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Number of Copies of Endogenous Rice Tungro Bacilliform (RTBV) From Local Rice in Indonesia

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Abstract: ***The focus of this study was to determine the position and the number of copies of RTBV endogenous fragments integrated into the genomes of local rice varieties. Local rice test consisted of Utri Merah, Pulut Sawijan, Srogel Abang, Bekongan, Blumbungan, and Tachung native 1 (TN1). The analysis showed that the RTBV genome was integrated into the local rice genome on the twelve chromosomes and at all loci. RTBV genome interration was demonstrated through the results of Polymerase Chain Reaction (PCR) analysis with 1200 base pairs (bp) genome length and blast protein analysis results with an alignment of 66.9% in P194 as Open Reading Frame (ORF) 3 RTBV, 93% in ORFy, and other various physical genome structures. The number of copies of RTBV endogenous fragments test varieties was very fluctuating and related to gene mutations and protein motifs. All test varieties had gene mutations, but in the Utri Merah, Pulut Sawit, Bekongan and Srogen Abang varieties which had a lower number of fragment copies in compared to the Blumbungan variety and TN1 were able to produce expressions of ile rich protein patterned genes that play a role in plant resistance. In contrast, Blumbungan and TN1 varieties despite having a high number of copies of the endogenous fragment of RTBV, gene expression has no protein ile-rich motifs***

Keywords: Local rice variety, endogenous RTVB, Endogenous RTBV copies, protein motifs

1. Introduction

Tungro is one of the most detrimental rice plants disease in Southeast Asia and South Asia. This disease has spread widely throughout the world, especially countries where the population cultivates rice. Tungro was first discovered in Indonesia in 1859 (Ou, 1984).

There are two types of tungro-causing viruses, namely the stem-shaped tungro virus RTBV and the tungro virus in the form of spherical Rice Tungro Spherical Virus (RTSV) (Hibino et al., 1978). RTBV virus particles have a length of 130 nm and a diameter of 30 nm, while RTSV is round with a diameter of 30 nm (Hibino et al., 1978; Hull, 1996). The two viruses are spread by green leafhoppers Nepothettix virescens which is spread semi-permanently from one plant to another. Rice plants infected with both viruses will show severe symptoms compared to being infected separately. Underdeveloped size, the presence of orange yellow leave, and the formation of rice tillers are a symptom of the two viruses (Hibino, 1983; Hibino, 1996). Conversely, being infected with the RTBV virus displaying mild symptoms and being infected with RTSV only shows symptoms of drwarfed size.

Several influecing fatotors of the emergence of tungro disease include rice seedlings infected with viruses, vulnerable rice varieties, vectors, poor control management, climate and weather conditions that support vector activity (Agrios, 2005; Zeigler and Savary, 2010). As a result, a decrease in production and losses of profit (Zeigler and Savary, 2010). Areas in Indonesia experienced increase in tungro disease include South Sulawesi with disease intensity from 10% to more than 50% (Pakki et al., 2010). In 1995, Surakarta experienced explosion of tungro disease in12,340 ha of rice fields. These cases calls for tungro disease control. However, the application of disease control methods to control tungro disease needs to consider the principles of ecological sustainability. One of these principles is through the method of using superior tungro resistant varieties (Koenning et al., 2001; Castagnone-Sereno, 2002; Cruz et al., 2003; Khus, 1980; Sitaresmi et al., 2013).

Double-stranded DNA similar to RTBV is known to be integrated in the host plant genome called endogenous-like RTBV (ERTBV) (Bhat et al. 2016). Furthermore, Kunii et al., (2004) reported that the presence of endogenous RTBV in the Asian rice genome had an impact on the level of resistance. Until now, the mechanism of virus genome integration into its host genome has not been generally understood, but there has been many reports from pararetroviruses that have genomes in the form of double-stranded Deoxyribo Nucleic Acid (DNA) (Hull and Mathews, 2004). The integration mechanism is thought to occur through end-joining non-homologous processes that do not involve the enzymatic machine retrotransposons (Feschotte and Gilbert, 2012). Kunii et al. (2004) stated that *Oryza sativa* and *Oryza rufipogon* from Asia (tungro endemic areas) had a greater number of endogenous segments of RTBV compared to other areas where rice tungro disease was not found. Therefore, the study of the number of fragments (copy number) and identification of integration positions are problems that need to be examined. Therefore, the objectives of this study are (1) To determine the position of the endogenous fragment of RTBV integrated in the local rice genome and (2) To analyze the number of copies Endogenous fragments of RTBV are integrated in the local rice genome.

