ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Genetic Variations of Tyrosinase (TYR) gene of Feather Colours in Local Indonesian Canary (*Serinus canaria*).

Mudawamah[1]*, MZ Fadli[2], and Aulanni'am[3].

[1]Faculty of Animal Husbandry, Islamic University of Malang, Malang, Indonesia.
[2]Faculty of Medicine, Islamic University of Malang, Malang, Indonesia.
[3]Faculty of Chemical, University of Brawijaya, Malang, Indonesia.

ABSTRACT

To full fill the demand of domestic market, breeders make selection and mating just based on variations in phenotype. However so far, no molecular genetic studies with relation to feather colours in local Indonesian Canary. The molecular genetic will inform the variations of genetics in genes relating with feather colours in birds, but there has been no attempt to local canary. One of the genes related to feather colour in locus C chicken (albino or non-pigmented) is tyrosinase. The benefits of this research were expected to contribute to the conservation and exploitation of natural resources in Indonesia, especially on genetic variation and candidate genes associated with the colour of canary feathers. Total DNA was extracted from blood canaries polymerase chain reaction amplified DNA product was sequenced. The results showed that the amplification of canary genomes with tyrosinase gene were 200 bp fragment length while the results of the sequence had a length between 578-158 bp fragments. The genetic variation of gene tyrosinase in local Indonesian canary was shown in genetic similarity of 87-100 % and haplotype diversity of 0,9951 with 20 types.

Keywords: Local canary, Feather Colour, Tyrosinase, *Serinus canaria*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Canary birds are the most species of birds in the family *Fringillidae*. One of the species has small body characteristics, with varying feather colour and birds chirping are *Serinus Canaria*. The original habitat of this bird is Canaria Island in Spain Europe. The natural habitat of this bird is in the open nature, non-migratory and monogamy [1-3]. Total amount of canary bird populations in the world and in Indonesia have been no reports however these birds went into the Least concern [4] because it is including in the category of birds with declining populations of the IUCN Red List (that is declining more than 30% in ten years or three generations).

Local Indonesian Canary is one of commodity that is able to drive the lower middle economic level. Recently, the demand of local canary with yellow, white, orange and mosaic colour were higher than the dark hair colour like as starblue and green. To fill market demands, breeders make selection and mating just based on variations in phenotype [5,6].

Selection and mating with phenotype variation should be supported by the study of genetic variation in relation with feather colour genes of local Indonesian canary. One of the genes related with feather colour in locus C of chicken (albino or non-pigmented) is Tyrosinase (TYR) [7-10]. In addition, the TYR gene is also related to skin colour in variety of animal species that synthesis of tyrosine kinase and protein tyrosine phospatase [11-17]. However, there is no report about the tyrosin gene in local canary.

It is necessary to observe the genetic diversity of various local canary feather colours as the basis for selection. It is expected to contribute to the conservation and exploitation of natural resources in Indonesia, especially on DNA variation and candidate of genes associated with feather colour canaries. The purpose of this study was to observe the genetic variation of different colours canary feathers by TYR gene.

MATERIALS AND METHODS

Blood sample

Blood samples were obtained from 28 samples of the local canary from Malang, East Java, Indonesia. Samples were derived from various types of canaries with different colours. They were starblue, green, yellow, white, green, yellow mosaic, mosaic starblue white, yellow and white mosaic. Of 0.1 cc blood samples taken at the right wing through the *vena axillaris* using a syringe that has been coated with 1 ml heparin (anti-coagulant). After blood sampling immediately be isolated or stored at 2-8 °.

