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***Research Article***

# POLYMORPHISMS IN GPX5 GENE AND ITS ASSOCIATION WITH PRODUCTION AND REPRODUCTION TRAITS IN NIANG MEGHA PIGS OF EASTERN HIMALAYA

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**ABSTRACT: The glutathione peroxidase 5 gene (GPX5) is closely associated with the Major Histocompatibility Complex (MHC), which directly affects the reproductive traits in swine. The study was aimed to investigate polymorphism and find out associations between individual genotypes of the glutathione peroxidase 5 (GPX5) gene with reproduction and production traits in Niang Megha pigs. Genomic DNA was extracted and genotyping was performed using PCR-RFLP technique. Polymorphisms at the Intron 1 of the GPX5 gene were determined by using PCR-RFLP with HinfI restriction enzyme and DNA sequencing analyses. Two alleles and three genotypes were identified by HinfI digestion of the GPX5 gene. In the SNP-HinfI (g.1896A>G) locus did not find a significant association between GPX5 polymorphism with reproduction and production traits. However, the least square mean (LSM) values for the genotype GG demonstrated superior litter size and body weight as compared to AA and AG genotypes in the studied population. These findings emphasize the importance of the porcine GPX5 gene in the performance traits of pig. Therefore, the porcine GPX5 gene may be used as a potential candidate gene for the genetic improvement of litter size traits in the pig breeding industry.**

**Key words:** Niang Megha Pig, GPX5, Polymorphism, Reproductive trait, Productive trait.

## INTRODUCTION

The state of Meghalaya is located in a Hilly region of North-East India. Pig rearing is an essential livestock module for the tribal population of Meghalaya. Among all the livestock species (9.06 million), pig (0.71 million) plays an important role in the livelihood of the tribal people of Meghalaya, accounting for 1.69% of the total livestock population (BAHS 2019). The total pig population of the region is mainly dominated by indigenous pigs, which are locally known as "Khasi local pig" or "Niang Megha".

It can sustain under low input management conditions and easily adapt to harsh climatic conditions. Indigenous pig breeds bear unique features such as better heat tolerance, disease resistance, good maternal qualities, early sexual maturity (Karunakaran *et al*. 2009) and good quality bristles (Mohan *et al.* 2014) compared with exotic and crossbreds. The local non-descript pigs contribute 65-75 percent of the total pig population in the North-East region (BAHS 2019), demonstrating the importance of pig rearing for farmers' livelihood systems in the region (Zaman *et al.* 2015, Talukdar *et al.* 2019). Pork production in India is limited, accounting for only 9% of all animal protein sources in the country (Panda *et al.* 2018). Among the several kinds of meat, pork is the most desired meat and contributes more than 70% to the total meat consumption in this region (Kumaresan *et al*. 2006b). The supply-demand gap for pork is higher in the Northeast (41.02 percent) than in the rest of the country (27.40 percent), which could be due to low-input backyard production systems and a lack of elite indigenous pig breeds (Mahajan *et al.* 2015).

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The reproductive traits in pig especially litter size is an utmost trait, as an increase in the number of litters per sow will increase economic returns for swine farming sector (Sato *et al.* 2016). The traditional selection criteria in pig breeding are laborious, expensive and mainly time consuming in the swine sector. Therefore, marker assisted selection employed to the identification of suitable markers for a reproductive trait is of great interest because these traits are sex-limited and have low heritability (Bidanel 2011). Several genes can be used as markers for reproductive traits in pig populations to use in marker assisted selection (MAS) programs (Bidanel 2011, Marantidis *et al.* 2013, Fang *et al.* 2014). The most promising candidate genes associated with reproductive traits in pigs are the oestrogen receptor gene (ESR), follicle-stimulating hormone beta subunit gene (FSHB), leptin and leptin receptor genes (LEP and LEPR), prolactin and prolactin receptor genes (PRL and PRLR), retinol binding protein 4 (RBP4), glutathione peroxidase 5 (GPX5), and several others (Onteru *et al.* 2009). Among them, the swine glutathione peroxidase 5 gene (GPX5) has numerous quantitative trait loci (QTL) linked to the major histocompatibility complex (MHC), which has been suggested to affect reproductive traits such as ovulation rate and litter size in swine (Buske *et al.* 2005). The swine GPX5 gene is of 1443 base pairs (bp) which encode a protein of 219 amino acids. It consists of 5 exons and has been mapped to swine chromosome region 7 (SSC7) (Bertani *et al*. 1999). To the best of our knowledge, polymorphisms in the GPX5 gene along with association studied with reproductive and productive traits were not studied in Niang Megha (NM) pig populations. Against this background, this study was aimed to evaluate the genetic polymorphisms as well as possible associations between individual genotypes with reproductive and productive traits in the GPX5 gene in Niang Megha pigs.