2. Methodology

The materials used are Ethanol absolute, agarose, Ethidium Bromide, double distilated H2O, PCR Buffer, 10 mM dNTP, primary pair, Taq DNA polymerase, MgCl2, liquid nitrogen, chloroform isoamilalkohol solution (chisam), extraction buffer, 3M sodium acetate, 2-propanol, 1x TAE buffer, Trish EDTA (TE) buffer, RNase; microtube, and local cultivar rice seeds. The primer used is ERTBV7 (TTTACTTGCCAAAACCAGC) which is produced by P.T Invitrogen.

The tools used are test tubes, erlenmeyer glasses, becker cups, measuring cups, sterilizers, ovens, magnetic stirers, micropipets, micropipette tips, ependorf tubes, mortars, rubber gloves, water baths, PCR machines, electrophoresis, UV transluminators (GelDoc), and nano drop. Test varieties originated from local Indonesia, namely Bekongan, Blumbungan, Srogel Abang, and Pulut Sawijan. While the comparison varieties are TN1 and Utri Merah.

Variables observed included the DNA amplicon PCR target (endogenous RTBV DNA) integrated in the local rice genome, the level of alignment of DNA sequences, the number of RTBV DNA copies in the rice genome, phylogenetic trees, and protein motifs. DNA tracking results confirmed to the database in GenBank, on the NCBI program, Rice Plant Biology, Gramene, Mega, Myhits, and NTSYS

3. Results

Amplification of RTBV DNA Fragments Integrated in the Rice Genome

Identification of RTBV genome fragments integrated in the rice genome was carried out using the ERTBV7 primer. Amplikon which was successfully amplified included the Utri Merah, Pulut Sawijan, Bekongan, Srogel Abang, Blumbungan and TN1 varieties with a genome weight of 1200 bp.

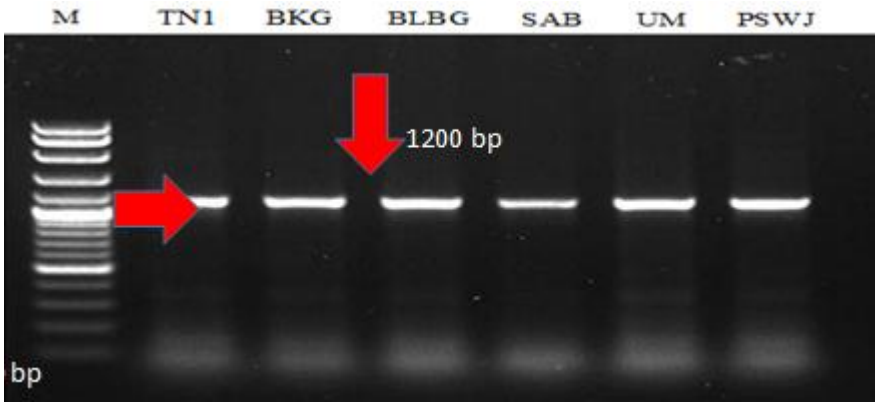

Figure 1: PCR Results of Test Varieties
Abbv.: M = Marker 1 kb; TN1 = Taichung Native 1; BKG = Bekongan; BLBG= Blumbungan: SAB = Srogel Abang; UM = Utri Merah; dan PSWJ = Pulut Sawijan

Alignment of Endogenous RTBV Sequences Analysis

The results of Blast Nucleotide analysis (Blanst N) indicate the local rice sequences of the test is parallel with the varieties of eRTBVL A1, eRTBVL C, IR36 ERTBV1, IR 36 ERTBV1, eRTBV C and eRTBVL A2 with percentages of identity sequentially 99%, 95%, 100% , 100%, 98% and 89%. Maximum score, Total score, Quary cover, E value varies depending on accession to the subject of similarity.

The results of Blast Protein analysis (Blast X) show that all ERTBV7 amplicons have a 66.9% alignment with 194K polypeptide bacilliform virus (accession number UniRef100Q86366); ORFy with specification quality length 391, P value 0 and identity at 93.35% (UniRef100Q5FV43); Retrotransposon protein, putative Ty3 Gypsy Subclass with specification quality length 566, P value 0 and similarity 92.05 (UniRef100Q101F5); putative polyprotein with specification length 444, P value 0 and similarity 90.77 (UniRef100Q7G647). The motives of proteins contained in the retrotransposon of each amplicon as presented in Figure 1, consisted of Reverse Transcripsion Long Terminal Repeat (RT LTR), Reverse Transcriptions (RVT), RNase H like and Caulimoviridae caulimoflower (Cauli VI). These results provide a strong sign that the RTBV genome as a virus is truly integrated into the rice genome and that that is integrated is ORF3 which encodes P194.

