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Retarded electrophoretic mobility of W chromosome specific DNA fragment from chicken

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ABSTRACT

In the study we report the unusually slow mobility of W chromosome specific DNA fragment. The primers designed to amplify a 481 bp DNA fragment of W chromosome of female chicken revealed its size on agarose gel electrophoresis to be 556 bp. The mobility of this fragment was still slower in polyacrylamide gel electrophoresis. This trend was observed in indigenous, exotic and Vanaraja varieties of chicken. This phenomenon of slow mobility of W chromosome specific DNA fragment was cross checked with the control DNA fragment of 256 bp. Delayed migration of PCR products was observed for other fragment of W chromosome repeat as well. Sequencing of PCR products revealed that the fragments were comprised of clusters of A (2-5) and T (2-5) sequences separated by (5-7) relatively G/C rich sequences. The anomalous behavior of slow migration of W chromosome specific fragments could be attributed to the occurrence of DNA curvatures because of the above mentioned sequence characteristics.

Contains 4 figures

Key words: W chromosome, chicken, retarded mobility, agarose, PAGE

In chicken male is homogametic (ZZ) and female is heterogametic (ZW) just opposite as in case of mammals. Thus, W chromosome, which is also one of the microchromosomes, is specific to female sex and essential in determination of gonadal sex. About 46 % of the W chromosome consists of 1.1 kb and 0.6 kb units of XhoI family repeats which are specific to the female genome of *Gallus g. domesticus, G. gallus, G. sonneratti, and G. varius* (Tone *et al*., 1984). The 0.6 kb and 1.1 kb XhoI fragments repeated 6000 times and 14000 times respectively in W chromosome of chicken. Taking advantage of the specificity of XhoI repeats to W chromosome, PCR based protocols have been developed for identification of sex of embryos and embryonic cells like blastodermal cells, amniotic fluid cells, etc., in various studies by amplifying DNA sequences of these repeats (Petite and Kegelmeyer 1995; Clinton, *et al*., 2001; Naito *et al*., 2003). In the present study while developing a simple PCR based protocol, we came across the retarded electrophoretic mobility of W chromosome specific fragment (481 bp) of XhoI repeat in chicken and hence the study examines its causes and behavior in agarose and polyacrylamide gel electrophoresis in three varieties of chicken.

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MATERIALS AND METHODS

White Leghorn (exotic), indigenous and Vanaraja varieties (hybrid of exotic and indigenous breeds) of chicken (*G. g. domesticus*) were used in the present study and blood samples from these chickens were collected aseptically in a sterile syringe from Jugular vein. Genomic DNA from whole blood of chickens was extracted using conventional Phenol Chloroform Isoamyl alcohol (PCI) method.

PCR primers i.e., SaC-F (5' TAACACGCTTCACTCACA 3') and SaC–R (5' ATGTTTGGACAGAGGTGC 3') were designed to amplify 481 bp single fragment of W chromosome specific DNA repeat (Xhol) sequence of chicken published in GenBank (NCBI) from nucleotide position of 135 to 615 (Kodoma *et al.,* 1987). Another set of published primers i.e., 18S R-F (5' AGCTCTTTCTCGATTCCGTG 3') and 18S R-R (5' GGGTAGACACAAGCTGAGCC 3') were used to generate a 256 bp of the 18S ribosomal gene (Clinton *et al*., 2001) from nucleotide position of 1267 to 1522 in both male and female sex as a control DNA fragment for comparative study. PCR reactions were carried out in 25 µl reaction volume having 12.5 µl Qiagen PCR master mix, 1 X Q-solution (Qiagen - USA), 100 ng of genomic DNA, 1.2 µM of SaC – F

