Porcine Research

Allelic diversity of growth hormone gene in a commercial pig population using single nucleotide polymorphism

[1]Ufuoma Godstime Sorhue, [1]Philip Okpako Akporhuarho, [1]Adimabua Mike Moemeka, [2]Ade Samson, [2]Ifeanyichukwu Udeh, [2]Sylvanus Ikenna Omeje

[1] Department of Animal Science, Delta State University Abraka, PMB 1, Abraka Nigeria;
[2] Department of Animal Production, Dennis Osadebey University, Anwai, Asaba Nigeria.
Corresponding author: U. G. Sorhue, gtsorhue@yahoo.com

Abstract. Due to the cost and technical skills required for molecular genetics studies, sequence data and genetic diversity information from livestock species are scarce in Nigeria. Our recent study was therefore conducted to identify single nucleotide polymorphisms (SNP) obtainable in a commercial pig population in Sapele, Delta State, Nigeria. We collected liver samples from 14 growing pigs that were randomly selected from a pig farm and stored using RNALater solution (Qiagen) in a -20^{o}c freezer prior to DNA extraction. DNA concentration and purity was checked using a spectrophotometer while PCR product was confirmed with gel electrophoresis. Primer was designed using the Primer 3 design tool as deployed on NCBI (NCBI 2022). The PCR product was cleaned and sequenced using BioEdit software, sequence data was aligned using MUSCLE algorithm on MEGA version X software, SNP calling, and frequency estimation was executed on GenAlEx 6.503 software. Single nucleotide polymorphisms were identified in 216 loci of the growth hormone gene beginning from locus 11 to locus 459. A maximum of three alleles were obtained in all polymorphic loci. The allelic frequencies obtained for locus 11 was C0.14:G0.86, C0.07:G0.79: T0.14 for locus 31 and G0.93:T0.07 for locus 459. Haploid diversity records of 2.15 ± 0.03, 1.30 ± 0.01, 0.37 ± 0.01, 0.21 ± 0.01, and 0.23 ± 0.01 for mean number of alleles, information index, population diversity and unbiased diversity of the population by locus. The SNP identified in this study showed that the diversity of the population studied is not genetically distant among individuals.
Key Words: DNA sequencing, frequency estimation, molecular markers, swine genomics.

Introduction. Genetic variations are the cause of swine genetic diversity. Single nucleotide polymorphism (SNP), which refers to variation at the level of a nucleotide, is the most common type of difference. A single nucleotide or base substitution may result in modifications to proteins that alter phenotypes (Pasupa et al 2020). The growth hormone gene (GH) promotes somatic growth and regulates various metabolic activities in vertebrate animals, which imply that it affects all aspect of animal production; it also promotes skeletal system growth and facilitates amino acid incorporation during protein synthesis (Wang et al 2014a). The GH located on chromosome pair 12, is a peptide hormone with about 190 residues which regulates activities in all mammals, it extends to over 2-3kb, and comprises five splits of exons and four introns. It has been reported that insertions of GH into growing pigs increased growth rate and the percentage of muscle and fat accretion decreased (Bižienė et al 2011). Genetic polymorphism of the growth hormone gene has been reported to be significantly associated with growth traits and is thus very useful in marker assisted selection for livestock species because growth is greatly correlated with litter size. Rothschild et al (2011) and Luo et al (2012) documented quantitative trait loci (QTLs) and SNPs related to economic traits that are in chromosome 12.

Single nucleotide polymorphism (SNP) has become a marker of choice, required for commercial diagnostic and parentage genotyping applications since automated genotyping systems have been developed to yield accurate genotypes (Chen et al 2007).

However, allele frequencies for commercial SNP markers in mixed breed pig populations have not been available, especially for commercial population in Nigeria. For the effective application of genomic selection, knowledge of population structure and genetic diversity levels is crucial (Grossi et al 2017). Results from the sequences generated in this population will therefore unravel the genetic distance among pigs and provide allelic frequencies in the Loci of the pigs, which can be used for marker assisted selection when genotyped. This study was therefore designed to identify the polymorphisms obtainable in the pig population studied and to identify novel single nucleotide polymorphisms (SNPs) in the experimental population.

