Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(4): 262-267 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i04.004

Research Article

Intensively detected allelic variations in the 7th exon of yak *FTO* gene

Fangfang Zhao, Lei Sun, Xin Cai*
School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, Sichuan 621010, China.

***Corresponding author**
Dr. Xin Cai
Email: caixin2323@126.com

Abstract: Single nucleotide polymorphisms (SNP) analysis of fat mass and obesity associated (*FTO*) gene has been associated with obesity in multiple populations. It was shown that *FTO* gene had been involved in the regulation of the activities of feeding, energy balance and fat storage. This experiment randomly collected 110 blood samples from Maiwa yak groups. To verified whether there were mutations among the nine exons in the *Bos grunniens FTO* gene, this study investigated the variations through PCR and sequencing. Only the 5th exon and 7th exon were found with variations. The 7th exon was chosen to experiment for more hotspots than the 5th exon. Six mutation hotspots were identified through sequence alignment. Haplotype frequencies and linkage disequilibrium (LD) coefficients of these SNPs were analyzed. In this data, only a pair of SNPs we found were strong linkage disequilibrium and one haploid type has the highest frequency. To sum up, the *FTO* gene of Maiwa yak in this study was determined extremely probable to be a housekeeping gene.
Keywords: Maiwa yak, *FTO* gene, SNPs, linkage disequilibrium

INTRODUCTION

Single nucleotide polymorphisms (SNPs) in the first intron of fat mass and obesity related (*FTO*) gene was found to be associated with body mass index (BMI) and predisposing to childhood and adult obesity [1]. The major effect of genetic variation in the region around SNP rs9939609 in children appeared to be associated with energy intake and preference for foods of high caloric density [2], which was also related to general fatness, body fat distribution, decreased insulin sensitivity and HDL-cholesterol [3]. Yajnik *et al*. [4] demonstrated that in Asian Indians, variants in the *FTO* gene predisposed to type 2 diabetes. Another study suggested that *FTO* expression was increased in skeletal muscle from type 2 diabetic patients [5]. Furthermore, the recently published data also showed that cells lacking *FTO* displayed decreased rates of mRNA translation, and increased autophagy, all of which were likely to contribute to the phenotype of stunted growth seen in humans and mice homozygous for loss-of-function mutations in *FTO* [6]. Taken together, the *FTO* gene could be an important candidate gene for regulation of energy homeostasis, body weight and food intake.

Body weight was very important for meat producing animals and efficiency of body weight regulation was dominated the energy intake and expenditure [7], while it was controlled by genetic and epigenetic effects, and environmental conditions. Genetic components was considered to play an important role in the regulation of energy balance, and many genes involved in energy homeostasis including the *FTO* gene identified [8].

Bos grunniens (yak) are the most important domesticated species which adapt to the alpine climates on the Qinghai–Tibetan Plateau for thousands of years. The amounts of yak inhabit these regions account for over 90% of the animals distributed all over of world [9]. The aim of this work was to investigate the naturally occurring variations in *FTO* gene of *Bos grunniens*.

MATERIALS AND METHODS

In total 110 yak individuals were randomly selected from a breeding population of Maiwa yak in Hongyuan county, Sichuan province of China. Blood samples were collected from these yak individuals and deposited in an ice box before transported to laboratory. Genomic DNA was extracted from blood using the phenol–chloroform method [10].

PCR was performed using the primers (Table 1) designed by Zhang *et al*. [11] to amplify ten fragments covering nine exons of yak *FTO* gene. PCR reaction of 25μl volume contained: 100 ng of each genomic DNA, 10 μM of each primer, 2.5μl of 10×Buffer (Mg^{2+} Free), 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.65U of Taq DNA polymerase (TaKaRa). The PCR protocol was 94°C for 5 min followed by 35 cycles of 94°C for 35 s, annealing

(Table 1) for 30 s [11], 72°C for 45 s and a final extension at 72°C for 10 min before saving at 12°C. PCR products were purified using a Qiagen QIAquick PCR purification kit and were sequenced on an ABI 3730 automated sequencer at BeiJing Zixi Bio Tech Co., Ltd, Beijing. Both strands of the PCR product were completely sequenced.

