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The Molecular and Cytogenetic Characterization of Benha Line Chickens

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A B S T R A C T

Benha line chickens (Line B) were originated by crossing the Egyptian native Golden Montazah cocks with the White Leghorn chickens hens. The main aim of this study was to estimate the genetic characterization of Benha Line Chickens compared to Golden Montazah and White Leghorn chickens as parents. In this study, the genomic DNA of B line, Golden Montazah and White Leghorn chickens were extracted to carry out Polymerase Chain Reaction (PCR) using five specific microsatellite markers (MCW0330, MCW0183, MCW0081, MCW0014 and ADL0278). These markers are specific for egg number, egg shell strength, clutch size, breast muscle weight (BMWT), body weight, egg production rate, heat stress and growth. Further, MCW0330 and MCW0081 primers are specific for resistance of Salmonella and Marek's (MD) diseases. Our results confirm that these specific microsatellite markers are specific for the Benha line chickens except the ADL0278 not specific for Benha line chickens. Further, 26 distinct alleles were detected at the five microsatellite markers. Amongst 26 distinct alleles, Benha Line was carried the largest number of specific alleles at five (20.8%), followed by Golden Montazah with two specific alleles (10.5%). In addition to, the polymorphic information content (PIC) and expected heterozygosity (He) for the three chicken populations were calculated. Benha Line chickens were the highest values of PIC and He per locus which were observed at MCW0183, MCW0330 and ADL0278, respectively, and the corresponding lowest were at MCW0014 and MCW0081, respectively. Moreover, chromosomal analysis was carried out and our results confirm that Benha Line chickens have a diploid number of 78 (2n = 78) chromosomes and the majority are microchromosomes.
Key words: Benha line chicken; weight; microsatellite markers; resistance; chromosomal analysis.

1.Introduction

Crossbreeding is one method that can improve economic traits in poultry. In Egypt, native chicken breeds had high non-additive genetic variance (Abdel Aal *et al*., 2016). Therefore, crossbreeding between native and exotic breeds in chicken will improve the heterozygosis of non-additive genes resulting the heterosis, which is important in combination of the different important characteristics of each breed (Willham and Pollak 1985; Hanafi and Iraqi, 2001; Taha and Abd El-Ghany, 2013).

Benha line chickens were constructed by crossing the Egyptian Golden Montazah cocks (50%) as native breed with the White Leghorn chickens hens (50%) as exotic breed following by three generations of "intense" mating (Iraqi, *et al.,* 2016 and El-Attrouny *et al*., 2017). A selection experiment was carried out during the period from November 2011 to May 2015 in the Faculty of Agriculture, Benha University.

The main objective of selection experiment to improve egg production and quality traits in Benha chicken (El-Attrouny *et al*., 2017). Further, t he genetic and phenotypic trends for egg production and quality traits were also estimated in Benha chickens (El-Attrouny *et al*., 2017).

Up to date, a few information is available on ‹the genetic diversity of Benha line chickens which have high egg production. Therefore, the current study was carried out toestimate the genetic characterization of Benha line chickens and importantly, genetic characterization contributes to breed definition, especially populations which are not well defined and provide an indication of their genetic diversity. As well as, it has potential to identify unique alleles in the breeds or lines studied (Roushdy *et al*., 2008).

Simple sequence repeats (SSRs) or microsatellites are molecular markers which are widely used in exploring genetic variation and phylogeny between populations of same species (Buchanan, *et al*., 1994; Machugh, *et al.*,1994) and are extensively used for assessing genetic structure, diversity, and relationships (Tautz, 1989). Microsatellites are characterized by tandem repeats of one to six bases (Muhammet and Mehmet, 2008). Compared to other types of molecular markers, microsatellites have many advantages such as being numerous and ubiquitous throughout the genome, showing a high degree of polymorphism, and codominant inheritance (Cheng and Crittenden, 1994).

Therefore, these markers are useful in estimating genetic relatedness and diversity in chickens which have been demonstrated in a number of indigenous breeds, inbred strains and in commercial chicken lines (Zhang, *et al*., 2002; Tadelle, 2003; Osman, *et al*., 2006; Tadano, *et al.*, 2007; Roushdy *et al*., 2008).