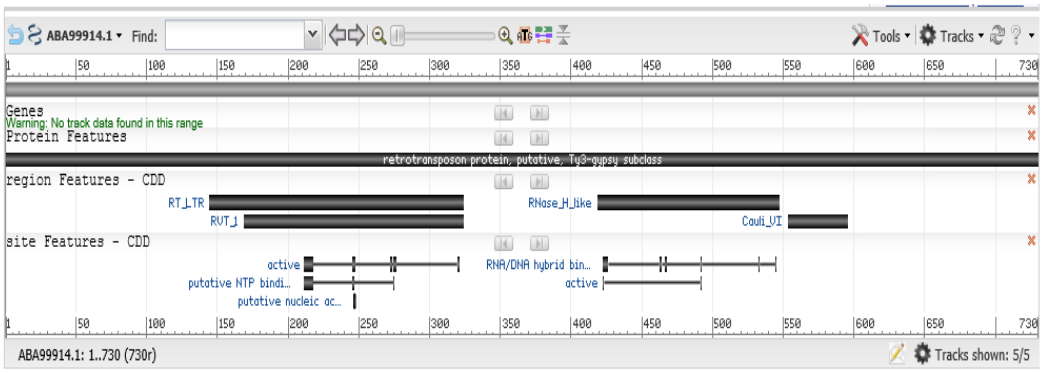

Figure 2. Protein Motifs Test Varieties amplified with ERTBVV7

The presence of the retrotransposon sequence on the ERTBV7 amplicon is interesting and important in relation to the mechanism of RTBV genome integration in the rice genome. Retrotransposes are DNA sequences that can move from one place to another in the genome of an organism (Havecker et al., 2004). Retrotransposons are categorized as class I transposons whose genetic elements are able to multiply by transcribing Ribo Nucleic Acid (RNA), then transcribed back into DNA with the help of the reverse transcriptase enzyme. These retrotransposons are similar to retroviruses that have the ability to double their genome RNA by converting RNA into more stable DNA to integrate with their host DNA (Hayward, 2017).

The sequence integration process occurs through repeated AT (ATrs) sequences. Chen et al., (2014) stated that, in the ATrs pararetrovirus it functions as a Hot spot and break point for endogenous integration of RTBV due to the absence of integrase enzymes. The results of a study conducted by Kunii et al. (2004) on whole sequences containing endogenous RTBV showed that 93% of the endogenous ends of RTBV were flanked by repeated AT sequences and strongly suspected of being associated with the integration of viral DNA into the rice genome. Therefore, the presence of repeated AT sequences in the whole sequence in each variety is the key to understanding RTBV genome integration into the rice genome. Based on the description of Kunii et al. (2004), all local test rice sequence (Utri Merah, Pulut sawijan, Bekongan, Srogel Abang, Blumbungan and TN1) has AT sequences that also flank the endogenous RTBV sequence. The number of repeated AT sequences ranges from 20-22 repetitions in each test rice variety.

Based on Gramene analysis, integrated Endogenous RTBV spread evenly on the 12 local rice chromosomes at different loci. The results of the analysis are presented in Table 1.

Table 1: *Endogenous* Position of RTVB in Rice Chromosoms and Locus

KRM	VARIETIES/LOCUS					
	UM	PSWJ	SAB	BKG	BLBG	TN1
1	1:36292200-36292833	1:36291796-36292831	1:30144046-30144687	1:30144044-30144687	1:14479751-14480256	1:14479751-14480256
2	2:35186693-35187258	2:35186693-35187256	2:35186218-35187256	2:35186129-35187258	2:35186129-35186764	2:35186129-35186764
3	3:27171327-27171345	3:22862893-22862910	3:13011330-13011356	3:13011330-13011356	3:32597095-32597116	3:10917102-10917119
4	4:19714735-19715300	4:19714735-19715298	4:19714260-19715298	4:19714171-19715300	4:19714171-19714806	4:19714171-19714806
5	5:14782108-14782130	5:14782108-14782130	5:14782082-14782108	5:14782082-14782108	5:14782082-14782104	5:14782082-14782104
6	6:1875058-1875733	6:1875206-1875644	6:1875058-1875644	6:1875058-1875733	6:1875098-1875733	6:1875098-1875733
7	7:6562913-6563499	7:6562915-6563506	7:6562915-6563556	7:6562913-6563556	7:6562913-6563216	7:6562913-6563222
8	8:4508255-4508739	8:4508212-4508650	8:21413902-21414609	8:21413813-21414609	8:21413813-21414448	8:21413813-21414448
9	9:2951413-2951849	9:14664905-14665468	9:14664501-14665468	9:14664501-14665470	9:14664501-14664976	9:14664501-14664976
10	10:20958480-20959601	10:20958482-20959115	10:195679-196275	10:20953168-20953755	10:20949090-20949618	10:20949090-20949618
11	11:13596093-13596658	11:13597946-13598264	11:13597141-13597895	11:13597141-13597895	11:13597946-13598279	11:13597946-13598279
12	12:20702847-20703412	12:20702849-20703412	12:20702849-20703679	12:20702847-20703679	12:20707343-20707940	12:20707343-20707940

Abbv.: KRM=Chromosom.

