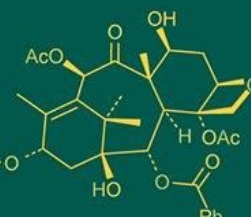
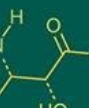
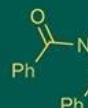


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(3): 564-568
www.biochemjournal.com
Received: 16-01-2026
Accepted: 19-02-2026

Seema Agarwal
Ranchi College of Veterinary
Science & Animal Husbandry,
Birma Agricultural University,
Ranchi, Jharkhand, India

Ravindra Kumar
Ranchi College of Veterinary
Science & Animal Husbandry,
Birma Agricultural University,
Ranchi, Jharkhand, India

Sudhanshu Shekhar
KVK, Ramgarh, Jharkhand,
India

Soumen Naskar
KVK, Ramgarh, Jharkhand,
India

Subhash Chandra
Ranchi College of Veterinary
Science & Animal Husbandry,
Birma Agricultural University,
Ranchi, Jharkhand, India

Tajwar Izhar
Ranchi College of Veterinary
Science & Animal Husbandry,
Birma Agricultural University,
Ranchi, Jharkhand, India

Corresponding Author:
Ravindra Kumar
Ranchi College of Veterinary
Science & Animal Husbandry,
Birma Agricultural University,
Ranchi, Jharkhand, India

Phenotypic, morphometric and genetic characterization of Khukhri, an indigenous chicken of Jharkhand

Seema Agarwal, Ravindra Kumar, Sudhanshu Shekhar, Soumen Naskar, Subhash Chandra and Tajwar Izhar

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3g.7926>

Abstract

In Jharkhand, indigenous poultry farming, also known as backyard poultry, is a widespread and culturally significant practice, particularly among the rural and tribal populations. The present study was conducted to survey, evaluate the performance under village and farm management conditions and molecular characterization of Khukhri chickens of Chotanagpur plateau of Jharkhand. The survey was conducted on 270 households and 540 native chickens. The phenotypic characters of native chickens studied showed the mix type predominant plumage colour. The overall shank colour was predominantly yellow (85.56%) followed by slaty-black. All the chickens (100%) had red earlobe, white skin and red comb. Adult body weight were observed to be 1552.18 ± 27.46 and 1182.04 ± 14.30 kg, however shank length (cm) were observed to be 9.85 ± 0.08 and 8.73 ± 0.05 for cock and hen, respectively. Twenty blood samples were collected in group 1 and 30 from Ranchi district in group 2. Twenty primers were screened, out of which 16 generated polymorphic amplification patterns. RAPD-PCR analysis generated a total of 172 bands, 168 of them were polymorphic. It was observed that primer OPA-16 amplified maximum number (16) of polymorphic bands and PBG-5 amplified lowest number (5) of polymorphic bands. Among the 16 primers used, three-fourth (75%) were highly polymorphic. The average number of amplified bands per primer ranged from 2.2 to 7.46 and the size of the amplified bands varied between 100 to 3000 base pairs. The mean value of Nei's gene diversity and Shannon's information index was 0.28 ± 0.01 and 0.43 ± 0.01 in group 1 and 0.29 ± 0.01 and 0.44 ± 0.01 in group 2 respectively.

Keywords: Khukhri, poultry, backyard, phenotypic, genetic characterization

Introduction

In Jharkhand, indigenous poultry farming, also known as backyard poultry, is a widespread and culturally significant practice, particularly among the rural and tribal populations. Farmers raise native breeds, along with some improved varieties, using low-input, free-range systems that provide nutritional security and supplemental income. While producing fewer eggs than commercial breeds, Khukhri meat and eggs are highly prized in local markets for their flavour. The native birds are slow grower and poor layers of small sized eggs but are good foragers, efficient mother, good sitter and require minimal care to grow. Their plumage color, long neck and long shank helps in protecting themselves against predators. The native breeds of chickens are the reservoir of genomes and major genes for improvement of high yielding exotic germplasm for tropical adaptability and disease resistance. In developing countries, the major threat to genetic diversity is due to introduction of high yielding germplasm in poultry sector by indiscriminate cross breeding of local breeds with less adapted exotic germplasm to evolve highly productive breed which adversely affect the conservation of the local ecology. Keeping in view the above facts the present study has been planned with objectives to characterize all the native breeds to find out the uniqueness among them so that those traits can be exploited to design breeding and conservation strategies (Prabhakaran and Valavan, 2020) [16]. Phenotypic and genotypic Genetic characterization of chicken is done to assess the origin of birds and the geographic distribution of their diversity by using different types of molecular markers. It provides new information to guide and prioritize conservation decisions for poultry as locally adapted chicken genetic resources

could become future assets in breeding programs and created new possibilities for the selection and rapid genetic improvement of chicken.