DNA Isolation

Canary Blood (0.1 ml) was added with RBC (Red Blood Cell) lysis with a ratio of 1:3 , incubated for 10 min at 25 ° C and then centrifuged (Hettich) at 3000 rpm for 10 minutes. Pellet was processed sequentially by added with 750 µl cell lysis solution, homogenized, divided into two microtube and then incubated for 15 min at 58°C in a water bath (Yamato BT 100). This solution was added with PCI solution (1 x vol), homogenized (Heraeus) for 1

min and centrifuged using cold micro centrifuge (refrigerated centrifuge Micro22R) at 13000 rpm for 10 min at 4 oC. The supernatant was transferred into a new microtube, added with CI (1 x vol), homogenized and centrifuged using micro centrifuge at 13000 rpm for 10 min at 4 ° C . The supernatant from this step was transferred to a new microtube, added with absolute ethanol (MERCK) as 2.5 times of supernatant volume, shaked and incubated in -20 ° C for 1 hour, then centrifuged at 13,000 rpm for 10 min at 4 ° C. Pellet was added with 500 ul of 70% ethanol, shaked gently and centrifuged at 13,000 rpm temperature of 4°C for 10 minutes. Then pellet was dried at 55^{o}C using the oven (Heraeus). This dried pellets, added with 70-100 ul TE buffet, heated 15 minutes using oven, and stored at -20 ° C for further testing. The quality of DNA was confirmed using 1.5 % Agarose Gel Electrophoresis .

Experimental

TYR Gene Amplification

The optimized PCR (Polymerase Chain Reaction) thermal cycles for the primer pair NS1 and GCfung was as follows: initial denaturation at 95°C for 4 min and 35 cycles of 95°C for 1 min, annealing at 50°C for 1 min and 10 sec, extension at 72°C for 2 min, then followed by a last extension at 72°C for 8 min. Each PCR reaction contained 5 µl of 10 × PCR buffer, 2 µl of MgCl2 (25 mM), 4 µl of dNTP (2.5 µM) mixture, 1 µl of BSA (1 µg/µl), 0.3 µM of each primer, 0.8 units *Taq* Polymerase, and 10 ng template DNA.

Before the amplification, primary design TYR gene with primary reference to the research results [18] with the sequence Forward 5'-TGC CAG GGG CTG GAC ATC CCC-3' Reverse 5'-GGG CCC CCA GCA GAT GAA GAA-3'. The next step was made the composition of the PCR with a volume of 20 ul per tube consisted of 7.4 ul ddH2O, 10 ul PCR kit (Go Taq Green master mix @) (10x taq polymerase buffer, dNTPs, MgCl2, primers, Taq DNA polymerase, ddH2O), 12:35 ul forward primer, reverse primer and 2 ul ul 0:35 genomic DNA canaries. The PCR program (Master Cycler Gradient Eppendorf) was used to amplify the TYR gene shown in Table 1.

Table 1: Program for PCR amplification of the TYR gene

The step of PCR	Temperature (^{o}C)	Time	Cycle
Predenaturasi	94	5 minute	1 time
Denaturasi	94	1 minute	
Annealing	62	45 second	35 cycle
Ekstensi	72	45 second	
The last extention	72	5 minute	1 time

Gel Band Purification and amplicon sequencing TYR gene

Before the sequencing, amplicon TYR gene performed gel band purification using the QIA quick gel extraction kit. Therefore, the results of gel band purification at $\pm$ 200 bp sequencing performed using the Qiagen Master Mix. This fragment was sent for sequencing to Macrogene Korean Company.

ISSN: 0975-8585

In this study, they used some software which were (1) to read the results of the sequence with sequence scanner software from macro gene korean company; (2) to determine the genetic distances using MEGA software version 5 [19], (3) to conduct a DNA sequence alignment with BioEdit [20], that was described the haplotype network with Network 4.6.0.0 software [21].

RESULTS AND DISCUSSION

Genome Isolation results

The isolation result of the genome in the various colours of canary that have been identified in accordance with Table 2.

Table 2: The Isolation Result of Genom Sample in Various Colour of Canaries

Number	Canary Feather Colour	Number	Canary Feather Colour
1	Starblue White Mosaic-3 (SWM3)	15	Yellow-4 (Y4)
2	Yellow-2 (Y2)	16	Yellow White Mosaic-3' (YWM3')
3	Starblue-1 (S1)	17	Green-2 (G2)
4	Yellow White Mosaic-2 (YWM2)	18	Yellow White Mosaic-2 (YWM2")
5	White-4 (W4)	19	White Starblue White Mosaic-2 (SWM-2)
6	Yellow-6 (Y6)	20	Yellow-1 (Y1)
7	Green-5 (G5)	21	Green-154 (G154)
8	Green-1 (G1)	22	White-1 (W1)
9	Yellow-3 (Y3)	23	White-5 (W5)
10	Green Yellow Mosaic-3 (GYM3)	24	Starblue-3 (S3)
11	White-3 (W3)	25	Yellow-5 (Y5)
12	Starblue-1" (S1")	26	Starblue White Mosaic-3 (SWM1)
13	White Starblue White Mosaic-4 (SWM-4)	27	Starblue2 (S2)
14	Yellow-7 (Y7)	28	Green-4 (G4)

