BIODIVERSITAS
Volume 25, Number 6, June 2024
Pages: 2547-2555

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250625

Identification and differentiation of rice ferritin gene in two different chromosomes in several local Indonesian rice varieties

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Manuscript received: 28 February 2024. Revision accepted: 18 June 2024.

Abstract. *Permatasari SE, Salamah A. 2024. Identification and differentiation of rice ferritin gene in two different chromosomes in several local Indonesian rice varieties. Biodiversitas 25: 2547-2555.* The presence of ferritin protein in rice plants indicates a regulatory response to environmental iron stress. It prevents the destructive effects of the chain reaction resulting from Fe^{2+} accumulation in the cell. This study aimed to detect and identify the location of the ferritin gene (*OsFER*) in eight local rice varieties in Indonesia. *OsFER2*, as the target gene was amplified using PCR, visualized by electrophoresis, and then sequenced. The sequencing results were analyzed using DNA Baser, BioEdit, and ClustalX2. The predicted proteins were visualized using the SWISS-MODEL server, Rice Genome Annotation Project Database, and chromosome map tools. The results show that all rice varieties studied have 100% alignment similarity and *OsFER* characteristics on chromosome 11 at LOC_Os11g01530 and chromosome 12 at LOC_Os12g01530, which have striking differences. The complete protein structure of the complex is found on chromosome 12, while only a portion of alpha-helix ferritin is found on chromosome 11. Differences in the position of the ferritin gene at different chromosomes impact the functional changes in the ferritin protein as an iron homeostasis key role. The two genes show different characteristics and are located at different genomic positions, suggesting a potential regulatory impact on the level of iron stress resistance in Indonesian rice varieties.

Keywords: Chromosome, ferritin gene, gene characterization, Indonesian local rice, varieties

INTRODUCTION

Iron (Fe) is an essential micronutrient with important roles particularly agricultural rice plants (*Oryza sativa* L.) in vital processes like photosynthesis and respiration. Rice plants require a precise concentration range 10^{-4} to 10^{-9}M for optimal growth (Harish et al. 2023). Iron deficiency can impair chlorophyll and induce chlorosis, disrupting photosynthesis, leading to senescence, and reducing rice crop production (Li et al. 2021; Kirk et al. 2022). On the other hand, excess iron can also cause poisoning when high concentrations of reactive iron is found in flooded field conditions (Das et al. 2020). Ferrous form iron dominates and influences the uptake pathways and cascade damaging mechanisms (Chen et al. 2021).

The toxicity of iron in plants arises due to the transformation of the oxidized state of iron, alternation from insoluble ferric (Fe^{3+}) to soluble ferrous oxide (Fe^{2+}). Ferrous ions could interact with hydrogen peroxide, forming highly reactive Hydroxyl radical species ('OH) through the fenton reaction (Li et al. 2023). Furthermore, ferrous ions produce superoxide radicals (O2'-) (Bou-Abdallah 2010). The cascading reactions initiated ultimately generate Reactive Oxygen Species (ROS). Excess ROS can disrupt cellular metabolism and degrade genetic material by causing irreversible oxidative damage to important biomacromolecules (Li et al. 2023; Zhang et al. 2023).

Ferritin plays a vital role in iron homeostasis as a reservoir for excess iron and a source of iron availability (Huan et al. 2020). Ferritin is conserved across nearly all organisms as a polymeric protein equipped to bind, absorb, and store iron through diverse mechanisms (Yu et al. 2017). Structurally, ferritin is a spherical protein composed of 12 or 24 subunits, forming a cavity (Pandey et al. 2018). Each organism exhibits unique ferritin specifications, particularly in subunit architecture. Notably, plants exhibit diverse ferritins and each is regulated by distinct genes. Four ferritin genes are indicated in *Arabidopsis thaliana* (L.) Heynh., encoding similar structures under diverse transcriptional regulations by specific conditions (Reyt et al. 2015). Whereas in rice plants, dominance is observed in two ferritin genes, identified into two ferritin regulations, namely *OsFER1* and *OsFER2* (Aung and Masuda 2020).

A comprehensive analysis of the rice whole genome identifies the *OsFER1* and *OsFER2* rice ferritins in duplicated regions within chromosomes 11 and 12. The coding sequence information of *OsFER1* (LOC_11gOs01530) with 537 bp length and 792 bp length for *OsFER2* (LOC_12gOs01530) was provided by the Rice Genome Annotation Project (http://rice.uga.edu/). The key distinction between the two ferritin genes in rice (*OsFER1* and *OsFER2*) lies in the presence of a deletion in *OsFER1*, two deletions located in the 50-UTR and immediately downstream from the start codon resulting in 15 single nucleotide changes (Stein et al.

2009). This explanation can elucidate the high similarity between the rice ferritin genes.

The specifications of ferritin genes in different locations may indicate distinct characteristics. These differences may be more pronounced at the variety level, meaning that they may be more noticeable between different varieties of the same plant species. Evaluating these differences becomes more reliable with evidence of variations in gene expression. In-depth identification is needed in the genomic arrangement of both ferritins by understanding the presence, location, and characteristics of the *OsFER* gene within the genome's complexity. It leads to predicting breakthrough in the appropriate view of the genome by comparing the gene product. The presence of ferritin genes on both rice chromosomes opens the potential for triggering different responses to iron stress. Local rice varieties are preferable to use over superior rice varieties giving more suitable for investigating alternative varieties with a high potential for superior iron toxicity resistance. Therefore, this study aimed to detect, identify, and characterize the differentiation of the *OsFER* gene in several local Indonesian rice varieties.