In addition to, many highly developed breeds with unique phenotypes tend to have low degrees of molecular diversity as detected by a panel of microsatellites (Wiener, *et al.,* 2004; Cañón *et al*., 2006; Peter, *et al.*, 2007). This emphasizes the importance of considering phenotypic and molecular diversity as separate and complementary criteria for conservation decisions. A breed with a unique phenotype may

be of value for conservation even if molecular diversity is small.

The objective of this research was to assess the genetic diversity of Benha line chicken, using Golden Montazah chicken and White Leghorn chicken as controlled populations, based on five specific microsatellite markers recommended by the Food and Agriculture Organization (FAO, 2011). This study served as an initial step in the plan for genetic characterization and conservation of the Benha line chicken genotypes, as well as Golden Montazah and White Leghorn chickens. Furthermore, chromosomal analysis of Benha line chickens was also performed using feather pulp cells.

2. Materials and Methods

2.1. Chicken Population:

This project started in 2008 and aimed to produce a synthetic line of chickens under hot climate conditions in Egypt. A description of the main features of the line Benha chickens (Line B) is carried out. It was founded in 2011 as a synthetic line between the Egyptian Golden Montazah and the White Leghorn (Figure 1). The procedure of foundation began mating Golden Montazah cocks to White Leghorn hens and it was followed by three generations of "inters" mating. Afterwards the line has been selected to highly egg production and quality according to the predicted breeding values based the BLUP procedure using animal model and morphological description of line- Benha by Iraqi, *et al.,* 2016 and El-Attrouny *et al*., 2017.

In this study used five Golden Montazah, seven white leghorn and seven Benha line chickens were selected randomly from Poultry Agricultural farm at Faculty of Agriculture, Benha University, Egypt

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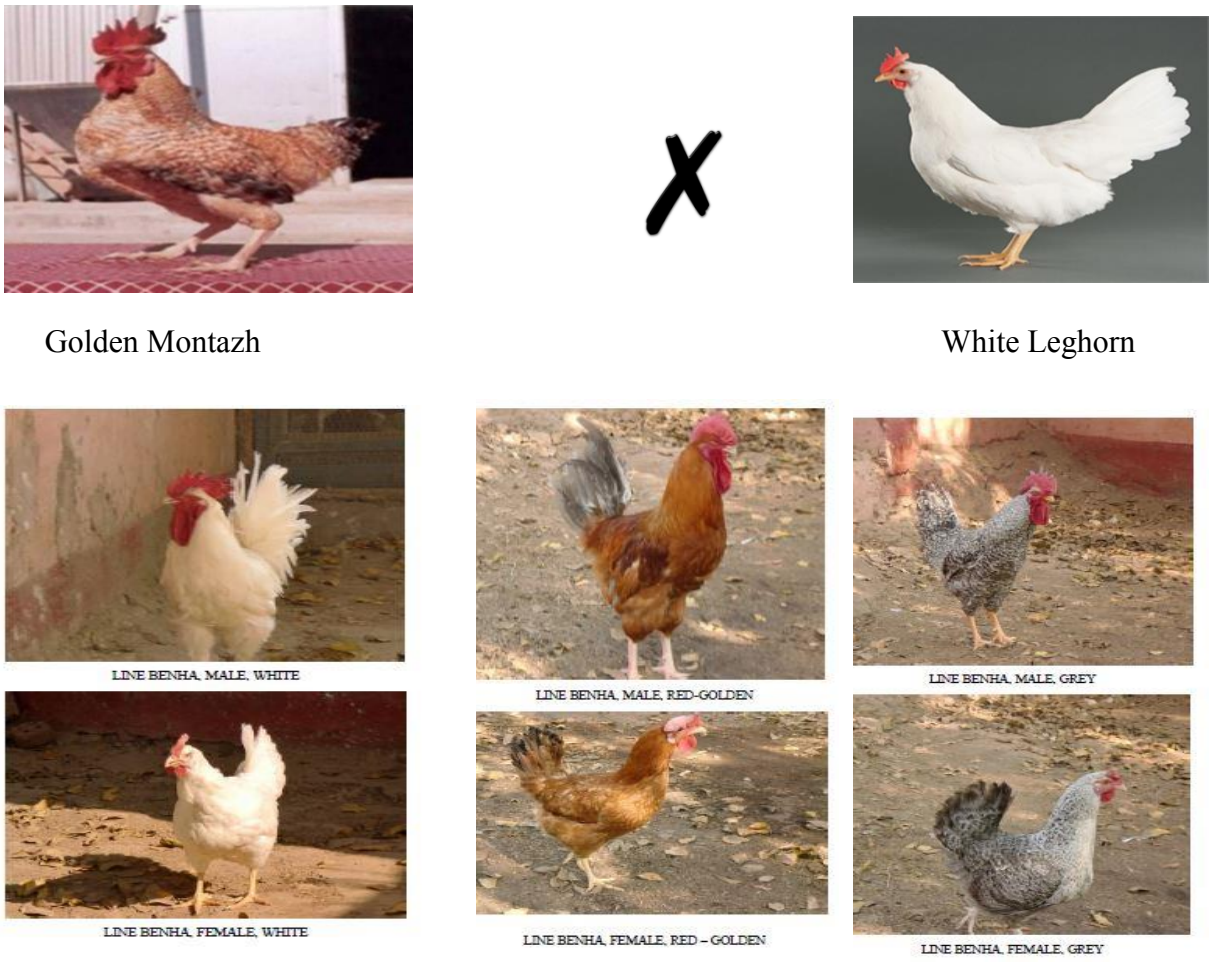

