Research Article

Inter-Simple Sequence Repeat loci Associations with Predicted Breeding Values of Body Weight in Kermani Sheep

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Abstract

The Kermani sheep is an important meat producing animal in Iran and its economic efficiency is mainly dependent on its growth and reproduction ability. Inter Simple Sequence Repeat (ISSR) is the genome region between microsatellite loci. The aim of this study was to evaluate the association of two different ISSR markers with predicted breeding values (PBV) of body weight trait in Kermani sheep. Blood samples were obtained from 240 Kermani sheep. Polymerase chain reaction (PCR) was performed using two ISSR primers (GA)9C and (AG)9C. The amplified PCR fragment sizes ranged from 100 to 3100 bp and primers amplified 28 (A1 to A28) and 36 (G1 to G36) fragments, respectively. In addition, breeding values for birth weight, weaning weight (at 3 months of age) and body weight at 9 months of age for all Kermani sheep were predicted by univariate analysis of the animal mixed model. Associations of the loci with predicted breeding values of body weights were evaluated using a general linear model. Five polymorphic ISSR loci, G14, G25, A8, A20 and A26 had significant associations with PBVs of body weight ($P<0.05$), whereas the animals with presence of the bands significantly had a lower PBV of body weight. It seems that the G14, G25, A8, A20 and A26 ISSR loci may involve or linked to some QTLs or major and minor genes affecting body weight of lambs and therefore, could be used as genetic markers in MAS.

Key words: Birth Weight; Genetic Markers; Iran; Microsatellite Repeats; Sheep; Polymerase Chain Reaction

Introduction

Researchers think that a kind of organism that has not sufficient genetic diversity is incapable to adapt with varying environs or competitors and parasites (1). In addition, the ability of a population to respond adaptively to environmental changes depends on its level of genetic variability or diversity (2). Thus, genetic diversity in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. We need to achieve a profound science about genetic resources to conserve the specific breed. Therefore, it is important to try to genetically characterize indigenous breeds (3) and the applications of molecular genetics have many important advantages (4). Farmers maintain and breed 27 sheep breeds and ecotypes that contain more than 50 million heads (5). One of the most important breeds of Iranian sheep is Kermani sheep (6). This local breed lives in the south-eastern of Iran and is a fat-tail breed and well adapted to a wide range of harsh environmental conditions in Kerman province. This breed is favorably able to cope with several environmental situations. Using molecular genetics methods similar to DNA markers is one of the best choices for faster and better accomplishment of animal breeding programs (1).

Inter Simple Sequence Repeat (ISSR) is the genome region between microsatellite loci. The ISSR is a molecular marker method which does

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not need the genome sequence information and leads to multi-loci and highly polymorphic patterns (7). Each ISSR band corresponds to a DNA sequence delimited by two inverted microsatellites. The ISSR loci are dominant markers with the assumption of only two alleles per locus. It has been shown that the ISSR markers are universal, quick, easy to apply, highly reproducible and polymorphous (8).

The ISSR method has been used in genetic diversity studies in several species such as cattle (9, 10), cattle, goat and sheep (2), sheep (5), fish (11), silkworm Bombyx mori (12), mouse (13) and so on. It seems that the ISSR markers could be used to find markers associated with major and minor genes controlling important traits. Until now, there is a very little data about association of ISSR markers with breeding values of traits in animals, thus the present study was conducted to evaluate the association of two different ISSR markers with predicted breeding values (PBV) of body weight trait in Kermani sheep.