Material and Method

Experimental site. The animals for this experiment were obtained from Songhai farm, Amukpe – Sapele, Delta State, Nigeria. The molecular biology work was conducted at Delta State University research laboratory from January to August 2019.

Ethics and approval. The ethics committee of the Department of Animal Science, Delta State University Abraka considered and approved this research with a registration number PhD - 080119.

Experimental animals. A total of fourteen (14) animals aging between 3-5 months were randomly selected from a population of eighty-six growing pigs in Songhai farm, Amukpe-Sapele Delta State. The animals were slaughtered, while liver samples were collected and stored using RNALater (Qiagen) solution in a -20^{o} freezer prior to DNA extraction. Total DNA was extracted using Bioline kit.

Polymerase chain reaction. Polymerase chain reaction (PCR) was carried out in a 50-mL volume containing 50 ng template DNA, 2mL primers (10mM each), 25mL of 26MasterMix (0.05units/mL Taq DNA polymerase, 4mM $MgCl_2$, 4mM dNTPs), and double-distilled (dd)H_2O. PCR reactions were performed under the following conditions: a 3-min hot start at 95^{o}C, 35 cycles of denaturation at 94^{o}C for 30 s, annealing at 60^{o}C for 30 s, extension at 72^{o}C for 90 s, and a final extension at 72^{o}C for 10 min. PCR products were loaded onto a 1% agarose gel and visualized using a gel imaging system.

Table1

Primer used for the analysis

	Sequence (5'->3')	*Tm*	*GC%*	*CH*	*GBAN*
FP	GGTACTCCATCCAGAACGCC	60.18	60.00	12	NC010454.4
RP	TTCTCCGGGTCGAGAGTGAA	60.25	55.00	12	NC010454.4

Note: Tm - melting temperature, GC% - guanine-cytosine content, CH - chromosome, GBAN - Gene Bank accession number, FP - forward primer, RP - reverse primer.

Table 1 shows the DNA primer of the growth hormone gene used for the study. The details of the primer from NCBI data base (NCBI 2022) shows it was the *Sus scrofa* growth hormone used for SNP analysis. It is the isolate TJ Tabasco breed of Duroc chromosome 12, Sscrofa11.1, somatotrophin precursor (gene bank accession no: NC010454.4), product length: 582.

Sequence data analysis. Initial sequence trimming and data cleaning was carried out on BioEdit software (Hall 1999). Sequence data was aligned using MUSCLE algorithm on MEGA version X software (Kumar et al 2018). The PCR products from pig samples were sequenced by the Invitrogen Trading Company (China). SNP calling and frequency estimation was executed on GenAlEx 6.503 software (Peakall & Smouse 2006, 2012)

Percentage of polymorphic loci were calculated with the following methods:

Na was obtained as the average number of different alleles;
Ne is the number of effective alleles = 1 / (Sumpi^2);
I is the Shannon's Information Index = -1*Sum (pi*Ln (pi));
h is Diversity = 1 - Sum pi^2, uh is Unbiased Diversity = (N / (N-1)) * h;

Where pi is the frequency of the ith allele for the population & Sum pi^2 Is the sum of the squared population allele frequencies.