Figure 1: Examples of different genotypes of chickens.

2. 2.Specific Microsatellite Genotyping:

In chickens, 29 markers belong to the list of microsatellite markers recommended by FAO (2011). Among these, five microsatellite markers distributed on 5 autosomes were chosen to evaluate the diversity. These markers named (MCW0330, MCW0183, MCW0081, MCW0014 and ADL0278) are shown in (Table 1).

PCR was carried out using Gene Taq Green PCR master mix (2X) (Genetix Biotech Asia Pvt. Ltd.). The PCR reagents were used as genomic DNA (1µl), forward primer (0.4µl), reverse primer (0.4µl), PCR master mix (5µl) and dH2O (5.7µl) for total volume 12.5µl.

All the PCR products were visualized on a 1 % agarose gel running at 130V for 25 min by a gel imager using a commercial imaging system (UVITEC). In addition, DNA ladder marker 1Kb was used to detect the PCR product. To detect the PCR product bands, the Image Lab software was used.

Table 1. Sequences of oligonucleotide primers used to amplify Five microsatellite loci of chicken (*Gallus gallus).*

Microsatellite marker name	Gene bank accession number	Chromosome	Primer sequence (5' -> 3') Forward Reverse	Annealing temperature (°c)	Allele Range size (bp)
MCW0330	G32085	17	TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC	61	256-300
MCW0183	G31974	7	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	61	296-326
MCW0081	---	5	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	62	112-135
MCW0014	---	6	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	56	164-182
ADL0278	G01698	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	58	114-126

2.3. Microsatellite DNA Polymorphism and Deviation from Hardy-Weinberg Equilibrium:

The number of alleles per locus, observed heterozygosity (*Ho*), and Nei's unbiased estimates of expected heterozygosity (*He*) were calculated using program Cervus 3.0.3 (Chacon-Cortes *et al*., 2012). The information content of each locus was calculated by the polymorphism information content (PIC) using program Cervus 3.0.3. Deviation from Hardy-Weinberg equilibrium were assessed across populations and for each locus based on exact tests as implemented in Cervus 3.0.3. Also, deviation from HWE at each locus in each population was obtained using program Genealex 6.4 (FAO/MoDAD, 2004; http://fao.org/dad-is).