Materials and Methods

Salting-out method with changing and optimization (14) was used for DNA extracting from the whole blood of 240 sheep. Two ISSR primers (GA)9C and (AG)9C (table 1) were used for amplification of desired fragments. The PCR products were electrophoresed on 1% agarose gel with 1×TAE buffer at 80V for 2h along with 0.1 kbladder (CinnaGen Co., Iran). The gels were stained with ethidiumbromide and visualized under UV light (BTS-20.M, UVItec Ltd., UK). ONE-Dscan software (Scanalytics, Inc., Fairfax, VA) was applied for size definition of the amplified fragments. Based on the presence or absence of the bands, the ISSR profiles were scored as 1 or 0, respectively, it is assumed that in any locus every created ISSR band is a dominant allele. For the ISSR markers, the locus was considered polymorphic when allelic frequencies is between 1 and 99% or frequency of dominant genotype (presence of the band) is higher than 2%. The data set and pedigree information used in the present study were collected for 16 year period from 1995 to 2011 from experimental flock at the Breeding Station of Kermani sheep, located in Shahrebabak city in Kerman province, south-east of Iran. Breeding values were predicted for body weights birth, 3 and9 months of age. Microsoft Excel and Access software were used for edition of data. Descriptive statistics of body weight data are presented in Table 2.

Table 1:Characterization of used ISSR primers for studied population of Kermani sheep.

Primer	Primer sequence (5'-3')	Annealing Temperature (°C)
(AG)9C	5'-AGA GAG AGA GAG AGA GAG C-3'	55
(GA)9C	5'-GAG AGA GAG AGA GAG AGA C-3'	55

Breeding values for birth weight, weaning weight (at 3 months of age) and body weight at 9 months of age were predicted by univariate analysis of the animal mixed model as follow:

$$y=Xb+Z_{1u1}+Z_{2u2}+e$$

Where, y was vector of the observations; b was vector of the fixed effects, including flock-year-season, sex (male and female) and birth type (1-3); u_1 and u_2 were vectors of random and maternal additive genetic effects; X, Z_1 and Z_2 were incidence matrices and e was vector of residual effects. The fixed effects of the model were previously determined by a general linear model analysis. The models were analyzed, based on Average Information algorithm of Restricted Maximum Likelihood (AI-REML) and using the Wombat software (15).

Table 2: Descriptive statistics of body weight records used for prediction of breeding values in Kermani sheep.

Trait	Birth BW (kg)	3 month BW (kg)	9 month BW (kg)
Number of records	2614	2312	1380
Mean	3.28	20.52	25.42
Standard deviation	0.49	5.26	6.65
Minimum	1.60	7.20	10.00
Maximum	4.80	36.00	52.00
Number of animals in pedigree	3498	3964	2894
Number of sires	63	54	33
Number of dams	951	862	620

Associations between predicted breeding values of body weight traits and the loci with genotypic frequencies of 5–95% were evaluated using a general linear model as follow:

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

Where, y_{ij} was a predicted breeding value of body weight for the jth animal; was overall mean; G_i was the effect of the ith genotype of the polymorphic ISSR locus (0 for absence or 1 for presence) and e_{ij} was residual effects. Normality of the residuals was evaluated using Kolmogorov-Smirnov test. The residuals of all traits had normal distributions and thus no data transformation was needed in the association analyses. Analyzing of the general linear models and computing of least square means were done using proc GLM of the SAS software (15) Furthermore, Bonferroni is more power when the number of comparisons is small, whereas Tukey procedure is more powerful when testing large numbers of means; hence Tukey procedure was used in this study.

Results

The amplified PCR fragment sizes ranged from 100 to3100 bp and (AG)9C and (GA)9C primers amplified 28 (A1 to A28) and 36 (G1 to G36) fragments, respectively (table 3). The (GA)9C and (AG)9C primers produced 29 (80.6%) and 24 (85.7%) polymorphic ISSR loci, respectively (table 4). Nei's gene diversity was 0.56 and 0.55 for (AG)9C and (GA)9C respectively. Shannon's index detected by (AG)9C (0.91) was higher than that of (GA)9C (0.89).

