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Efficiency of RAPD marker in providing polymorphism in different Chicken breeds by using the OPA1 Primer

Zubair Shafi Dar* and S.S. Pawar

Department of Zoology, Govt. Vidarbha Institute of Science and Humanities (Autonomous) Amravati.

Abstract: A study was carried out to determine the efficiency of Random Amplified Polymorphic DNA marker in generating polymorphism in various chicken strains. A single marker is used to see the polymorphism in the 10 chicken breeds. In OPA1primer, total number 76 fragments were reported out of which 23 fragments show high polymorphism. The number of different bands is 23 which indicate the 100% polymorphism. The study found that RAPD was effective at detecting polymorphism in various chicken breeds. However, a large number of primers are required to detect acceptable polymorphism in different populations.

Keywords: Polymorphism, Chicken, Breeds, RAPD

Introduction

Animal husbandry has played an important role in poultry science over the last few decades. Diversified geographically, India is rich in a variety of indigenous chicken resources. The majority of chickens are low- to medium-performance native and decorative breeds. They are generally conserved in small populations. Due to the decreased population size of native breeds, this can't fulfil market needs, so the introduction of commercial breed is available with limited conservation measures.

Numerous novel nucleic acid fingerprinting techniques have emerged as a result of the modern molecular genetics field's rapid development. Genetic relationships between livestock populations, including poultry, can currently be established using molecular markers like DNA fingerprinting and restriction fragment length polymorphism (RFLP) (Hillel *et. Al.,* 1992). RAPD analysis, also known as randomly amplifying polymorphic DNA sequences, or arbitrarily priming PCR (AP-PCR) typing (Welsh and McClelland, 1990; Williams *et al.,* 1990), Similar to other molecular markers, it has been demonstrated that they open up fresh perspectives on how to conduct genetic evaluations of local breeds. They are most often used to study the genetic relatedness of livestock breeds (Kemp and Teale, 1994; Appa *et. al.,* 1996) Moreover, they conduct studies to deepen our knowledge of the genetic diversity, marked assisted selection methods, parentage testing, breed relatedness, and species identification. A RAPD-PCR has also shown

commitment for genetic variation of related species which has been used to obtain data on population dynamics (Clark and Lanigan, 1993).Subsequently, the purposes of this research were to genetically characterize of different breeds by using RAPD

2. Materials and methods

The experimental work of the present study was conducted at Department of Zoology, Govt. Vidarbha Institute of Science and Humanities, Amravati on ten chicken strains, Molecular genetics analyses DNA extraction individual blood samples were collected from 10 birds (10 birds/strain), the blood sample (approximately 2 ml) was taken via the brachial vein from each individual of each bird. Each and every chicken used was normal, healthy, and fertile. DNA was extracted from whole blood following the instruction of Nucleo-pore Genetix Brand DNA Sure Purification Kit. The quantity and quality of the isolated DNA was determined by spectrophotometer at 260 nm and agarose gel electrophoresis.

Locations:

Chicken population were Surveyed in the study

Breed	Abbreviation	location	Sample size	Altitude	Group	Longitude	Latitude
B-300	B3	Nagpur	40	310 m	Low	79°5'17.36"E	21°8'44.88"N
Aseel	A1	Dharni	30	323 m	Low	76°52'59.99"E	21°32'59.99" N
Kavery	K1	Yavatmal	35	445 m	Low	78°12'04.07"E	20°38'87.94"N
Kadaknath	K2	Amravati	25	343 m	Low	77°46'46.37"E	20°56'14.72" N
Desi	D1	Chikhaldara	42	1188m	High	77°32'68.12"E	21°40'30.12" N
Hy.Cross	HC	Amravati	28	343 m	Low	77°46'46.37"E	20°56'14.72" N
Boiler	B2	Nagpur	45	310 m	Low	79°5'17.36"E	21°80'44.88"N
K.F	KF	Kulgam	22	1739m	High	33°64'49.91"E	75°01'80.31"N
L.L	L1	Anantnag	7	1601m	High	33°72'97.29"E	75°14'97.80"N
RIR	R1	Nagpur	26	310 m	Low	79°5'17.36"E	21°8'44.88"N

Original location of the breeds. The process of determining altitude, longitude, and latitude using sampling locations as the standard.