Amplification results of PCR genes TYR

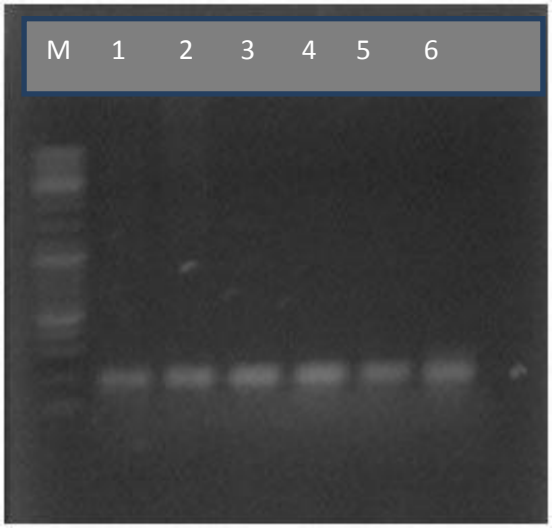

Figure 1: Amplification results of PCR with the TYR gene on agarose gel 1%

M: Marker; 1: Green-2 (G-2); 2: Green-155 (G-154); 3: Yellow-2 (Y-2) 4: starblue-1 (S-1); 5: White Mosaic starblue-2 (SWM-2); 6: Mosaic White Yellow-2 (YWM-2)

ISSN: 0975-8585

Identifying indonesian local canary of genetic molecular based on TYR gene has been chosen because it has specific sequence varies in chicken feathers colour, so it can be used in this study [7-9, 23, 24]. The length of the TYR gene was successfully amplified at ±200 bp on white star blue mosaic, yellow green mosaic, white mosaic of yellow, green, yellow, white and star blue of canary feather colour. One of the PCR succeed can be seen in Figure1.

The next step was sequencing to confirm the Amplification results of PCR gene TYR on various feather- colours of canary. These Sequencing results read scanner sequence software from Macrogene Korean Company.

Sequencing results of amplification PCR using gene TYR

From 28 canaries samples were successfully did by PCR from TYR gene obtained 25 samples, which successfully did by sequence from various feather colour of local canary with the scanner sequence software (Table 3). Table 3 shows that the length of DNA fragments that result sequencing lowest is 160 bp and the highest is 578 bp. Furthermore, the sequencing results, the bases were selected in order to obtain high purification of the base amount that can be further analyzed for type and haplotype networks as well as similarity.

Table 3: Fragment length DNA sequences results from TYR gene on Various canary feathers colour.

No.	Feather colour codes	Fragmen length of DNA (bp) sequencing result with TYR gene	∑ Basa analyzed
1	SWM3	276	118
2	Y2	244	115
3	S1	192	125
4	YWM2'	223	136
5	W4	169	116
6	Y5	162	116
7	G5	444	116
8	G1	239	118
9	Y3	239	116
10	GYM2	243	113
11	W3	158	114
12	S1''	162	116
13	SWM2	160	111
14	Y7	158	118
15	Y4	301	119
16	YWM3'	234	115
17	G2	571	115
18	YWM2''	401	115
19	SWM2	573	115
20	Y1	571	112
21	G154	399	118
22	W1	578	135
23	W5	237	115
24	S3	288	118
25	Y5	485	115

ISSN: 0975-8585

Genetic Variations of Tyrosinase (TYR) gene in Various Canary Feathers Colour

Based on DNA sequence alignment with BioEdit [20], there was only 21 samples out of 25 samples were successfully carried out sequentially. Based on haplotype network analysis results with network software 4.6.0.0, the value of haplotype diversity as big as 0.9952, divided into 20 haplotypes with the details as Table 4 and Figure 2.