The results of associations between predicted breeding values (PBV) of body weight traits and the loci with genotypic frequencies of 5–95% are presented in Tables 5 and 6. Most of the studied loci did not show any significant association with predicted breeding values of body weight at various ages. Only five polymorphic ISSR loci, G14, G25, A8, A20 and A26 had significant associations with PBVs of body weight ($P < 0.05$). The locus G14 with estimated length of 790–840 bp (Table 2) had a significant association with PBV of weaning weight, whereas the presence of G14 band significantly ($P = 0.025$) decreased the PBV of weaning weight (Table 5). Another ISSR locus, G25 with estimated 1610–1700 bp length (Table 2) was another ISSR locus detected by (GA)9C primer, with significant association with PBV of birth weight. The animals with G25 band significantly had a lower PBV of birth weight (Table 5). Three ISSR loci, detected by (AG)9C, A8, A20 and A26, with estimated lengths of 610–650, 1410–1500 and 2110–2200 bp, respectively (Table 2) had significant associations with PBVs of body weight. In comparison of least square means, the animals with A8, A20 or A26 bands, significantly ($P = 0.016$) had a lower 9 month and birth weights PBVs, respectively (Table 6). In all polymorphic ISSRs with a significant association with body weight PBV (G14, G25, A8, A20 and A26), the presence of the band significantly decreased the PBV of body weight.

Discussion

In this study, for both Nei's gene diversity and Shannon indices, gene diversities detected by (AG)9C were higher than that of (GA)9C. For other Iranian Mehraban sheep, Zamani et al. (5), reported Shannon's information indices of 0.25 and 0.20 and Nei's gene diversity indices of 0.14 and 0.11, for (AG)9C and (GA)9C markers, respectively, which are noticeably lower than the gene diversity of Kermani sheep in this study. The lower genetic diversity detected in Mehraban sheep (5) was probably due to the low geographical distances of Mehraban sheep flocks in Hamedan province. Genetic variation in Bovinae, quantity and quality of amplified DNA fragments, using ISSR-PCR method, in Mongolian yaks (Bos grunniens) and fifteen cattle breeds were evaluated. Results showed that 53 fragments out of 55 were polymorphic and there were some differences in quantity and quality of observed fragments in yaks and cattle breeds. Generally, more than 90% of the fragments were common in all investigated breeds, but differed in their frequency (16). However, results of Askari et al. (2) showed 60 polymorphic fragments with some differences in quantity and quality of observed fragments in those three species (cattle, goat and

Table 3: Lengths of ISSR loci, detected by (GA)9C and (AG)9C primers in Kermani sheep.

Locus name	(GA)9C ISSR									
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9	G_{10}
length	100-210	220-250	260-300	310-340	350-380	390-410	420-440	450-490	500-540	550-600
Locus name	(GA)9C ISSR									
	G_{11}	G_{12}	G_{13}	G_{14}	G_{15}	G_{16}	G_{17}	G_{18}	G_{19}	G_{20}
length	610-650	660-700	710-780	790-840	850-900	910-1000	1010-1040	1050-1100	1110-1140	1150-1200
Locus name	(GA)9C ISSR									
	G_{21}	G_{22}	G_{23}	G_{24}	G_{25}	G_{26}	G_{27}	G_{28}	G_{29}	G_{30}
length	1210-1300	1310-1400	1410-1500	1510-1600	1610-1700	1710-1800	1810-1900	1910-2100	2110-2200	2210-2300
Locus name	(GA)9C ISSR									
	G_{31}	G_{32}	G_{33}	G_{34}	G_{35}	G_{36}				
length	2310-2500	2510-2600	2610-2700	2710-2800	2810-3100	>3100				
Locus name	(AG)9C ISSR									
	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}
length	100-210	310-340	350-380	420-440	459-490	500-540	550-600	610-650	660-700	710-780
Locus name	(AG)9C ISSR									
	A_{11}	A_{12}	A_{13}	A_{14}	A_{15}	A_{16}	A_{17}	A_{18}	A_{19}	A_{20}
length	790-840	850-900	910-1000	1010-1040	1050-1100	1110-1140	1150-1200	1210-1300	1310-1400	1410-1500
Locus name	(AG)9C ISSR									
	A_{21}	A_{22}	A_{23}	A_{24}	A_{25}	A_{26}	A_{27}	A_{28}		
length	1510-1600	1610-1700	1710-1800	1810-1900	1910-2100	2110-2200	2210-2300	>3100		