Materials and methods

The present study was conducted to survey, evaluate the phenotypic and molecular characterization of Khukhri chickens. The survey was conducted on 270 households and 540 Khukhri chicken populations of Chotanagpur plateau of Jharkhand. Thirty blood samples were collected from 50 unrelated native chickens (2 ml each) from the wing veins. RAPD analysis was conducted with The 22 decamer primers (Table 1) of arbitrary sequences with high GC content (60-70%) were used. The amplifications were carried out in final volume of 25 µl containing 5 µl genomic DNA, 2.0 µl DNTPs (2.5mm), 1 µl primer (10pm), 0.25 µl of Taq polymerase and 2.5 µl of 10X reaction buffer with MgCl₂. Amplifications were performed on DNA thermal cycler. Presence of band was designated as '1' and absence as '0'. The matrix data obtained was fed to the POPGENE software (version 1.32) and the results were obtained for observed and effective number of alleles, Nei's gene diversity, Shannon's Information index, gene identity and genetic distance. Genetic diversity within each population was determined as the observed and expected number of alleles (Kimura and Crow, 1964) ^[11], Nei's genetic diversity and Shanon's Information Index (Lewontin, 1972) ^[12] using Popgene software (Yeh *et al*, 1999) ^[21].

Results and discussion

The overall value regarding predominant plumage colour were observed to be mix and red followed by brown, white, black and white, shank colour observed to be mostly yellow,

ear lobe red, skin colour white, comb red and comb type mostly single followed by pea and rose (Table 1) Phenotypic characteristics was in accordance to the findings of Tania *et al.* (2005) ^[20] in Kashmir Faverolla birds, Banerjee (2012) ^[4] in native chickens of West Bengal. The overall average body weight of male and female chicken was 1552.18±27.46 and 1182.04±14.30 g. Shank length observed to be 9.85±0.08 and 8.73±0.05 respectively for male and female (Table 1).

The lesser frequencies were black (10.00%), brown (8.33%) and white (8.33%) (Table 2) Out of 22 only 16 primers were capable of generating polymorphic amplification patterns and hence, they were used in the subsequent analysis with 50 DNA samples. A total of 172 bands were generated from these primers and of these, 168 bands (97.674%) were polymorphic. It was observed that primer OPA-16 amplified maximum number (16) of polymorphic bands and PBG-5 amplified lowest number (5) of polymorphic bands. Among the 16 primers used, three-fourth (75%) were highly polymorphic. The average number of amplified bands per primer ranged from 2.2 to 7.46 and the size of the amplified bands varied between 100 to 3000 base pairs (Table2). The per cent polymorphism observed in the present study was in close agreement with the findings of Saleem (2019) who reported 96.96% polymorphism in three Indian breed of poultry. However lower percent of polymorphism was documented by Jallad *et al.* (2013), Balozza *et al.* (2013) ^[3], Ibrahim *et al.* (2015) ^[8], Abdulrazaq and Suliaman (2016) ^[1], Shivaprasad *et al.* (2016) ^[19], Fadhil *et al.* (2016) ^[6], Gorkhail *et al.* (2020) ^[7] and Sang-Hwan Kim, (2021) ^[9]. However, Karabag and Balcioglu, (2010) ^[10] observed higher polymorphism (99.49%) than the present finding in Japanese quail.