2.4.*Analysis of Molecular Variance (AMOVA):*
To quantify the extent of molecular variation, locus-by-locus analysis of molecular variance (AMOVA) was performed using Genealex 6.4. In the current study both *F*ST and *R*ST were used to determine the potential differences between the two statistics. *F*- and *R*-statistics were obtained using AMOVA approach as implemented in Genealex 6.4 (FAO/MoDAD, 2004; http://fao.org/dad-is).

2.5.Study of Benha Line chromosomes from Feather Pulp:

Chromosomal analysis was done on Benha Line chickens at (2- 3) weeks of age. The feather pulp cells of Benha Line can be performed as soon as pin feathers are present at the base of growing feathers. The Chromosomal protocol was performed according techniques in animal cytogenetics book (Popescu, *et al*., 2000).

3. Results and Discussion

3.1.The specific microsatellite markers were identified in Benha line chickens:

To identify the characterization of Benha Line chickens, our results were compared with Golden Montazah and White Leghorn as parent of Benha Line chickens (Table 2). The MCW0330 primer which is linked for ten genes; that may encode to proteins of egg number, breast muscle weight (BMWT), body weight (21days) and feather pecking (FP). Our results confirm that the MCW0330 primer is specific for Benha Line chickens.

Further, the MCW0183 primer which is specific for twenty- four genes; that may encode to proteins of the antibody response to *E. coli*, body weight (35- 56, 63, 70, 77 and 84 days), egg shell strength, cluch size and Carcass weight. Our results confirm that the MCW0183 primer is specific for the Benha line chickens.

Moreover, the MCW0081 primer is specific for eight genes; that may encode to proteins of egg shell thickness, egg production rate and body weight (42 days) and growth rate (1-8 days). Further, this primer is specific for Marek`s disease- related traits (MD) and resistance of Salmonella. Our results confirm that the MCW0081 primer is specific for the Benha Line chickens.

In addition, the MCW0014 primer is specific for two genes; that may encode to proteins of total white fat weight, heat stress and related to adhesion of cellular cells in the muscle cell. Our results confirm that the MCW0014 primer is specific for the Benha Line chickens.

The last primer was used the ADL0278 which is specific for five genes; that may encode to proteins of carcass weight and egg after taste. Our results confirm that the ADL0278 primer is not specific for Benha Line, Golden Montazah and White Leghorn chickens.

According, the main features of Benha line which were high egg production, high resistance for Pasteurellosis and Salmonella diseases, high fertility and hatchability. Also, it had low mortality rate (1%) during the production period (El-Attrouny *et al*., 2017). Therefore, our recent findings confirm that the Benha Line chickens are specific for egg production and Marek`s disease- related traits (MD) and resistance of Salmonella.

Table 2: The specific microsatellite markers were identified in Benha line chickens.

	112-135bp			164-182bp			296-326			256-300			114-126		
	Chromosome No.5			Chromosome No.6			Chromosome No.7			Chromosome No.17			Chromosome No.8		
	B. Line	G.M. Line	W.L Line	B. Line	G.M. Line	W.L Line	B. Line	G.M. Line	W.L. Line	B. Line	G.M. Line	W.L Line	B. Line	G.M. Line	W.L. Line
Marek's disease-related traits	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Egg shell thickness.	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Egg production rate	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Carcass weight	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Body weight (42 days)	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Body weight (56 days)	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Body weight (77 days)	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Catenin alpha 3	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Total white fat weight	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Egg shell strength	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Cluch size	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Egg number	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-

3.2. Within Chickens breed diversity:

The number of alleles for each microsatellite markers and the number of specific alleles for each breed are summarized in Table 3. A total of 26 distinct alleles were detected at the five microsatellite markers in 19 birds. Amongst 26 distinct alleles, Benha Line, Golden Montazah and White Leghorn possessed 24, 19 and 18 alleles respectively.

Seven of all 26 alleles (26.9%) were unique to only one breed. Benha Line was carried the largest number of specific alleles at five (20.8%), followed by Golden Montazah with two specific

alleles (10.5%). No found specific alleles belonged to White Leghorn.

