International Journal of Livestock Research, Vol. 11 (4) Apr' 2021
http://www.ijlr.org
eISSN: 2277-1964

Original Research

Metaphor Gel Electrophoresis: A Tool for High Resolution Genotyping with Microsatellite Marker in Pati; The Local Indigenous Duck of Assam

Purabi Kaushik*, Kabita B Kalita, R. J. Deka and Jnyanashree Saikia

College of Veterinary Science, Assam Agricultural University, Khanapara, Assam, INDIA

*Corresponding Author: purabiicar@gmail.com

How to cite this paper: Kaushik, P., Kalita, K., & Deka, R. (2021). Metaphor Gel Electrophoresis: A Tool For High Resolution Genotyping with Microsatellite Marker In Pati;The Local Indigenous Duck Of Assam. *International Journal of Livestock Research*, 11(4), 129-132. https://doi.org/10.5455/ijlr.20200826061050

Received : Aug 13, 2020
Accepted : Mar 08, 2021
Published : Apr 30, 2021

Copyright © Kaushik *et al., 2021*

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0). http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Microsatellites are highly polymorphic, so they have been very useful in population genetics studies. High level of allelic variation, co-dominant mode of inheritance and potential for automated analysis make them an excellent tool for a number of approaches like genotyping, mapping, and genetic characterization. The resolution capability of Metaphor agarose gel is very fine. Study was conducted on 50 local pati ducks. Blood sample was collected from 5 different areas of Assam. Presently, a panel of 17 informative duck microsatellite markers were identified from database as reported by Alyethodi and Kumar (2010) and Huang et al. (2005). MAGE was successful in clearly separating the alleles with a size difference of very small difference. In microsatellite analysis for large number of samples MAGE can be considered very economical for analysis of polymorphisms.

Keywords: MAGE, Microsatellite marker, Pati Duck, PCR

Introduction

Ducks are the second most popular species after chicken in our country contributing significantly to the overall poultry production. As per FAO (2) statistics, the duck meat production increased from 0.026 million tonnes to 0.15 m tonnes. Converged use of various conventional molecular and health care technologies will augment duck egg and meat production. Ducks are easy to rear compared to chicken. They can be reared by providing only a small water channel. Though the egg production and meat productivity of Pati duck is not too much, but there is significant scope to increase its productivity by cross breeding. Pati ducks have disease resistivity more compared to other breeds of duck found in Assam. Microsatellites are codominant in nature and are highly polymorphic. Small amount of blood samples or alcohol preserved tissue is adequate for analyzing them. The length polymorphism of a microsatellite marker is commonly detected through polymerase chain reaction (PCR). The amplification is done using pairs of specific SSR primers flanking tandem arrays of microsatellite repeats and followed by electrophoresis (Rahman *et al.*, 2002b). MetaPhor agarose gel electrophoresis (MAGE) is another approach to separate alleles of microsatellite markers (Abdurakhmonov *et al.*, 2007, Mahhamed *et al.*, 2008). MetaPhor agarose (FMC or Cambrex Corporation, USA) is an intermediate melting temperature agarose (75°C) that provides twice the resolution capabilities of the finest sieving agarose products. MetaPhor agarose gels (2% to 4%) made in either TAE or TBE and stained with ethidium bromide are ideal for resolving SSRs (Anonymous, 2004). The present research work was conducted to optimize and establish a more rapid and efficient method for genotyping with microsatellite markers in Pati ducks of Assam.