sheep). They found that in most fragments, sheep had the highest frequencies rather than the others that confirm our results in this paper. The haplotype analysis of ISSR markers revealed that, some of them to be significantly less frequent in each species. Furthermore, in these three species some unique haplotypes were introduced in their paper. The studied markers in the present study produced 53 polymorphic ISSR loci (83% of all detected loci). This finding agrees with previous reports on high genetic variability of ISSR loci. Zamani et al. (7)studied Iranian Mehraban sheep breed and produced 51 polymorphic ISSR loci. In a study on 19 breeds and a breeding type of cattle, the (AG)9C and (GA)9C primers detected 66 ISSR loci of which 64 (97%) were polymorphic (10). In another study, the (GA)9C and (AC)9C primers detected 60 ISSR loci with 100–3100 bp sizes and26.7–81.7% polymorphism, in different populations of cattle, goat and sheep (2). In Markhoz mohair goat also 82% of discovered ISSR loci were polymorphic (17). The ISSR method has been also applied for study of genetic variability in other species such as silkworm B. mori

.**Table 4:** Frequencies of the observed ISSR bands, detected by (GA)9C and (AG)9C in Kermani sheep

Locus name	(GA)9C ISSR									
	G_1	G_2	G_3	G_4	G_5	$^*G_6$	G_7	$^*G_8$	$^*G_9$	$^*G_{10}$
Number	2	3	3	4	3	5	3	6	36	16
Percent	0.83	1.25	1.25	1.67	1.25	2.08	1.25	2.50	15	6.67
Locus name	(GA)9C ISSR									
	$^*G_{11}$	$^*G_{12}$	$^*G_{13}$	$^*G_{14}$	$^*G_{15}$	$^*G_{16}$	$^*G_{17}$	$^*G_{18}$	$^*G_{19}$	$^*G_{20}$
Number	26	33	35	51	37	23	10	5	9	7
Percent	10.83	13.75	14.58	21.25	15.42	9.58	4.17	2.08	3.75	2.92
Locus name	(GA)9C ISSR									
	$^*G_{21}$	$^*G_{22}$	$^*G_{23}$	$^*G_{24}$	$^*G_{25}$	$^*G_{26}$	$^*G_{27}$	$^*G_{28}$	$^*G_{29}$	$^*G_{30}$
Number	5	14	13	25	17	26	98	100	73	35
Percent	2.08	5.83	5.42	10.42	7.08	10.83	40.83	41.67	30.42	14.58
Locus name	(GA)9C ISSR									
	$^*G_{31}$	$^*G_{32}$	$^*G_{33}$	$^*G_{34}$	$^*G_{35}$	G_{36}				
Number	49	18	19	68	12	3				
Percent	20.42	7.50	7.92	28.33	5.00	1.25				
Locus name	(AG)9C ISSR									
	A_1	A_2	A_3	$^*A_4$	$^*A_5$	$^*A_6$	$^*A_7$	$^*A_8$	$^*A_9$	$^*A_{10}$
Number	1	2	3	5	10	10	24	41	30	49
Percent	0.42	0.83	1.25	2.08	4.17	4.17	10	17.08	12.50	20.42
Locus name	(AG)9C ISSR									
	$^*A_{11}$	$^*A_{12}$	$^*A_{13}$	$^*A_{14}$	$^*A_{15}$	$^*A_{16}$	$^*A_{17}$	$^*A_{18}$	$^*A_{19}$	$^*A_{20}$
Number	31	10	14	30	32	110	70	41	45	69
Percent	12.92	4.17	5.83	12.50	13.33	45.83	29.17	17.08	18.75	28.75
Locus name	(AG)9C ISSR									
	$^*A_{21}$	$^*A_{22}$	$^*A_{23}$	$^*A_{24}$	$^*A_{25}$	$^*A_{26}$	$^*A_{27}$	A_{28}		
Number	60	80	65	50	5	12	5	3		
Percent	25	33.33	27.08	20.83	2.08	5	2.08	1.25		