0.4 μM of 18S R - F & R primers. Optimized PCR reaction conditions were standardized at 94 °C for 2 min followed by 25 cycles of 94 °C for 5 s, 54 °C for 5 s and 72 °C for 5 s. Final extension step of 72 °C for 5 min was used for all reactions. PCR reactions were carried out in Thermal Cycler (Model 2720 Applied Biosystems, Foster city, USA). After amplification, PCR products were analyzed on 1.5 % agarose gel in 1 X TAE buffer and bands were visualized under U.V. light after ethidium bromide staining. The PCR products were also resolved on 6 % non-denaturing polyacrylamide gel electrophoresis in 1 X TBE and silver stained for visualization of bands. The 100 bp DNA ladder (ready to use GeneRuler™ Fermentas-Life Sciences USA) was used for determining the size of amplified products. The size of the PCR products was determined with the help of Kodak 1D software.

W chromosome specific PCR products of SaC - F and SaC - R primers amplified from White leghorn, indigenous and Vanaraja chicken varieties were purified using sample exonuclease shrimp alkaline phosphatase digestion method. Purified PCR products were directly sequenced in either directions using ABI prism 3100 genetic analyzer to ascertain the specificity of primers and to compare the nucleotide sequence between these three varieties of chicken.

RESULTS AND DISCUSSION

All the PCR products amplified from primers specific to W chromosome and 18S ribosomal gene were tested individually using DNA samples from adult male and female chickens. The W chromosome specific primers amplified PCR product only in female chicken whereas 18S ribosomal gene primers generated a 256 bp PCR product both in male and female chicken. The PCR products of W chromosome belonging to all three genetic groups have migrated unusually slowly in contrast to the normal migration of 18S gene fragments (Fig. 1) as revealed by their estimated size of 556 bp using Kodak 1 D software as against their actual size of 481 bp in all three genetic groups. In order to investigate the electrophoretic behaviour of other W chromosome specific XhoI repeats, primers designed by Clinton *et al.* (2001) to amplify 415 bp W chromosome specific DNA fragment was also used for comparison purpose (Fig. 3.) Here also, the mobility of W chromosome sequence was delayed and the size determined from 1D Kodak software was 474 bp against the actual size of 415 bp, however Clinton *et al.* (2001) did not report this finding in their study. Subsequently, PCR products of SaC, and

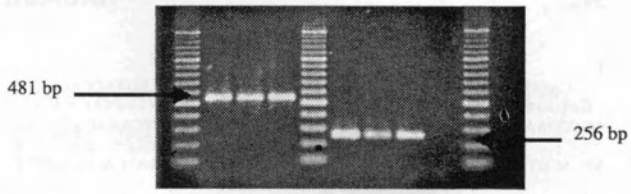

Fig. 1. Agarose gel (1.5 %) electrophoresis of SaC and 18S R gene PCR products, Lanes 1, 5 and 10 molecular size markers (100 bp ladder); lane 2 - 4 SaC PCR products and lane 6-8 18S R gene PCR products; lanes 2 and 6 – indigenous chicken, lanes 3 and 7 Vanaraja chicken and lane 4 and 8 white leghorn chicken; lane 9 – negative control.

18S R gene belonging to all three genetic groups were resolved on 6 % native PAGE to see the differences in migration to that in agarose gel electrophoresis (Fig. 2). Interestingly, migration of the W chromosome specific (SaC) PCR products was still slower whereas that of 18S R gene fragment remained same. It was

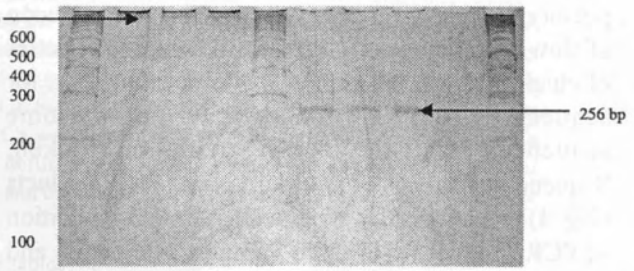

Fig. 2 Non denaturing PAGE (6 %) electrophoresis of SaC and 18S R gene PCR products, lanes 1, 5 and 10 molecular size markers (100 bp ladder); lanes 2-4 SaC PCR products and lanes 6-8 18S R gene PCR products; lanes 2 and 6–indigenous chicken, lanes 3 and 7 Vanaraja chicken and lanes 4 and 8 white leghorn chicken; lane 9 – negative control.