Consequently, the specific alleles were observed in three loci of all five microsatellite markers (60.0%). Across the breeds, the average number of alleles per locus was 5.22±2.17 (26/5) with the range from 4 (MCW0014, MCW0183 and MCW0330) to 9 (ADL0278). The mean number of alleles in Benha Line chickens was 4.8±1.18 with the range from 4 (MCW0081, MCW0014, MCW0183 and MCW0330) to 8 (ADL0278). While, the mean number of alleles in Golden Montazah was 3.8±0.84 and in White Leghorn it was 3.6±1.14.

Table 3: The Number of specific alleles for three chicken genotypes.

Locus	Number of alleles (specific alleles)			Total number of alleles (specific alleles) for individual site
	Benha Line	Golden Montazah	White Leghorn	
MCW0081	4(2)	3(1)	2(0)	5(3)
MCW0014	4(1)	3(0)	3(0)	4(1)
MCW0183	4(0)	4(0)	4(0)	4(0)
MCW0330	4(0)	4(0)	4(0)	4(0)
ADL0278	8(2)	5(1)	5(0)	9(3)
Total	24(5)	19(2)	18(0)	26(7)
Mean±S.D	4.8±1.18	3.8±0.84	3.6±1.14	5.22±2.17

3. 3.Chicken Breed genetic differentiation:

Heterozygosity is known as gene diversity. In this study, the heterozygosity was estimated within the three chicken populations based on a five specific microsatellite markers showing substantial number of detected alleles and polymorphic information content (PIC). PIC was an ideal index to measure the polymorphism of allele fragments. According to Botstein *et al*. (1980), PIC>0.50 indicates a highly informative locus, 0.25<PIC< 0.50 indicates a reasonably informative locus, and PIC<0.25 indicates a slightly informative locus.

Therefore, the PIC and expected heterozygosity (He) for the three chicken populations are summarized in Table 4. The average polymorphism information content (0.372) and the average expected heterozygote frequency (0.494) of White Leghorn chicken and Benha Line were 0.320 and 0.409 were the highest, and those of Golden Montazah were 0.297 and 0.369, respectively, which were the lowest.

As to Benha Line chickens, the highest values of PIC and He per locus were observed at MCW0183, MCW0330 and ADL0278 (0.489 and 0.369), respectively, and the corresponding lowest were at MCW0014 (0.245 and 0.215) and MCW0081 (0.336 and 0.279), respectively.

In terms of Golden Montazah chickens,

the highest values of PIC and He were both observed at MCW0014 and MCW0183 (0.365 and 0.480), and the lowest value of PIC and He were both observed at ADL0278 (0.215 and 0.245). While, MCW0081 and ADL0268 showed the highest value of PIC and He (0.500 and 0.375) both in White Leghorn chickens, which mean that the two chicken breeds were heterozygous at this locus. However, the value of PIC and He was (0.369 and 0.489) at MCW0014, MCW0183 and MCW0330, which meant that at this locus 50% White Leghorn chicken were homogeneous.

Table 4. Polymorphism information content (PIC), Expected heterozygosity (He) for three chicken genotypes.

Locus	Benha Line		Golden Montazah		White Leghorn	
	H_e	PIC	H_e	PIC	H_e	PIC
MCW0081	0.336	0.279	0.320	0.269	0.500	0.375
MCW0014	0.245	0.215	0.480	0.365	0.489	0.369
MCW0183	0.489	0.369	0.480	0.365	0.489	0.369
MCW0330	0.489	0.369	0.320	0.269	0.489	0.369
ADL0278	0.489	0.369	0.245	0.215	0.500	0.375
Means	0.409	0.320	0.369	0.297	0.494	0.372

Genetic difference was observed (overall F*st* = -0.081, in Table 5); that is, around 8.1% of the microsatellite variation among the three chicken breeds was due to breed differentiation. Only one loci contributed significantly to this differentiation. It can also be seen that the deficit of heterozygotes was very high (0.775) ($p<0.001$) and Except for five loci (MCW0081, MCW0014, MCW0183, MCW0330 and ADL0278).