.Polymorphic loci *

(12) and mouse (13).Use of genetic markers to account for genetic variation of quantitative traits increases the precision of genetic selection, called marker assisted selection (MAS). The studies on MAS generally tend to focus on mapping a few QTLs to identify the genes of QTL. These studies often involve SNP testing in candidate genes based on their physiological action (7). In the recent years, genomic selection is increasingly applied in animal breeding programs. Breeding values in genomic selection are generally predicted based on SNPs or other DNA markers. The ideal method to predict BVs based on genomic data is to calculate the conditional mean of BV given the genotype of the animal (7). The ISSR markers are useful to find markers associated with major and minor genes controlling important traits. Several studies have been conducted on associations of ISSR markers with important characteristics of plants including chickpea (18), wheat (19), mulberry (20), strawberry (21) and so on, but a few studies were done on animals, especially silkworm, B. mori (22) and sheep (7). The present study was probably the first study on ISSR markers associations with production traits of Iranian Kermani sheep. In this study, five ISSR

Table 5: Associations of the (GA)9C loci with genotypic frequencies of 5–95% with breeding values of body weight traits in Kermani Sheep.

Locus	Birth BW			Weaning BW			month BW 9		
	LS means (kg)		P value	LS means (kg)		P value	LS means (kg)		P value
	+	-		+	-		+	-	
G_{9}	-0.220	-0.236	0.458	0.899	0.789	0.488	0.541	0.846	0.698
G_{10}	-0.012	-0.012	0.895	-0.691	-0.683	0.876	-1.334	-0.406	0.404
G_{11}	-0.009	-0.021	0.754	-0.797	-0.599	0.301	-0.744	-0.861	-0.886
G_{12}	-0.010	-0.025	0.768	-0.601	-0.883	0.301	-0.724	-0.901	0.864
G_{13}	-0.045	-0.022	0.255	-0.699	-0.899	0.401	-0.601	-0.755	0.853
G_{14}	0.008	-0.025	0.405	**-0.912**	**-0.550**	**0.031**	-0.970	-0.638	0.622
G_{15}	-0.011	-0.021	0.965	-0.697	-0.699	0.898	-0.899	-0.599	0.801
G_{16}	-0.015	0.009	0.801	-0.701	-0.812	0.715	-0.301	-1.403	0.302
G_{17}	-0.035	-0.012	0.245	-0.689	-0.889	0.399	-0.589	-0.744	0.832
G_{22}	-0.041	0.031	0.501	-0.401	-1.111	0.080	-1.080	-0.555	0.703
G_{23}	0.041	-0.060	0.201	-0.301	-1.304	0.306	-1.608	-0.061	0.501
G_{24}	0.011	-0.029	0.288	-0.599	-0.698	0.497	-0.695	-0.793	0.899
G_{25}	**-0.069**	**0.048**	**0.034**	-0.801	-0.689	0.759	-1.689	-0.197	0.117
G_{26}	0.010	-0.031	0.634	-0.584	-0.645	0.699	-1.354	-0.098	0.198
G_{27}	-0.121	0.112	0.128	-0.674	-0.058	0.532	-0.853	-0.596	0.567
G_{28}	-0.132	0.103	0.498	-0.587	-0.735	0.207	-1.411	-0.352	0.312
G_{29}	-0.008	0.001	0.433	-0.875	-0.763	0.624	-0.999	-0.201	0.104
G_{30}	-0.045	0.033	0.202	-0.587	0.638	0.792	-1.243	-0.399	0.401
G_{31}	-0.007	-0.010	0.896	-0.785	-0.801	0.444	-0.554	-0.886	0.692
G_{32}	-0.017	0.001	0.599	-0.777	-0.884	0.399	-0.789	-0.999	0.892
G_{33}	0.132	-0.154	0.397	-0.532	-0.692	0.778	-1.001	-0.099	0.678
G_{34}	0.004	-0.111	0.332	-0.792	-0.629	0.412	-0.532	-0.801	0.798
G_{35}	0.001	-0.015	0.862	0.657	-0.699	-0.488	0.543	-0.754	0.965

