JOURNAL OF LIFE SCIENCE AND ENVIRONMENTAL PROTECTION
https://www.worldlinkpub.com/journals/journal-of-life-science-and-environmental-protecti/

ISSN: 3141-5989

POLYMORPHISM IN THE HEAT STRESS PROTEIN OF INDIGENOUS PIG POPULATIONS OF NIGERIA

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Received: 12 May 2026
Accepted for publication: 15 June 2026
Published: 01 July 2026

ABSTRACT

This study investigated the expression patterns of HSP90 and HSP110 genes, which encode heat shock proteins, using 60 genomic DNA samples isolated from indigenous pigs in Nigeria. The polymorphism information content (PIC) of each marker was evaluated to determine its level of informativeness. DNA samples were collected from apparently healthy indigenous pigs in Kaduna, Benue, and Ogun States under field heat-stress conditions, with ambient temperature and relative humidity recorded at the time of sampling. Polymerase chain reaction (PCR) amplification was performed using primers specific for each heat shock protein gene, and the PCR products were analyzed by polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. Allelic variation was assessed using Microsatellite Analyzer software to generate allele frequencies. The results revealed alleles A and C as the predominant alleles for markers SSR1(HSP90) and SSR2 (HSP110), respectively. The effective number of alleles and expected heterozygosity were higher for SSR2 (He = 0.42) than for SSR1 (He = 0.38). PIC values ranged from 0.56 (HSP90) to 0.60 (HSP110), indicating that both markers were informative (PIC $\geq$ 0.52). This study provides the first molecular characterization of HSP90 and HSP110 gene polymorphisms in Nigerian indigenous pigs under tropical heat-stress conditions, suggesting their potential as reliable genetic biomarkers for thermotolerance selection and future breeding programs aimed at improving heat resilience in tropical livestock.

Keywords: Heat shock proteins, Indigenous pigs, Polymorphism, Thermotolerance

1.0 INTRODUCTION

The ability of livestock to adapt and survive heat stress is directly impacted by heat stress (HS), which has become a significant problem in the era of climate change (Islam *et al*., 2024). It has been demonstrated that when animals are unable to mitigate the effects of heat stress (HS) load, they may die from hyperthermia (Prates, 2025). To preserve an animal's health, performance, adaptability, and survivability, the impacts of HS must be mitigated. High ambient temperatures are the primary factor limiting animal production in tropical and subtropical locations, whereas extremely low temperatures in temperate regions are similarly detrimental to pigs (Haebeeb *et al*., 2023). The sensation of discomfort and physical strain caused by exposure to extremely hot environments is known as thermal stress. Heat stress in extreme stress in spring season is considered forms of thermal stress.

The genes for Heat Shock Proteins (HSPs), including HSP90 and HSP110, are members of the molecular chaperone family and are markedly increased in response to physiological and environmental stress (Tella, 2025). By generating intracellular and extracellular signals that control cellular processes and general metabolism outside the thermoneutral range, these genes are essential for helping pigs survive heat stress (Tella, 2025). Furthermore, they support the correct folding and unfolding of damaged proteins during heat stress, which helps maintain cellular balance and enhances the pig's ability to adapt to harsh environmental conditions (Patir and Upadhyay, 2020). HSP genes provide defense against cerebral ischemia, circulatory failure, and hyperthermia when over expressed in response to heat stress (Patir and Upadhyay, 2020; Tella, 2025). Cellular protection (cytoprotection), immunological response, protein synthesis, cytoskeletal protection, protein translocation and steroid hormone receptor regulation, transportation, protein refolding, safeguarding proteins from cellular stress, inhibitory apoptosis, and adaptation during and after thermal assault are all specifically dependent on the HSP 90 gene (Chakafana *et al*., 2021).