Results and Discussion

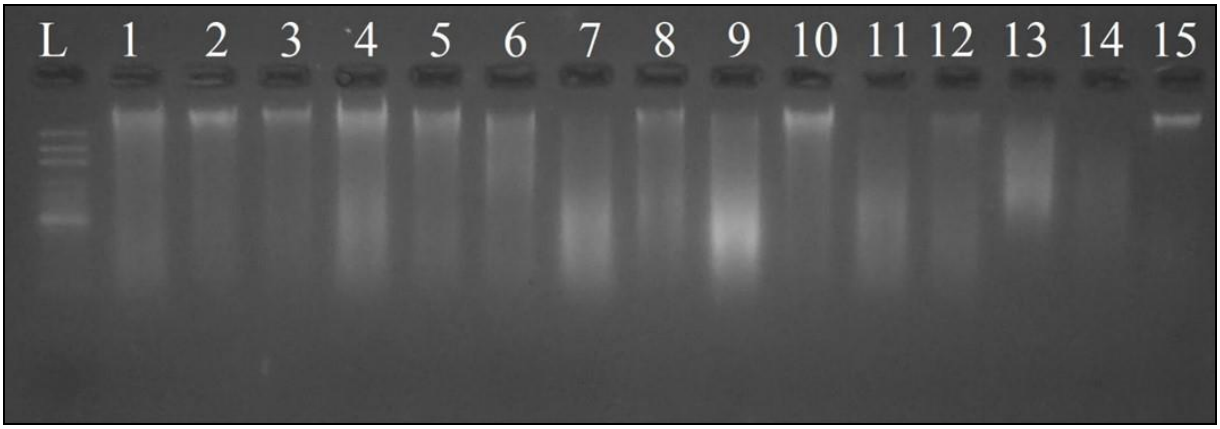

Figure 1. Plate I: Genomic DNA (L – 100bp ladder).

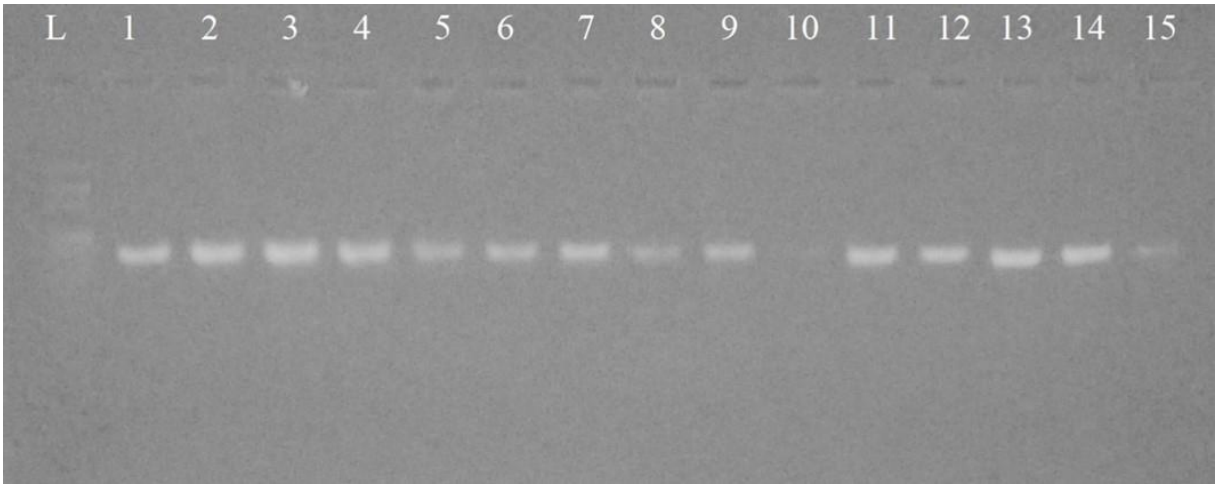

Figure 2. Plate II: PCR product.

Table 2

Mean and Standard Error of Haploid Diversity over Loci for the Population

Population		*N*	*Na*	*Ne*	*I*	*H*	*Uh*
	Mean	14	2.153	1.303	0.372	0.210	0.226
1	SE	0.000	0.025	0.018	0.012	0.008	0.009

Note: N - sample size, Na - number of alleles, Ne - number of effective alleles, I - information index, u - diversity, uh - unbiased diversity by locus.