MATERIALS AND METHODS

Time and place

The research was conducted for 6 months period, from July to November 2023 in the Molecular Biology Preparation Laboratory and Integrated Instrumentation Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, West Java, Indonesia.

Plant materials

The samples used in this study were eight rice varieties consisting of six Java local rice varieties Inpari 42, Situbagendid, Wayapo, Ciherang, and Sunggal from Jember, Indonesia and Cupatmangu variety from Bandung, Indonesia; Kangkung variety from Kalimantan local rice variety; and Sigupai variety as Aceh's local rice variety.

Procedures

DNA isolation

DNA isolation was performed according to the Viogene isolation kit. Fresh leaf tissue of 100 mg was ground in liquid nitrogen, transferred to microcentrifugation tubes, added with buffer PX1 and RNAse, and incubated at 65°C for 30 minutes. PX2 buffer was added and incubated on ice for 10 minutes before centrifuging at 13,000 rpm for 2 minutes. The supernatant was collected, PX3 buffer and ethanol were added, and the mixture was centrifuged for 1 min at 10,000 rpm. The supernatant was discarded and WS buffer was added twice into the mini-column tube for washing before centrifuged for 1 min at 13,000 rpm. Next, the mini-column tube was transferred to a new 1.5 mL tube, and 20 uL TE buffer was added, allowed to stand for 5 minutes, and centrifuged at 13,000 rpm for 1-2 minutes to elute the DNA. The DNA sample was stored at -20°C.

DNA amplification and electrophoresis

Polymerase Chain Reaction (PCR) was used for DNA amplification with specific primers for the *OsFER2* gene fragment, which is about 600 bp in size. The forward primer had the base sequence 5'CAGCCATTCGAGGAGCT CAA-3' and 5'GCAACGTTGTCACGGTCAAA- 3' as the base sequence of the reverse primer (Sequence ID: AF519571.1) (Gross et al. 2003). The PCR reaction mixtures were performed in a volume of 20 µL containing 10 µL of GoTaq Green Master Mix, 7 µL of nuclease-free water, 1 µL of 10 mM forward primer, 1 µL of 10 mM reverse primer, and 1 µL of DNA template. GoTaq Green Master Mix is a pre-mixed solution containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer. The PCR program consisted of a pre-denaturation step for 3 minutes at 95°C; 40 cycles of denaturation for 10 seconds at 95°C, annealing for 20 seconds at 53°C, and extension for 45-30 seconds at 72°C; and following by a final extension for 5 minutes at 72°C. DNA amplified from PCR products was visualized using 1.5% agarose gel electrophoresis and run on 75V for 30 minutes. Furthermore, the electrophoresis result was visualized by using a UV transilluminator to determine the band size of the target gene.

DNA sequencing

The *OsFER2* gene fragment was sent to PT Genetika Science Indonesia for sequencing. DNA sequence result was obtained in the form of the *.abi file and then analyzed using some software tools.

Data analysis

Raw ferritin gene sequence read from each rice variety needs to be adjusted by checking the electropherogram to generate the contig. DNA Baser generated consensus nucleotide sequences from forward and reverse sequence reads, and CLUSTALX2 was used to align the sample sequences. Gene characterization and differentiation of the obtained sequences were detected and identified using a BLAST search of the NCBI GenBank database. BioEdit software was used to translate the nucleotide sequences into amino acids of the different ferritin sequences. Furthermore, based on the obtained amino acid sequences, 3D protein structures were visualized using the SWISS-MODEL server (https://swissmodel.expasy.org). The chromosomal coordinates of the protein sequences were visualized using the Rice Genome Annotation Project Database (http://rice.uga.edu/).

RESULTS AND DISCUSSION

DNA amplification

The *OsFER2* gene target was successfully amplified using PCR and a specified primer (Figure 1). All varieties showed double bands on the electrophoresis visualization results. They showed high similarity in both the brightness and thickness of the band with a size of 500-600 bp. The other band, around 800 bp, appeared more faded, indicating the detection of the ferritin gene at a more extended sequence length, but at a lower concentration. Therefore, to verify whether the two bands, 600 bp and 800 bp, share

similarity in sequences, the representative from the Ciherang variety was used for sequencing analysis for both DNA fragment's lengths (Figure 2). The comparison showed that both fragments show similarity, but 800 bp had a longer sequence.

Sequence analysis

The sequencing results from gene amplification with the *OsFER2* primer consist of exon 1, intron, and exon 2 regions and have a high similarity in nucleotide length for each rice variety used. The resulting nucleotide sequence for the main band is approximately 500 to 600 bp with 579 bp length for Impari 42, 530 bp for Cupatmangu, 500 bp for Situbagendid, 573 bp for Wayapo, 577 bp for Kangkung, 579 bp for Sigupai, 574 bp for Ciherang, and 500 bp for Sunggal. The alignment results showed that all rice varieties had complete similarity and did not show any nucleotide polymorphism. Furthermore, the alignment of the *OsFER2* gene in each variety showed a coherent alignment (Figure 3).

