Identification of 19-bp indel of the Pleomorphic Adenoma Gene 1 in Bali cattle population

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Abstract. Pleomorphic adenoma gene 1 (PLAG1) is a zinc finger transcription factor gene located on bovine chromosome 14 (BTA14) affecting body size and reproduction traits in cattle. The objective of this study was to identify 19-bp indel of the PLAG1 gene in Bali cattle population. A total of 96 blood samples of Bali Cattle were collected from Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BPTU-HPT) Denpasar. Genomic DNA was extracted from blood samples and used to detect 19-bp indel of the PLAG1 gene using following primer pair 5'-TCCGAACAACAGGTGAGGGAGAAAT-3' and 5'-CCACTTCAGG-GGTGCTCTAGGTTTG-3'. The polymerase chain reaction (PCR) products using DNA pool samples were sequenced to validate the PCR product and to find out novel polymorphism in Bali cattle population. The result showed that there was no variation found in Bali cattle population based on 19-bp indel of the PLAG1 gene, which is indicated by 123 bp DNA band. However, sequence analysis of the PLAG1 gene resulted in a novel single nucleotide polymorphism (SNP) at nucleotide number 32235 of the PLAG1 gene that changed guanine (G) to adenine (A). This novel SNP could be furthermore genotyped and it might be a potential candidate marker for body size and reproduction traits in Bali cattle.

1 Introduction

Cattle is one of livestock commodity producing meat who are considerable contributing to national beef supply. In 2019, beef production reaches 504,802.29 tons which contributes around 10.32% of total meat production in Indonesia. Beef is the second highest meat commodity after broiler in fulfilling meat national needs [1]. Cattle plays an important role in national meat supply. Therefore, it is very important to improve productivity and population of Indonesian local cattle, especially whose genetically superior such as Bali cattle *(Bos javanicus*).

Bali cattle is Indonesian native cattle breed which is hypothesized to be originated from domestication of wild Banteng (*Bibos banteng syn Bos sondaicus*) long time ago. Some

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advantages of Bali cattle are more adaptable with Indonesian tropical environment, high efficiency of production and reproduction such as high fertility (80-85%), calving interval around 12 to 14 months, and high carcass percentage. Moreover, sexual maturity of Bali cattle is started at the age of 18 months, the estrus cycle in heifer ranges from 16 to 23 days, its estrus time is 36 to 48 hours with 18 to 27 hours of fertile period, and Bali cattle's fertility is much higher than *Bos taurus* cattle [2]. In addition, body weight and measurement traits are the easiest growth traits to be observed consisting of birth weight, actual body weight, withers height, body length, chest girth, waist height, and chest depth [3]. Those traits are well-known as quantitative traits which are economically and biologically important traits and able to be used as indicators in selecting superior Bali cattle [4]. The development of recent molecular technique allows human being to improve native cattle production genetically through marker assisted selection.

A candidate gene well-known involving in cattle growth is Pleomorphic adenoma gene 1 abbreviated PLAG1. It is also known as a gene controlling growth and body size of cattle due to directly affecting *Insulin-like growth factor* 1 (IGF1) serum [5-6]. Previous study reported that a 19-bp indel namely rs523753416 located in intron 1 of the PLAG1 gene is associated with growth and body measurement traits of Chinese cattle population [7]. It is also associated with birth weight of Indonesian Peranakan Ongole cattle [8]. In addition, PLAG1 gene affects carcass traits of Nellore cattle [9]. The PLAG1 gene is not well-studied in Indonesian cattle. Therefore, the objective of this study was to identify 19-bp indel of the PLAG1 gene in Bali cattle population.

2 Materials and methods

2.1 Bali cattle population and DNA extraction

A total of 96 Bali cattle were used in this study. They were 2 to 3 years old which are originated from BPTU-HPT Denpasar, the Province of Bali. The blood samples were collected from *vena jugularis* by using 18G vacutainer needle and 3 ml tube containing EDTA. Those blood samples were then used to extract genomic DNA based on high concentrated salt method described by Montgomery and Sise [10]. Moreover, extracted genomic DNA was quantified to evaluate DNA concentration and purity using NanoPhotometer (P-Class®, Implen, Munchen, Jerman). The genomic DNA was stored at -20^{o}C until used.