Materials and Methods

For the present study, a total 50 number of Pati ducks were selected which includes 10 number from Sivasagar district, 20 numbers from Nalbari district, 10 numbers from Udalguri district and another 10 from duck farm of CVSc. Approximately 1 ml of venous blood was collected aseptically from the wing vein of the birds in vaccutainer tube containing EDTA (2.7%). The samples were properly labelled and transported immediately in ice box to the Department of ILFc, C.V.Sc, A.A.U, Khanapara and later stored in deep freezer at -20°C temperature till the isolation of genomic DNA. Genomic DNA was isolated from 100µl of whole blood. The concentration of genomic DNA was estimated by Picodrop spectrophotometer (Model no.-PICOPET01). The concentration of the genomic DNA was estimated at OD 260 nm. The ratio of OD values at 260 nm and 280 nm was used as a criterion to check the purity of the extracted genomic DNA. Then PCR for amplification was performed for the samples with already established microsatellite primers. For the gel preparation, 3% Metaphor agarose was slowly added to the chilled 1x TBE buffer with continuous swirling. It was soaked in that cold buffer for about 10-15 minutes before heating in the microwave (to prevent it from foaming and not to over boil the gel). Just prior to pouring the gel, ethidium bromide (0.3 µg/ml) was mixed to the dissolved agarose. Once the molten gel was solidified after being poured into a cast, it was kept at 4°C for 20 minutes before use to obtain a better resolution and for easier handling of the gel (Anonymous, 2004). After adding 3 µl 6x loading dye to the 8 µl PCR product, 10 µl of it was loaded on the Metaphor agarose gel submerged in chilled 1x TBE buffer and electrophoresed for about 1-2 hours at 6 V/cm using horizontal electrophoresis system. After electrophoresis, the finely resolved products were visualized under UV transilluminator. The data was analysed for further genome mapping studies.

Results and Discussion

MAGE produced very good results. Polymorphism among the primers can be very distinctly noticed. Allele separation is distinct. Very small size differences can be analysed doing MAGE. It can also resolve PCR products that differ in size by 2% (Anonymous, 2004). In the present research work we have effectively employed MetaPhor agarose gel electrophoresis for genotyping with microsatellite markers in Pati duck. Ethidium bromide is commonly used to stain nucleic acid (Sambrook and Russel, 2001) in Metaphor gels for visualization of SSR products (Fig. 1 and 2). Gels can be stained either before or after electrophoresis. To stain prior to electrophoresis, ethidium bromide was added to the dissolved Metaphor agarose just before pouring the gel. The ethidium bromide stain runs in the opposite direction of the DNA therefore the upper and lower portions of the gels appeared differentially stained especially when gels were electrophoresed at high voltages for a short period of time. Therefore, for even staining, ethidium bromide could be included in the electrophoresis buffer. Analysis generated a total of 41 microsatellite alleles across all studied loci by using 17 microsatellites. Since, this study utilized the same set of primers as reported by Alyethodi *et al.* (2010) the results have been compared with these. Highest numbers of alleles 5 (A/B/C/D/E) were recorded at locus CAUD 04 and CAUD 05. The number of alleles at various polymorphic microsatellite loci

ranged from 1 to 5. The allele frequency ranges from 0.04 to 1. Polymorphic patterns were observed in 12 out of 17 microsatellite loci studied in case of Pati ducks. The average number of alleles per locus is 2.41. Based on these studies the microsatellites used to characterize the pati ducks can be useful for distinguishing it from the other native Indian ducks. To achieve better resolution, it is better to use fresh Metaphor gels.