Eight rice varieties were analyzed using the complete sequence of chromosomes 11 and 12 in the *Oryza sativa japonica* group. The *OsFER2* gene of the varieties Impari 42, Cupatmangu, Sigupai, and Ciherang had a query coverage of 99% using the complete sequence of chromosome 11 (AP014967.1). In contrast, the varieties Situbagendid, Wayapo, Kangkung, and Sunggal had a query coverage of 100%. The varying percent identity was obtained for each rice variety (Table 1). Alignment results with the complete chromosome 12 sequence (AP0149968.1) show that each variety has two regions with different query coverage and percent identity (Table 2).

According to BLAST NCBI, ferritin in all varieties exhibited different attachment positions on chromosomes 11 and 12. Based on the alignment of all varieties that showed complete similarity, the Kangkung variety was selected as a representative sample for comparing the ferritin gene characteristics on both chromosomes. This decision was based on its most extended nucleotide sequence, which had a query cover value of 100% and a percent identity approaching 100% (Tables 1 and 2).

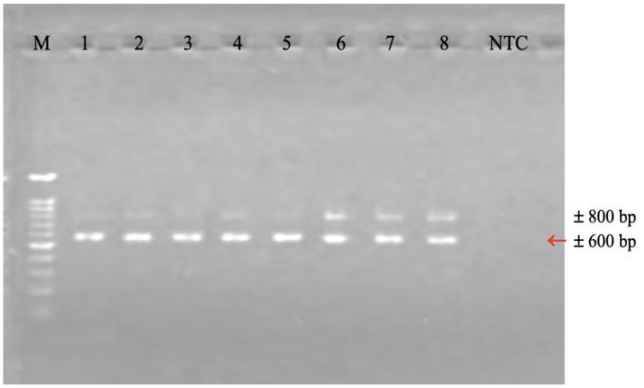

Figure 1. The visualization from electrophoresis on PCR results showed that all samples had double bands with the brightest DNA band size at 500-600 bp: M. 100 bp DNA ladder; 1. Impari 42; 2. Cupatmangu; 3. Situbagendid; 4. Wayapo; 5. Kangkung; 6. Sigupai; 7. Ciherang; 8. Sunggal, and NTC: Non-Template Control

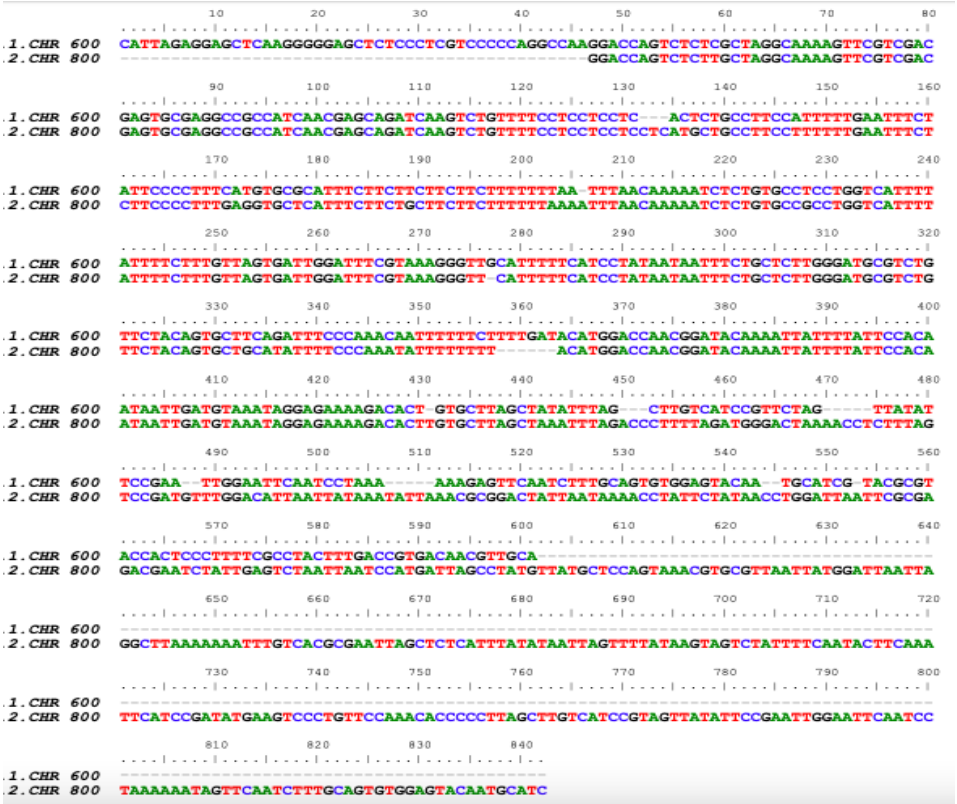

Figure 2. Alignment of 600 bp and 800 bp fragments of *OsFER2* gene from Ciherang variety