2.2 Amplification of the PLAG1 gene and sequencing analysis

Amplification of the PLAG1 gene was conducted using primer pairs previously reported by Xu et al. [7] as follows: 5'-TCCGAACAACAGGTGAGGGAGAAAT-3' as forward primer and 5'- CCACTTCAGGGGTGCTCTAGGTTTG-3' as reverse primer. A polymerase chain reaction (PCR) was carried out in total volume of 25 µL containing 12.5µL Go Taq® Green Master Mix (Promega, Madison, USA), 9.5 µL nuclease free water, 1 µL each primer, and 1 µL genomic DNA template. The PCR was initiated by pre-denaturation at 95°C for 5 minutes and followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Final extension was applied at 72°C for 10 minutes to complete the reaction. Furthermore, the PCR product was checked on 2% agarose gels stained by ethidium bromide using submarine electrophoresis system (Mufid ex, Advance, Japan) at 110 volt for 35 minutes. Then, it was visualized on the Glite UV Gel Doc System® (Pacific Image Electronic Co., Ltd., New Taipei City, Taiwan). In addition, three

DNA pool samples containing three different individual genomic DNA was sequenced using 1st BASE sequencing service (APICAL SCIENTIFIC Laboratory, Selangor, Malaysia).

2.3 Data analysis

The PLAG1 gene sequence obtained in this study was analyzed using BioEdit Sequence Aligment Editor Version 7.2.5 to extract and to get clear the PLAG1 gene sequences. To identify 19-bp indel and other polymorphism within the gene, all sequences were aligned using Clustal Omega software which is accessible online (https://www.ebi.ac.uk/Tools/-msa/clustalo/).

3 Results and discussion

3.1 Amplification of 19-bp indel of the PLAG1 gene

Amplification of the PLAG1 gene fragment in Bali cattle population resulted 123 bp PCR product (Figure 1). This result was in line with those predicted for detection 19-bp indel of the PLAG1 gene. It showed that the DNA isolated from Bali cattle blood was adequate to be used for PCR. The success of DNA amplification depends on the accuracy of the primers used to anneal with the right site of the DNA template [11]. In the PCR, the function of oligonucleotide primer is to hybridize with the DNA template, to define the specific fragment of DNA to be amplified, and also at the same time to provide a hydroxy group (-OH) at the 3' end which is needed for the DNA extension [12].

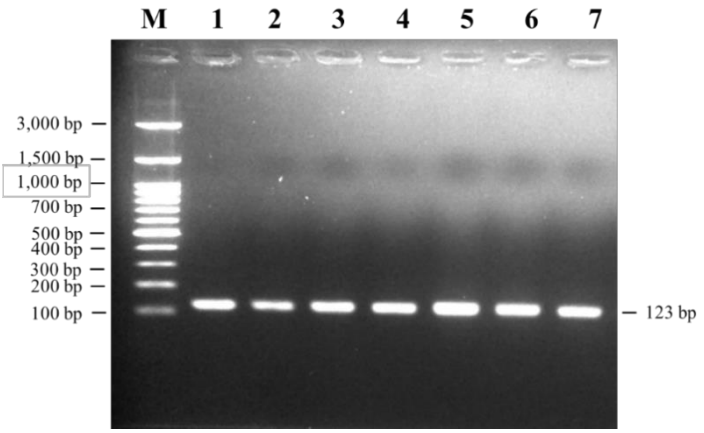

Fig. 1. The PCR product of 19-bp indel of the PLAG1 gene. M is 100 bp marker ladder; 1-7 are individual Bali cattle sample with DD genotype.

