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Molecular characterization of Mizoram local pigs (Zovawk) using microsatellite markers

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ABSTRACT

Zovawk are indigenous pigs of the state of Mizoram, India. The 22 microsatellite markers recommended by Food and Agricultural Organization of United Nations (FAO) for Swine were used to investigate genetic diversity and population structure. The loci used for investigation are informative. The number of observed alleles (N_a) detected ranged from 2 to 11, with an overall mean of 5.54±2.558. In total 122 alleles were observed in across loci. The effective number of alleles (N_e) ranged from 1.0444 to 7.4405 with a mean of 2.71±1.472. The frequency of allele values ranged from 0.0200 to 0.9783. The PIC value ranged from 0 to 0.8489. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.46±0.252 and 0.54±0.192 respectively. The within breed estimate indicate heterozygosity shortage of 0.1526. The Hardy-Weinberg equilibrium test revealed that 11 loci out of 22 deviated from equilibrium. Shannon's information index (I) was sufficiently high with a mean of 1.12. The bottleneck analysis revealed that population has not undergone any recent reduction.
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KEYWORDS

Mizoram local pig (Zovawk);
Heterozygosity;
Microsatellites;
Polymorphic information content;
Shannon's information index;
Bottleneck.

INTRODUCTION

In northeast India, Mizoram is a small and remote state where pig rearing is a tradition. The indigenous pig of Mizoram is locally known as Zovawk. The population size is small and outnumbered by crossbreed population. Their coat colour is black with white patch on the legs; they are short legged with small and erect ears, face is narrow and long tapering towards the snout which is moderately curved (Figure 1). The average litter size at birth of Zovawk is 6.59±0.58[15]. Zovawk enjoy popularity amongst the pig farmers in the state for its ability to sustain in low input system.

To date no molecular level studies are reported on this valuable pig germplasm of North-East region. In view of this the present investigation has been planned to study the genetic diversity and population structure within Mizoram local pig population using 22 highly polymorphic microsatellite markers.

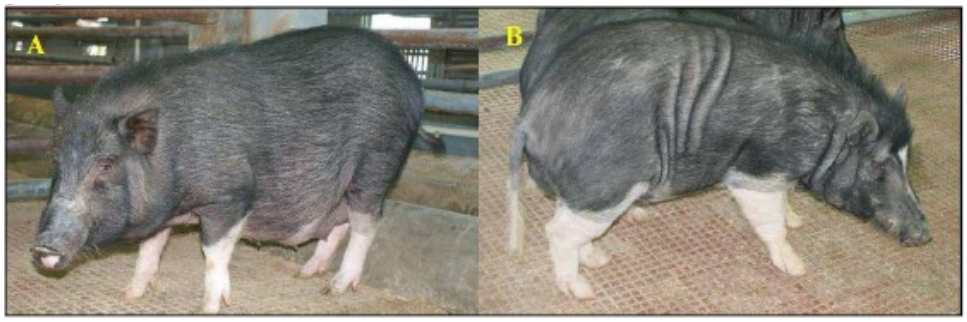

Figure 1 : Typical Mizoram local pigs (Zovawk) (A) Sow and (B) Boar

MATERIALS AND METHODS

A total of 40 blood samples of Mizoram local pig were randomly collected in an EDTA (10.8 mg) coated BD vacutainers (6 ml) from different districts of Mizoram and immediately samples were placed on ice and transported to the laboratory and stored at 4°C until use. Genomic DNA was isolated from whole blood samples of swine by using standard phenol-chloroform method[9] with few modifications. The quantity and quality of isolated DNA were confirmed. The concentrated samples were diluted to reach appropriate concentrations for the purpose of PCR amplification.

A total of 22 microsatellite markers were selected from the list recommended by Food and Agricultural Organization of United Nations (FAO) for Swine[3] based on their level of polymorphism, allele size range and reliability of allele calling to evaluate genetic diversity and structure in Zovawk pigs. The forward primer of each marker was fluorescently labeled with either FAM, NED, PET or VIC dye. All microsatellite markers were first checked under single locus amplification conditions to evaluate their performance in the multiplex.

