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Molecular characterization of Doom pigs using microsatellite markers

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Assessment of genetic diversity was carried out in Doom pig using 22 microsatellite markers, recommended by Food and Agriculture Organization (FAO). All the studied loci were highly polymorphic and a total of 120 alleles were observed across the investigated loci. The range of alleles was found to be 4 to 10 with a mean of 5.4±1.65. The frequency distribution of microsatellite alleles in the population was from 0.02 to 0.6667. The observed and expected heterozygosity values were 0.62±0.287 and 0.67±0.142, respectively. The polymorphic information content (PIC) was 0.63±0.143. Microsatellite analysis revealed moderate to less genetic diversity in the Doom pig population. The overall mean of within-population inbreeding estimate (F_{IS}) was 0.089. The Shannon's information index (I) was sufficiently high with a mean of 1.36. The bottleneck analysis revealed that population has not undergone any recent reduction.

Key words: Heterozygosity, microsatellites, polymorphic information content (PIC), bottleneck, doom pig.

INTRODUCTION

Pig rearing is an integral part of the tribal population of North-East India, which accommodates 28% of the country's pig population. Assam possesses two distinct varieties of indigenous pigs namely Doom and Assam Local pig.

The Doom variety of pig is found in Dhubri, Goalpara and Bongaigaon districts of Assam and they are comparatively larger than the Assam Local pigs. Their coat colour is black with thick line of hair on the crest extending up to the lumbar region (Figure 1).

Due to the large body size, high prolificacy and ability to be sustained in low input system, Doom pigs enjoy greater popularity amongst the pig farmers in the state of Assam. These pigs can also be considered as a potential source of new allelic combinations. To date, no molecular level studies are reported on this valuable pig germplasm of North-East region. Considering the importance and utility, the present study has been planned to investigate genetic diversity and structure within Doom pig population using 22 polymorphic microsatellite markers.

MATERIALS AND METHODS

Sample collection

A total of 40 blood samples of Doom pig were randomly collected

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Figure 1. Typical Doom pigs (A) sow and (b) boar.

in an EDTA (10.8 mg) coated BD vacutainers (6 ml) from Dhubri, Goalpara and Bongaigaon districts of Assam (Figure 2) and immediately samples were placed on ice and transported to the laboratory and stored at 4°C until use.

Genomic DNA isolation and quantification

Genomic DNA was isolated from whole blood samples of swine by using standard phenol-chloroform method (Sambrook et al., 1989) with minor 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.

Microsatellite selection and analysis

A total of 22 microsatellite markers were selected for the present investigation based on their level of polymorphism, allele size range and reliability of allele calling to evaluate genetic diversity and structure in Doom pig of Assam. 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 Wallin et al., 2002. Based on the guidelines of Henegariu et al. (1997) and Loffert et al. (1999), 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 s), annealing (48 to 62°C for 30 s) and extension (72°C for 45 s) using Applied Biosystems (Model #: 9902) Veriti^{TM} 96- well thermal cycler.

Genotyping and data analysis

The genotyping was carried out on an automated DNA sequencer

Figure 2. Figure showing the breeding tract of Doom pig (kindness to Google earth map).

(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-Weinberg equilibrium (HWE), observed (H_o) and expected (H_e) heterozygosity, F-statistics and Shanon information index (I) using POPGENE version 1.32 (Yeh et al., 1999). Polymorphic information content (PIC) was calculated according to Nei (1978). The BOTTLENECK (version 1.2.03) (Cornuet and Luikart, 1996) 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 Doom pig are presented in Table 1. All the 22 loci investigated were polymorphic in nature. The number of observed alleles (N_a) ranged from 4 (TNFB, SO107, SW72, SO008, SO225, SO90, SO226 and SO386) to 10 (SW936), with an overall mean of 5.4±1.65 and the total number of alleles in this population was found to be 120. However, the effective number of alleles (N_e) ranged from 1.383 (SO226) to 7.014 (SW96) with a mean of 3.51±1.504. Overall allele frequency ranged from 0.020 (at locus SO086) to 0.666 (at loci SW957 and SO218). The PIC value ranged from 0.262 (SO226) to 0.842 (SW936) with a mean of 0.63±0.143. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.62±0.287 and 0.67±0.142, respectively which ranged from 0 (SO226) to 0.947 (SO010) and 0.277 (SO226) to 0.857 (SW936), respectively. The chi-square (χ^2) test for Hardy-Weinberg equilibrium revealed that 13 out of 22 loci deviated from equilibrium. Shannon's information index (I) value ranged from 0.581 (SO226) to 2.093 (SW936) with a mean value of 1.3611.

