J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Penguatan Risbang No. 60/E/KPT/2016

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
43(4):315-322, December 2018
DOI: 10.14710/jitaa.43.4.315-322

Genetic polymorphism of Pit-1|*Hinf*I gene in Grati-Ongole Grade cattle at Indonesian Beef Cattle Research Station

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Received March 23, 2018; Accepted September 09, 2018

ABSTRAK

Gen *pituitary transcription factor* (Pit-1) merupakan salah satu gen yang dianggap bertanggung jawab terhadap pertumbuhan pada sapi. Polimorfisme gen Pit-1|*Hinf*I di ekson 6 (g.1256G>A) diketahui berhubungan dengan sifat pertumbuhan maupun susu pada sapi. Penelitian ini bertujuan untuk mengidentifikasi polimorfisme gen Pit-1|*Hinf*I pada sapi PO Grati. Sebanyak 107 sampel DNA sapi PO Grati digunakan dalam penelitian ini. Identifikasi genotipe dilakukan dengan metode PCR-RFLP. Pada penelitian ini, hanya genotipe AB dan BB yang berhasil teridentifikasi dengan frekuensi masing-masing sebesar 0,009 dan 0,991, sedangkan frekuensi alel A dan B masing-masing sebesar 0,005 dan 0,995. Frekuensi genotipe yang teramati pada populasi ini, tidak menyimpang dari HWE. Nilai Ho, He dan PIC masing-masing sebesar 0,009; 0,009 dan 0,009. Kesimpulan dari penelitian ini adalah gen Pit-1|*Hinf*I pada sapi PO Grati bersifat monomorfik.

Kata Kunci : Bos indicus, gen Pit-1, PCR-RFLP, polimorfisme gen, sapi PO

ABSTRACT

The pituitary transcription factor (Pit-1) gene is one of the considered genes that responsible to growth in cattle. A specific Pit-1|*Hinf*I gene polymorphism located within exon 6 (g.1256G>A) has been shown to be associated with growth and milk traits in cattle. This study aimed to identify the Pit-1|*Hinf*I gene polymorphism in Grati-Ongole Grade cattle (Grati-OG cattle). A total of 107 genomic DNA of Grati-OG cattle were used in this study. The detection of polymorphism was performed by PCR-RFLP method. Only AB and BB genotypes were succesfully identified with the frequency of 0.009 and 0.991, respectively. Whereas, the frequency of A and B alleles were 0.005 and 0.995, respectively. The observed genotype frequencies in this population do not deviated from HWE. The value of Ho, He and PIC were 0.009, 0.009 and 0.009, respectively. In conclusion, the Pit-1|*Hinf*I gene polymorphism in Grati-OG cattle is monomorphic and hence it should not be used in further association studies.

Keywords: Bos indicus, gene polymorphism, PCR-RFLP, Pit-1 gene, Ongole Grade cattle

INTRODUCTION

Growth traits is one of the most important and preferable economic traits for beef production. Most economic traits are controlled by a large number of genes which each contribute a small effect to the trait, the loci responsible for such traits being known as quantitative trait loci (QTL) (Curi *et al.,* 2006). Recently, molecular genetics technologies allow the identification of genetic variation at QTL associated with desirable economic traits in many domesticated animals including in cattle. Furthermore, this technologies could be used to explore the genetic potential of the cattle breeds. Selection to the DNA level of the loci affecting growth traits will be more precise and accurate.

The pituitary transcription factor (Pit-1) is a specific transcription factor in pituitary cells that bound elements to activate the expression of prolactin (PRL), thyrotropin β-subunit and growth hormone (GH) genes (Tuggle and Trenkle, 1996). The Pit-1 encoded by Pit-1 gene or also known as POU1F1 gene. The bovine Pit-1 gene has a length of 18,093 bp, consist of 6 exons and 5 introns (NCBI, 2018) and has been mapped to the centromeric region of bovine chromosome 1 (Moody *et al.,* 1995). Deficient in Pit-1 synthesis decreases in proliferation of cell lines producing PRL and GH (McCormick *et al.,* 1990). A specific Pit-1|*Hinf*I gene polymorphism located within exon 6 (g.1256G>A) has been identified as a silent mutation (Dierkes *et al.,* 1998). This polymorphic site has been widely studied and shown to be associated with growth traits in several beef cattle (Zhao *et al.,* 2004; Xue *et al.,* 2006; Carrijo *et al.,* 2008; Yang *et al.,* 2011) and milk yield and quality in dairy breed cattle (Renaville *et al.,* 1997; de Mattos *et al.,* 2004; Yan *et al.,* 2011). However, there were no association found in several studies (Di Stasio *et al.,* 2002; Dybus *et al.,* 2003; Zhao *et al.,* 2004; Trakovická *et al.,* 2015). This varied results suggest that the Pit-1|*Hinf*I gene polymorphism need to be further investigated in other breeds including Indonesian local cattle.