3.PCR amplification and electrophoresis analysis

For resultant RAPD profiles from chicken DNA, 10 decamer primer (OPA1) obtained from Biogene Technologies in this study (Table 1). The preference of primer was made based on the degree of polymorphism found in the tested samples, as well as the specificity and repeatability of amplified products. The equal amounts of individual DNA of samples were used for genotypes investigation. The PCR programme conditions included a first denaturation step at 95°C for 5 minutes, then 40 cycles, each of which included 94°C for half minutes, annealing at 35°C for 1 and half minutes, extension at 72°C for 2 minutes, and culminated with a final extension cycle at 72°C for 10 minutes. PCR products were

separated on 1.5% agarose gel stained with 1 µl of ethidium bromide at 100 V for 60 minutes and visualized under U.V. trans-illuminator. PCR reaction mixture contained 75 ng genomic DNA, 1.5 µl 10X enzyme buffer containing MgCl2, 0.2 µl Taq DNA polymerase, 2 µl dNTPs, 0.5 µl primer (10 pmol) and sdH2O was added to the mix to reach a total volume of 15 µl.

Table 1: Primer Sequence of Primer with GC Content

S.No	Primer Name	Sequence (5 -3')	GC Content %	No. of amplified bands	No. of polymorphic bands	Percent Polymorphism
1	OPA01	CAGGCCCTTC	70	76	23	100

Data analysis

The amplified products were given a score of 1 or 0 depending on whether or not bands representative of all the DNA samples were present (for RAPD). The 2010 version of Gel Analyzer software was used to process the identified DNA bands on the agarose gel for data analysis.