The *FTO* gene sequences of 110 yaks were aligned and edited in Clustal X with parameters set to default [12]. Polymorphic sites of nucleotide of *FTO* gene were explored and analyzed by using MEGA 4 [13].

Table 1. *FTO* gene primers were used for PCR amplification.

Loci	Primers sequences (5'-3')	Annealing temperatures (°C)	Region
1	F:CTTTCCGAGGGAGAATGG	61.8	Part of 5' flanking, the 1st exon and part of 1st intron
	R:GACCCCAGCTACAAGTGC		
2	F:GTTTGTTAATTGGCTGTACC	62.0	Part of 1st intron, the 2nd exon and part of 2nd intron
	R:AATCTCTCTAGACCACAG		
3	F:GCTTTGCTTTTGTTCTAGTGG	68.0	Part of 1st intron, the 2nd exon and part of 2nd intron
	R:TTGGCTTTCTCCTTGGCAG		
4	F:TGCAGGTAGAGACCATCCA	65.0	Part of 3rd exon and part of 3rd intron
	R:TCATGTTATGTTCGGGGCT		
5	F:GATGAAACATTCCTGAC	57.0	Part of 3rd intron, the 4th exon and part of 4th intron
	R:GCTTTGATCCTTGCATTACC		
6	F:GGTTCTTGTCATTCTTCTTG	57.2	Part of 4th intron, the 5th exon and part of 5th intron
	R:TCTAGTTAGATGGAAGCAAT		
7	F:AGCATAGGCTGAGTTGTGA	64.0	Part of 5th intron, the 6th exon and part of 6th intron
	R:TACCTCGTTGTGGATTTCTT		
8	F:TACTGGAGGAGAACTGAAT	60.0	Part of 6th intron, the 7th exon and part of 7th intron
	R:GCACAACATCCCAAGAAA		
9	F:TACTGTCCTTATTTGCTTATG	58.4	Part of 7th intron, the 8th exon and part of 8th intron
	R:AAAGCCCTCATTTTCCAG		
10	F:AGTTGCCTGAGTATGGGTCTT	64.6	Part of 8th intron, the 9th exon and part of 3' flanking
	R:ATAGCCTTTCCTCCTCCACC		

SNP genotypes for each yak individual were identified by examining the sequencing chromatograph. Gene frequency, genotype frequency and deviation from Hardy–Weinberg equilibrium (HWE) for each SNP site were analyzed by using the online program SNPStats (http://bioinfo.iconcologia. net/snpstats/start.htm). This program was also used to measure the pairwise linkage disequilibrium (LD) pattern between SNPs based on multi-allelic D′ and r, and to estimate the dominant haplotype for all SNPs identified in the gene *FTO*.

RESULTS AND DISCUSSION

The 1st to 10th fragment of yak *FTO* gene amplified was 218 bp, 283 bp, 384 bp, 350 bp, 210 bp, 326 bp, 262 bp, 252 bp, 259 bp and 261 bp, respectively. Electrophoresis pattern for PCR product of the 7th and 10th exon of *FTO* gene was shown in Figure1 and Figure2, respectively.

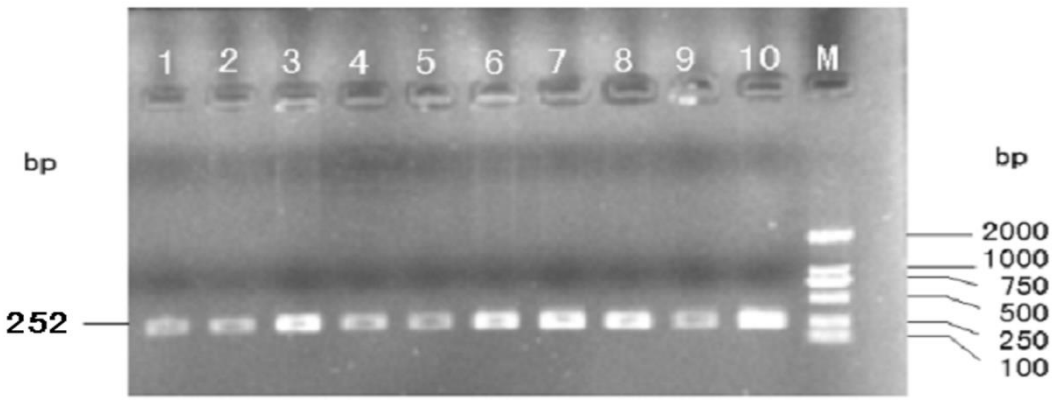

Figure 1: PCR amplification of 7th exon of *FTO* genes from 110 Maiwa yaks.
M stands for DL2000 DNA Marker and the numbers 1～10 indicate different individuals of Maiwa yaks.