The within population inbreeding (F_{IS}) estimates revealed deficiency of heterozygosity at 10 loci which ranged from 0.108 (SO335) to 0.848 (SW911). Only 12 loci revealed negative F_{IS} values indicating the absence of inbreeding in these loci. The mean F_{IS} value observed was 0.089. Though positive F_{IS} values were observed at 10 loci, only 8.9% of inbreeding was recorded in Doom pig.

Three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model

Table 1. Microsatellite analysis in Doom pig population.

Panel	Locus	Size range (bp)	Parameter							
			Na	Ne	PIC	Ho	He	I	F_{IS}	HWE
Panel 1	SW936	91-113	10	7.0145	0.8422	0.8636	0.8574	2.0933	-0.0072	73.94**
	SW353	143-173	6	3.5000	0.6713	0.9286	0.7143	1.4439	-0.3000	18.93^{NS}
Panel 2	TNFB	167-181	4	3.5556	0.6674	0.7500	0.7188	1.3209	-0.0435	4.75^{NS}
	SW24	94-110	5	3.5714	0.6756	0.8000	0.7200	1.4185	-0.1111	11.5^{NS}
	SO355	243-265	6	4.6512	0.7516	0.7000	0.7850	1.6264	0.1083	27.20*
	SO107	145-161	4	2.5641	0.6422	0.7000	0.6100	1.1097	-0.1475	3.3^{NS}
Panel 3	SO218	193-201	5	2.0769	0.4850	0.5556	0.5185	1.0507	-0.0714	5.51^{NS}
	SW72	223-245	4	3.2667	0.6413	0.2857	0.6939	1.277	0.5882	26.8**
Panel 4	SO228	100-116	6	3.8647	0.7089	0.9000	0.7412	1.5558	-0.2142	34.77^{NS}
	SW122	173-185	7	5.1429	0.7784	0.9167	0.8056	1.7553	-0.1379	24.47^{NS}
Panel 5	SO008	100-130	4	2.4590	0.5362	0.4667	0.5933	1.0851	0.2135	9.03^{NS}
	SW957	236-242	5	2.0836	0.4877	0.4444	0.5201	1.0487	0.1454	24**
	SO225	273-293	4	3.1934	0.6295	0.8235	0.6869	1.2508	-0.1990	16.69*
	SO010	112-156	5	2.6350	0.5527	0.9474	0.6205	1.1420	-0.5268	15.37^{NS}
Panel 6	SO070	169-185	5	3.3333	0.6555	0.5000	0.7000	1.3705	0.2857	20.08*
	SW911	108-128	5	3.7674	0.6933	0.1111	0.7346	1.4555	0.8487	56.57**
	SO086	153-177	7	3.9683	0.7133	0.9200	0.7480	1.5830	-0.2299	93.31**
Panel 7	SO90	162-184	4	1.7131	0.3920	0.25	0.4163	0.8346	0.3994	22.12**
	IGFI	244-251	8	6.3297	0.8226	0.4167	0.842	1.9355	0.5052	138.75**
Panel 8	SO386	250-320	4	2.6309	0.5457	0.9286	0.6199	1.1003	-0.4979	40**
Panel 9	CGA	181-105	8	6.2439	0.8194	0.5312	0.8398	1.9051	0.3674	118.86**
Panel 10	SO226	197-209	4	1.3838	0.2620	0	0.2773	0.5819	1	163.89**
Mean overall loci			5.45 ± 1.654	3.588 ± 1.504	0.635 ± 0.143	0.624 ± 0.287	0.671 ± 0.142	1.361 ± 0.368	0.0898	

*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 or excess of heterozygotes, HWE, Hardy-Weinberg equilibrium; I, Shannon's Information Index.

Table 2. Bottleneck analysis in Doom pig population.

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	9.39	14	0.01382	2.416 (0.00785)	0.00759
TPM	9.33	12	0.13391	1.071 (0.14201)	0.04672
SMM	9.59	7	0.14331	-0.781(0.21726)	0.56987

IAM, Infinite allele model; TPM , Two phase model; SMM , Stepwise mutation model.

(SMM) were used for Bottleneck analysis (Table 2). In Doom pig population, under Sign test, the expected number of loci with heterozygosity excess was 9.59 (SMM) which is higher than the observed number of loci 7 (SMM) with heterozygosity excess. The expected number of loci (10.64 and 9.33) with heterozygosity excess was significantly (P>0.05) higher than the observed number of loci (8 and 12) 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 (-0.781). Under Wilcoxon rank test,