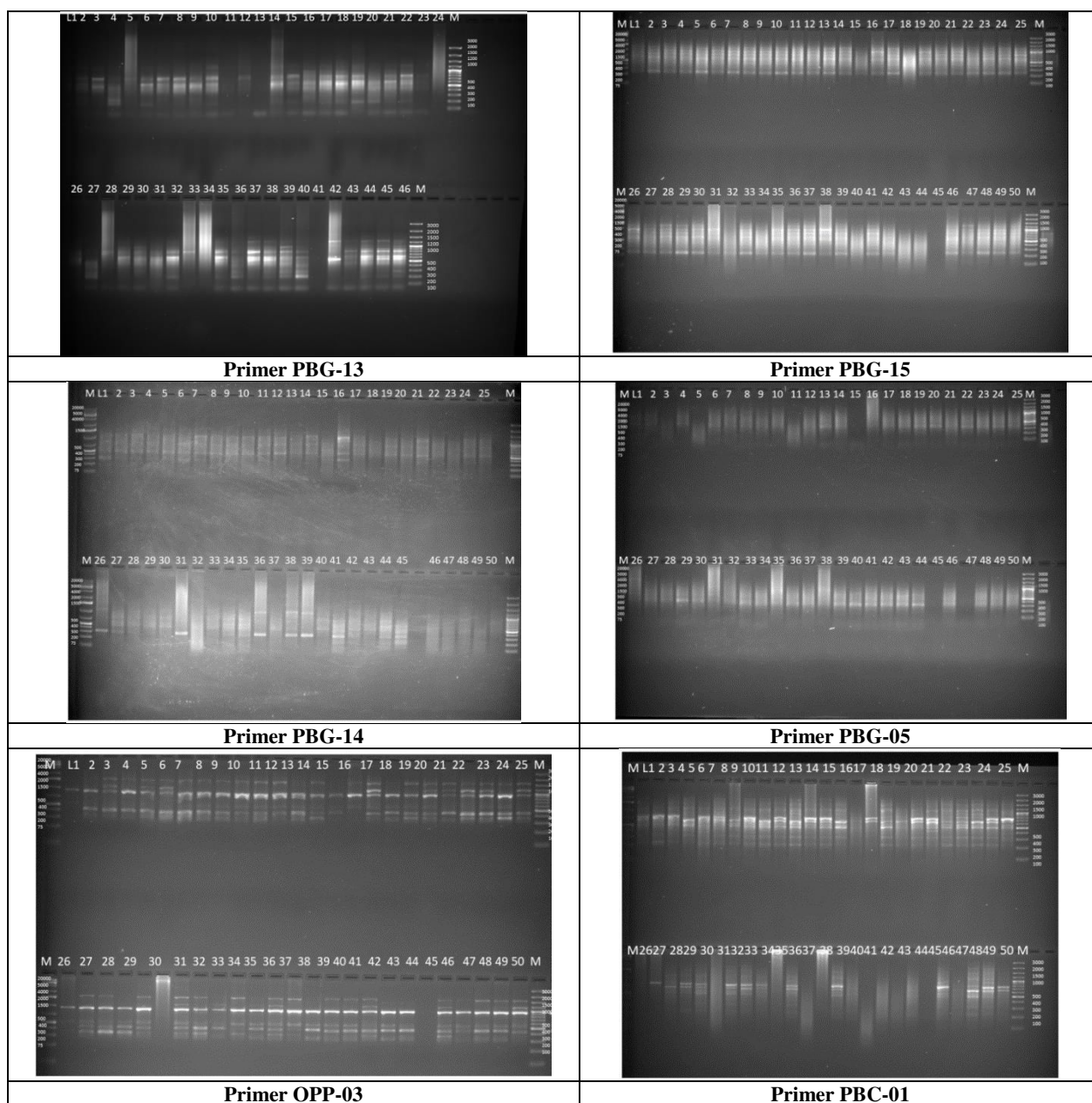
Table 1: Phenotypic traits of khukhri chicken of Chotanagpur plateau of Jharkhand

Parameters	Overall (540)	
	Male (n=180)	Female (n=360)
1. Plumage color (%)		
White	9.44(17)	12.22(44)
Black	6.67(12)	19.44(70)
Brown	11.11(20)	52.78(190)
Black and white	12.78(23)	8.61(31)
Mix	27.22(49)	3.06(11)
Red	32.78(59)	3.89(14)
2. Shank color (%)		
Yellow	97.78(176)	85.56(308)
Slaty black	2.22(4)	14.44(52)
3. Ear lobe color (%)		
Red	100(180)	100(360)
4. Skin color (%)		
White	100(180)	100(360)
5. Comb color (%)		
Red	100(180)	100(360)
6. Comb type (%)		
Single	51.67(93)	54.44(196)
Pea	33.33(60)	34.17(123)
Rose	15(27)	11.39(41)

Morphometric traits	Sex	Overall (n=540)
Adult Body weight (g)	Male (180)	1552.18±27.46
	Female (360)	1182.04±14.30
Shank length (cm)	Male (180)	9.85±0.08
	Female (360)	8.73±0.05

Table 2: Sequence of primers, maximum, minimum, average number of bands, polymorphic bands, percent polymorphism and their size range from the random primers