Ongole Grade cattle (or in Indonesian called as Peranakan Ongole/OG cattle) is one of the most popular Indonesian local breeds cattle. This breed is formed from hybridization result between Ongole cattle (*Bos indicus*) x Java cattle or Banteng (*Bos javanicus*) since the "Ongolization Program" in 1915 that imposed by Duth colonial goverment, where the Ongole cattle was imported from Nellore province of India (Hardjosubroto, 1994). The phylogenetic analysis has cluster OG cattle into *Bos indicus* clade (Hartati *et al.,* 2015). This breed is very popular and has a large contribution in the provision of national meat of Indonesia. The OG cattle breeding programs have been conducted by Indonesian Beef Cattle Research Station (IBCRS), Ministry of Agriculture of Indonesia in Grati since 2004 (so-called as Grati-OG cattle). However, the selection method used is still conventional method and has not used genetic markers. So that, the selection system is has not optimal yet. The use of molecular technology approach is expected to accelerate the production of superior breeding stocks in IBCRS.

Many loci that responsible to growth traits need to be further investigated in OG cattle such as Pit-1 gene. The genetic polymorphism of Pit-1 gene in several Indonesian local beef cattle (such as Bali, Madura, Pesisir, Aceh and Katingan beef cattle) has been reported by Jakaria and Noor (2015). However, it has not been reported in OG cattle. Thus, the aim of this study was to identify the Pit-1|*Hinf*I gene polymorphism in Grati OG cattle.

MATERIALS AND METHODS

Animals and DNA Samples

A total of 107 individuals of Grati-OG cattle used in this study were from the Superior Breeding Stock Management Unit (UPBU) at the BCRS, Ministry of Agriculture of Indonesia located in Grati-East Java. Blood samples were collected from jugular vein into 3-mL vaccutainer tubes containing K3E EDTA as anticoagulant. DNA isolation were performed using DNA extraction kit (Qiagen, Taiwan) from whole blood samples and then stored at -20°C for further use.

PCR Amplification

In this study, a pair of primers designed by Moody *et al.* (1995) were used to amplify of 1301 bp targeted fragments of Pit-1 gene (Pit-1|*Hinf*I) by PCR method. Detailed primers information is given in Table 1. PCR reaction was performed in a total volume of 10 µL containing of approximately 10-12 ng/µL of genomic DNA, 5 µL of MyTaq^{TM} HS Red Mix, 2x (Bioline, USA) , 0.2 µM of each primers and ddH_2O to a final volume of 10 µL. PCR conditions were as follows : pre-denaturation at 95°C for 1 min; followed by 40 cycles of amplification at 95°C for 15 s,

Table 1. The Information of Primers used to Amplify Pit-1|*Hinf*I Gene in Grati-PO cattle (Moody *et al.* 2005)

Locus	Location of Polymorphic Site	Primer Sequences (5'- '3)	GenBank Acc. No.	Amplicon Size (bp)	Annealing Temperature (°C)
Pit-1|*Hinf*I	Exon 6	F : CAATGAGAAAGTTGGTGC R : TCTGCATTCGAGATGCTC	Y15995.1	1301	63.5

63.5°C annealing for 15 s and 72°C extension for 10 s; and final extension at 72°C for 5 min.

PCR-RFLP Genotyping

Detection of alleles and genotypes were performed by PCR-RFLP method using *Hinf*I restriction enzyme (Promega, USA). The digestion mixtures and conditions were performed according to manufacturer's instructions. The amplified and digested DNA fragments were separated on 1% and 2% agarose gels, respectively and then stained with GelRed®10,000X in water (Biotium, USA). Visualization of fragment band patterns in gels were performed under G-BOX Gel Documentation System (Syngene, UK). The targeted Pit-1|*Hinf*I polymorphism is located at nucleotide position 1256 within exon 6 where G to A (g.1256G>A), according to Dierkes *et al.* (1998) as presented in Figure 1.