Result and Discussion

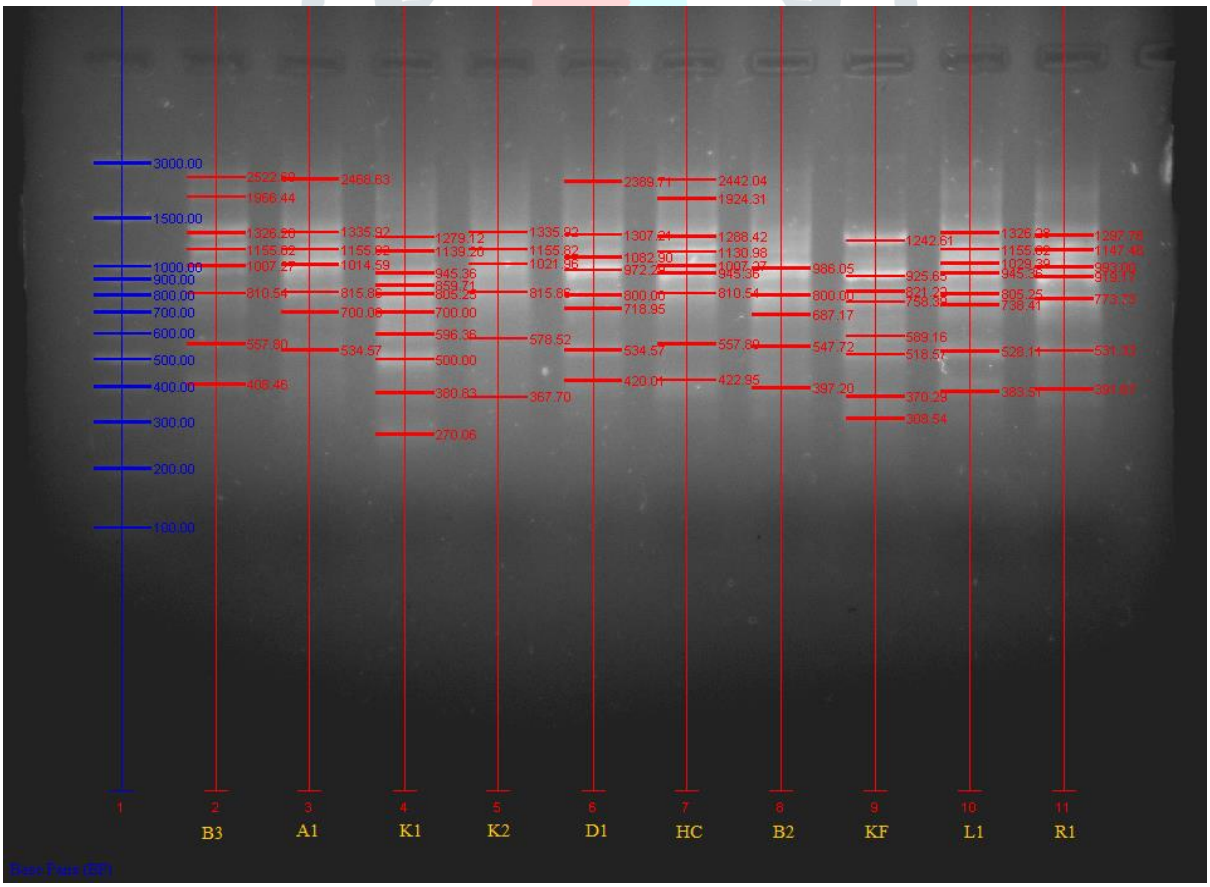

Figure 1: RAPD for Different breeds of Chicken

The Breed differences were seen in the polymorphism within the populations. Out of the 70 total bands, 36 bands exhibit polymorphism. In comparison to the native breed, the exotic breeds generally displayed more similarity. The lane 4th and 9th breed showed greatest within population similarity.

Different primers are anticipated to give different numbers of amplicons because the influence of the annealing site on the template DNA determines how much DNA is amplified from arbitrary sequence primers (Shivaraman *et. al.*, 2001).

The findings showed that such breeds had a very high level of intra- and inter-varietal genetic change. Sharma et al. (2001) used various parts of eggs and various RAPD markers to identify polymorphism in various breeds. The information from RAPD showed that there were only minor differences between native Chinese chickens and both broilers and layers, and that the gene diversity within a population was high in native Chinese chicken, intermediate in broilers, and low in layers (Zhang *et. al.*, 2002b)

While Dokki-4 and Rohd Island Red-based crosses that produce the Golden and Silver Montazah strains (Mahmoud *et. al.*,1974a,b). Furthermore, the Nichols and Mamourah strains were crossed to create the El-Salam strain (Abd El Gawad *et. al.*, 1983). Figure 1 displays a typical example of the banding pattern profiles that were obtained for various native chicken strains using the distances coefficient in 3D scaling. The genetic relatedness between the strains was also estimated using the RAPD patterns produced by various arbitrary primers. The benefit of this method is that RAPD primers enable polymorphism detection in the absence of any prior knowledge of the target genome.

Since the hereditary resemblance within populations is estimated using frequency bands. The methods used to identify chickens are intended to identify the inclusion of particular DNA sequences or combinations of sequence data that distinguish them. In order to choose one or a combination of a few primers that will produce banding patterns specific to a particular genotype, one must first analyse a large number of primers. For the purpose of identifying different varieties, some authors proposed a key of bands produced by using numerous different random primers (Abdullah *et. al.*, 2000, Jayanti *et. al.*, 2000).

Conclusion:

High polymorphism and genomic diversity in chickens indicate a solid genetic foundation. The findings regarding the genetic composition of these populations of chickens have conservation-related ramifications. We can conclude from the assessments conducted for this study that method can be successfully used to identify differences between different populations of animals that belong to the same species. However, additional research involving numerous, thoroughly sampled intrinsic chickens from various regions of the country as well as more RAPD markers are needed in order to discuss the intricate population structures.

Our future research based on integrating RAPD and microsatellite indicators (markers) will give more information about various chicken breeds. Finally, satisfactory nuclear DNA level variation among

various populations of chickens in the area was discovered using RAPD markers. This article data of RAPD may be a reliable source of information about the variety of chicken in this area.

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