## MATERIALS AND METHODS

### Geographical location and climatic description

The animals were selected randomly from organized farmers' herd and Nucleus Pig Breeding Farm of ICAR Research complex (RC) for North Eastern Hilly (NEH) region, which is situated at 25°41' N latitude and 91°55' E longitude with an altitude of 1010 meters above mean sea level. The region is situated in subtropical Eastern Himalayan Hilly climate and temperature rises in the summer season (May to August), ranging between 28°C to 29.3°C, and experiences the highest rainfall from May to September with annual precipitation ranging from 2500 to 3000 mm. Whereas, the winter season (November to February), is very cold with temperatures ranging from 12.3°C to 5.5°C (Chakraborty *et al.* 2014).

### Management of animals

The experimental pigs were housed according to their sex, age and physiological condition and maintained under an intensive management system in the nucleus pig breeding farm. The pregnant sows were relocated to a farrowing pen twenty to thirty days before the estimated farrowing date. Lactating sows were fed with a standard diet (3300 ME/kg, 22.0% crude protein). The pigs were dewormed and vaccinated regularly, and other treatments were given as required.

Majority of the farmers had housing with locally available materials such as wooden plunks or timber, bamboo sheets, and roof was made either of bricks, RCC and corrugated galvanised iron/asbestos sheet (Shadap *et al.* 2017). Generally, the farmers reared pigs with zero inputs like kitchen waste products as well as vegetable waste mixed with rice polish/ wheat bran, sweet potato, banana pseudo stem and colocasia. These feeds are mixed and boiled to improve digestibility and to breakdown toxins from some feeds to make the feed more palatable (Talukdar *et al.* 2019).

### Ethical approval

The experimental plan of the study was duly permitted by the Institutional of Animal Ethics Committee (IAEC) of the ICAR Research complex (RC) for North Eastern Hilly (NEH) region, Umiam, Meghalaya, India (Approval ID and date: RC/IAEC/2020/2; 11/6/2020).

### Experimental animals and data collection

Fifty-two animals were randomly selected in three successive years (2019-2021) at organized farmers' herd

**Table 1. Primer sequences of the GPX5 gene in Niang Megha pig.**

| Primer set | Sequence (5'-3') | Region covered | Amplicon size (bp) | Annealing temperature (°C) | Reference |
|---|---|---|---|---|---|
| 1. | F-TTC ATG TAG AAC TTA TTT CTG<br>R-TGA CTT ACC CAT TCT TCA G | Intron 1 | 501 | 52.0°C | Buske *et al.* (2006) |

*(GPX5 = Glutathione peroxidase 5; F = Forward Primer; R = Reverse Primer; bp = base pairs).

**Table 2. Genetic parameters of the GPX5 gene in Niang Megha pig.**

| SNP | Genotypes | Genotypic frequency | Allele | Allelic frequency | $N_e$* | I* | Nei* | PIC* | $X^2$* value | HWE test* |
|---|---|---|---|---|---|---|---|---|---|---|
| SNP- Hinf1 (g.1896A>G) | AA (11) | 0.140 | A | 0.375 | 1.882 | 0.661 | 0.468 | 0.359 | 5.084 | Disequi-librium |
| | AG (17) | 0.469 | | | | | | | | |
| | GG (24) | 0.390 | G | 0.625 | | | | | | |

$N_e$*:Effective number of alleles; I*:Shannon's information index; Nei*:Expected heterozygosity; PIC*: Polymorphism information content; $X^2$*: Chi-square value; HWE*: Hardy-Weinberg dis-equilibrium.

**Table 3. Least squares mean (LSMEANS) and standard errors (SE) for reproductive traits of different genotypes of GPX5 gene in Niang Megha pig.**

| SNP | Genotypes | AP (Days) | AFC (Days) | AFF (Days) | FI (Days) | LB (No.) | LW(No.) | LWB (Kg) | LWW (Kg) |
|---|---|---|---|---|---|---|---|---|---|
| SNP-Hinf1 (g.1896A>G | AA (11) | 220.38±2.57 | 250.12±1.67 | 370.02±2.41 | 212.94±3.86 | 5.80±0.32 | 5.42±0.61 | 3.49±0.37 | 27.82±2.31 |
| | AG (17) | 218.10±2.71 | 250.16±3.11 | 367.22±1.72 | 216.40±2.11 | 5.26±0.51 | 5.21±0.27 | 3.56±0.23 | 28.61±1.30 |
| | GG (24) | 220.31±1.23 | 252.37±1.32 | 369.54±0.81 | 221.13±3.10 | 6.11±0.24 | 5.71±0.42 | 3.49±0.17 | 31.47±1.56 |

AP=Age at puberty; AFC=Age at first conception; AFF=Age at first farrowing; FI=Farrowing intervals; LB=Litter size at birth; LW= Litter size at weaning; LWB=Litter weight at birth; LWW =Litter weight at weaning.