Table 4: Haplotype type of Various Canary Feathers Colour

No	Haplotype type
1	Hap_1: 1 [Y4]
2	Hap_2: 1 [YWM3]
3	Hap_3: 1 [G154]
4	Hap_4: 1 [SWM4]
5	Hap_5: 1 [G1]
6	Hap_6: 1 [SWM3]
7	Hap_7: 1 [GYM2]
8	Hap_8: 2 [Y3 Y2]
9	Hap_9: 1 [G5]
10	Hap_10: 1 [G2]
11	Hap_11: 1 [Y6]
12	Hap_12: 1 [W5]
13	Hap_13: 1 [Y5]
14	Hap_14: 1 [S3]
15	Hap_15: 1 [Y4]
16	Hap_16: 1 [SWM2]
17	Hap_17: 1 [W4]
18	Hap_18: 1 [Y7]
19	Hap_19: 1 [W3]
20	Hap_20: 1 [Y1]

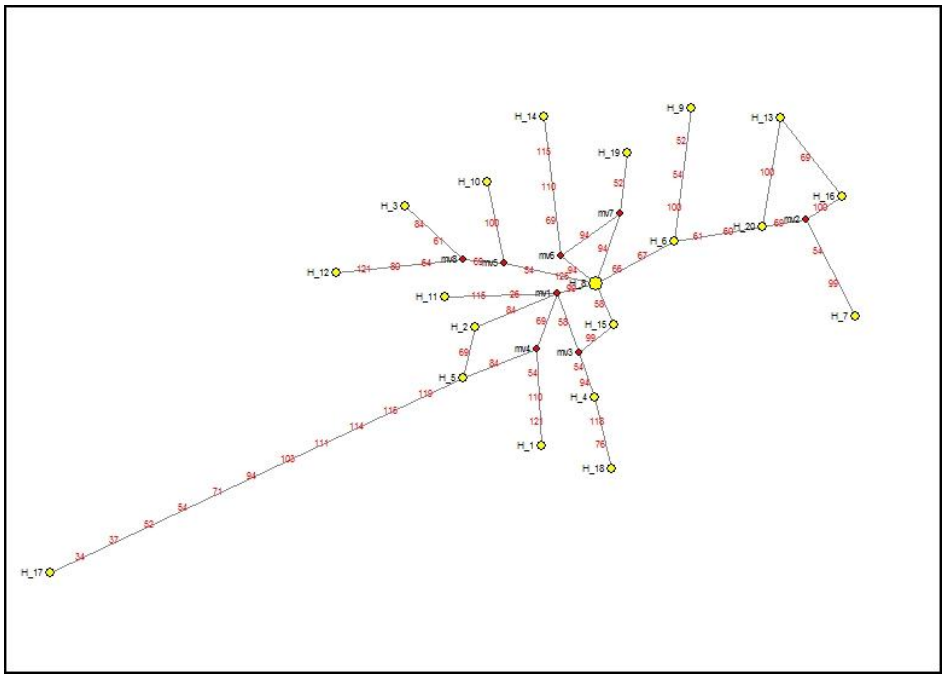

Figure 2: Results of Network Haplotype in various canary feathers colour

ISSN: 0975-8585

Based on the analysis based on haplotypes (Table 4) show that the composition of the DNA of various coat color canaries are very varied. This means that the results of amplification with gene TYR are able to describe the variation of various coat color canaries. Supported the research that each canary feathers in different colors have different haplotypes (90%).

Based on Figure 2, the highest homology appears in G1-YWM3 walnuts (H5-H2) and Y4-Y3, Y2 (H15-H8) and the lowest homology G1-W14 (H5-H17). This suggests that the color green feather (G) or dark colors tend to have much relationship with bright colors (W), otherwise the color green (G) tend to have a close relationship with a mosaic of light and dark (YWM).