Data Analysis

Polymorphism indexes such as genotype frequency, allele frequency, expected heterozygosity (He) and observed heterozygosity (Ho) were calculated according to Nei and Kumar (2000) and Polymorphism Information Content (PIC) was calculated according to Botstein *et al.* (1980) by direct counting. The genotypic frequency distribution for its deviation to Hardy-Weinberg Equilibrium (HWE) was analyzed by chi-square test.

RESULTS AND DISCUSSION

Detection of genotypes and alleles in this study were performed by PCR-RFLP method. In this study, the targeted fragment of Pit-1|*Hinf*I gene was succesfully amplified with amplicon size of 1301 bp. The Pit-1 digestion with *Hinf*I endonuclease resulted in three fragments (260, 617 and 424 bp) and four fragments (260, 617, 379 and 45 bp) assigned as A and B alleles, respectively. However, from 107 individuals of Grati-OG cattle studied, only two types of genotype were detected i.e. BB and AB genotypes

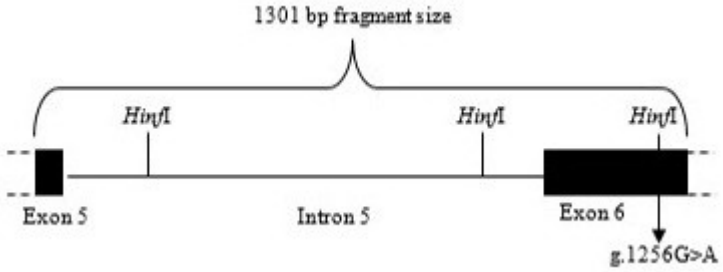

Figure 1. Schematic Representation of the Position of *Hinf*I Restriction Site and Targeted Polymorphic Site in Amplified Fragment of Pit-1 Gene as Illustrated by Dierkes *et al.* (1998). The targeted fragments are flanked by a pairs of primer including parts of exon 5, intron 5 and exon 6. The restriction site of *Hinf*I (5' G↓ANTC 3') are shown. In the A allele, nucleotide transition (G>A) at the position of 1256 causing *Hinf*I restriction enzyme do not cut the fragment at this position. Broken lines indicate that exons 5 and 6 extend up- and downstream.

characterized by four fragments (260, 617, 379 and 45 bp) and five fragments (260, 617, 424, 379 and 45 bp), respectively (Figure 2). The 45 bp fragment is not visible in the gel.

The genotype frequencies, allele frequencies and HWE test of Pit-1|*Hinf*I gene in Grati-OG cattle are shown in Table 2. This present study showed that the BB genotype and the B allele were most frequent (0.991 and 0.995), whereas the AA genotype did not detected. The A allele is very rare (0.005) which is only detected from one individual bearing the AB genotype. According to Nei (1987) and Allendorf and Luikart (2007), Such condition of this Pit-1|*Hinf*I gene is stated to be monomorphic. Although, it still at Hardy-Weinberg Equilibrium (HWE).

The genetic indexes such as observed heterozigosity (Ho), expected heterozigosity (He) and polymorphism information content (PIC) in this study showed that Ho, He and PIC value of Pit-1|*Hinf*I gene in Grati-OG cattle population were similar (0.009). This Ho and He value was lower than in other Indonesian local breeds cattle were 0.037 to 0.130 and 0.036 to 0.139, respectively (Jakaria and Noor, 2015).

The polymorphism of Pit-1|*Hinf*I gene in exon 6 (g.1256G>A) has been reported in numerous breeds of *Bos taurus*, *Bos indicus*, *Bos javanicus* and *Bos primigenius* species and its crossbreeds/hybrids cattle (Table 3). Based on these literatures, it could be understood that the frequency of A allele tends to be higher in *Bos taurus* and crossbreed/hybrid groups than in *Bos indicus*, *Bos javanicus* or *Bos primigenius* groups. Furthermore, the crossbreeds cattle that are influenced by *Bos taurus* cattle genetic have increasing proportion of A allele and commonly the frequency exceeds that in *Bos taurus* groups. This suggests that crossbreeding with *Bos taurus* cattle may alter the proportion of alleles and increases the genetic diversity in Pit-1|*Hinf*I gene of animals.