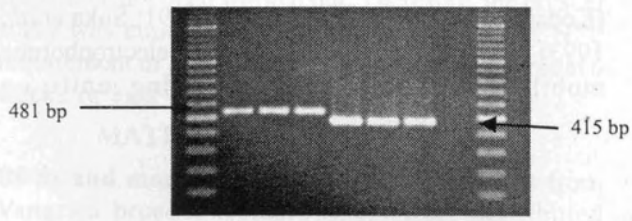

Fig. 3. Agarose gel (1.5 %) electrophoresis of SaC and XhoI repeat PCR products, lanes 1 and 9 molecular size markers (100 bp ladder); lane 2-4 SaC PCR products and lanes 5-7 XhoI PCR products; lane 2 and 5 – indigenous chicken, lane 3 and 6 Vanaraja chicken and lane 4 and 7 white leghorn chicken; lane 8 – negative control

observed that the 18S ribosomal sequence exhibited the same mobility (256 bp) in both agarose and polyacrylamide gel electrophoresis. The migration of

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TAACACGCTTCACTCACAAAGCACGCATTTTCACCCCGAAAGTACCACCTTTC
AGCCGAAAATTACGCTTTTTCCTCCAGAAAATACCACTTCTCAAACAGAAAT
ATCACGTTTTCGCCAAGAAAATAGCACCATTCACCCCAAAATCACGCGTTTTC
TCTCCAGAACTACCACTTTTCTCACGGAAATCACACATTTTCTTCCCGAAAGT
ACCACCTTGCACACGAAAATCACGCATTTTCTGCGCGAAACAACCCCATTTC
ACCCCAAAATCAGTCTTTTTCTTCCGGAAAATACAACTTTTCTAACGGAAACC
ATGCACTGTCACTCCGAAAATACAAGTTTTTGCCCGAAAATCACGCATTTTCC
CTTCGTAAATTCCCCATGTCGCCAGAAAATAATGCATTTCCTTACCGTAAATG
CCCCTTTTCACCCAAAAATCACGCATTCCCCCCGAAAAAACGCACCTCTGTCC
AAACAT **481**

Fig. 4. Nucleotide sequences of part of XhoI repeat unit family of W chromosome of chicken used in the study

polyacrylamide gel electrophoresis. The migration of SaC PCR products in agarose gel electrophoresis was slower and their migration was still reduced under polyacrylamide gel electrophoresis. This phenomenon of slow migration was noticed in all the three varieties of chicken tested. Therefore, it was decided to go for sequencing of PCR product of W chromosome sequences of all the three varieties of chicken. Sequencing analysis of W chromosome PCR products (Fig. 4) reveled that length as well as base composition of PCR product in all three varieties of chicken and from that of blast searches (NCBI) were identical with clusters of A (2-5) and T (2-5) sequences separated by (5-7) relatively G/C rich sequences. From the literature, it was revealed that the slow migration of W chromosome specific fragments could be attributed to the occurrence of DNA curvatures/bent due to arrangement of nucleotides as mentioned above (Kodama *et al*., 1987; Saitoh *et al*., 1991; Suka *et al*., 1993). Early studies reported delay in electrophoretic mobility of XhoI family repeating units on polyacrylamide gel electrophoresis only; however we observed retarded mobility of XhoI family repeat of W chromosome in agarose gel electrophoresis as well. This particular property of slow mobility of DNA fragment is not just restricted to XhoI family repeats of chicken alone; it was observed recently in chromo-helicase DNA (CHD) binding gene fragments of Kiwi birds under PAGE and this property was exploited for developing PCR based sexing protocol for kiwi birds (Huynen *et al*., 2006).

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