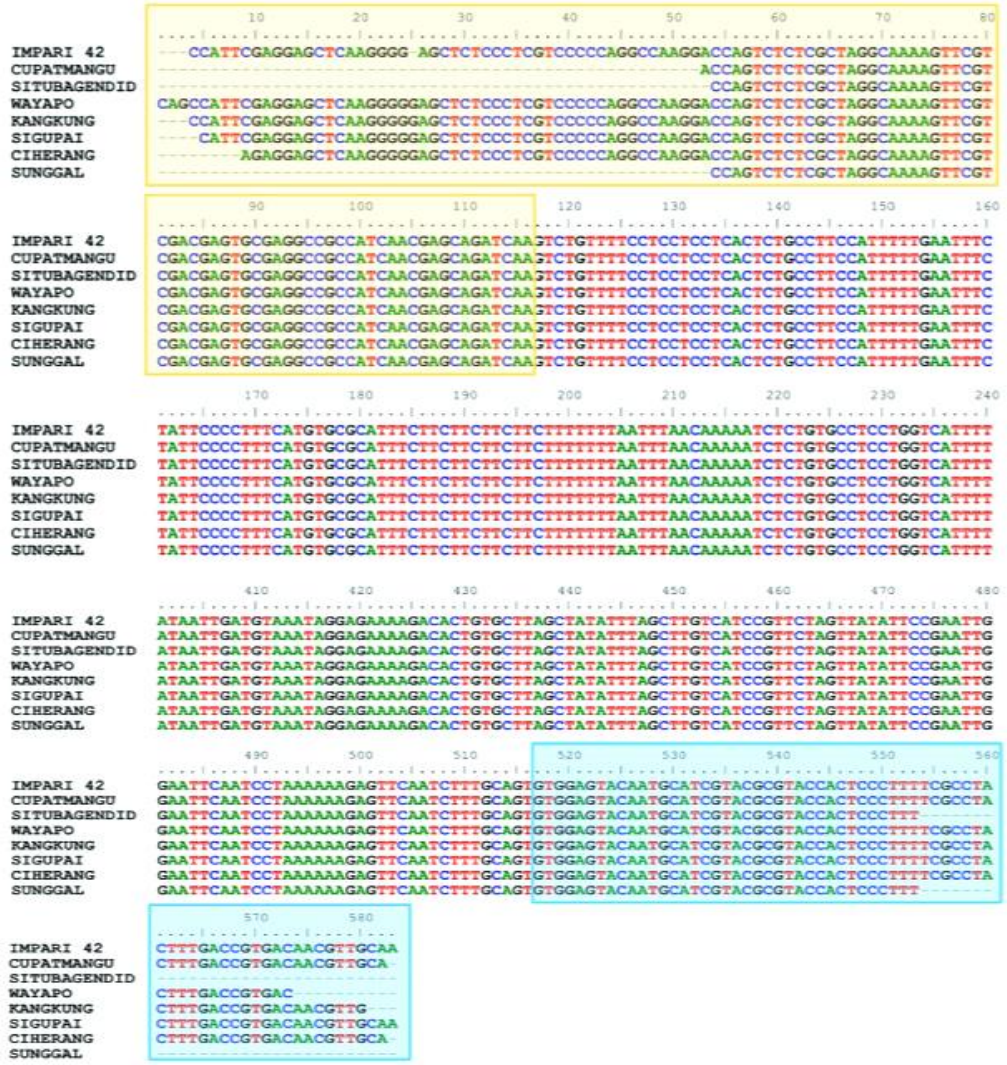

Figure 3. Multiple alignments of eight Indonesian rice *OsFER2* gene with 100% similarity alignment. The nucleotide sequences of exon 1 are in the yellow box and exon 2 is in the blue box

The NCBI BLAST alignment graph was used to differentiate the character of the attachment of the query gene to ferritin, which is located on chromosomes 11 and 12. The analysis revealed that the Kangkung variety ferritin gene is located at the locus LOC_Os11g01530 on chromosome 11. The ferritin gene on chromosome 11 comprises seven exons with six intron sequences and has genome sequences ranging from 305,050 to 307,728. The query sequence for Kangkung variety overlaps significantly with a portion of Exon 1 and the first intron adjacent to exon 2.

Exon 1 of the ferritin gene on chromosome 11 begins at position 307,728 (X1 Start) and ends at position 307,366 (X1 End) (Figure 4). The Kangkung variety gene query overlaps with exon one at position 307,478 (Q-X1 Start) and extends to the end of the exon. Additionally, the query aligns more with the first intron for 400 bp until the beginning of the exon two sequence. Exon 2 of the ferritin gene on chromosome 11 begins at nucleotide position 306,900 and overlaps with the Kangkung variety Query. The overlap ends in the middle of exon two at position 306,883, while the end of exon 2 is at position 306,902. The overlap of the Kangkung sequence in Exon 1 is only 112 nucleotides, with 400 nucleotides in the intron and 64 nucleotides overlapping with exon 2.

The ferritin gene is located at the LOC_Os12g01530 locus on chromosome 12 of the Kangkung variety. It extends from nucleotide position 320,871 to 323,630 and consists of eight exons with seven intron segments. The first exon of the ferritin gene on chromosome 12 extends from position 320,871 (start Q-X1) to position 323,630 (end X1). The ferritin sequence of the Kangkung variety can be divided into two alignments: a more extended region one alignment and a shorter region two alignment (Figure 4). The region 1 sequence is 577 bp long and is aligned at positions 323,444 to 323,332. The remaining sequence overlaps with the intron until nucleotide 323,002, with 112 bp aligned with exon 1 of the ferritin gene on chromosome 12. The second region, spanning 135 bp aligns with the first intron at position 322,542. It is flanked by exon 2 with the start of the exon two sequence at position 322,689. It

overlaps with exon 2 for 128 bp because the overlap of the alignment with exon 2 ends at position 322,561, whereas the end of the exon is located at position 322,542. Therefore, region 2 has a 128 bp alignment of the Kangkung gene with the ferritin gene on chromosome 12. The Kangkung sequence alignment overlapping exons one and two can be expressed as a polypeptide sequence that assembles the ferritin protein. However, the intron sequence cannot be interpreted.