**Table 4. Least squares mean (LSMEANS) and standard errors (SE) for productive traits of different genotypes of GPX5 gene in Niang Megha pig.**

| SNP | Genotypes | BW (30 d) | BW (60d) | BW (90 d) | BW (120d) | BW (150 d) | BW (180 d) | BW (210 d) | BW (240 d) |
|---|---|---|---|---|---|---|---|---|---|
| SNP-Hinf1 (g.1896A>G) | AA (11) | 3.48±0.62 | 5.71±0.11 | 9.52±1.47 | 14.39±2.26 | 20.12±1.14 | 25.32±1.14 | 31.14±1.02 | 37.45±0.21 |
| | AG (17) | 3.71±0.23 | 5.42±0.60 | 9.30±2.14 | 14.60±1.78 | 19.76±2.10 | 25.10±0.96 | 30.58±0.72 | 37.31±0.62 |
| | GG (24) | 4.01±0.56 | 6.12±0.33 | 10.68±1.20 | 15.02±0.53 | 20.89±1.60 | 27.23±0.71 | 33.22±0.10 | 37.11±0.32 |

*BW: Body weight (kg)

(*n*=13) and nucleus pig breeding farm (*n*=39) of ICAR Research complex (RC) for North Eastern Hill (NEH) region, Umiam, Meghalaya. The data for reproductive and productive traits were taken from pedigree sheet of the animals. The reproductive traits such as age at puberty (AP), age at first conception (AFC), age at first farrowing (AFF), farrowing intervals (FI), litter size at birth (LB), litter size at weaning (LW), litter weight at birth (LWB), litter weight at weaning (LWW); and data on body weight (BW) were recorded at monthly interval from birth up to 8 months of age.

**Genomic DNA extraction**

Genomic DNA was isolated from blood samples using the Qiagen DNeasy Blood and tissue kit, diluted to working concentration (30 ng $\mu l^{-1}$) and stored at -20°C, which were used as templates for polymerase chain reaction (PCR) amplification.

**Targeted regions and PCR amplification**

Based on the reference sequence (AF124818.1) of the swine GPX5 gene, a reported set of PCR primer (Buske *et al.* 2006) was used to amplify the targeted region encompassing intron 1, of the GPX5 gene in pig population. The primer sequence, product size, amplified region, and annealing temperature are presented in

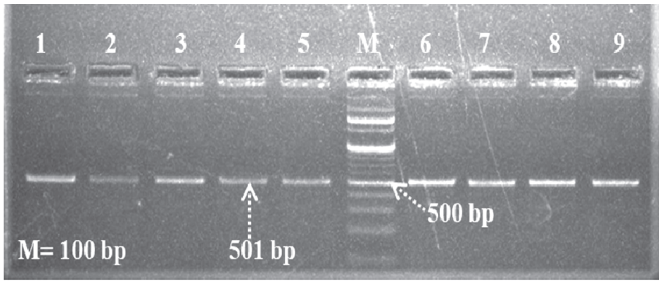


**Fig. 1. PCR amplification product of the GPX5 gene in Niang Megha pig.**

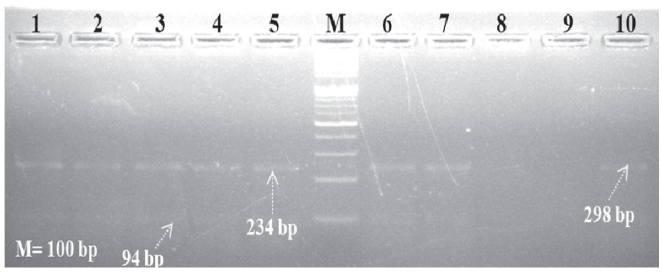


**Fig. 2. Electrophoresis pattern of polymorphisms of the GPX5 gene in Niang Megha pig.**

Table 1. PCR amplification of locus was carried out in 25 µl volume with 30 ng $\mu l^{-1}$ genomic DNA, 1.0 µM of each forward and reverse primers, PCR Master Mix (2X) (Thermo Fisher) of 12.5 µl, and 8.5 µl of water. PCR was carried out in thermal cycler (Eppendorf) in the following stages: initial denaturation at 95°C for 3 min, followed by 37 cycles of denaturation at 95°C for 30 sec, annealing at 51°C for 45 sec, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products