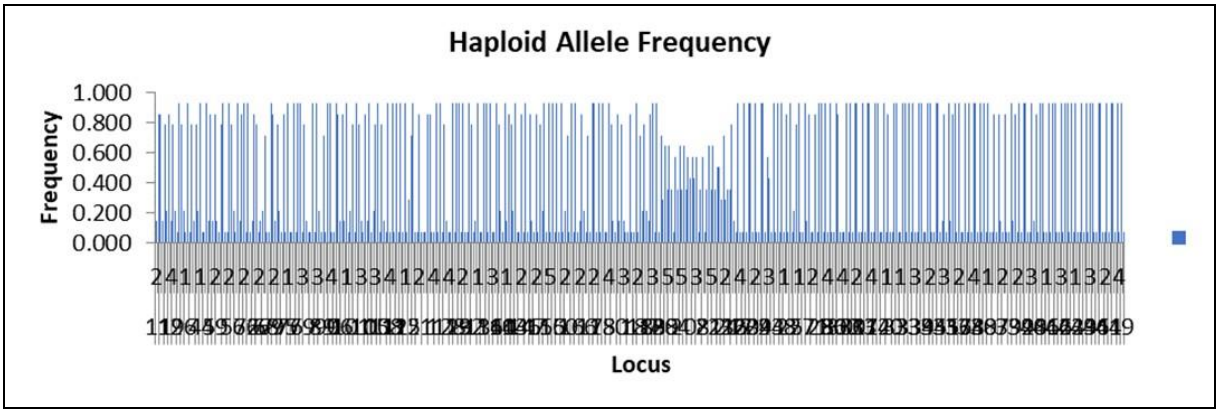

Figure 3. Allelic frequency with graph over loci for haploid data.

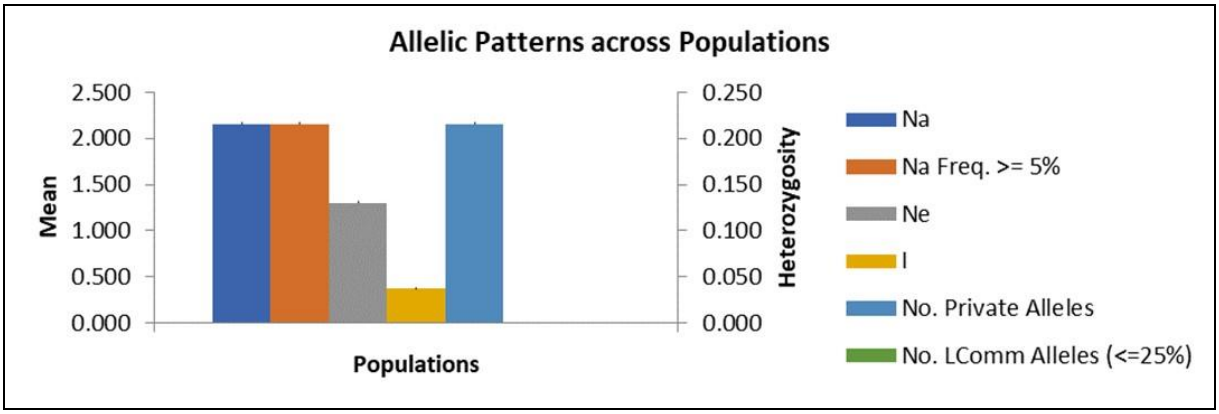

Figure 4. Allelic patterns across population.

