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Evaluation of genetic diversity of Indigenous poultry (Khukhri) found in Chotanagpur Plateau of Jharkhand

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ABSTRACT

Indigenous poultry farming plays an important role in livelihood and nutritional security of rural and tribal populations of Jharkhand. The present study was undertaken to evaluate the phenotypic performance and genetic diversity of Khukhri chicken of the Chotanagpur plateau. A field survey was conducted on 270 households covering 540 birds. Phenotypic characterization revealed predominance of mixed plumage with yellow shank colour (85.56%) and uniform red earlobe, comb and white skin. However, variability in comb type was observed with single comb (≈52%) being predominant. Adult body weight of males and females averaged 1552.18 ± 27.46 g and 1182.04 ± 14.30 g, while shank length was 9.85 ± 0.08 cm and 8.73 ± 0.05 cm, respectively. Genetic diversity was assessed using RAPD markers on 50 birds. Out of 22 primers, 16 produced polymorphic bands generating 172 bands, of which 168 (97.67%) were polymorphic. Nei's gene diversity ranged from 0.28 ± 0.01 to 0.29 ± 0.01, and Shannon's information index ranged from 0.43 ± 0.01 to 0.44 ± 0.01 across populations. The study indicated moderate genetic variability within Khukhri chicken populations. However, the observed variability in comb type suggested that further studies focusing on uniform sub-populations (particularly single-comb birds) are necessary for breed standardization and conservation planning.

Keywords: Khukhri, Indigenous chicken, Genetic diversity, Phenotypic traits, RAPD

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 colour, 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. 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. Keeping in view the above facts, the present study was planned with an objective 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).

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MATERIALS AND METHODS

The study was conducted in the Chotanagpur plateau region of Jharkhand. A total of 270 households were surveyed, covering 540 indigenous chickens. Data on plumage colour, shank colour, comb type, earlobe colour, and morphometric traits were recorded. Blood samples were collected from 50 unrelated birds (2 ml each) from wing vein. Genomic DNA was extracted and subjected to RAPD-PCR analysis using 22 decamer primers with 60–70% GC content. PCR amplification was performed in 25 µl reaction volume. Out of 22 primers, 16 producing

clear and reproducible bands were used for analysis. Bands were scored as present (1) or absent (0). Genetic diversity parameters such as number of alleles, Nei's gene diversity, and Shannon's index were estimated using POPGENE software. Considerable variation in comb type was observed in the population. Since single comb birds constituted the majority (~52%), interpretation of results has been discussed with emphasis on this predominant type, while acknowledging heterogeneity in the population. Genetic diversity within each population was determined as the observed and expected number of alleles (Kimura and Crow, 1964), Nei's genetic diversity (Nei, 1972) and Shanon's Information Index (Lewontin, 1972) using Popgene software (Yeh *et al*. 1999).

RESULTS AND DISCUSSION

Phenotypic characteristics: The phenotypic characterization of Khukhri chicken revealed considerable variability in plumage colour, with mixed and red colours being predominant, followed by brown, white and black combinations. Shank colour was mainly yellow (85.56%), while earlobe, comb and skin colour were uniformly red and white, respectively.

However, substantial variation was observed in comb type, where single comb (≈52%) was predominant but pea (≈34%) and rose (≈14%) combs were also present (Table 1). This indicated that the population lacked phenotypic uniformity, which is a key requirement for breed stabilization. Phenotypic characteristics was in accordance with the findings of Tantia *et al.* (2005) and Banerjee (2012) in Kashmir Faverolla birds, and native chickens of West Bengal, respectively.

Similar phenotypic variability has been reported in indigenous chickens of Zambia and other tropical regions, where heterogeneous traits are attributed to natural selection, uncontrolled mating, and adaptation to diverse agro-climatic conditions (Liswaniso *et al.* 2024). Such variability suggested that these populations are still in a transitional stage rather than representing a well-defined breed.

Morphometric traits: The average adult body weight of males (1552.18 ± 27.46 g) and females (1182.04 ± 14.30 g), along with shank length (9.85 ± 0.08 cm and 8.73 ± 0.05 cm), indicated moderate growth performance typical of indigenous chickens (Table 1).

Comparable findings have been reported in African and Asian indigenous chicken populations, where birds exhibit lower productivity but superior adaptability to harsh environments, disease resistance, and scavenging ability (Rachman *et al*. 2024). These traits make indigenous chickens particularly suitable for low-input backyard systems.