Multiplex PCR has been used for multicolor fluorescence genotyping. Based on the guidelines of Henegariu[4] and Loffert[6] the initial parameters of multiplex PCR were set up. The basic PCR reaction mixture (15 µl) containing 20-50 ng of template DNA; 1.5 mM $MgCl_2$; 5 picomoles each of forward and reverse primers; 1 unit of taq DNA polymerase and 200 mM dNTPs was prepared. Amplification was carried out with initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation (95°C for 30 sec), annealing (48°C to 62°C for 30 sec) and extension (72°C for 45 sec) using Applied Biosystems (Model #: 9902) Veriti™ 96- well thermal cycler.

The genotyping was carried out on an automated DNA Sequencer (ABI PRISM 3130XL). The resulting data were analyzed using standard software Gene Mapper™ version 4.0 (Applied Biosystems Inc., California, USA) to generate genotype calls for each locus by using GS 500 (- 250) LIZ as size standard.

Genetic diversity was determined as allele frequencies, effective number of alleles (N_e), test of Hardy-Weingberg equilibrium (HWE), observed (H_o) and expected (H_e) heterozygosity, F-statistics and Shanon information index (I) using POPGENE v 1.32[14]. Polymorphic information content (PIC) was calculated according to Nei[7]. The BOTTLENECK v 1.2.03[1] analysis was performed to know whether this pig population exhibits a significant number of loci with excess of heterozygosity.

RESULTS

The results of genetic diversity in Mizoram local pig are presented in TABLE 1. All the 22 loci investigated were polymorphic in nature. The number of observed alleles (N_a) ranged from 2 (S0218 and SW352) to 11 (SW936), with an overall mean of 5.54±2.558 and the total number of alleles in this population was found to be 122. However, the effective number of alleles (N_e) ranged from 1.0444 (S0218) to 7.4405 (SW936) with a mean of 2.71±1.472. Overall allele frequency ranged from 0.02 (at loci SW936, TNFB, SW24, S0107, S0010 and S0086) to 0.9783 (at locus S0218). The PIC value ranged from 0.0416 (SO218) to 0.8489 (SW936) with a mean of 0.48±0.212. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.46±0.252 and 0.54±0.192, respectively

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TABLE 1 : Microsatellite analysis in Mizoram local pigs (Zovawk)

Panel	Locus	Parameters							
		N_a	N_e	PIC	H_o	H_e	I	F_{IS}	HWE
Panel 1	SW936	11	7.4405	0.8489	0.8400	0.8656	2.1734	0.0296	60.68**
	SO005	5	2.3803	0.5474	0.6154	0.5799	1.1787	-0.0612	12.52^{NS}
	SW353	7	3.8020	0.6740	0.3333	0.7370	1.5580	0.5477	96.1**
Panel 2	TNFB	10	5.1440	0.7611	0.6000	0.8056	1.8473	0.2552	63.68*
	SW24	10	3.9308	0.7075	0.6800	0.7456	1.7823	0.0880	64.94*
	SO355	6	2.4491	0.4861	0.3478	0.5917	1.1417	0.4121	66.61*
	SO107	7	3.4819	0.6672	0.8000	0.7128	1.5455	-0.1223	29.31^{NS}
Panel 3	SW72	7	2.1515	0.4704	0.4000	0.5352	1.1723	0.2526	83.6**
	SO218	2	1.0444	0.0416	0.0435	0.0425	0.1047	-0.0222	0^{NS}
Panel 4	SO228	4	1.5547	0.3359	0.4167	0.3568	0.7263	-0.1679	1.47^{NS}
	SO227	5	1.7561	0.3902	0.4167	0.4306	0.8379	0.0323	13.86^{NS}
	SW122	5	2.6241	0.5759	0.8333	0.6189	1.2161	-0.3464	10.05^{NS}
Panel 5	SO008	3	1.9459	0.4235	0	0.4861	0.8240	1.0000	37.06**
	SW957	4	1.5484	0.3301	0.1250	0.3542	0.7097	0.6471	61.93**
	SO225	3	1.7194	0.3915	0.4800	0.4184	0.7525	-0.1472	0.9^{NS}
	SO010	6	3.9557	0.6948	0.8400	0.7472	1.4968	-0.1242	16.15^{NS}
Panel 6	SO070	4	1.5070	0.3172	0.1667	0.3364	0.6879	0.5046	52**
	SW911	4	2.1533	0.4765	0.5417	0.5356	0.9566	-0.0113	5.74^{NS}
	SO086	6	2.3719	0.5128	0.6000	0.5784	1.2177	-0.0373	27.28*
Panel 7	IGFI	3	1.9702	0.3557	0.3043	0.4924	0.7533	0.382	5.72^{NS}
Panel 8	SO386	8	3.2542	0.6206	0.2917	0.6927	1.4897	0.5789	88.94**
Panel 9	SW352	2	1.6000	0	0.5000	0.3750	0.5623	-0.3333	0^{NS}
Mean overall loci		5.54 ± 2.558	2.71 ± 1.472	0.48 ± 0.212	0.46 ± 0.252	0.54 ± 0.192	1.12 ± 0.491	0.1526	

***Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$); ^{NS}Not significant ($P \geq 0.05$).; N_a, Number of alleles; N_e, Effective number of alleles; PIC, Polymorphic information content; H_o, Observed heterozygosity; H_e, Expected heterozygosity; F_{IS}, Deficit and excess of heterozygotes, HWE, Hardy-Weinberg equilibrium; I, Shannon's information Index.**

which ranged from 0 (S0008) to 0.84 (SW936 and S0010) and 0.0425 (S0218) to 0.8656 (SW936) respectively. The chi-square (χ^2) test for Hardy-Weinberg equilibrium revealed that 11 out of 22 loci deviated from equilibrium. Shannon's information index (I) value was sufficiently high with a mean value of 1.12 ±0.491.

The within population inbreeding (F_{IS}) estimates revealed deficiency of heterozygosity at 12 loci and 10 loci revealed negative F_{IS} values indicating the absence of inbreeding in these loci. The mean F_{IS} value observed was 0.1526. Though positive F_{IS} values were observed at 12 loci, only 15.26 per cent of inbreeding was recorded in Zovawk pigs.

Three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) were used for Bottleneck analysis (TABLE 2). In Mizoram local pig population, under Sign test, the expected number of loci with heterozygosity excess was 12.71 (SMM) which is higher than the observed number of loci 3 (SMM) with heterozygosity excess. The expected number of loci (12.39 and 12.55) with heterozygosity excess were significantly (PÃ0.05) higher than the observed number of loci (10 and 7) with heterozygosity excess under IAM and TPM respectively. Standard difference test (T2 statistics) in this population provided the significant gene diversity deficit under the one mutation model SMM (-7.4). Under Wilcoxon rank test, probability values of 0.64881 (IAM), 0.99668 (TPM) and 1.00000 (SMM) were found to be significant. The mode shift analysis[5] revealed L-shaped curve (Figure 2) indicating no mode-shift in the frequency distribution of alleles revealing that

TABLE 2 : Bottleneck analysis in Mizoram local pigs (Zovawk)

Model	Sign rank test - Number of loci with heterozygosity excess			Standardized differences test - T2 values (probability)	Wilcoxon test - Probability of heterozygosity excess
	Expected	Observed	Probability		
IAM	12.39	10	0.20465	-0.440 (0.32992)	0.64881
TPM	12.55	7	0.01440	-3.153 (0.00081)	0.99668
SMM	12.71	3	0.00002	-7.400 (0.00000)	1.00000

IAM - Infinite allele model; TPM - Two phase model; SMM - Stepwise mutation model

the population has not undergone any recent and/or sudden reduction in the effective population size and remained at mutation-drift equilibrium.