It has been demonstrated that the genomic foundation for thermotolerance selection in tropical animals subjected to heat

Cite this article: Ubu, I. and Osaiyuwu, O. H. (2026). Polymorphism in the heat stress protein of indigenous pig populations of Nigeria *Journal of Life Sciences and Environmental Protection,* 1(1):34-40

stress is provided by the HSP90 gene. It is generally acknowledged that the biological response to heat stress includes alterations in gene expression. Even yet, the most well-researched examples of genes whose expression is impacted by heat shock are the HSPs. Many HSPs, such as HSP32, HSP40, HSP60, HSP70, HSP90, HSP110, and many more, are found to have increased expression during hyperthermic stress (Omotoso *et al*., 2019; Al-jaryan *et al*., 2023). HSPs are essential for intracellular transport, maintaining proteins in an inactive state, and preventing protein degradation (Horowitz *et al*., 2022). Additionally, HSPs function as molecular chaperones by participating in the assembly of proteins without being integral to the final protein structure (Tella, 2025). HSPs play crucial roles in cell-cycle control signaling and the protection of cells against apoptosis. This study is crucial as it addresses a significant challenge in livestock production, heat stress (HS) and its impact on pigs adaptability, survivability, and productivity.

Pigs play an essential role in the livelihoods of smallholder farmers, particularly in Nigeria, where they serve as a primary source of food and income. Their ability to thrive in harsh terrains and climatic conditions makes them indispensable to rural economies, particularly for marginalized farmers and landless laborers who rely on them for their livelihoods. However, with the increasing threat of climate change, rising global temperatures have become a primary concern for livestock production (Ubu *et al*., 2025). Heat stress negatively affects pigs by compromising their health, reproduction, and overall productivity. To mitigate these challenges, understanding the role of heat shock proteins (HSPs), particularly HSP90 and HSP110, is essential (Gaughan *et al*., 2023). These proteins play a crucial role in helping pigs cope with heat stress by regulating protein folding, cellular protection, and immune response (Jemmali *et al*., 2018).

By studying the expression of HSP genes in indigenous pig populations, researchers can identify genetic markers associated with thermo-tolerance. This knowledge can be applied in breeding programs to enhance resilience to heat stress, ensuring sustainable livestock production in regions vulnerable to extreme climate. Additionally, insights gained from this research can inform management strategies aimed at improving pig welfare and productivity under challenging environmental conditions. Ultimately, this study contributes to the broader goal of developing climate-resilient pigs farming systems and sustainability, which are crucial for ensuring food security and alleviating poverty in regions experiencing heat stress. By equipping farmers with the knowledge and tools to breed and manage pigs more effectively, the study supports sustainable agricultural practices that benefit both animals and the communities that depend on them. However, there is paucity in research of this very kind on Nigerian indigenous pigs. It is in this light that the study seeks to investigate polymorphism in the Heat Stress Protein (HSP 90 and HSP110) of indigenous pig populations of Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Location

The study was conducted in the humid forest agro-ecological zone of Nigeria cutting across Ogun State (Latitude 7°0'N and Longitude 3°30'E), River State (Latitude 4°45' and Longitude N7°0'E) and Imo State (Latitude 5°28'N and Longitude 7°4'E). Humid forest agro-ecological zone of Nigeria has high annual rainfall of 2000 – 4000 mm around August/September, supporting lush vegetation, high humidity of about 78-89 %, and temperatures around 25-28°C, with contrasting climate change (Wukipedia.com). Pigs are predominantly raised in this region using tradition or semi-intensive management practices, and they are known to possess distinctive adaptations suited to this environment. The locations for the study were chosen specifically due to the high prevalence of Nigeria indigenous pigs these areas.

2.2 Experimental Animals

A total of 60 Nigerian pig ecotypes were randomly sampled with 20 from each of the sample populations.

2.3 Determination of Ambient Temperature and Relative Humility

The ambient temperature (°C) and relative humidity (RH, in percent) were recorded before blood collection. Moral *et al*. (2023) equations were used to determine the temperature humidity index (THI): $THI = 0.8*T + RH * (T – 14.4) + 46.4$, where T = Ambient Temperature (°C); RH = Relative Humidity (%).