Genetic diversity analysis: RAPD analysis revealed a high level of polymorphism (97.67%) (Table 3), indicating substantial genetic variability within the Khukhri population (Table 2). The values of Nei's gene diversity (Table 5)

Table 1. Phenotypic traits of Khukhri chicken of Chotanagpur plateau of Jharkhand

Traits	Categories	Overall (540)	
		Male (n=180)	Female (n=360)
1. Plumage colour (%)	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)
		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)
	Parameters	Sex	Overall (n=540)
Morphometric traits	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

(0.28–0.29) and Shannon's information index (0.43–0.44) further support moderate genetic diversity (Table 4). 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 breeds of poultry. However, lower percent of polymorphism was documented by Gorkhali *et al.* (2020). But, Karabag and Balcioglu, (2010) had observed higher polymorphism (99.49 %) in Japanese quail than in the present findings.

Recent genomic studies have consistently shown that indigenous chicken populations possess high genetic diversity compared to commercial breeds due to minimal artificial selection and continuous natural adaptation (Wu *et al*. 2024). Similarly, studies using SNP markers in indigenous chickens have also reported considerable heterozygosity and polymorphism, highlighting their importance as genetic reservoirs (Wu *et al*. 2024).

The high polymorphism observed in the present study indicated the presence of diverse alleles, which can be exploited in breeding programmes. However, it also reflects lack of genetic uniformity.

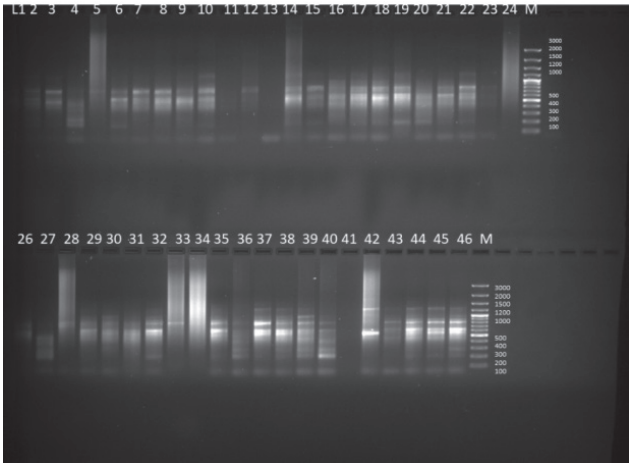
Population structure and genetic relationship: The genetic similarity between the two populations was found to be high (97.67%), indicating low genetic differentiation despite geographical separation. This suggests gene flow between populations or a common genetic origin. Similar findings have been reported in indigenous chicken ecotypes from different agro-ecological zones, where low differentiation indicated that populations behave as a single genetic pool due to random mating and absence of structured breeding programmes (Sovi *et al. 2024*). Recent studies have emphasized that indigenous chickens act as reservoirs of genetic diversity and adaptive traits, but require systematic selection and breeding to develop into well-defined breeds (Wen *et al*. 2025). In this contrast, the observed genetic variability in Khukhri chicken is advantageous for future selection and breeding programmes. Therefore, understanding genetic diversity using molecular markers is crucial, as it will help in designing effective breeding strategies and conservation plans for indigenous poultry resources (Goli *et al*. 2024).

The number and percentage of polymorphic bands observed in two different sub populations are given in Table 3. In population 1, the polymorphism loci were 152 with 87.86 % polymorphism. Whereas, in population 2, the polymorphic loci were 159 with 91.99 per cent polymorphic. High polymorphism also holds true when compared as grouped population of Bokaro and Ramgarh and Ranchi.

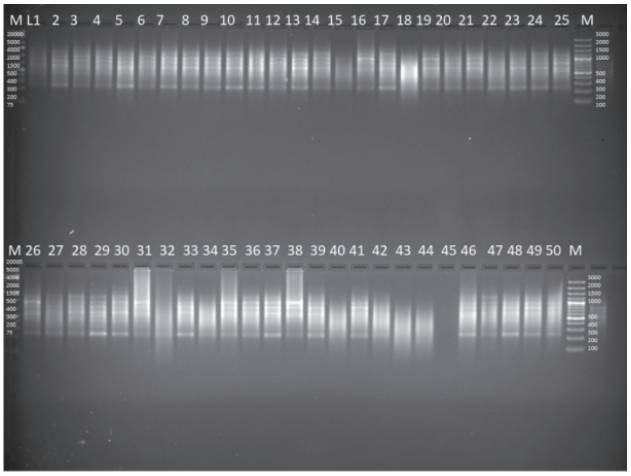
The mean observed number of alleles was 1.87 ±0.02 and 1.91 ± 0.02 in population 1 and 2, respectively. But, the respective mean effective number of alleles were 1.48 ±0.02 and 1.49 ± 0.02. It was observed that mean value of Nei's

Table 3. Polymorphic bands observed in two different sub populations.

