Genetic characterization of dahlem red layers by PCR-RAPD

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ABSTRACT
A total of one hundred Randomly Amplified Polymorphic DNA (RAPD) markers were utilized to detect the DNA polymorphisms in a total of 48 full sib mated, half sib mated and non-in bred groups of Dahlem Red birds. Twenty one per cent of the primers tested yielded distinct polymorphic RAPD profiles. Out of 341 amplified bands 204 bands (59.82 %) were found to be polymorphic. The genetic similarity ranged from 83.94 to 87.90 % and, 81.14 to 85.25 % with in group based on band sharing and frequency respectively. Full sib group showed higher genetic similarity then non-in bred group. The genetic similarity based on band frequency ranged from 88.07 to 90.18 %, based on band frequency varied from 72.78 to 79.53 percent. The full sib and half sib groups showed maximum genetic similarity. The genetic distance between full sib and non-inbred groups was found to be the maximum (0.3176), while it was minimum between full sib and half sib groups.

Key words: Dahlem red, Inbred, Non-inbred, RAPD-PCR.

INTRODUCTION
India is the largest among developing countries in the world with ever increasing demand for poultry products. Dahlem Red is a red-feathered breed, laying tinted eggs with good egg weight and known for its high disease tolerance and immune competence (Kundu et al., 1999). It is used to produce improved germplasm suitable for rural/backyard poultry (Sharma and Hazary, 2002).

The development of molecular genetic techniques has been created new possibilities for the selection and rapid genetic improvement of chicken. The molecular markers possess unique properties that make them more useful for genetic analysis then other markers. Several molecular markers such as Restriction Fragment Length Polymorphisms (RFLP), Microsatellites (Weber and Mary, 1989), Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP), Amplified Fragment Length Polymorphisms (AFLP, Vos et al., 1995) and Random Amplified Polymorphic DNA (RAPD, Williams et al., 1990), are available for the dissection of complex genetic traits, for explosion.

RAPD has several advantages in comparison to other markers, as the technique is easy, faster and no need of prior knowledge of the sequence to be amplified (Williams et al., 1990). The variation in these primers is due to single base mismatches between the primer and template (Williams et al., 1993)

The present investigation was undertaken for the molecular genetic characterization of Dahlem Red population using RAPD markers as the information available is scanty.

MATERIALS AND METHODS
Dahlem Red population maintained at the Project Directorate on Poultry (PDP), Rajendranagar, Hyderabad, were utilized for the present study. A total of 48 birds chosen randomly to form three groups (full sib, half sib and non-inbred) with equal males and females in each group for the RAPD analysis. A total of 100 decamer RAPD primers numbered serially from PDP40F to PDP115F and PDP155F to PDP 180F were utilized. Genomic DNA was isolated from the blood samples as per Sambrook and Russell (1989) with slight modifications. The quality of isolated genomic DNA was checked by agarose gel electrophoresis, concentration and purity were determined by spectrophotometrically with an optical absorbance at 260 and 280 nm.

The RAPD analysis was carried out using the genomic DNA of the individual birds as well as pooled DNA samples from full sib mating, half sib mating and non-inbred mating populations, pooled DNA samples for each sex in each full sib mating, half sib mating and non-inbred mated birds prepared by mixing equal quantities of DNA from individual birds. The amplifications were carried out in a thermal cycler (PTC 200 MJ Research) with a final volume of 12.5ml PCR reaction mixture containing 1ml of (25 pM primer), 1ml (200 mM) of the dNTPs, 1.5ml (2 mM) Magnesium chloride, 1ml (1.25 U) Taq DNA polymerase, 1.25ml (10X) PCR buffer and 1ml (100 ng) genomic DNA and remaining 5.75ml of distilled water. The PCR reaction conditions employed were initial denaturation at 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 36°C for 1min, extension at 72°C for 2 min and a final extension for 10 min. The PCR amplified
products were resolved on 1.75% agarose gel along with 1Kb+100 bp and 1Kb ladders as size markers and the gel was visualized under gel documentation system (BioRad) and analyzed by Quality One software. The annotated pictures were further lane-matched and compared by diversity database software along with the molecular weight standard for scoring the bands.

The scoring and matching of bands in gels were done using Quantity One and Diversity database software (BioRad) along with the standard size ladder. For presence only cleared resolvable and distinct bands were recorded. The presence of a band was scored as one and the absence as zero and such data were entered into a binary character matrix. The sizes of the amplified PCR products were determined with the help of a computer program. The genetic similarity within and between the groups was calculated using the following measures.