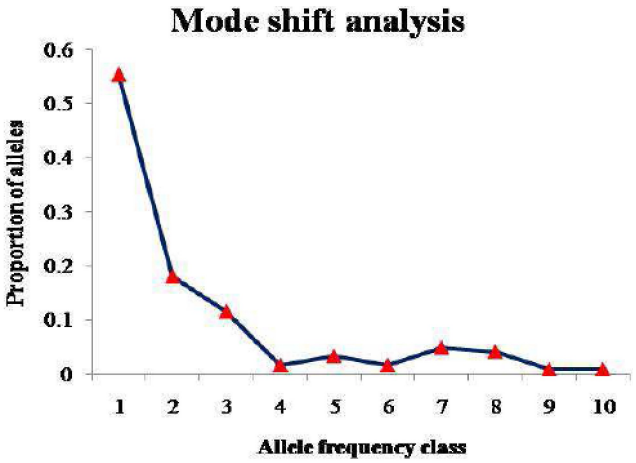

Figure 2 : Graphical representation of allele proportions and their contribution in Mizoram local pigs (Zovawk)

DISCUSSION

The number and sizes of microsatellite alleles observed in this study fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans of FAO[3]. The mean number of alleles observed (5.54) in the study is less than the mean number reported for North Indian (7.92), Northeast Indian pig (7.84) types[8] and Brazilian (8.96) pig breeds[10]. Zaman *et al.*[16] reported the mean observed alleles (N_a) of Ghungroo pig of North Bengal as 4.90±2.567 with a total of 103 alleles. The mean number of effective alleles (2.71) is almost similar with the mean number reported in Brazilian pig[10] breeds viz., Landrace (2.70); Monterio (2.34); Moura (2.32); MS60 (2.56) and Piau (2.94). However, contrary to the present finding higher mean number of effective alleles were reported in Ghungroo pig[16]. The pig population under study showed low effective number of alleles than the observed number of alleles which might be due to very low frequency of most of the alleles at each locus and few alleles might have contributed to the major part of the allelic frequency at each locus.

The PIC value (0.48) in Zovawk pigs is corroborates with the mean PIC (0.49±0.171) in Meghalaya local pigs[17] and in Brazilian pig breeds mean PIC value of 0.655 have earlier been reported[10] using 28 different microsatellite markers, which is higher with present investigation. However, most of the loci possessed high PIC values (above 0.5) signifying that these markers are highly informative for characterization of Zovawk pigs. The mean observed and expected heterozygosity (0.46 and 0.54) in the present study is lower with the mean number of observed (0.584) and expected (0.685) heterozygosity in Brazilian pig breeds[10]. The present findings of observed heterozygosity is also lower than the reported[11] value in Southern African domestic pigs viz., Landrace (0.522); Large White (0.584); Duroc (0.504); Namibia (0.518); Mozambique (0.609); Kolbroek (0.537) and Kune-Kune (0.508).

The deficiency of heterozygotes (15.26 per cent) in Zovawk pig population is higher than the heterozygote shortfall observed in Duroc pig 5.1 per cent; Landrace pig 3.8 per cent; Large White pig 6.5 per cent; Pietrain pig 6.1 per cent[12] and lower as compared to heterozygote shortfall reported in Bae pig 22.6 per cent; Canastra pig 23 per cent UDB pig 22.8 per cent; Duroc pig 25.0 per cent[2]. The present findings of F_{IS} value supports random mating in the studied population. The deviation of 11 out of 22 loci from equilibrium may be due to consequences of small population size.

The Zovawk pig population is non-bottlenecked as evident from the quantitative graphical method[1]. The population has not undergone any recent and/or sudden reduction in the effective population size and remained at mutation-drift equilibrium. In the present

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study, no mode-shift was detected in the frequency distribution of alleles and a normal L-shaped curve was observed.

In Conclusion, the investigation stands first in genetic characterization of Zovawk pig population in North-East India using microsatellite markers and results revealed the polymorphic nature of microsatellite loci screened in Zovawk pig. The population has not undergone any reduction at least in the recent past. The significant level of variability in this population is indicative of valuable genetic diversity.

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