loci (G14, G25, A8, A20and A26) had significant associations with PBVs of body weight traits, whereas the animals with presence of the bands significantly had a lower PBV of body weight that confirmed results of Zamani et al. (7) for Iranian Mehraban sheep Hence, it seems that the G14, G25, A8, A20 and A26ISSR loci may involve or linked to some QTLs or major and minor genes affecting body weight of lambs and therefore, could be used as genetic markers in MAS. However, sequencing of the G14, G25, A8, A20 and A26 ISSR loci in future studies may help to map major and minor genes affecting body weight of sheep. In conclusion, the ISSR loci are highly polymorphic and could be used for genetic diversity studies. Five ISSR loci detected by (GA)9C and (AG)9C primers (G14, G25, A8, A20 and A26) are likely to contain or linked to some QTLs or major and minor genes affecting body weight and therefore could be used in marker assisted selection programs.

Conflicts of interest

The author declares that he has no conflict of interest.

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Table 6: Associations of the (AG)9C loci with genotypic frequencies of 5–95% with breeding values of body weight traits in Kermani Sheep.

Locus	Birth BW (LS means (kg		P value	Weaning BW LS means (kg)		P value	month BW 9 LS means (kg)		P value
	+	-		+	-		+	-	
A_5	-0.145	-0.113	0.699	0.899	0.489	0.488	-3.845	-1.793	0.438
A_6	-0.145	-0.101	0.641	0.501	0.783	0.199	-1.999	-1.878	0.473
A_7	-0.205	-0.111	0.254	0.499	0.799	0.301	-3.001	-2.986	0.602
A_8	-0.121	-0.112	0.825	0.554	0.872	0.112	**-3.499**	**-1.792**	**0.018**
A_9	-0.103	-0.201	0.504	0.706	0.798	0.412	-2.619	-2.577	0.899
A_{10}	-0.076	-0.099	0.165	0.432	0.765	0.073	-2.013	-2.101	0.674
A_{11}	-0.098	-0.087	0.789	0.754	0.587	0.389	-2.811	-2.413	0.481
A_{12}	-0.211	-0.197	0.772	0.768	0.754	0.901	-2.202	-3.565	0.345
A_{13}	-0.091	-0.152	0.401	0.799	0.701	0.711	-2.601	-2.886	0.875
A_{14}	-0.009	-0.012	0.735	0.747	0.654	0.621	-2.612	-2.799	0.786
A_{15}	-0.201	-0.245	0.751	0.764	0.798	0.802	-2.897	-2.815	0.827
A_{16}	-0.231	-0.121	0.101	0.768	0.801	0.901	-2.998	-2.588	0.602
A_{17}	-0.211	-0.201	0.765	0.599	0.766	0.399	-3.124	-2.087	0.198
A_{18}	-0.109	-0.099	0.803	0.702	0.588	0.699	-2.877	-2.321	0.498
A_{19}	-0.117	-0.109	0.863	0.634	0.687	0.764	-2.964	-3.001	0.545
A_{20}	-0.125	-0.198	0.501	0.699	0.701	0.901	**-2.001**	**-3.121**	**0.045**
A_{21}	-0.178	-0.154	0.711	0.701	0.801	0.648	-2.455	-2.999	0.299
A_{22}	-0.124	-0.137	0.514	0.755	0.638	0.613	-2.913	-2.012	0.214
A_{23}	-0.131	-0.121	0.697	0.714	0.725	0.865	-3.401	-2.746	0.154
A_{24}	-0.149	-0.125	0.768	0.799	0.706	0.412	-3.256	-2.679	0.401
A_{26}	**-0.168**	**-0.049**	**0.014**	0.659	0.648	0.811	**-2.999**	**-2.001**	**0.041**

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