2.4 Blood Sample collection

About 0.5 ml of blood was collected from the coccygeal vein of the tail-head of 60 Nigerian indigenous pig populations with the use of 1-inch, 18-gauge classic needle and syringe, one per animal. The area to be sampled was swapped first with alcohol by dipping a cotton ball into 70% rubbing alcohol and used to clean the area in an upward motion only away from the anal region to prevent the samples from being contaminated. The blood collected was dropped on FTA classic cards and allowed to dry before being dropped in well labeled envelopes to differentiate the samples. Samples were afterwards conveyed to African Biotechnology Laboratory Ibadan, Oyo State for Laboratory Analysis.

2.5 DNA Extraction, PCR Amplification and Visualisation

About 200µL of the blood sample was utilized for DNA extraction using Bioline International's Isolate II Genomic DNA extraction Kits. With 100µL of elution buffer, the final elution was diluted. According to protocol, the purified DNA sample was also stored at -20ºC for long-term storage. The presence of genomic DNA in the final eluted solution from the last DNA extraction stage was confirmed using agarose gel electrophoresis. The samples were run alongside a DNA ladder at 100 volts for 30 minutes on a 0.75 percent agarose gel containing ethidium bromide. The concentration of extracted DNA was determined using an Ultra-Violet Spectrometer from PG Instruments Ltd. The primer sequences were stated below

HSP90 Forward 5’ TCATCGGAGATGCAGCCAAGAA 3’
Reverse 5’ AGATCTCCTCGGGGAAGAAGGT 3’
HSP110Forward 5’ AAATAAGTCGACATGCCTGAGCAAACCCAG 3’
Reverse 5’ CTTCATCTGCAGTTAGTTAGTCTACTTCTTCCAT 3’

Using the programmed Thermocycler, the amplification process was carried out in 200ul microcentrifuge tubes (Mastercycler pro by Eppendorf). 15 microliters of PCR master mix, 1microliter each of forward and reverse primers, 3 microliters of DNA template, and 10 microliters of sterilized distilled water were used to make a 30-microliter reaction mix. The components were mixed properly prior to centrifugation for 5 seconds at 10,000 (rpm). Reactions to amplify all target regions were conducted with a final volume of 20 µl: 80 ng of the DNA template, 0.165 µM of each primer, 1.5 mM of the reaction buffer containing $MgCl_2$, 0.2 mM of each dNTP, and 0.650 units of Taq DNA polymerase. Polymerase chain reactions (PCR) were performed using a standard PCR program in a Bio-Rad thermocycler model T100. The denaturation step was conducted at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, an annealing step at a temperature of 30°C based on each primer pair, and a final extension at 72°C for 50 s. Finally, the PCR products were visualized in a 5% agarose gel and purified using the ExoSAP-IT enzyme.

2.6 Data Analysis

The banding pattern on the gel was converted to numerical values, with 1 representing the presence of a band and 0 representing the lack of a band. The software NTSYS-pc, version 2.0, was used to estimate genetic relatedness between genotypes using Jaccard's similarity coefficient, and UPGMA was used to cluster the genotypes (unweighted pair group method using arithmetic averages). The strength of clusters was assessed using Boot program and bootstrap methodology

3.0 RESULTS

3.1 Gel Image

Figure 1 and 2 revealed PCR –SSR1 and SSR2 techniques used to genotype and detect polymorphisms of HSP 90 and HSP 110 genes in indigenous pigs of Nigeria. The PCR of all tested 60 pigs DNA gave specific sequence repeatedly at the expected band size 400, 300 and 200 bp respectively for HSP 110 gene while HSP 90 gave specific sequence at the expected band size (300-bp) in the indigenous pigs of Nigeria. The ladder 39 – 58 of electrophoresis gel for HSP 110 gene are upregulated and those animals utilized HSP 110 for adaptation to heat stress condition.