Nucleotide sequence of the Amplification Results with TYR gene on various canary feathers colour

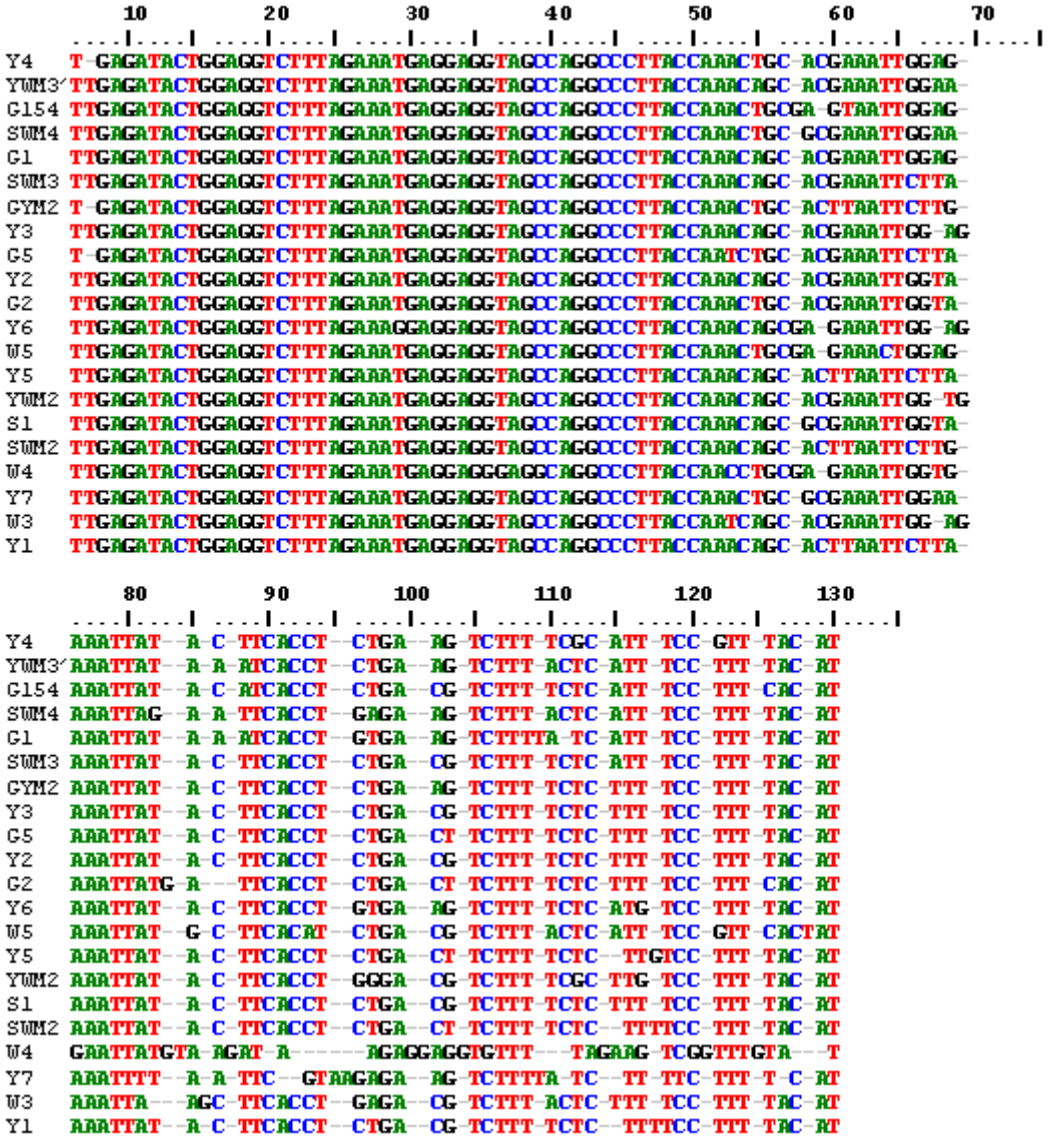

Figure 3: Nucleotide sequence of various canary feathers colour

ISSN: 0975-8585

From the results of these sequences (Figure 3) with Local BLASTn on the TYR gene obtained similarity values as shown in Table 5. Figure 3 showed that sequence various between canary was in the second order, That was senyawa basa-N tirosin, sequences 55-70, 80-130. In another hand, there was only individual W4 (white 4) which shown a very different sequence while compared with other canaries in sequence 80-130. It was estimated that there was high mutation in individual W4 (white colour) that related with TYR gene. [22] TYR gene related with plumage colour white geese.