The genotypic distribution pattern of Pit-1|*Hinf*I gene found in Grati-OG cattle was tend to

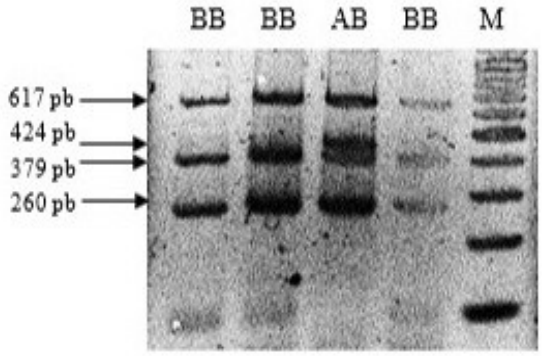

Figure 2. Genotype Visualization of Pit-1|*Hinf*I Gene in Grati-PO Cattle in a 2% Agarose Gel. AA = AA genotype ; AB = AB genotype ; M = 100 bp DNA ladder. The 45 bp fragment is not visible in the gel.

Table 2. Genotype and Allele Frequencies in the Pit-1|*Hinf*I Gene in Grati-PO Cattle

Genotype			Allele		HWE	
Name	n	Frequency	Name	Frequency	χ^2test	χ^2tab
AA	0	0.000	A	0.005	0.002	3.841
AB	1	0.009	B	0.995		
BB	106	0.991				

Total sample; 107 n = number of individuals; HWE = *Hardy-Weinberg Equilibrium*; if χ^2test <χ^2tab (α=0.05) means the genotype frequency is in HWE

Table 3. Genotype and Allele Frequencies of Pit-1|*Hinf*I Gene in Numerous Cattle Breeds

Species	Breeds	N	Genotype Frequency			Allele Frequency		References
			AA	AB	BB	A	B	
Bos taurus	Holstein	214	-	-	-	0.150	0.850	Woolard *et al.* (1994)
	Holstein	181	0.04	0.31	0.65	0.200	0.800	Ozdemir (2012)
	Hereford	45	-	-	-	0.210	0.790	Moody *et al.* (1995)
	Qinchuan	67	0.03	0.40	0.57	0.232	0.768	Zhang *et al.* (2009)
	Piemontese	287	-	-	-	0.250	0.750	Di Stasio *et al*. (2002)
	Angus	416	0.11	0.44	0.45	0.331	0.669	Zhao *et al.* (2004)
	Limousine	130	0.07	0.41	0.52	0.270	0.730	Dybus *et al.* (2003)
	Sarabi	82	0.45	0.34	0.21	0.622	0.378	Nahavandi *et al.* (2010)
	Golpaygani	57	0.61	0.26	0.12	0.755	0.254	Nahavandi *et al.* (2010)
	Hanwoo	816	0.54	0.37	0.09	0.724	0.726	Han *et al.* (2010)
Bos indicus	Grati-OG	107	0	0.01	0.99	0.005	0.995	This study
	Indian Gyr	51	0	0.04	0.96	0.020	0.980	Mukesh *et al.* (2008)
	Ongole	42	0	0.10	0.90	0.048	0.952	Mukesh *et al.* (2008)
	Brazilian Gyr	40	0	0.10	0.90	0.050	0.950	de Mattos *et al.* (2004)
	Deoni	48	0	0.11	0.89	0.054	0.946	Mukesh *et al.* (2008)
	Brahman	324	0.01	0.11	0.89	0.059	0.941	Beauchemin *et al.* (2006)
	Hariana	42	0	0.23	0.77	0.114	0.886	Mukesh *et al.* (2008)
	Sahiwal	45	0	0.26	0.74	0.130	0.870	Mukesh *et al.* (2008)
	Nanyang	100	0.21	0.51	0.28	0.465	0.535	Xue *et al.* (2006)
	Talishi	70	0.61	0.31	0.08	0.770	0.230	Nahavandi *et al.* (2010)
	Nellore	79	0.80	0.20	0	0.897	0.103	Curi *et al*. (2006)
	Pesisir	100	0.01	0.13	0.86	0.075	0.925	Jakaria and Noor (2015)
	Aceh	25	0	0.08	0.92	0.040	0.960	Jakaria and Noor (2015)
Bos javanicus	Bali	245	0	0.04	0.96	0.018	0.982	Jakaria and Noor (2015)
Bos primigenius	Podolica	104	0.14	0.32	0.54	0.300	0.700	Selvaggi and Dario (2011)
Bos taurus x *Bos indicus*	F1 AxQ[1]	36	0.11	0.44	0.44	0.333	0.667	Zhang *et al.* (2009)
	F1 DxQ[2]	42	0.07	0.21	0.71	0.178	0.822	Zhang *et al.* (2009)
	F1 LxQ[3]	47	0.04	0.28	0.68	0.181	0.819	Zhang *et al.* (2009)
	½ Angus[4]	245	0.30	0.69	0.01	0.641	0.359	Curi *et al.* (2006)
	Canchim	30	0.80	0.17	0.03	0.883	0.117	Curi *et al.* (2006)
	½ Simmental[5]	30	0.73	0.27	0	0.867	0.133	Curi *et al.* (2006)
Bos indicus x *Bos javanicus*	Madura	68	0	0.07	0.93	0.037	0.963	Jakaria and Noor (2015)