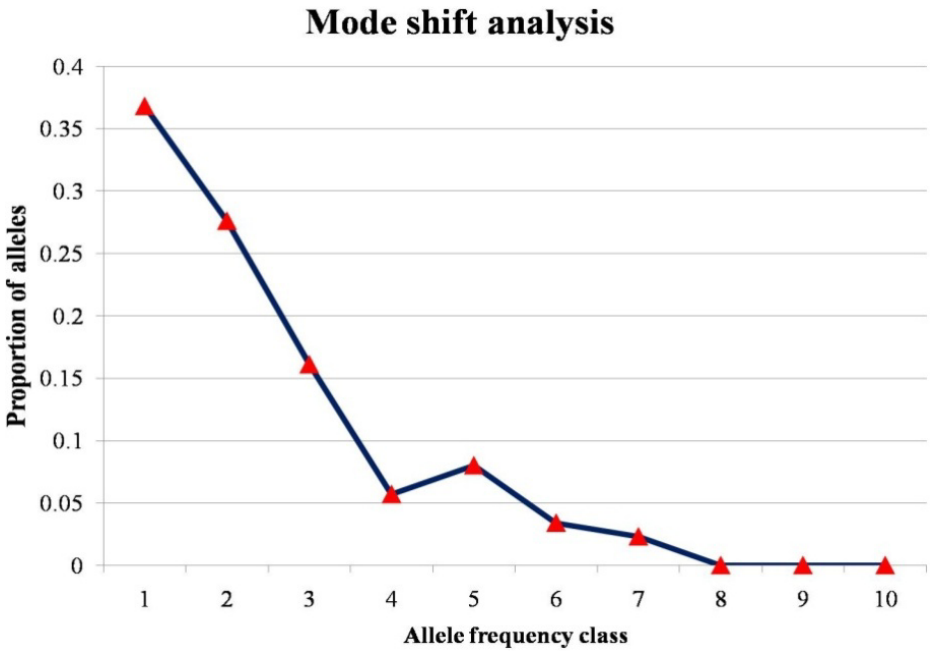

Figure 3. Graphical representation of allele proportions and their contribution in Doom pig population.

probability values of 0.00759 (IAM), 0.04672 (TPM) and 0.56987 (SMM) were found to be non-significant. The mode shift analysis (Luikart et al., 1998) revealed L-shaped curve (Figure 3) indicating no mode-shift in the frequency distribution of alleles revealing that the population has not undergone any recent and/or sudden reduction in the effective population size and remained at mutation-drift equilibrium.

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 (FAO, 1998). The mean number of alleles observed (5.40) in the study is less than the mean number reported for Suwo pigs (6.40) (Zaman et al., 2013d), North Indian (7.92) and Northeast Indian pig (7.84) types (Rajeev et al., 2001), Brazilian (8.96) pig breeds (Sollero et al., 2010) and higher than the mean number reported for Ghungroo pigs (4.90) (Zaman et al., 2013a), Niang Megha pigs (3.9) (Zaman et al., 2013b). Moreover, the mean number of observed alleles in Mali pigs (5.63) (Zaman et al., 2013c) and Zovawk pigs (5.54) (Zaman et al., 2014) were corroborates with the present findings. However, the mean number of effective alleles (3.58) is higher than the mean number reported in Brazilian pig (Sollero et al., 2010) breeds viz., Landrace (2.70); Monterio (2.34); Moura (2.32); MS60 (2.56) and Piau (2.94). The pig population under study showed lower 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 range of PIC between 0.137 and 0.874 with the mean of 0.655, reported in Brazilian pig breeds (Sollero et al., 2010) using 28 different microsatellite markers, is in close agreement with the present results which ranged from 0.262 to 0.842 with a mean of 0.635. Most of the loci possessed high PIC values (above 0.05) signifying that these markers are highly informative for characterization of Doom pig. The mean observed and expected heterozygosity (0.62 and 0.67) in the present study is in agreement with the mean number of observed (0.584) and expected (0.685) heterozygosity in Brazilian pig breeds (Sollero et al., 2010). The present findings of observed heterozygosity is higher than the reported value (Swart et al., 2010) in Southern African domestic pigs namly, 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 mean within population inbreeding estimate (F_{IS}) was 0.089. The deficiency of heterozygotes (8.9%) in Doom pig population is comparable to heterozygote shortfall observed in Duroc pig 5.1%; Landrace pig 3.8%; Large White pig 6.5%; Pietrain pig 6.1% (Vicente et al., 2008) and not significant as compared to heterozygote shortfall reported in Bae pig 22.6%; Canastra pig 23% UDB pig 22.8%; Duroc pig 25.0% (Silva et al., 2011). The

present findings of F_{IS} value support random mating in the studied population. The deviation of 13 out of 22 loci from equilibrium may be due to consequences of small population size.

The Doom pig population is non-bottlenecked as evident from the quantitative graphical method (Cornuet and Luikart, 1996). 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 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 Doom pig populations in North-East India using microsatellite markers and the PIC values observed in the present study is indicative of the fact that the markers used are highly informative for characterization of diversity in Doom pigs. 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. The needful strategy has to be taken to maintain the existing genetic variation and its sustainable utilization.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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