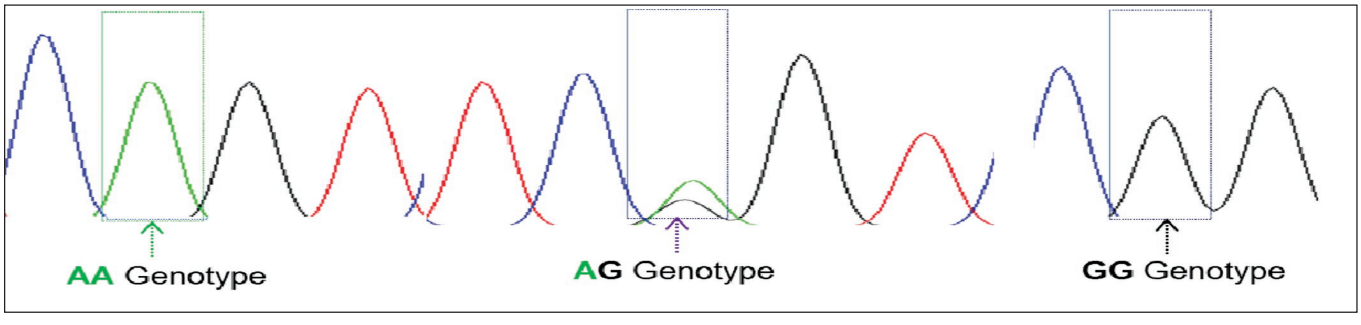


**Fig. 3. Chromatogram showing polymorphism at SNP-Hinf1 locus (g.1896A>G) of GPX5 in Niang Megha pig.**

were detected by electrophoresis using a 1.8 % (Fig. 1) and 3.0 % (Fig. 2) agarose gel in 0.5×TBE (tris–borate–EDTA buffer) mixed with 0.5 µg/ ml ethidium bromide stain and was visualized under GeNei™ Imaging System.

**Digestion of PCR product by RE**

The restriction digestion (RE) was carried out in 20 µl of reaction mixture of each sample containing 10 µl of PCR product, 9.0 µl of 10 X buffer, and 1.0 µl of Hinf1 enzyme (Promega). The reaction mixture was incubated overnight at 37 °C, and the digested products were separated by 3.5% agarose gel and visualized on a U.V. transilluminator. The gels were photographed using GeNei™ Imaging System.

**Statistical analysis**

The population parameters (gene and genotypic frequencies, effective allele number (Ne), Shannon index (I), expected heterozygosity (Nei), and the polymorphism information content (PIC) were estimated by PopGene version 1.32 (Yeh *et al.* 1999). Associations among genotypes with reproductive traits *viz.* age at puberty (AP), age at first conception (AFC), age at first farrowing (AFF), farrowing intervals (FI), litter size at birth (LB), litter size at weaning (LW), litter weight at birth (LWB), litter weight at weaning (LWW); and production traits were analyzed using a general linear model (GLM) procedure of SPSS Version 16.0.

The following model was used for the analysis of the variance of each trait:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where

$Y_{ij}$ = Adjusted value of type traits of $j^{th}$ animal to $i^{th}$ genotype

$\mu$ = Overall mean

$G_i$ = Fixed effect of $i^{th}$ genotype

$e_{ij}$ = Random error associated with $Y_{ij}$ observation and supposed to be NID (0, $\sigma^2 e$)

## RESULTS AND DISCUSSION

**GPX5 SNP identification and allele frequencies**

The selected amplified PCR products were sent for purification and custom sequencing from both ends (5' and 3'ends). Sequences were analysed with the chromas software (version 2.6.6), and each edited sequence was

aligned with the corresponding reference sequence (AF124818.1) using ClustalW multiple sequence alignment program (https://www.ebi.ac.uk/Tools/msa/) to identify SNP. To detect this SNP, PCR-RFLP was carried out; one SNP locus was firstly found, namely, SNP-HinfI (g.1896A>G) (Fig. 3). The SNP-HinfI locus (g.1896A>G) was located at intron 1 and mutated from A to G, after digestion by the HinfI, which could be generated fragments with lengths of 298, 234, 94 bp and fragments with lengths of 64, 53, 33 and 23 bp were not visible on an agarose gel. This finding appeared in conformity with the earlier observation of Terman *et al.* (2013) in the Poland pig populations. Nomenclature of detected polymorphism was done according to Bertani *et al.* (1999) *viz.* 1B1B genotype (GG), 1B2B (AG) and 2B2B (AA). The study indicated that allele G at g.1896A>G locus was predominant in the Niang Megha pig population. Chi-square tests revealed that g.1896A>G locus did not meet with the Hardy-Weinberg equilibrium ($p< 0.01$) in the studied population.