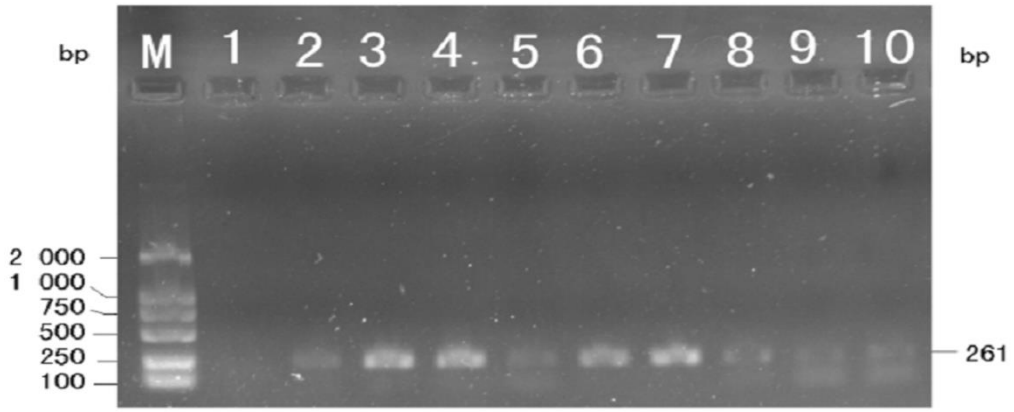

Figure 2: PCR amplification of fragment 10 of *FTO* genes from 110 Maiwa yaks.
M stands for DL2000 DNA Marker and the numbers 1～10 indicate different individuals of Maiwa yaks.

Mutation analysis revealed that the ten sequenced fragments of *FTO* gene for 110 Maiwa yaks exhibited specific variation patterns, for all variation sites were intensively detected in the 7th exon of *FTO* gene, namely SNP1, SNP2, SNP3, SNP4, SNP5 and SNP6. Parts of SNPs were shown in Figure3 A and Figure3 B. These SNPs were deposied in GenBank with the accession numbers. Among the six SNPs, homozygous genotypes were found to be dominant in all genotypes and the frequency of each homozygous genotype was higher than 0.9. The dominant homozygote allele in SNP1, SNP2, SNP3, SNP4, SNP5 and SNP6 was G, G, G, A, G and T, respectively. Genotypes of each SNP and corresponding frequencies were listed in Table 2. All these SNP indicated no significant deviation from HWE (P>0.05), therefore, this yak population sampled in this study were in an equilibrium state (Table 2).

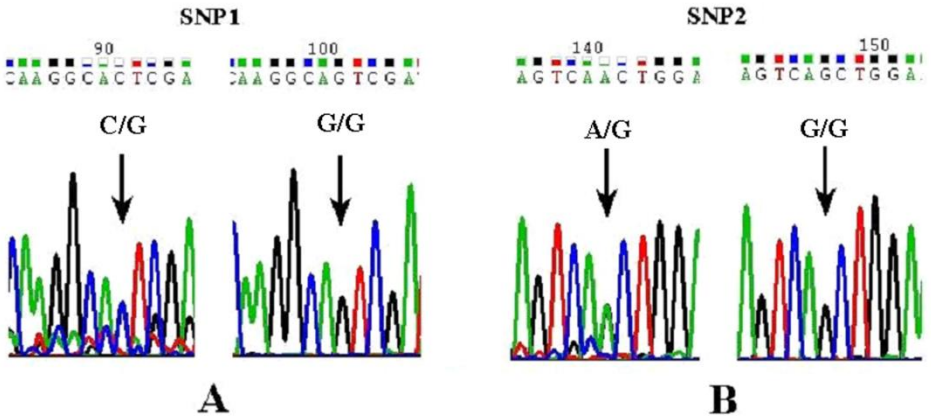

Figure 3: **Part loci of mutations found in 7th exon of Maiwa yak *FTO* gene and the detected genotypes. A indicates two genotypes: C/G and G/G detected from SNP1; B indicates two genotypes: A/G and G/G detected from SNP2.**

Table 2: The allele frequencies and genotype frequencies of SNPs detected in the *FTO* gene.