In accordance with the analysis of existing genetic differentiation between the possible pairs of genetic groups, the F*st* value showed slightly high differentiation (0.035) between MCW0081and three chicken genotypes which was the highly significance. The lowest F*st* value (0.027) was observed between MCW0014 and three chicken genotypes.

The heterozygote deficit within population (F*is*) for the three chicken genotypes are summarized in Table 5. Comparatively, the F*is* for MCW0183 and MCW0330 primers was the highest (1.0) followed by ADL0278 primer (0.723), MCW0014 primer (0.772) and MCW0081 primer (0.276). Many cases of F*is* for Benha Line (7), Golden Montazah (5) and White Leghorn (7) were statistically significant ($p<0.01$). In all three chicken populations, both all primes showed heterozygosity very high significance ($p<0.01$).

Table 5. The results from F-statistical analysis and number of populations.

Locus	F_{it}	F_{st}	F_{is}
MCW0081	0.302^{***}	0.035^{***}	0.276^{***}
MCW0014	0.766^{***}	-0.027^{ns}	0.772^{***}
MCW0183	1.00^{***}	-0.149^{ns}	1.00^{***}
MCW0330	1.00^{***}	-0.113^{ns}	1.00^{***}
ADL0278	0.686^{***}	-0.133^{ns}	0.723^{***}
Mean	0.757^{***}	-0.081^{ns}	0.775^{***}

F_{it} = The global heterozygote deficit across three populations; F_{st} = Genetic differentiation; F_{is} = The heterozygote deficit within population (inbreeding coefficient). *** $p<0.001$

According our data, the AMOVA analysis of MCW0081 showed that most of genetic variation (3%) was observed among the populations, whereas the variance among populations of MCW0014, MCW0183, MCW0330 and ADL0278 was 0.0%.

Further, the AMOVA analysis of MCW0081 showed that less of genetic variation (27%) was observed among the individuals, whereas the most genetic variance among individuals was100% for the MCW0183 and MCW0330 markers.

According our results, we can predict that many genetic defects can be diagnosed by associated molecular markers. These markers can be used to screen the population and identify carriers of the undesirable alleles. The use of carriers for mating, especially with each other, should be avoided when possible.