From ladder 1 – 38 and 58 – 95 of electrophoresis gel for HSP 110 genes are down regulated and those animals are resistant to heat stress with less utilization of HSP 110. The ladder with thick/ double bands is upregulated for HSP 90 and these ladders are alleles of the same genes while thin /one band indicates single allele and downregulated. It could be deduced that those animals used more of HSP 90 genes for adaptation than HSP 110 genes.

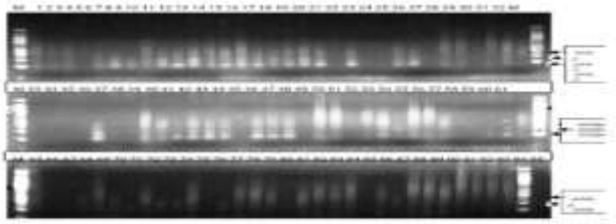

Figure 1: Electrophoresis gel for DNA of HSP-90 gene in indigenous pig of Nigeria

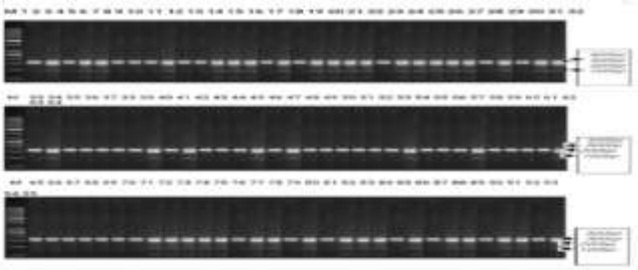

Figure 2: Electrophoresis gel for DNA of HSP-110 gene in indigenous pig of Nigeria

3.2Allelic Frequency

The allele frequencies of A and C indicated by markers 1 and 2 (HSP90 and HSP110 genes) are shown in Table 1. Superior allele C, at 72 %, was identified by Single Sequence Repeat; SSR_1 (HSP 90 gene), while recessive allele A, at 28 %, was also identified by the same marker. Superior and recessive alleles A and C were identified similarly by Single Sequence Repeat; SSR_2 (HSP 110 gene), with corresponding frequency values of 76% and 24%, respectively. The results indicate that the SSR_1 marker (corresponding to the HSP90 gene) corresponds to the major allele C. In contrast, marker SSR_2 identified allele A (HSP 110 gene) on the same pigs, with a slightly higher percentage (72 versus 75) for allele C as indicated by the SSR_1 marker (HSP 90 gene).

Table 1: Alleles frequencies of the gene in Nigerian indigenous Pigs

Marker	Allele	Allelic frequency
SSR_1	A	0.2777
	C	0.7223
SSR_2	A	0.7557
	C	0.2443

Note: SSR_1 = HSP 90 (Marker 1), SSR_2 = HSP 110 (Marker 2), A= Allele A, C= Allele C

3.3 Genetic Variability

Table 2 shows the genetic variation statistics within Nigerian pig ecotypes. It showed that the observed number of alleles for all loci (SSR_1 and SSR_2) was the same, but the adequate number of alleles differed. The effective number of alleles in the Nigerian pig ecotypes was 1.67 for the SSR_1 marker and 1.59 for SSR_2. It can be deduced that the adequate number of alleles for all loci of SSR_1 was higher than that of SSR_2. The mean adequate number of alleles for SSR_1 and SSR_2 revealed differences in the genetic expression patterns of both markers in the same Nigerian pig ecotypes under study. Shannan's information index value of 0.59 (SSR_1) was higher than 0.55 (SSR_2).