SNP	Allele	Allele Frequency	Genotype	Genotype Frequency	P-value*
SNP1	G	0.9864	G/G	0.9727	1
	C	0.0136	G/C	0.0273	
SNP2	G	0.9955	G/G	0.9909	1
	A	0.0045	G/A	0.0091	
SNP3	G	0.9818	G/G	0.9636	1
	A	0.0182	G/A	0.0364	
SNP4	A	0.9773	A/A	0.9545	1
	C	0.0227	A/C	0.0455	
SNP5	G	0.9591	G/G	0.9181	nd
	T	0.0409	G/T	0.0819	
SNP6	T	0.9818	T/T	0.9636	1
	C	0.0364	T/C	0.0364	

FTO gene was ubiquitously expressed in human tissues, and highly expressed in arcuate, paraventricular, dorsomedial, and ventromedial nuclei, all being sites of critical importance for the control of energy balance, fat storage and metabolism [1,6,14], and its ancestor originated from 450 million years ago and highly conserved across most organisms [15]. In this work, only six mutation sites were detected in the 2805 bp sequences covering nine exons of yak *FTO* gene. Therefore, *FTO* was more a housekeeping gene than mutant gene, and it could be explained by this view that *FTO* gene has been subjected to high levels of continuous purifying selection during yak evolution. As a result, mutations and variations that would influence the activity of metabolism had been subjected to purifying selection mechanism during yak evolution [9,16].

The pairwise linkage disequilibrium (LD) patterns between SNPs were analyzed based on multi-allelic D′ and r (Figure4). Five pairs of SNPs, namely SNP1-SNP4, SNP1-SNP6, SNP3-SNP4, SNP3-SNP6 and SNP4-SNP6, demonstrated higher multi-allelic D' but lower r value, which suggested that genetic recombination be still likely to occur between these pairwise SNPs. SNP2 kept week multi-allelic D' and r (r value close to 0) with SNP1, SNP3, SNP4 and SNP6, respectively, suggesting SNP2 be a possible recombination hotspot. As both higher D' (0.6575) and r^2 value (0.56813) were detected between SNP1 and SNP3, linkage disequilibrium might be maintained between these two variation site and genetic recombination was less likely to occur.

The degrees of LD were different in various populations and different regions of genes [17]. Domestication and breeding improvement were involved in the selection for specific alleles at candidate genes, resulting in reduced genetic diversity and increased LD relative to unselected genes [18, 19]. In this work, less LD was identified in *FTO* gene of yak than that of bovine [11], which could be explained by the fact that yaks have been grazed livestock for thousands of years and less domestication, artificial selection or breeding improvement were imposed on yak population during their raising and management.