**Genetic parameter of GPX5 gene polymorphisms**

Statistical analyses showed that the frequencies of genotypes and alleles were different at SNP- HinfI in resource population (Table 2). As shown in table 2, three genotypes were found for the SNP-HinfI locus in Niang Megha Pig. Consequently, the population indices of this SNP, including Ne, I, Nei, and PIC, were analysed based on genotypic frequency numbers (Table 2). The classification of PIC value demonstrated that SNP-HinfI locus specified value of 0.359, which indicated a moderate level of genetic diversity. The GPX5 gene in the Niang Megha pig population was observed to be away from Hardy Weinberg equilibrium and heterozygote deficiency was also observed.

**Association of GPX5 genetic variants with reproduction and production traits**

It is worth pointing out that the study of association has been conducted using GPX5 genotypes on the reproductive and productive traits of the Niang Megha pigs were presented in Tables 3 and 4. However, we did not find any significant effect. Hence, we can assume that the analyzed marker (SNP-HinfI) may not have any important effect on the performance traits in the studied population. Other results also revealed that the GPX5 genotypes were significantly ($p\leq0.01$) associated with the better quality of semen in boars, although no significant correlation with litter size in sows (Mackowski *et al.* 2004, Buske *et al.* 2006). Furthermore, in Italian Large White pigs, GPX5 gene variants had linked with a variety of functional teats (Dall'Olio *et al.* 2012). In contrast to our finding, Polasik *et al.* (2017) observed significant ($p\leq0.01$) differences with litter size, and 1B1B sows genotype had the largest litter sizes as compare to 1B2B and 2B2B genotypes in large white x landrace crossbred sows.

In this study, we explored a total of one SNP locus in GPX5 *i.e.* SNP-HinfI (g.1896A>G) located in the Intron 1 and evidence was provided constantly to prove that intron played an important role in regulating post-transcriptional mechanism, mRNA splicing, and other modes of gene regulation although Intron did not code protein (Statello *et al.* 2021). So, the SNP located in the Intron could be significant for the function of protein into full play. Data analysis revealed that all genotype distributions of the SNP locus were in Hardy-Weinberg disequilibrium, which implies significant ($p<0.05$) differences in genotypic and allelic distributions within the studied population. Thus, genetic variations could be probably affected by artificial selection, because selection may significantly change the genotypic and allelic distribution of the GPX5 gene. He, Ne, and PIC were used to measure genetic variation in the population, with PIC and He having higher values indicating significant levels of genetic variation. It was an intermediate polymorphism ($0.25<PIC<0.5$) at the SNP-HinfI locus in the studied breed.

Association analysis did not find a significant effect on any reproductive and productive traits in the Niang Megha pig population. The present findings agree with Buske *et al.* (2006), who found no significant difference in the two extreme groups for litter size in sows. Our findings fully support the observation of Zhang *et al.* (2010), who reported that GPX5 variants were not significantly associated with the individual birth weight at 0 and 30 days in the F1 hybrid pig (wild boars x Large White pigs) population. Furthermore, Barranco *et al.* (2016) also investigated the presence of GPX5 in seminal plasma and discovered that boars with high GPX5 levels had higher farrowing rates and litter sizes than boars with low GPX5 levels. Recently, Michos *et al.* (2021) reported that no significant relationship of GPX5 with litter sizes but significant ($p>0.04$) and positively correlated with farrowing rates (6.7%) in pigs. As a result, we can conclude that the GPX5 is an ideal biological marker for analysing paternal traits (sperm quality) rather than maternal traits (litter size) in sows (Buske *et al.* 2005, Kmiec *et al.* 2007).

## CONCLUSION

Results of the present study indicated that the SNP-HinfI (g.1896A>G) polymorphism is not associated with reproductive and productive traits in the Niang Megha pigs' under study. Therefore, SNP-HinfI polymorphism may not be suitable in marker assisted selection programs to improve performance traits, although in other pig populations this SNP has been applied to this purpose. This is the first report that analyses the allele frequency distribution of the SNP-HinfI polymorphism in the studied breed. Ever since the present study had formulated the results based on a relatively small sample size, therefore, further investigations are required to establish the correlation of this SNP with litter size traits in larger population and diverse commercial pig breeds.

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