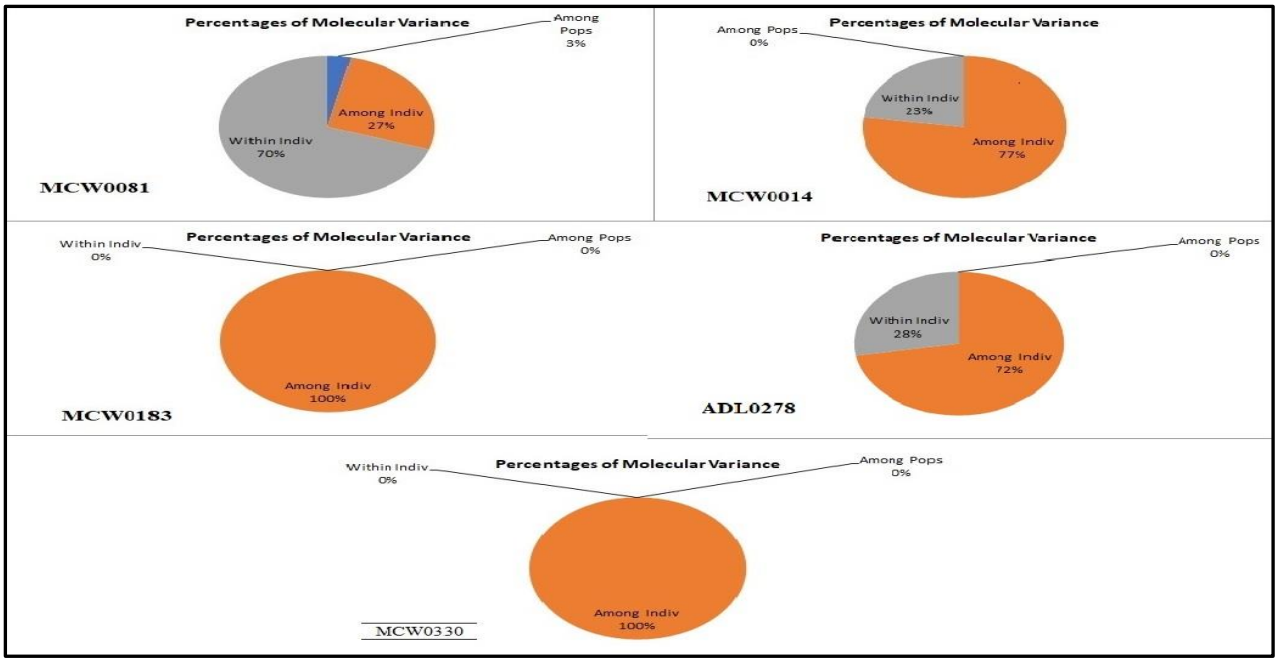

Figure 2: The analysis of molecular variance (AMOVA) within and among three populations.

4. Chromosomal Analysis of Benha Line Chickens:

The Benha Line chicken was checked for chromosomal analysis. We detected 39 pair of chromosomes and ZW as shown in figure 3. Further, the majority of Benha line chromosomes are microchromosomes.

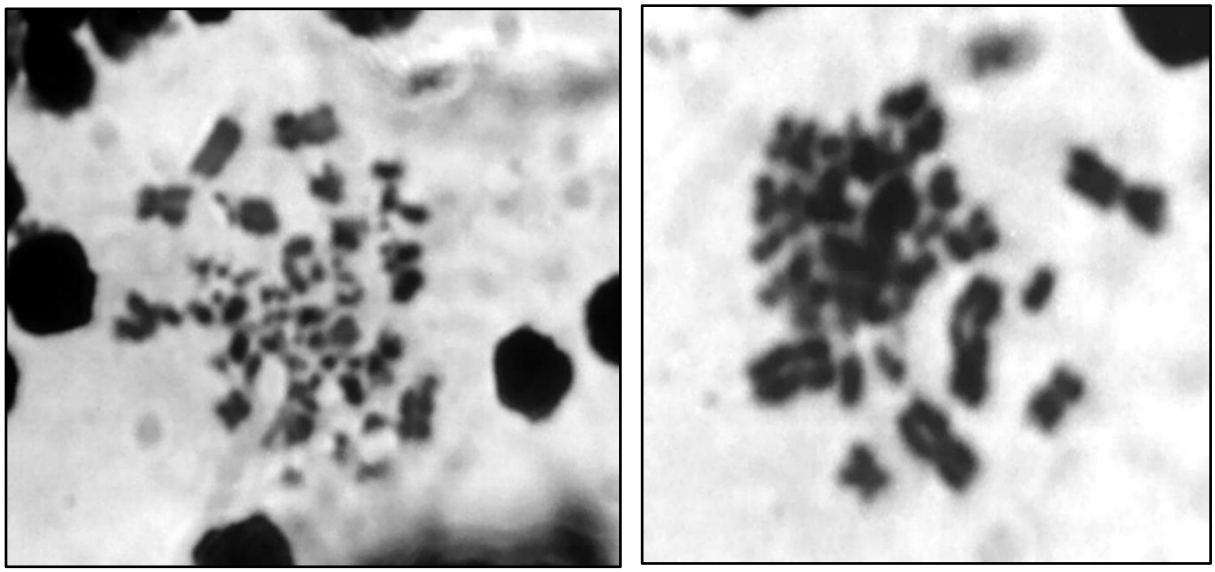

Figure 3: Chromosomal analysis of Benha Line chickens.

5. Conclusion

According to our previous results that confirmed that Benha line chickens (Line B) are high egg production, high resistance for Salmonella diseases, high fertility and hatchability. Also, our current results confirm that Benha Line chickens are more specific for egg production, breast muscle weight (BMWT), body weight at different ages and resistance for Salmonella and Marek's disease (MD). Furthermore, Benha line chickens have good

adaptation for hot climate conditions due to resistance of heat stress.

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