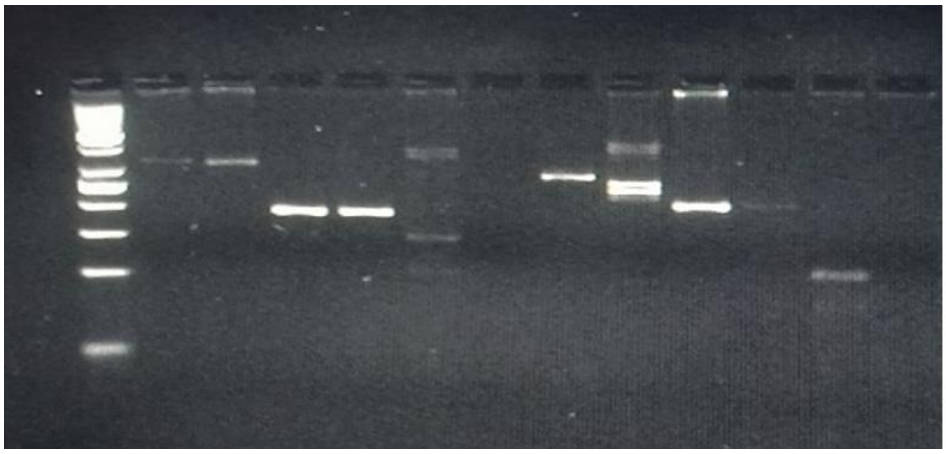

Figure 1: MAGE of primers 1-8

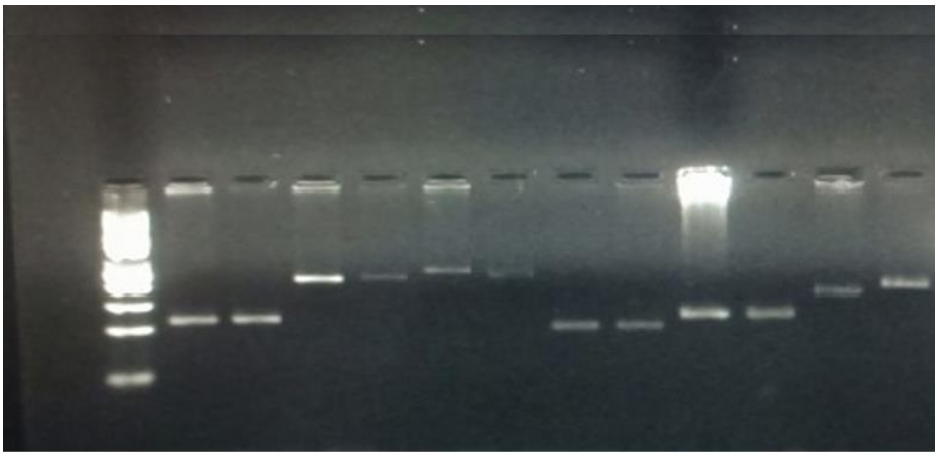

Figure 2: MAGE of primers 9-17

Conclusion

Metaphor Gel Electrophoresis can be successfully used for characterisation of poultry breed and the findings clearly demonstrate that the resolution of MAGE is high to distinguish SSR alleles and is ideal for the researchers currently involved in genotyping with microsatellite markers.

Acknowledgement

The author wishes to express her deepest sense of gratitude to College of veterinary science, AAU, Khanapara for allowing and helping her to continue the research work.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Abdurakhmonov, I. Y., Buriev, Z. T., Saha, S., Pepper, A. E., Musaev, J. A., Almatov, A., and Abdukarimov, A. (2007). Microsatellite markers associated with lint percentage trait in cotton, *Gossypium hirsutum*. *Euphytica*, 156:141–156.
2. Alyethodi, R.R., Kumar, S., (2010). Genetic Characterization of Moti Indian Native Duck Using Microsatellite Markers. *Journal of Applied Animal Research.* 38:2, 223-227.
3. Anonymous. (2004). MetaPhor agarose. Cambrex Bio Science Rockland, Inc., USA. Available at www.cambrex.com/bioproducts.
4. Huang, Y., Cheng, X., and Tang, B., (2005). Characterization of 35 novel microsatellite DNA markers from the duck (*Anas platyrhynchos*) genome and cross-amplification in other birds. *Genetics Selection Evolution*. 37: 455–472.
5. Muhammad Asif, Mehboob-ur-Rahman, Javed Iqbal Mirza1 and Yusuf Zafar (2008) High resolution metaphor agarose gel electrophoresis for genotyping with microsatellite markers. *Pak. J. Agri. Sci*., 45(1) 75-79.
6. Rahman, M., Malik, T.A., Aslam, N., Asif, M., Ahmad, R., Khan, I.A. and Zafar, Y., (2002). Optimization of PCR Conditions to Amplify Microsatellite Loci in Cotton (*Gossypium hirsutum* L.) Genomic DNA. *International Journal of Agriculture & Biology 2*(4): 282-284.
7. Sambrook, J. and Russell, D.W. (2001) Molecular cloning: a laboratory manual. Vol. 2, 3rd edn. Cold Spring Harbor Laboratory Press, New York.

****