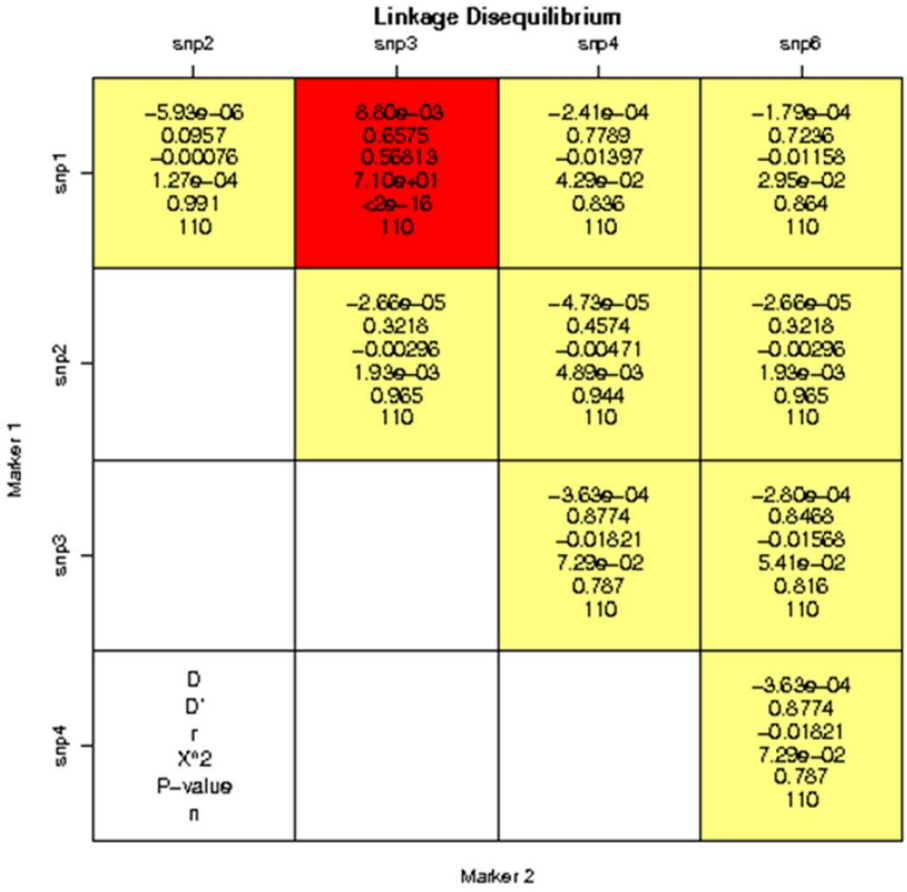

Figure 4: **Linkage disequilibrium of SNP1 to SNP6 were analyzed. D, D', r, x^2, p-value were calculated separately. SNP5 was not related to any SNP.**

Eight SNP haplotypes were found in haplotype analysis (Figure5). The dominant haplotype was GGGAGT with the frequency of 0.9272, while half of the haplotypes were rare in the yak population, with the frequecies lower than 0.01.

Haplotype frequencies estimation (n=110)									
	snp1	snp2	snp3	snp4	snp5	snp6	Total	group.G.G	Cumulative frequency
1	G	G	G	A	G	T	0.9272	0.9272	0.9272
2	G	G	G	C	T	T	0.0227	0.0227	0.95
3	G	G	G	A	T	C	0.0182	0.0182	0.9681
4	G	G	A	A	G	T	0.0091	0.0091	0.9773
5	C	G	A	A	G	T	0.009	0.009	0.9863
6	C	G	G	A	G	T	0.0046	0.0046	0.9909
7	G	A	G	A	G	T	0.0045	0.0045	0.9955
8	G	G	G	A	C	T	0.0045	0.0045	1

Figure 5: Haplotypes of SNP1 to SNP6 were analysed. Type 1 to type 8 were different haplotypes. Data of Total in the picture showed the frequences of each haplotype.

CONCLUSIONS

Knowledge of *FTO* gene we obtained is strong associated with body mass index (BMI) and predisposing to obesity. For that matter, we carried out a study to investigate the variants of *FTO* gene in 110 Maiwa yaks. With found variation sites, low LDs were investigated in the pairwise linkage disequilibrium (LD) patterns analysis between SNPs. The dominant homozygote allele type was detected. To sum up, *FTO* gene of yaks we conjectured were more a housekeeping gene than mutant gene, which has been subjected to high levels of continuous purifying selection during yak evolution.

Therefore, it is conceivable that mutations detected in the *FTO* gene have an effect on its activity and function. Further analysis is needed to investigate how these variations may affect *FTO* activity and influence whole cattle energy metabolism.

ACKNOWLEDGMENTS

This work was supported by the grants from China Postdoctoral Science Foundation funded project (No.20090461336) and China Postdoctoral Special Science Foundation funded project (No.201003703).

