was very similar.

DISCUSSION

This study aimed to analyze the genetic diversity and population structure through 12 MS markers for Korea Chicken 22 breeds. F-statistic were estimated in a fixation index as $F_{st}$, $F_{it}$ and $F_{is}$ among the 12 MS markers. The estimated mean value of the $F_{st}$, $F_{it}$ and $F_{is}$ were 0.183, 0.231 and 0.058, respectively. The five out of 12 markers named ADL0293, LEI0094, MCW0029, MCW0123 and MCW0145 showed a negative number (Table 1). However, all the others
showed a positive number. The $F_{is}$ represents a degree of nonrandom mating (deviation from Hardy–Weinberg equilibrium) (Suh et al., 2014). And the expected probability of identity values of 12 MS markers were calculated in random individuals ($PI$), random half-sib ($PI_{half-sibs}$) and random sibs ($PI_{sibs}$), which were estimated as $3.65 \times 10^{-17}$, $1.13 \times 10^{-12}$ and $2.81 \times 10^{-6}$, respectively. Overall, the total expected probability ($PE$) of identity values was 98.11% for the discrimination of Korean chicken breeds.

White leghorn (NF, NK) exhibited a lower degree of genetic diversity [NF: MNA = 3.75, $H_{exp} = 0.468$, $H_{obs} = 0.392$, PIC = 0.413; NK: MNA = 3.92, $H_{exp} = 0.471$, $H_{obs} = 0.366$, PIC = 0.419] than all other breeds in all measures of genetic diversity whereas a high degree of diversity was observed in ROSS (MNA = 7.00, $H_{exp} = 0.710$, $H_{obs} = 0.683$, PIC = 0.666) (Table 2). Heterozygosity was observed for White leghorn as quite low compared to other breeds which may be due to inbreeding among closely related breeds. In previous study, even though they have been obtained with different marker sets, white leghorn exhibited a lower degree of genetic diversity (MNA = 3.43, $H_{exp} = 0.416$, $H_{obs} = 0.326$, PIC = 0.371) (Suh et al., 2014). The current result was similar to the one reported by Suh et al. (2014) and another study, the values for $H_{exp}$ and PIC reported by Kong et al. (2006) for Korean chicken breeds ($H_{exp} = 0.630$ and PIC = 0.552), Suh et al. (2014) for Korean chicken breeds ($H_{exp} = 0.696$ and PIC = 0.653), Seo et al. (2017) for Korean chicken breeds ($H_{exp} = 0.694$ and PIC = 0.650) and Choi et al. (2019) for Korean chicken breeds ($H_{exp} = 0.620$ and PIC = 0.558) were almost similar to the values obtained for the present analysis. These results indicated Korean chicken breeds have kept a high level of genetic diversity.

Fig. 1 illustrates the population relationships based on PCoA using individual multilocus genotypes of the 12 MS markers. Clearly, based on the first component, Leghorn (NF, NK) was confirmed to be separated from the other groups. Cornish (NS, NH) was confirmed near the KNC, HL and LO by the second component. And it showed that HL and LO are genetically very close by the variance of first and second components. And the neighbor network analysis of the 22 breeds confirmed the FCA results as the Korean chicken breeds segregated in a similar pattern (Fig. 1, 2). As a result of checking the genetic distance between groups by phylogenetic tree, it was confirmed to be the nearest genetic distance (0.0515) for Hy-line Brown (HL) and Lohman Brown (LO) and the farthest genetic distance (0.726) for HanHyup A (HA) and Leghorn K (NK).

This study is the analysis based on the 12 MS marker polymorphisms of the genetic diversity in the 22 Korean chicken breeds. Our results indicated that these multiplex PCR marker sets will have considerable applications in population genetic structure analysis. In addition, since the MS markers in this study are highly polymorphic, they can also be applied for the conservation, traceability and future improvement of these Korean chicken breeds.

CONCLUSION

In conclusion, we analyses the genetic diversity and population structure through 12 microsatellite (MS) markers for 22 Korean Chicken breeds. The reliability and power of identification using 12 MS markers were improved, and the genetic diversity and probability of individual discrimination were confirmed through statistical analysis. As a result of the genetic distance between groups by phylogenetic tree, it was confirmed to be the nearest genetic distance (0.0515) for Hy-line Brown (HL) and Lohman Brown (LO) and the farthest genetic distance (0.726) for HanHyup A (HA) and Leghorn K (NK).

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ETHICS APPROVAL

The study was approved by the Hankyong National University Animal Ethics Committee (No. 2018-2).

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SUPPLEMENTARY MATERIALS

Supplementary material can be found via https://doi.12750/JARB.36.3.154.

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