Table 1. BLAST NCBI results of *OsFER2* in eight rice samples against *Oryza sativa japonica* group in Chromosome 11 complete gene (AP014967.1)

Description	Total score	Query cover (%)	E value	Acc. length	Percent identity (%)	Range cover
Impari 42	1430	99	0	579	96.65	1-577; 307.478-306.901
Cupatmangu	1493	99	0	530	99.81	1-480; 307.429-306.901
Situbagendid	1439	100	0	500	100	1-500; 307.428-306.929
Wayapo	1474	100	0	573	99.83	1-573; 307.481-306.900
Kangkung	1461	100	0	577	99.83	1-577; 307.478-306.902
Sigupai	1471	99	0	579	99.83	1-577; 307.477-306.901
Ciherang	1430	99	0	574	99.83	2-573; 307.472-306.901
Sunggal	1428	100	0	500	100	1-500; 307.428-306.929

Table 2. BLAST NCBI results of *OsFER2* in eight rice samples against *Oryza sativa japonica* group in Chromosome 12 complete sequence (AP014968.1)

Description	Total score	Query cover (%)	E value	Acc. length	Percent identity (%)	Range cover
Impari 42	1430	99	0	579	95.12	Range 1: 1-444; 323.444-323.002 Range 2: 441-577; 322.693-322.500
Cupatmangu	1493	99	0	530	94.78	Range 1: 1-396; 323.395-323.002 Range 2: 393-529; 322.693-322.560
Situbagendid	1439	100	$3e^{-170}$	500	94.76	Range 1: 1-395; 323.394-323.002 Range 2: 392-500 ; 322.693-322.588
Wayapo	1474	100	0	573	95.37	Range 1: 1-448; 323.447-323.002 Range 2: 445-573; 322.693-322.568
Kangkung	1461	100	0	577	95.34	Range 1: 1-445 ; 323.444-323.002 Range 2: 442-577 ; 322.693-322.561
Sigupai	1471	99	0	579	95.33	Range 1: 1-444; 323.443-323.002 Range 2: 441-577; 322.693-322.560
Ciherang	1430	99	0	574	95.28	Range 1: 1-440; 323.438-323.002 Range 2: 437-573; 322.560-322.693
Sunggal	1428	100	$3e^{-170}$	500	94.76	Range 1: 1-395; 323.394-323.002 Range 2: 392-500; 322.693-322.588

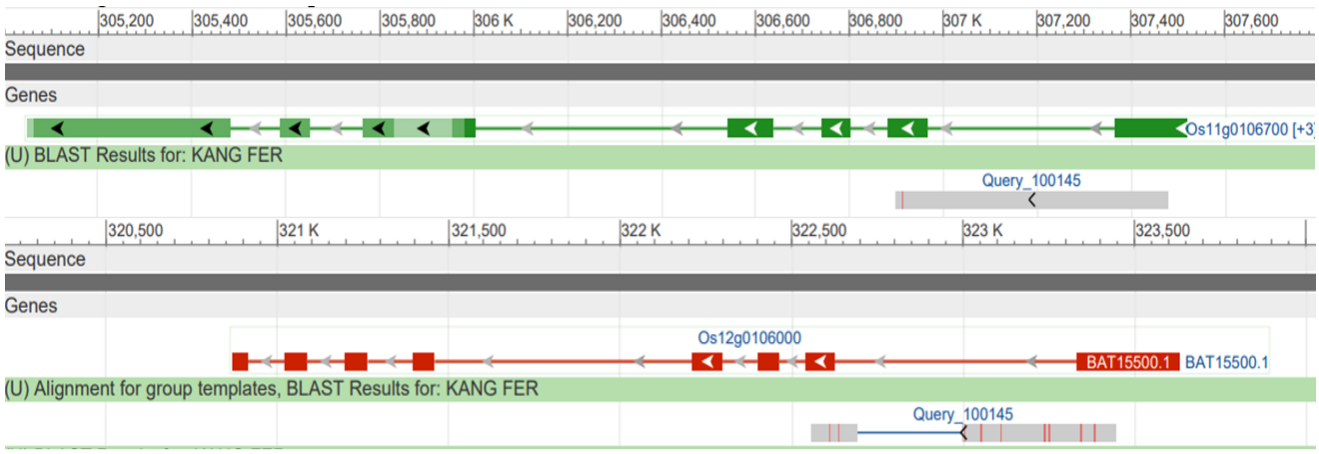

Figure 4. Alignment Kangkung's sequence (query_100145 - grey bar) among Niponbare's ferritin gene at chromosome 11 (green bar) and chromosome 12 (red bar)