Primers	Sequence	Maximum No. of Band	Average	Polymorphic band	Min. No. of Band	Band Size (bp)	Polymorphism (%)
OPA-11	AACGCGTCGG	13	4.94	13	1	300-3000	100
OPA-14	TCTGTGCTGG	10	6.38	10	6	400-1600	100
OPA-16	AGCCAGCGAA	16	5.74	16	1	100-2800	100
OPA-7	GAAACGGGTG	15	6.52	15	2	200-2000	100
OPM-9	GTCTTGCGGA	12	4.66	12	1	200-2000	100
PBG-3	GAATGCAGCG	9	4.94	8	2	300-1500	88.89
PBG-13	CAGGACATCG	11	5.9	11	3	100-1500	100
PBG-15	GCGAACCTGG	11	6.78	11	1	100-1400	100
PBG-14	GACGGAAGAG	7	3.46	7	1	400-1200	100
PBG-5	CTGAGGAGTG	5	2.62	5	1	400-1500	100
OPP-3	CTGATACGCC	8	5.36	6	4	300-1600	75
OPP-11	AACGCGTCGG	10	5.56	10	1	400-2000	100
PBC-1	AGTCCTCGCC	14	7.46	13	1	200-1700	92.86
PBC-8	CGCTGTCGCC	9	6.06	9	1	300-1700	100
OPM-10	TCTGGCGCAC	13	2.2	13	1	400-2000	100
PBG-7	ACCGGGAACG	9	3.4	9	1	100-2000	100
Total		172		168			97.67

**Plate 1:** Indigenous chicken genomic (50 samples) amplification with different primers

The number and percentage of polymorphic bands observed in two different sub populations are given in Table 3. In population 1 the polymorphic loci were 152 and 87.86% was polymorphic. Whereas, in population 2 the polymorphic

loci were 159 and per cent polymorphic loci was 91.91. High polymorphism also holds true when compared as grouped population of Bokaro and Ramgarh and Ranchi.

Table 3: Polymorphic bands observed in two different sub populations

S. No.	Population	Polymorphic loci	% Polymorphic loci
1	1 (RB)	152	87.86
2	2 (R)	159	91.91

The mean observed number of alleles was 1.87 ± 0.02 in population 1 and 1.91 ± 0.02 in population 2. Whereas, the mean effective number of alleles was 1.48 ± 0.02 in population 1 and 1.49 ± 0.02 in population 2. It was observed that, mean value of Nei's gene diversity (*h*) in population 1 and population 2 was 0.28 ± 0.01 and 0.29 ± 0.01 respectively. However, mean value of Shannon's information index (*I*) in population 1 and population 2 was 0.43 ± 0.01 and 0.44 ± 0.01

respectively. Low within population genetic diversity was reflected through effective number of allele and Shannon's information index (Table 4). Lower Nei's genetic diversity than the present study was observed by Ahmed and AL-barzinji (2019) and Gorkhail *et al.* (2020) [7]. Higher Shannon index was reported by Karabag and Balcioglu (2010) [10] and lower Shannon index than the present findings was observed by Gorkhail *et al.* (2020) [7].

Table 4: Genetic diversity of two different poultry population. (N=50)

Population	Sample size	na	ne	h	I
1(RB)	20	1.87 ± 0.02	1.48 ± 0.02	0.28 ± 0.01	0.43 ± 0.01
2 (R)	30	1.91 ± 0.02	1.49 ± 0.02	0.29 ± 0.01	0.44 ± 0.01

na = Observed number of alleles, ne = Effective number of alleles [Kimura and Crow (1964)] [11], h = Nei's (1973) gene diversity [14], I = Shannon's Information index [Lewontin (1972)] [12]

Nei's (1972) [13] genetic identity (above diagonal) between both the populations was found to be 0.9767 and genetic distance (below diagonal) was 0.0235 (Table 5). So, there was 97.67% genetic similarity and 2.35% difference between the two populations. The present findings were in close conformity with findings of Dehghanzadeh *et al.* (2009) [5] and Ibrahim *et al.* (2015) [8] but did not agree with

the findings of Baloza *et al.* (2013) [3], Radwan *et al.* (2015) [17], Abdulrazaq and Suliaman (2016), Shivaprasad *et al.* (2016) [19], Fadhil *et al.* (2016) [6], Ahmed and AL-barzinji (2019), Gorkhail *et al.* (2020) [7] and Kim (2021) [9] who reported lower genetic similarity. However, higher genetic similarity than the present study was reported by Olowofeso *et al.* (2006) [15].