The conventional PCR was tested with gel electrophoresis and is shown in plate 1 and plate 2 from Figure 1 and Figure 2. From the results, the genomic DNA reveals bands for all samples, except for sample number 10 where bands were not seen, which is indicative of low or nonexistence of DNA in the samples. The nucleotide sequences were converted to numerals with codes given to each haplotype: 1 = Adenine, 2 = Cytosine, 3 = Guanine, 4 = Thymine, 5 = Neutral as shown in Figure 3. Single nucleotide polymorphisms were identified in 216 loci in the pig population beginning from locus 11 to locus 459. A maximum of three alleles were recorded in all polymorphic loci. The allelic frequencies obtained in ratios for locus 11 was C0.14:G0.86, 0.07C:0.79G: 0.14T for locus 31 and 0.93G: 0.07T for locus 459. Table 2 shows the mean haploid diversity over loci for the population; the mean number of alleles is 2.15±0.03, while 1.30, 0.37, 0.21 and 0.23 represents the number of effective alleles, information index of the population, population diversity and unbiased diversity of the population by locus respectively. The allelic patterns across populations are shown in Figure 4 above; the analysis of sequence data revealed a total of 216 polymorphic loci of five haplotypes. The allelic frequency showed 31 loci having 3 SNP in one locus while the other 185 SNP are comprised of two and one SNP per locus. Wang et al (2014b) identified five SNP loci in the GH gene viz; CA12G, T45C, G84A, G93A, and C133T in two different breeds of pigs while three SNP loci were also discovered in region CA12G, T45C, and G93A for another breed of pig in India which is far lesser than the 216 polymorphic positions identified in the current study. Although the SNPs observed in this study are putative readings that require genotyping using high resolution melting for confirmation. Wang et al (2014b) also reported that factors such as geographical distribution and breeding methods could be responsible for differences in SNPs among pig breeds and genotypes. SNPs have been found to significantly affect quantitative traits at locus 12 and locus 45 in pig breeds in India. The GH gene fragments have also been reported to be more divergent compared

to fragments of other genes. Singh et al (2014) reported SNPs in the exon 5 of the GH in different breeds of goats and suggested its use for marker assisted selection. The Shannon information index of 0.372 obtained in this population is lower than 1.576 and 1.315 reported by Joshi et al (2012) for Murrah buffalo in India. The value of 2.15 and 1.30 reported for mean number of alleles and effective number of alleles our study is lower than the range of 3.50-12, and 1.77 obtained by Silva et al (2011) for commercial pigs in Brazil. Same trend was observed for polymorphic information index. Kharzinova and Zinovieva (2020) also reported higher values of NE, NA and allelic diversity for Russian pigs. The values in our study were lesser than values reported in literatures for allele diversity profiles because the population studied in our recent experiment was restricted to a single commercial population of crossbred pigs; this is because genetic differences in populations are due to the variances existing in that population (Sorhue et al 2014). Hence, within population diversity is expected to generate lower genetic diversity index than between populations. Since SNPs have been reported to affect economic traits in pigs (Tian et al 2014), it is necessary to conduct more genetic diversity studies using single nucleotide polymorphisms to improve the accuracy of genomic selection.

Conclusions. The study has identified 216 SNP loci in the growth hormone gene of the domestic pig (*Sus scrofa*), revealing the allelic frequencies across the pig population studied. The SNP identified in this study if genotyped can be used for marker assisted selection of farm animals for effective prediction of genomic selection. From this study, we have also identified the number of SNPs available in a commercial pig population in this part of the world as well as establishing allelic frequencies of commercial pig population consisting of crossbred animals.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Ufuoma Godstime Sorhue, Department of Animal Science, Faculty of Agriculture, Delta State University, Abraka, Nigeria, e-mail: gtsorhue@yahoo.com
Philip O. Akporhuarho, Department of Animal Science, Faculty of Agriculture, Delta State University, Abraka, Nigeria, e-mail: okpakophilip@gmail.com
Adimabua Mike Moemeka, Department of Animal Production, Faculty of Agriculture, Dennis Osadebey University, Asaba, Nigeria, e-mail: mikeadison1@gmail.com
Ade Samson, Department of Animal Production, Faculty of Agriculture, Dennis Osadebey University, Asaba, Nigeria, e-mail: adesamson@dou.edu.ng
Ifeanyichukwu Udeh, Department of Animal Science, Faculty of Agriculture, Delta State University, Abraka, e-mail: drudeh2005@yahoo.com
Ikenna Sylvanus Omeje, Department of Animal Science, Faculty of Agriculture, Delta State University, Abraka, e-mail: siomeje@gmail.com

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