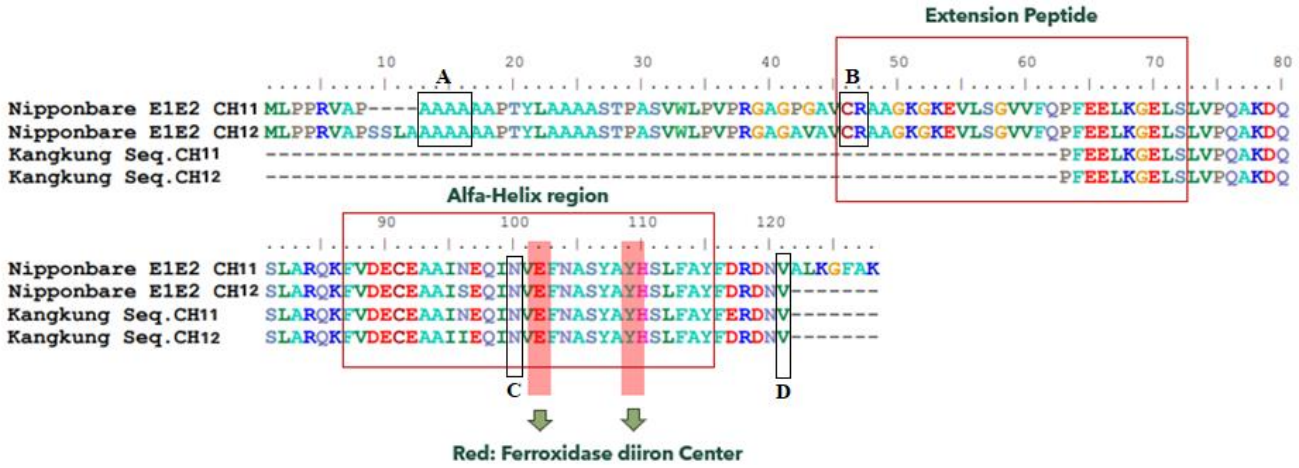

Figure 5. Alignment of Kangkung's protein sequence and Niponbare's exon 1 and exon 2 ferritin gene at chromosomes 11 and 12

The sequence of the Kangkung rice variety overlaps with the ferritin gene on chromosomes 11 and 12. Therefore, to compare the sequences on chromosomes 11 and 12 between the Kangkung and Niponbare varieties, the sequence of amino acids in exons 1 and 2 was analyzed. The results of the polypeptide alignment revealed amino acid differences in Niponbare chromosomes 11 and 12 (Figure 5). Three black boxes (A, B, and C) indicate deletions and amino acid changes. In the Kangkung variety, amino acid changes have occurred. Aspartic acid (D) becomes glutamic acid (E) in chromosome 11 (D black box), and serine (S) becomes isoleucine (I) in chromosome 12 (C black box). Both chromosomes 11 and 12 in The Kangkung variety have a mature region consisting of part of the extension peptide in exon one and the fully expressed alpha-helix region in exon 2. The ferroxidase diiron center, which is the first active site, is located in exon two within the alpha-helix region (Nguyen et al. 2022).

These polypeptide variations do not significantly alter the main function of ferritin as they do not affect the active site. The Kangkung variety sequence contains an extension peptide and an alpha-helix region with a ferroxidase diiron center in the exon two alpha-helix region. Therefore, it is still possible that the ferritin in the Kangkung variety has retained its function. Specifically, the amino acid sequence of the Kangkung variety showed the following changes: the substitution of aspartic acid (D) with glutamic acid (E) at position 214 on chromosome 11 and the substitution of serine (S) with isoleucine (I) at position 228 on chromosome 12 do not impact the mature region of the ferritin protein, which includes the extension peptide and the alpha-helix region. The Kangkung variety's ferritin protein appears functional, as evidenced by the extension peptide, which aids in ferritin assembly, and the alpha-helix region, which houses the ferroxidase diiron center responsible for iron oxidation.

Based on the comparison of polypeptide alignment, there are differences in the amino acid sequence (Figure 5). However, they do not significantly affect the main function and structure of the ferritin. In addition to overlapping sequences with Nipponbare's exon one and exon 2, the Kangkung variety also contains fewer extension peptide regions and entire alfa-helix regions with two amino acids as active sites (ferroxidase diiron centers), which is supported by the protein structure visualization generated using the SWISS-Model Server program (Figure 6).

Visualization using SWISS-Model on the full cds-ferritin on chromosomes 11 and 12 of Nipponbare reveals significantly different structures. The ferritin protein on chromosome 11 consists of six exons and lacks the complex channel structure present in ferritin on chromosome 12, which has seven exons. A complete complex ferritin channel comprises a total extension peptide, a four-helix region with the ferroxidase diiron center, a ferrihydrite nucleation center, and an iron ion channel, all supporting the overall ferritin protein channel complex. In contrast, even though chromosome 11 ferritin possesses an extension peptide and a four-helix region, it only has one active site, the ferroxidase diiron center.

The protein structure of the Kangkung variety on chromosome 11 is not much different from the structure of the ferritin protein on chromosome 12, which can be seen in the overlapping sequences of exons 1 and 2. In essence, the polypeptide sequences of Kangkung on both chromosomes have similar exon 1 and exon 2 components, even to the point of being identical. The only minor difference is in the shorter extension peptide, and both have a complete coiled helix structure (Figure 6). Both also have the active site ferroxidase diiron center in the longer coiled alfa-helix.

Discussion

The critical role of iron (Fe) in rice plants occurs in a proper proportional system that is not deficient or excessive. Deficiency can lead to ferroptosis and premature senescence, while in excessive conditions, it can lead to iron toxicity (Pradhan et al. 2020). It is crucial to control the amount of ferrous ions in cells. By producing ferritin, cells can establish iron homeostasis by maintaining the dynamic balance of iron as iron release-reservoir and avoiding the toxicity from iron ion reactivity (Guo et al. 2022; Li et al. 2023). Organisms need to retain the ferritin gene in response to Fe stress, predominantly plants, which are often exposed to more sensitive ferrous forms when there is excessive waterlogging (Pandey et al. 2018; Pais et al. 2023).