Table 5: Similarity value with Local BLASTn at MC1R Canary

Hpt	Sample																				
	Y4	YWM3'	G154	SWM4	G1	SWM3	GYM2	Y3	G5	Y2	G2	Y6	W5	Y5	YWM2	$S1^2$	SWM2	W4	Y7	W3	Y1
Y4	0																				
YWM3'	94	0																			
G154	94	92	0																		
SWM4	92	94	90	0																	
G1	94	96	92	92	0																
SWM3	93	93	91	80	92	0															
GYM2	94	90	91	88	90	94	0														
Y3	97	94	93	91	94	95	93	0													
G5	92	90	89	88	88	96	94	93	0												
Y2	92	94	92	91	92	97	93	98	95	0											
G2	93	92	91	90	90	92	91	93	94	95	0										
Y6	94	92	92	92	93	91	89	94	87	92	87	0									
W5	92	90	93	90	94	89	100	92	87	90	90	92	0								
Y5	90	89	88	100	100	95	95	92	95	94	92	88	100	0							
YWM2	94	90	91	89	92	92	91	95	91	95	91	92	90	92	0						
$S1^2$	94	93	92	92	92	96	92	97	94	99	94	92	91	93	94	0					
SWM2	91	89	90	100	89	95	97	94	95	94	92	88	100	98	92	93	0				
W4	90	96	92	93	96	96	94	96	94	96	90	90	92	96	90	92	96	0			
Y7	97	97	95	100	95	96	100	95	96	95	97	94	95	100	96	97	100	93	0		
W3	95	91	88	92	91	90	88	94	90	92	90	90	90	87	92	92	89	96	94	0	
Y1	91	91	90	87	89	97	97	94	95	96	92	88	100	98	92	95	98	96	100	89	0

Table 5 showed that Y5 has the same similarity with SWM-4, Y7, and G1 as well as W5, Y7 has the same similarity with SWM-4, Y5 and SWM-2 as well as Y1, W5 has the same similarity with Y5, SWM-2 and Y1, GYM-2 has the same similarity SWM-2 with W5 and Y7, Y1 has the same similarity with W5 and Y7.

From the results of research using TYR gene (non-pigment gene) showed a tendency variety or individual polymorphism of various canary feathers colour, it can be seen from: the tendency variety sequence results amplification TYR gene with the length between 158-573 bp, haplotype variety was 0.9951 which was divided into 20 haplotypes, the tendency variety based on the similarities value with Local BLASTn values was ranging between 87% - 100%.

In addition, the varieties value of sequence results from amplification TYR gene with a length was between 158-573 bp. The length of the fragments from this research, it was still accordance with the results of Real Time PCR gene TYR in Korean quail feathers colour with fragments length 158 and 213 bp [17] and PCR gene TYR in exon 2 of silver fox. These results of the research was rather different from [11] which amplification TYR in albino mice with fragments length between 366-433 bp.

Haplotype variety was 0.9951, which was divided into 20 haplotypes. This statement was supported with the result of the research by [10] that DNA microsatellite analysis

results with gene TYR in chickens Japanese games (coloured plumage) with a white chicken recessive was obtained 28 linkage groups.

This Variety was based on the similarities value with Local BLAST with values ranging between 87% -100%. The Results of this research supported by [10] and [24] similarities values gene TYR on chicken feathers colour was 90%, while the silver fox has the closest genetic relationship (homologous) was 100% [24].

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that TYR gene (non-pigment genes) can be used to detect identifier molecular genetic through variety or individuals polymorphism from various canary feathers colour, it can be seen from the band length variety amplification result between 158-573 bp, haplotype variety was 0.9951 which was divided into 20 haplotypes, the variety of similarities value between 87% -100%.