REFERENCES

1. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM; A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. Science, 2007;316: 889-894.
2. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN; An obesity- associated *FTO* gene variant and increased energy intake in children. N Engl J Med, 2008;359: 2558-66.
3. Kring SI, Holst C, Zimmermann E, Jess T, Berentzen T, Toubro S, Hansen T, Astrup A, Pedersen O, Sørensen TI; *FTO* gene associated fatness in relation to body fat distribution and metabolic traits throughout a broad range of fatness. PLoS One, 2008;3: 8-e2958.
4. Yajnik CS, Janipalli CS, Bhaskar S, Kulkarni SR, Freathy RM, Prakash S, Mani KR, Weedon MN, Kale SD, Deshpande J, Krishnaveni GV, Veena SR, Fall CH, McCarthy MI, Frayling TM, Hattersley AT, Chandak GR; *FTO* gene variants are strongly associated with type 2 diabetes in south asian indians. Diabetologia, 2009;52: 247-252.
5. Amélie B, Etienne L, Emmanuelle M, Sandra P; *FTO* is increased in muscle during type 2 diabetes, and its overexpression in myotubes alters insulin signaling, enhances lipogenesis and ROS production, and induces mitochondrial dysfunction. Diabetes ,2011;60: 258-268.
6. Gulati P, Cheung MK, Antrobus R, Church CD, Harding HP, Tung YC, Rimmington D, Ma M, Ron D, Lehner PJ, Ashcroft FM, Cox RD, Coll

AP, O'Rahilly S, Yeo GS; Role for the obesity-related *FTO* gene in the cellular sensing of amino acids. Proc Natl Acad Sci. U. S. A. 2012;7: 2557-2562

7. Muller MJ, Bosy WA, Heymsfield SB; Is there evidence for a set point that regulates human body weight? F1000 Med Rep, 2010;2: 59.
8. Zhang C, Wang Y, Chen H, Lan X, Lei C, Fang X; Association between variants in the 5-untranslated region of the bovine *MC4R* gene and two growth traits in Nanyang cattle. Mol Biol Rep, 2009;36: 1839-1843.
9. Cai X, Mipam TD, Zhang HR, Yue BS; Abundant variations of *MC4R* gene revealed by phylogenies of yak (*Bos grunniens*) and other mammals. Mol Biol Rep, 2011;38: 2733-2738.
10. Müllenbach R, Lagoda PJ, Welter C; An efficient saltchloroform extraction of DNA from blood and tissues. Trends Genet, 1989;5: 391.
11. Zhang B, Zhang Y, Zhang LZ, Wang J, Li Z,Chen H; Allelic polymorphism detected in the bovine *FTO* gene. Mol Biotechnol, 2011;49: 257-262.
12. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG; The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. Nucleic Acids Res, 1997;25: 4876-4882.
13. Kumar S, Dudley J, Nei M, Tamura K; MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform, 2008;9: 299-306.
14. Fredriksson R, Hägglund M, Olszewski PK, Stephansson O, Jacobsson JA, Olszewska AM, Levine AS, Lindblom J, Schiöth HB; The obesity gene, *FTO*, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. Endocrinology, 2008;149: 2062-2071.
15. Sanchez-Pulido L, Andrade-Navarro MA; The *FTO* (fat mass and obesity associated) gene codes for a novel member of the nonheme dioxygenase superfamily. BMC Biochem, 2007;8: 23.
16. Hughes DA, Hinney A, Brumm H, Wermter AK, Biebermann H, Hebebrand J, Stoneking M; Increased constraints on *MC4R* during primate and human evolution. Hum Genet, 2009;124: 633-647.
17. Nakamoto K, Wang S, Jenison RD, Guo GL, Klaassen CD, Wan YJ, Zhong XB; Linkage disequilibrium blocks, haplotype structure, and htSNPs of human *CYP7AI* gene. BMC Genet, 2006;7: 29.
18. Zhou M, Lei M, Rao Y, Nie Q, Zeng H, Xia M, Liang F, Zhang D, Zhang X; Polymorphisms of vasoactive intestinal peptide receptor-1 gene and their genetic effects on broodiness in chickens. Poult Sci, 2008;87: 893-903.
19. Yamasaki M, Tenaillon MI, Bi IV, Schroeder SG, Sanchez-Villeda H, Doebley JF, Gaut BS, McMullen MD; A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. Plant Cell, 2005;17: 2859-2872.