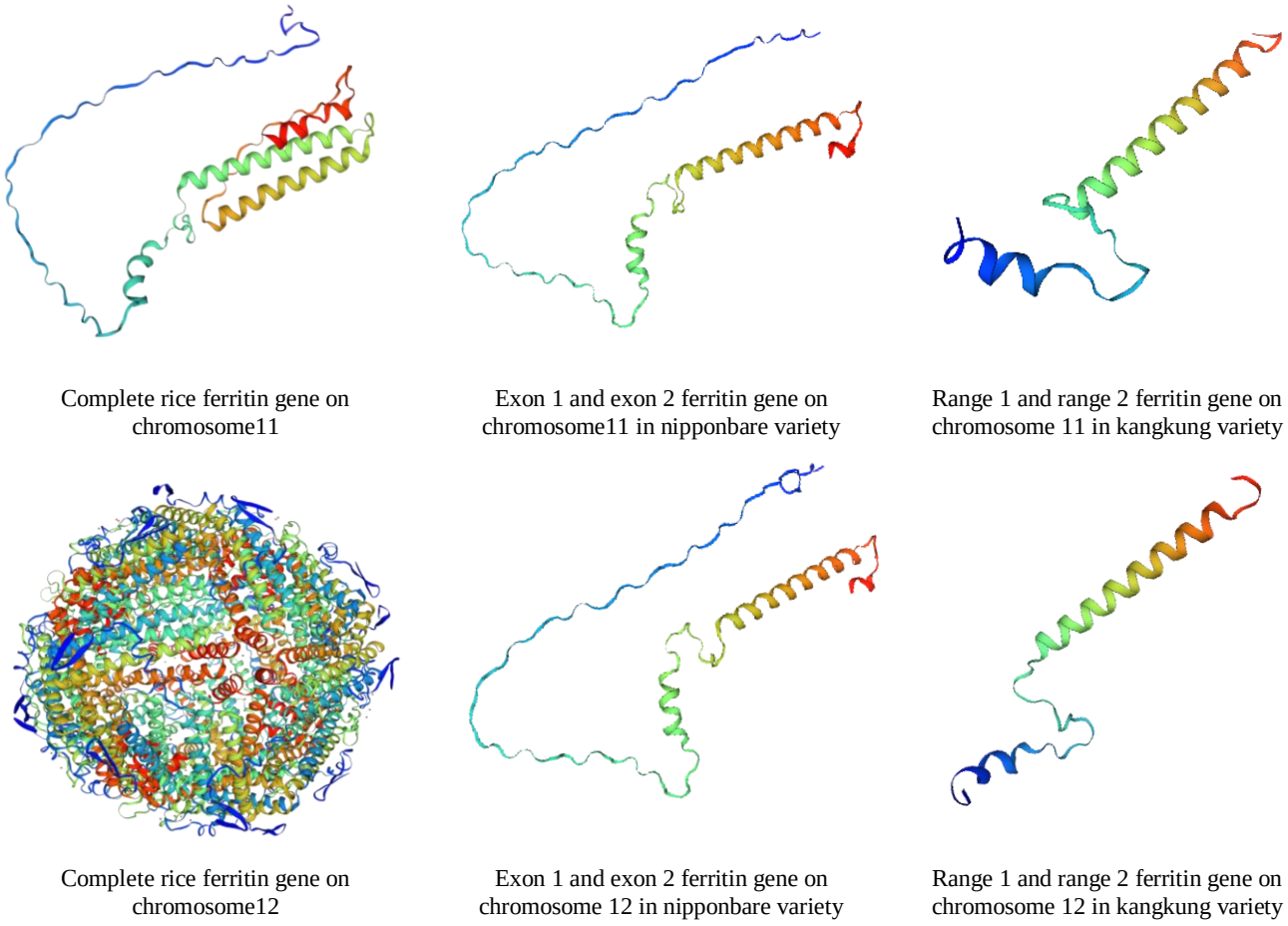

Figure 6. The visualization of the structure of ferritin protein on chromosomes 11 and 12 in the Nipponbare and Kangkung varieties

Ferritin in rice plants is well-regulated at the subspecies level, as demonstrated by the lack of significant differences in the characteristics of the ferritin gene among the rice varieties sampled (Pandey et al. 2018). The double bands obtained from the electrophoresis result from PCR product (Figure 1) indicate that the *OsFER* gene is a gene family in which two genes have some conserved sequence part(s). The band sizes obtained were approximately 600 bp for the short band and approximately 800 bp for the longer band. Based on a Rice Genome Annotation Project, the ferritin genome in *Oryza sativa indica* group has a size of 2448 bp on chromosome 11 and 3958 bp on chromosome 12. Similar results were also found in the multi-band electrophoretic analysis of the rice ferritin gene in the study by Stein et al. (2009). This phenomenon could be an example of partial gene duplication, which has also been found in *japonica* rice but with a slightly different ferritin gene length. The difference in length of the ferritin gene sequence could be an indicator of partial duplication of a gene, which is a common occurrence in plants.

The result of multiple alignment sequences of *OsFER2* gene from eight Indonesian rice varieties shows 100% similarity (Figure 3), indicating that they have the same nitrogen base sequence and tend to be expressed into the same product, ferritin. The alignment of the polypeptide sequence of the Kangkung variety with Nipponbare's reference sequence also yields a similar result. Notably, there are several differences in amino acid readings, particularly in the second exon. For instance, aspartic acid (D) changes to glutamic acid (E) in alignment with Nipponbare's ferritin on chromosome 11, and serine (S) changes to isoleucine (I) (Figure 5). Despite the presence of amino acid changes, the structure and function of ferritin remain unaffected as these changes are not located in the active site, suggesting that the ferritin gene is well conserved in rice plants. Ferritins are a group of proteins responsible for maintaining iron homeostasis in all living organisms except yeast (Briat et al. 2010; Chikoti et al. 2020).