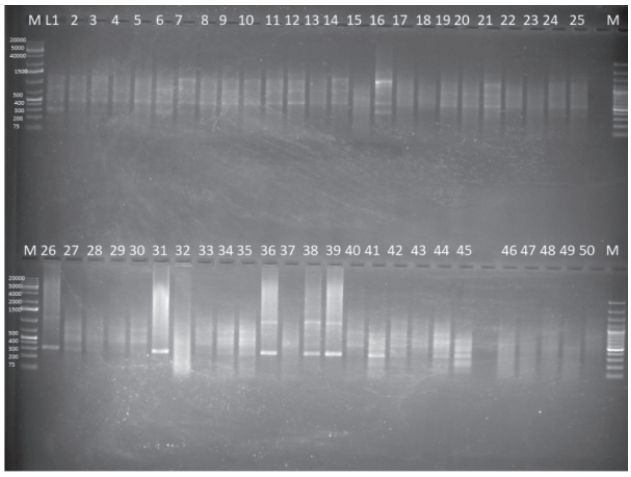
S. No.	Population	Polymorphic loci	% Polymorphic loci
1	1 (RB)	152	87.86
2	2 (R)	159	91.91



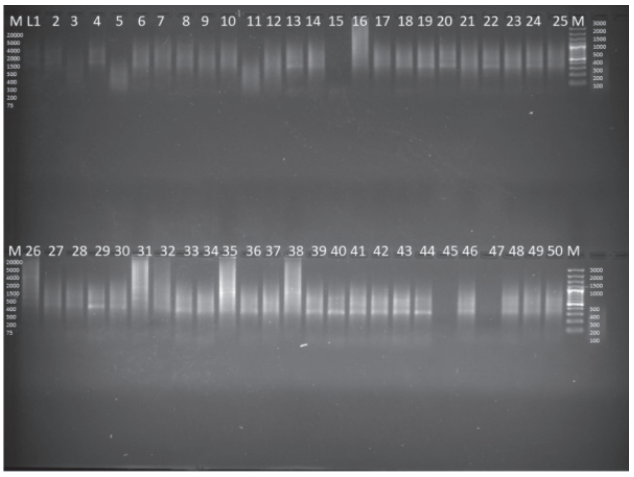
Primer PBG-13



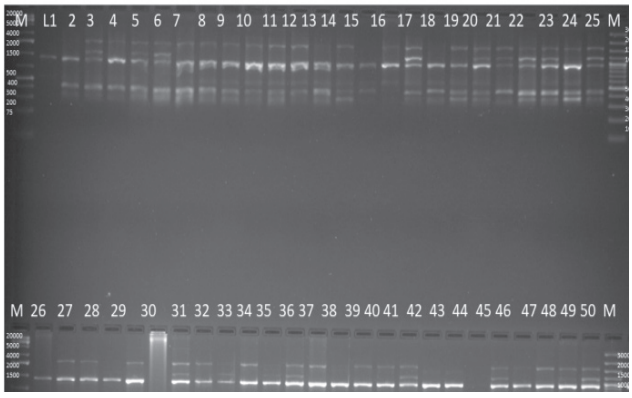
Primer PBG-15



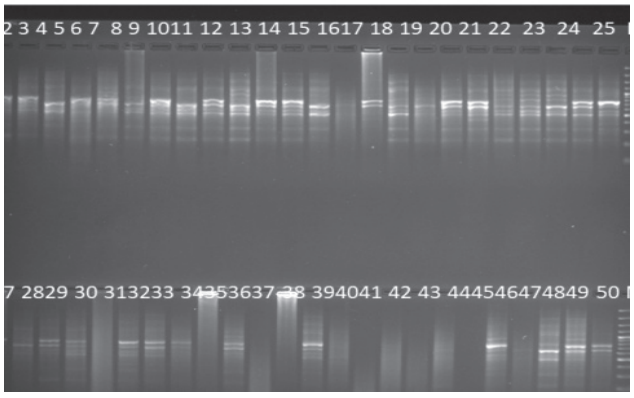
Primer PBG-14



Primer PBG-05



Primer OPP-03



Primer PBC-01

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

gene diversity (h) in population 1 and population 2 was 0.28 ± 0.01 and 0.29 ± 0.01 respectively. However, their mean value of Shannon's information index (I) were 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). Higher Shannon index was reported by Karabag and Balcioglu (2010) while lower value was observed by Gorkhail *et al*. (2020).

Nei's (1972) 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 Ibrahim *et al.* (2015) but did not agree with the findings of Radwan *et al.* (2015),

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)]; h = Nei's (1973) gene diversity; I = Shannon's Information index [Lewontin (1972)]

Table 5. Nei's (1972) 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	****

Ahmed and AL-barzinji (2019), Gorkhail *et al.* (2020) and Kim (2021) who reported lower genetic similarity. However, higher genetic similarity than the present study was reported by Olowofeso *et al.* (2006).

It may be concluded 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.

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