Table 2: Genetic variation statistics in Nigerian Pig ecotypes

Marker	Sample size	Na	Ne	I
SSR_1	60	2	1.6702	0.5909
SSR_2	60	2	1.5858	0.5562
Mean	60	2	1.6280	0.5736

Note: Na = Observed number of alleles, Ne = Effective number of alleles [Kimura and Crow (1964)], I = Shannan's Information Index [Lewontin (1972)], SSR1 = HSP90 (Marker 1), SSR2 = HSP110 (Marker 2)

3.4 Heterozygosity for all Loci in pig populations

Table 1 presents the result of heterozygosity for all loci in Nigerian pig ecotypes**.** The results revealed that the observed heterozygosity values were significantly greater than the expected heterozygosity values for the two markers (HSP 90 and HSP 110 genes) tested. The highest expected heterozygosity value (Nei) of 0.40 was observed in marker 1 (HSP90 gene) compared to a lower value of 0.37 in marker 2 (HSP110 gene) in Nigerian pig ecotypes. This result, however, revealed differences in the expression patterns of markers 1 and 2 corresponding to HSP90 and HSP110 genes, respectively.

Table 2: Heterozygosity for all loci in Nigerian Pig Ecotypes

Marker	Sample size	Ho	He	Average heterozygosity	Nei
SSR_1	60	0.5111	0.4057	0.3732	0.4012
SSR_2	60	0.4881	0.3735	0.3863	0.3694
Mean	60	0.5000	0.3896	0.3793	0.3853

Note: Ho: observed heterozygosity, He: Expected heterozygosity, Nei: Nei's (1973) expected heterozygosity

4.0 DISCUSSION

As members of the molecular chaperone family, Heat Shock Protein (HSP) genes specifically, HSP90 and HSP110 are extensively expressed in response to physiological and environmental stress. By initiating intracellular and extracellular signaling pathways that control cellular processes and general metabolism outside their typical thermal range, these genes are essential for helping animals adapt to heat stress (Tella, 2025). Proteins encoded by HSP genes lessen heat stress-induced cellular damage. Several stimuli, including high temperatures, physical effort, and oxidative stress, cause them to become activated. In reaction to such difficulties, these proteins are released both inside and outside the cell (Gori *et al*., 2022). Extreme heat triggers an increase in HSP gene expression, which stimulates the synthesis and maturation of new proteins to replace those weakened by stress. Furthermore, when repair is not feasible, HSPs help refold broken proteins or guide them to breakdown pathways. By preventing programmed cell death, the accumulation of these proteins in stressed cells not only facilitates protein repair but also significantly contributes to cell survival (Silanikavo, 2020). The current study found that Nigerian pig ecotypes differed in the expression of two important markers, i.e, HSP90 and HSP110. Results indicated that HSP110 (SSR_2 marker) had a stronger expression pattern than HSP90. Furthermore, HSP90 (0.59) had better Shannon's information index values than HSP110 (0.55), and HSP90 (1.67) had a larger effective number of alleles than HSP110 (1.59). These results are consistent with other studies that have found the HSP90 family to be essential for cellular thermo tolerance (Gaffin *et al*., 2019). A well-known indicator of the cellular stress response, this gene family is among the most evolutionarily conserved and plays a crucial role in protein folding within cells.

Animal lifespan, survivability, and improved heat stress response have all been linked to polymorphisms in the HSP90 gene (Sonna *et al*., 2022). Specific single-nucleotide polymorphisms (SNPs) in the HSP90 promoter region have been linked to important reproductive traits, including pregnancy rates and calf weaning weights (Baena *et al*., 2018) as well as shorter productive lifespans in dairy cows (De Vries, 2020). Similar to this, studies on Holstein cows have identified regulatory single-nucleotide polymorphisms (SNPs) in genes such as HSP90A1A and ATPIA1, which play a role in thermoregulation (Kapila *et al*., 2023). Additionally, SNPs linked to heat tolerance have been found within the HSF1 gene (Marco *et al*., 2022). SNPs in the 5′ regulatory region of HSP90 have been shown to affect semen quality features, including sperm motility and concentration, in pigs. Polymorphisms in the HSP90 gene are associated with varying degrees of heat tolerance in chickens (Aryani *et al*., 2019).