In achieving its function as a key role in iron homeostasis, ferritin in rice is regulated by the *OsFER* genes. There are two types of ferritin in rice plants, namely *OsFER1* and *OsFER2* (Briat et al. 1999; Stein et al. 2009; Nguyen et al. 2022). *OsFER1* and *OsFER2* have a difference of 15 nitrogen bases due to two deletions at two sites in *OsFER*. According to Stein et al. (2009), the two genes are regulated in different locations, specifically on rice chromosomes 11 and 12. So far, there is no clear indication of the specification of ferritin on chromosomes 11 and 12 that refers to the characteristics of *OsFER1* or *OsFER2*. Based on the BLAST alignment of ferritin in all eight rice

varieties studied, there is a tendency gap to identify specific characteristics of ferritin on different chromosomes.

Regardless of the designation of *OsFER1* and *OsFER2*, the comparison of characteristics showed differences in the ferritin product on chromosomes 11 and 12. Chromosome 11 has eight exons, with a shorter sequence while chromosome 12 has eight exons with a longer DNA sequence (Figure 4). Complete exon ferritin comprises a terminal peptide region and a mature region. Mature regions can be categorized into extension peptide and helix regions (A, B, C, D, and E helix regions) (Nguyen et al. 2022). The extension peptide on chromosomes 11 and 12 of all rice varieties studied is on exon 1 and then the first helix region starts from exon 2 in the form of a coiled alfa-helix region. Within the alfa-helix region in exon 2, there is an active site at the polypeptide sequence 102 in the form of glutamic acid (E) and 109 in the form of tyrosine (Y). These two amino acids are the ferroxidase diiron center, which plays a role in the function of oxidizing ferrous ions (Fe^{2+}) into the more stable ferric form (Fe^{3+}) (Pullin et al. 2021). By preserving its amino acid sequence, the ferritin ferroxidase center retains its essential function of oxidizing metal ions, ensuring proper iron homeostasis (Huan et al. 2020).

Based on the results of the comparison of the characterization of ferritin on different chromosomes, the different active sites are responsible for the differences in structure and function. Ferritin on chromosome 11 has a simpler structure than that expressed on chromosome 12 (Figure 6). Ferritin on chromosome 12 appears to be a collection of many ferritins on chromosome 11 that form a pore or channel. The complex structure of ferritin on chromosome 12 could be formed due to a complete active site. The main active site is amino acids that act as an iron ion channel. Meanwhile, the ferroxidase diiron center for stabilizing iron reactivity catalyzed ferrous ions' oxidation (Mehlenbacher et al. 2017). Furthermore, the presence of the ferrihydrite nuclease center supports the last stages of iron binds and sequestration by transforming ferric iron intermediates into mineral ferrihydrites (Zhang et al. 2021; Masison and Mendes 2023). The complete function of ferritin, composed of sequences encoded amino acids that act as an iron ion channel, supported the ferrihydrite nuclease center and the ferroxidase diiron center. These three sites work together to store, absorb, and bind iron in various mechanisms (La et al. 2018; Blankenhaus et al. 2019).

The ferroxidase diiron center active site was found in all eight varieties studied and all sequences overlapped with ferritin on chromosomes 11 and 12 (Figure 5). Based on the identification of ferritin in Nipponbare variety on chromosome 11, it only has the active site of the ferrihydrite nucleation center (Kawahara et al. 2013; Sakai et al. 2013). However, the results obtained in this study found the active site of the ferroxidase diiron center, which should not be owned by ferritin on chromosome 11 based on the gene bank. The result obtained from the eight Indonesian rice varieties used here aligns with the research by Nguyen et al. (2022), which compared various ferritin variations that showed *OsFER1* and *OsFER2*, which have ferroxidase diiron centers. So, there is an indication of a change in the characterization of the same gene at different chromosome locations (Hahn et al. 2006). The results of this study can confirm that ferritin on chromosome 11, in addition to the active site of the ferrihydrite nucleation center, also has the ferroxidase diiron center. Meanwhile, the complete complex ferritin forms the ferritin channel architecture with full function as a key role protein in iron homeostasis in cells.

In conclusion, specificity potential of ferritin gene variation using *OsFER2* primers at different chromosomal locations especially at chromosomes 11 and 12 in eight rice varieties was obtained. The placement of the ferritin gene on different chromosomes might influence plant functions beyond iron regulation. Since genes are often not isolated entities and are able to interact with other genes or factors to produce various effects and environmental cues, the chromosomal location could alter these interactions, potentially leading to unforeseen consequences. As for the potential for other genes or factors to interact with the ferritin gene and impact its function. This is an important area of research that should be explored further. This study offers valuable knowledge on plant iron uptake and storage mechanisms, paving the way for future research on broader applicability across crops and environments.

ACKNOWLEDGEMENTS

We thank all parties who have contributed to support this research activity. This research was funded by the PUTI Postgraduate Research Grant 2023 Universitas Indonesia, Depok, Indonesia for AS with contract number NKB-219/UN2.RST/HKP.05.00/2023.

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