The PLAG 1 gene is located on chromosome 14 in cattle and has 4 exons [13]. Identification of 19-bp indel of the PLAG 1 gene in Bali cattle found only one genotype (DD) indicated by 123 bp DNA band (Figure 1). In identification of 19-bp indel in PLAG1 gene, the expected PCR products are 142 bp DNA band for homozygous wild type (WW), 142 and 123 bp for heterozygous (WD), and 123 bp for homozygous deletion (DD) [7]. Bali cattle genotype which tends to be less varied (monomorphic) is due to the phenomenon of phenotypic plasticity as a result of natural selection [14]. In addition, the ability of an individual to display more than one morphology is a response to environmental changes which is known as phenotypic plasticity [15].

3.2 Analysis of the PLAG1 gene sequence

Sequence alignment analysis of the PLAG1 gene fragment identified an intronic single nucleotide polymorphism (SNP) located at nucleotide number 32235 of the PLAG1 gene. This SNP was a kind of substitution mutation that changed Guanine (G) to Adenine (A) (Figure 2A and 2B). The 19-bp indel did not vary since only DD variant found which were proved by identical PCR products size for all samples used in this study (Figure 1) and identical nucleotide sequences based on alignment analysis (Figure 2C). Previous study reported that there are three variants for 19-bp indel location [7]. Moreover, polymorphisms in the PLAG1 gene are associated with body size and growth traits of beef cattle [7,16-18]. Therefore, the novel SNP located at nucleotide number 32235 of the PLAG1 gene could be promising marker to be genotyped in Bali cattle since PLAG1 gene is well-studied gene affecting cattle growth. Unfortunately, there was no restriction enzyme site for this novel SNP, direct sequencing could be the right approach to genotype this SNP.

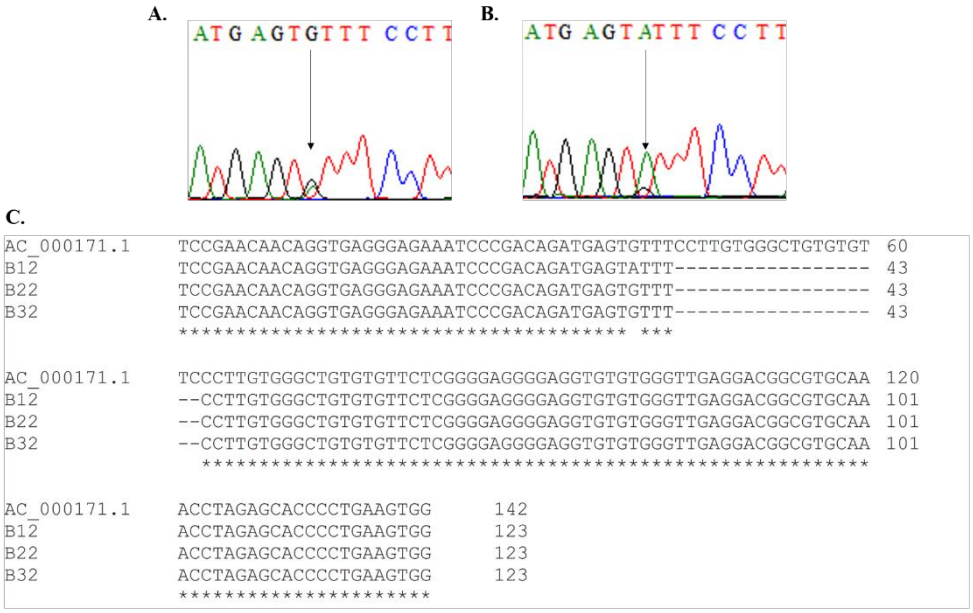

Fig. 2. A novel SNP of the PLAG1 gene fragment identified in this study. A. G variant at nucleotide number 32235 of the PLAG1 gene, B. A variant at nucleotide number 32235 of the PLAG1 gene, C. Alignment of PLAG1 sequences.

4 Conclusion

The 19-bp indel of the PLAG1 gene did not vary where only DD genotype identified in Bali cattle population. However, a novel SNP located at nucleotide number 32235 of the PLAG1 could be promising genetic marker to be genotyped for association study.

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