Functional variations of the HSP90 gene also have a significant impact on cellular responses to stress and mRNA stability (Ali *et al*., 2024). Genetic equilibrium can be influenced by factors such as mutation, migration, genetic drift, and selection, which alter genotypic and allelic frequencies over generations. SSRs in HSP90 and HSP110 genes exhibiting high heterozygosity indicate genetic variability, which is essential for selection and breeding programs. A heterozygosity level greater than 0.70 is considered high polymorphism (Romero *et al*., 2019). However, in the present study, both HSP90 and HSP110 showed heterozygosity levels below 0.50, indicating moderate genetic variation. For association studies, markers having a minor allele frequency (MAF) greater than 0.10 are frequently deemed appropriate.

The results of this study are likely influenced by the sampling approach, which focused on phenotypic extremes, as indicated by the observed MAF values, deviations from Hardy-Weinberg equilibrium, and the presence of just two genotypes. Compared to HSP90 (72) and HSP110 (75), the allele frequencies were somewhat higher. The genetic diversity of the HSP90 gene has been highlighted by earlier reports of similar results in chickens (Ali *et al*., 2024), humans (Sonna *et al*., 2022), and poultry (Aryani *et al*., 2019). Diversity in the HSP110 gene has been linked to improved survival, adaptation, and thermotolerance in several species (Kishore *et* *al*., 2023). Genetic diversity among various ethnicities is suggested by the differences in HSP110 genetic profiles (Hung *et al*., 2023). The HSP90 gene exhibited higher genetic variability in this study than the HSP110 gene, indicating that it is more important for Nigerian pig ecotypes tolerance to heat stress. The identified genetic variations between these two genes may serve as valuable genetic resources for breeding initiatives that enhance livestock's survival, adaptability, and thermotolerance, particularly in hot and humid tropical climates (Zabinsky *et al*., 2019). Furthermore, in animals subjected to heat stress, these genetic variations may contribute to the development of medication resistance and disease tolerance (Worku *et al*., 2023). Other HSP genes or loci are similar in mammalian species. For example, an earlier study by Glowatzki-Mullis *et al*. (2018) reported that the HSP90 gene in mammalian species exhibited a high degree of relatedness. HSP genes were highly conserved in both protein-coding and regulatory sequences, exhibiting ordinary homology. Amino acid sequences of the HSP90 gene were highly conserved among HSP sub-families (Kim *et al*., 2018).

5.0 CONCLUSION

The genes are conserved across a wide range of animals. As such, they can serve as biomarkers for selection and breeding programs aimed at enhancing thermo-tolerance in various livestock animals, particularly pigs under thermal stress. The expression patterns could be further investigated for potential specific effects on the thermo tolerance performance of Nigerian pig ecotypes in hot tropical environments.

ACKNOWLEDGMENTS

I acknowledge invaluably contribution of all the laboratory staff of the African Biotechnology Laboratory, Ibadan, Oyo State, Nigeria.

CONFLICT OF INTEREST

There is no conflict of interest in this research

ETHICAL APPROVAL

This study on the expression analysis of heat shock protein (HSP) genes in extensively reared indigenous Nigerian pig ecotypes has been reviewed and approved by three supervisors of Joseph Sarwuan Tarka University Makurdi. The research complies with the ethical guidelines for animal welfare and experimentation, ensuring minimal discomfort to the animals involved. All procedures, including blood sample collection, were conducted following the principles outlined in the guidelines for the Care and Use of Laboratory Animals and the International Ethical Standards for the Use of Animals in Research. The animals used in this study were maintained under standard husbandry conditions, with access to appropriate nutrition, suitable housing, and veterinary care by the owners. No invasive procedures or long-term suffering were induced as part of the research. Additionally, animal owners and caretakers provided informed consent before sample collection, ensuring transparency and compliance with ethical standards.

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