ACKNOWLEDGMENT

Authors thanks to Laboratory of Halal Centre Islamic University of Malang, Bio molecular and Biochemistry Laboratory University of Brawijaya Malang, Indonesia. Thanks to Novi Arfarita for English improvement and and also input of the writing of this article. This study was supported by Fundamental Program of DITLITABMAS DITJEN DIKTI, Republic of Indonesia.

REFERENCES

[1] Austad SN. ILAR Jl 2011; 52 (1): 89-96.
[2] Mota PG, Cordosa GC. Biol J Linnead Soc 2007; 92: 183-194.
[3] Leitner S, Marshall RC, Leister B, Catchpole CK. Ethology 2006; 112: 554-563.
[4] Burdfield I, Pople R, Hagemeijer W, Nagy S. Bird Population Trends and Threat in Europe. Bird life International, European Divission Office Wageningen Netherlands 2004; 1-24.
[5] Mudawamah, Fadli MZ, Aulanni'am. The Plumage Colour Pattern *Heredity* and Reproductive Performance of Starblue and Green Canary (*serinus canaria*). Proceedings. The 3rd International Seminar on Tropical Animal Production.October 15-16, 2002. Faculty of Animal Science.Gadjah Mada University.Yogjakarta.
[6] Mudawamah, Fadli MZ, Aulanni'am. The Phenotypic and Genotypic Character of Mosaic White Yellow Feathered of Canary (*Serinus canaria*). Proceeding.International Conference on Cellular and Molecular Biology Science. February, 26-28, 2010. Singapore.
[7] Mochii M, IIO, A Yamamoto H, Takeuchi T, Eguchi G. Cell Res 1992; 5: 162-167.
[8] Tobita-Teramoto T, Jang GY, Kino K, Salter DW, Brumbaugh J, Akiyama T. Poultry Sci 2000; 79: 46-50.
[9] Chang CM, Coville JL, Coquerelle G, Gourichon D, Oulmouden A, Tixier-Boichard M. BMC Genom 2006; 7:19.
[10] Sato S, Otake T, Suzuki C, Saburi J , Kobayashi E. Poultry Science Association Inc, 2007.

[11] Blaszczyk WM, Arning L, Hoffmann K-P, Epplen JT. Pigment Cell Res 2005; 18: 144-145.
[12] Bentem SDLFV, Hirt H. Trend Plant Sci 2008; 30 (10): 2-6.
[13] Hubbard JK, Uy JAC, Hauber ME, Hoekstra HE, Safran RJ. Trends Genet 2010; 26 (5): 232-239.
[14] Zhang H, Wang Z, Wang A, Li H. J Animal Sci Biotechnol 2013; 3: 26.
[15] Luo C, Qu H, Wang J, Wang Y, Ma J, Li C, Yang C, Hu X, Li N, Shu D. BMC Genom 2013; 14:334.
[16] Fagerland H AS, Austbe L, Fritsvoid C, Alarcon M, Rimstad E, Falk K, Taksdal T, Koppang EO. Veterin Res 2013; 44 (107): 2-7.
[17] Xu Y, Zhang XH, Pang YZ et al. Asian Austr J Anim Sci 2013; 26 (11): 1518-1522.
[18] Boichard MT, Faugeras R, Coville JL, Chang CM, Coquerelle G. Genotyping Test for Feather Colour Genes in Chicken 2012; 15: 591-597.
[19] Koichiro T, Dudley J, Nei M, Kumar S. Molecular Evolutionary Genetics Analysis (MEGA) version 4. Center of Evolutionary Functional Genomics, Biodesign Institute, Arizona State University 2007.
[20] Hall TA. Nucl Acids Symp Ser 1999; 41: 95–98.
[21] Wang Y, Li SM, Huang J, Chen SY, Liu YP. Asian Austr J Anim Sci 2014;27(6) : 778-783.
[22] Bandelt H-J, Forster P, Rohl A. Mol Biol Evol 1999; 16: 37-48.
[23] Brumbaugh JA, Bargar TW, Oetting WS. J Heredity 1983; 74: 331-336.
[24] Liu Z, Gong Y, Duan L, Feng M, Zhu W, Ge M, Liu X, Zhao J, and Ghuo X. Asian J Animal Veter Adv 2013; 8 (5): 754-760.