The genetic similarity estimated as per the Nei’s method (1978) as the proportion of bands shared between two individuals \( N_{ab} \) to the total number of bands scored \( N + N_{i} \) for the same two individuals. Hence,

\[
BS_{ab} = \frac{2N_{ab}}{(N + N_{i})}
\]

The within group genetic similarity (WF) was estimated using the equation given by Raman et al. (2001) and Singh and Sharma (2002).

\[
WF_{ij} = \frac{1}{N} \sum V_{i}
\]

Where, \( V_{i} \) is the proportion of individuals possessing the \( i^{th} \) band across all the individuals and \( N \) is the total number of bands amplified.

The genetic similarity between two lines known as genetic identity index (Yu and Pauls 1993; Zhang et al., 1995) was obtained from the following formula.

\[
BF_{ij} = \frac{1}{N} \sum \left( \frac{2(V_{i}^{(1)}V_{i}^{(2)})}{(V_{i}^{(1)})^{2}+(V_{i}^{(2)})^{2}} \right)
\]

Where, \( V_{i}^{(1)} \) and \( V_{i}^{(2)} \) are the frequency of occurrence of the \( i^{th} \) band in group 1 and 2, respectively and \( N \) is the total number of bands scored.

An index of genetic distance \( D (BF) \), between two populations was calculated by the following equation given by Kuhnlein et al. (1989)

\[
D (BF)_{ij} = - \ln (BF_{ij})
\]

Where \( (BF)_{ij} \) is the genetic identity index of two groups.

RESULTS AND DISCUSSION

The gel images showing the RAPD-PCR fingerprints amplified by the primers PDP50F and PDP71F; PDP73F and PDP168F; PDP75F and PDP157F; and PDP63F, PDP77F, PDP94F, PDP96F, PDP54F, PDP72F, PDP50F and PDP71F are presented in figures 1, 2, 3 and 4 respectively.

21 out of 100 primers produced reproducible fingerprinting patterns, showing polymorphism. Similar results were reported by Deepak Sharma et al. (2001) in White Leghorn, Rhodes Island Red, Red Cornish, White Plymouth Rock and Kadaknath breeds. An annealing temperature of 36°C for one minute was yielded good results, as also reported by Plotsky et al. (1995) and Sharma et al. (2001) in chicken lines. An optimum primer concentration of 25 pico moles per reaction was used to obtain a balance of both high and low molecular weight products.

A total of 341 bands were amplified by 21 primers and, the number of bands amplified by the primers varied from 12 to 23. The average number of bands per primer and the percentage of polymorphic bands obtained in the present study were higher than those reported by Sharma et al. (2001).

The PCR reaction conditions employed in the present investigation favored the amplification of longer fragments of DNA ranging from 100 bp to 3.40 kb. Similar results were also reported by Deepak Sharma et al. (2000).

Results revealed that the three Dahlem Red groups investigated shared a proportion of RAPD fragments among themselves indicating, high degree of homology and the regions are conserved among the groups.

The analysis of fingerprints revealed that the full sib group was unique by the presence of a1866 bp band on amplification by the primer PDP157F (Fig.3) and 496 bp and 380 bp fingerprints on amplification by the primer PDP176F. Therefore, the primers PDP157F and PDP176F could distinguish the full sib group from the half sib and non-inbred groups studied. The half sib group was unique by the presence of 1788 bp fingerprint, for the primer PDP50F. (Fig.1). The non-inbred group could be distinguished by the presence of a unique 421 bp for the primer PDP54 F and PDP73 F. (Fig.2).

The overall mean genetic similarity within the groups, by the band sharing method was 83.94 per cent in non-inbred and 87.90 per cent in full sib group, which revealed the existence of higher intra-group genetic similarity. These findings are in accordance with those reported by Ahlawat et al. (2004).

The percentage of genetic similarity between the groups ranged from 88.07 per cent to 90.18 per cent, which was in accordance with the earlier studies of Deepak Sharma et al. (2000).

The mean genetic similarity within the group based on band frequency was 81.14% in non-inbred and
85.21 per cent in full sib group. Similar results were observed by Raman et al. (2001).

The mean genetic similarity between the three groups based on the band frequencies was 72.78 per cent between full sib and non-inbred and 79.53 per cent between full sib and half sib. Similar results were observed by Sharma et al. (2001).

Genetic distances revealed that full sib and half sib groups were nearest (0.2290) from each other, while the full sib and non-inbred groups were farthest (0.3176) from each other. This may due to increased in homozygosity in full sib and half sib groups for the five generations of inbreeding. Almost similar results were reported by Raman et al. (2001).

It was concluded that the RAPD markers were able to detect the polymorphism between the different groups of Dahlem Red and could establish the genetic relationships. The data resulting from RAPD assays can be extended to further dissect traits in a more refined way to extract knowledge on specific genes and genetic pathways using other molecular methodologies.

REFERENCES