Table 5: Nei's (1972) [13] Original measures of genetic identity (above diagonal) and genetic distance (below diagonal) for two poultry populations

Population ID	1 (RB)	2 (R)
1 (RB)	****	0.9767
2 (R)	0.0235	****

In conclusion it was observed that khukhri poultry of Jharkhand is a coloured bird having good potential with characteristic feature and well adopted to grow under backyard condition in agro-climatic condition of Jharkhand. Out of 22 primers, 16 were found to be polymorphic in the native chicken population of Chotanagpur plateau of Jharkhand. It may be concluded that the different population of native chicken population of are related at the RAPD marker levels.

Acknowledgements

The authors are thankful to the Ranchi College of Veterinary Science, Birsa Agricultural University and ICAR-AICRP on Poultry Breeding for their support in generating records and providing facilities for the current study.

References

1. Abdulrazaq HS, Ali Suliaman NM. Polymorphism between five local chickens in Erbil using RAPD. ZANCO J Pure Appl Sci. 2016;58(1):65-69.
2. Ahmad LS, Al-Barzinji YMS. Genetic diversity among local quail using RAPD-DNA marker. Rev Cient Vet. 2019;29(3):178-185.
3. Baloza SH, Hemeda SA, Sosa GA, Shady AA. Assessment of genetic variability among Fayoumi, Rhode Island Red and their crosses using RAPD-PCR. Benha Vet Med J. 2013;25(2):232-239.
4. Banerjee S. Morphological characterization of indigenous chickens of Sikkim and West Bengal, India. Anim Genet Resour. 2012;51:57-71.
5. Dehghanzadeh H, Mirhoseini SZ, Romanov MN, Ghorbani A. Evaluation of genetic variability and distances among five Iranian native chicken populations using RAPD markers. Pak J Biol Sci. 2009;12(11):866-871.
6. Fadhil IA, Dakheel MH, Hussein TH. Detection of genetic diversity through two poultry breeds using RAPD-PCR technique. J Babylon Univ Pure Appl Sci. 2016;24(9):2661-2668.
7. Gorkhail NA, Dhakal R, Sapkota S, Koirala P, Pokhrel P, Pokhrel BR, *et al.* Polymorphism of Sakini chicken population from different locations/altitudes of Nepal

- using RAPD markers. SAARC J Agric. 2020;18(2):115-124.
8. Ibrahim AM, Sabry AM, Hassan MM, El-Hallous EI, Mohamed AA. Genetic characterization of local chicken from Taif region in Saudi Arabia using RAPD markers. Int J Biosci. 2015;6(10):142-148.
 9. Kim SH. Identification of genetic association among different colors of Korean native chicken breeds through RAPD-PCR method. J Anim Health Prod. 2021;9(1):33-39.
 10. Karabag K, Balcioglu MS. Genetic diversity among selected Japanese quail (*Coturnix coturnix japonica*) lines using RAPD markers. J Appl Anim Res. 2010;38:149-152.
 11. Kimura M, Crow JF. The number of alleles that can be maintained in a finite population. Genetics. 1964;49:725-738.
 12. Lewontin RC. The apportionment of human diversity. Evol Biol. 1972;6:381-398.
 13. Nei M. Genetic distance between populations. Am Nat. 1972;106:283-292.
 14. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 1973;70:3321-3323.
 15. Olowofeso O, Wang JY, Zhang P, Dai GJ. Genetic analysis of Haimen chicken populations using decamer random markers. Asian Australas J Anim Sci. 2006;19(11):1519-1524.
 16. Prabhakaran R, Valavan ES. Breeding strategies for native chicken production in India. J Entomol Zool Stud. 2020;8(3):193-199.
 17. Radwan LM, Mahrous MY, El-Denary ME. Assessing genetic variability in egg production and egg quality traits of four Egyptian local strains of chickens using RAPD and SSR techniques. Egypt J Anim Prod. 2015;52(1):55-61.
 18. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.
 19. Shivaprasad C, Gupta BR, Chatterjee RN. Genetic characterization of Dahlem Red layers by PCR-RAPD. Indian J Anim Res. 2016;50(6):877-880.
 20. Tantiya MS, Ganai N, Vij PK, Viji RK, Ahlawat SPS. Chicken breeds of India: Kashmir Faverolla. Karnal: National Bureau of Animal Genetic Resources; 2005.
 21. Yeh FC, Boyle T, Rongcai Y, Ye Z, Xian JM. POPGENE version 3.1. Edmonton: University of Alberta; 1999.