n : number of sample; [1]Angus x Qinchuan; [2]Germany Yellow x Qinchuan; [3]Limousine x Qinchuan; [4]Angus x Nellore; [5]Simmental x Nellore

be similar with other Indonesian local breeds cattle such as Bali, Pesisir, Aceh, Madura cattle (Jakaria and Noor, 2015) and especially with 16 Indian local cattle breeds that are all *Bos indicus* breeds cattle (Mukesh *et al.,* 2008). Furthermore, the B allele is predominant allele in Indonesian local breeds and *Bos indicus* cattle (Table 3). This similarity pattern may be caused OG cattle as well as Grati-OG cattle are categorized in *Bos indicus* breeds and descendant of Indian Ongole cattle as supported by the history of "Ongolization Program" (Hardjosubroto, 1994) and molecular evidence, where the Indian Ongole cattle was the anchestor of OG cattle (Hartati *et al.,* 2015). Thus, the high frequency of the B allele in this study confirmed that the B allele was tend to be almost fixed in Zebuine cattle (*Bos indicus*). Interestingly, it does not occur in Nellore (Curi *et al.,* 2006) which has the common anchestor as OG cattle (Hartati *et al.,* 2015). Likewise in other two *Bos indicus* breeds, Nanyang (Xue *et al.,* 2006) and Talishi cattle (Nahavandi *et al.,* 2010) in which the frequency of A allele is considerably higher than other *Bos indicus* cattle. Moreover, when analysis within Indian *Bos indicus* breeds is categorized based on cattle utility, the frequency of A allele in dairy (0.074) and dual purpose (0.085) were significantly higher than for draft purposes (0.029) ($P<0.05$) (Mukesh *et al.,* 2008). Grati-OG cattle in Indonesia that is utilized as draft or beef purpose tend to have same allelic distribution with draft-purposed cattle in India in which the A allele is rare. These incidents may be further investigated to know the evolutionary process and the effects of allelic variants based on their utility traits in *Bos indicus* cattle.

The same value between Ho and He indicated as randomly mating within Grati-OG cattle population (Frankham *et al.,* 2002) and this is supported by the HWE test. However, a very low heterozigosity and PIC value indicated that the Pit-1|*Hinf*I gene has a poor diversity and less informative in Grati-PO cattle population. Hence, it should not be used in association studies with growth or any certain traits in the Grati-OG cattle population. Nei (1987) stated that the value of heterozygosity depends on the number of samples, the number of alleles and the frequency of alleles. Decreased heterozygosity could lead to loss of genetic variation in a population and even fixed alleles (Russell, 2010). The strategies to increasing gene diversity of Pit-1|*Hinf*I are through crossing between individuals within and outside the population of Grati-OG cattle or crossbreeding with other breeds to increase the frequency of the favorable allele or genotypes. It should consider the breeding objectives itself.

CONCLUSION

In conclusion, the Pit-1|*Hinf*I gene correspond to g.1256G>A in Grati-Ongole Grade cattle (*Bos indicus*) is found to be monomorphic and less informative as a genetic marker. Hence, it can not be used in further association analysis between marker and certain phenotypic traits including growth traits. Further investigation of Pit-1|*Hinf*I gene polymorphism in other OG cattle population especially raised by farmers are needed to confirm for its polymorphism status.

ACKNOWLEDGMENTS

This research was funded by Kerjasama Penelitian, Pengkajian dan Pengembangan Pertanian Strategis (KP4S) Research Program (Grant no. 90.22/HM.230/H.1/05/2017.K) from Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture of Indonesia. The authors are grateful to Lusiana, Viana Rahmawati and Septiana Tri Nugraheni for laboratory assistance and to all people who involved in blood sampling assistance.

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