The results of nucleotide and endogenous amino acid alignment analysis of RTBV showed that there was a mutation in the form of insertion and nucleotide lesion in the ERTBV sequence of each test rice variety. Most nucelotide

mutations in Utri Merah occur at positions 598-630 pb, Pulut Sawijan at 518-550 pb, Srogel Abang at 517-550 pb, Bekongan at 607-638 pb, Blumbungan at 818-191 pb and 194-202 pb (Figure 2). Blumbungan and TN1 experienced insertions at 606-636, 606-636 respectively. Amino acid dilution in Utri Merah occurs at 211 and 218 pb and 221-229 pb, Pulut Sawijan at 184 pb and 194-204 pb, Srogel Abang at 184-192 and 194-203 pb, Bekongan at 214-221 pb and 224 -233 pb, Blumbungan at 225-233 pb. TN1 and Blumbungan were inserted in 213-232 pb, 214-223 pb respectively (Figure 3). Insertions, mutations and lesions in nucleotide and amino acid sequences will affect the functioning of a gene in an organism's biological system (Yan Du et al., 2017). Insertion, conversion, duplication and dilution also greatly influence the interaction of viruses and hosts during the process of infection and replication (Michael *et al*, 1992). When the host genome experiences insertion, mutation and dilution in a very crucial position for the viral infection process, there will be incompatible interactions with the host and cause the virus to fail to infect, so that the host plant is categorized as resistant (Hwang et al., 2013). Therefore, the number of insertions, mutations and dilutions in the Utri Merah genome is thought to induce the Utri Red resistance to the tungro virus.

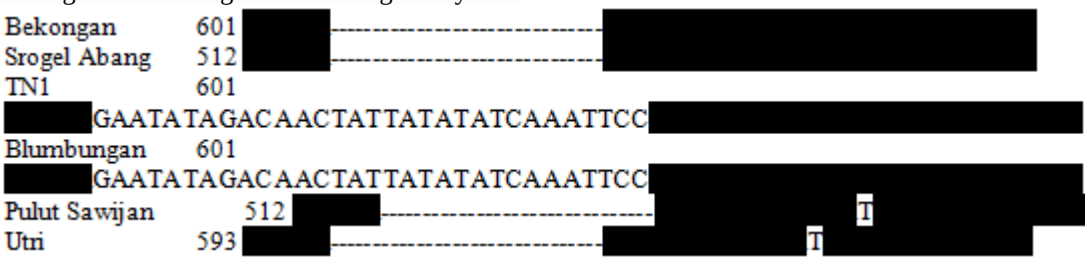

Figure 3: Position of Nucleotide Mutations in test varieties

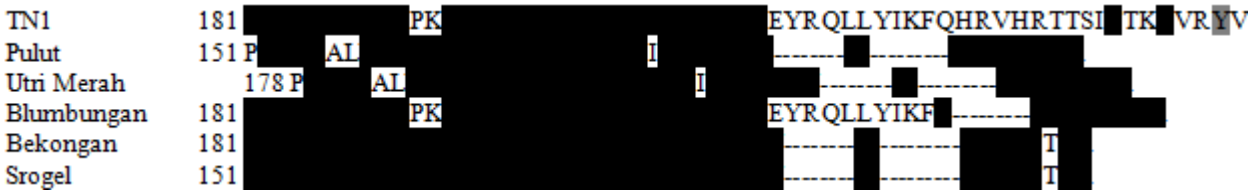

Figure 4: Position of Amino Acid Mutations in Test Varieties

Analysis of the number of copies of RTBV fragments in the rice genome

The number of copies of the endogenous fragments of RTBV on each chromosome in each test rice variety is presented in Table 2.

Table 2: Number of endogenous fragments of RTBV in Test Rice Varieties

Chromosoms	Local Rice Varieties						Total
	UM	PSWJ	SAB	BKG	BLBG	TN1	
1	22	18	19	22	28	26	135
2	12	7	8	8	12	12	59
3	6	4	3	3	4	3	23
4	12	14	12	12	21	21	92
5	5	2	3	3	3	3	19
6	12	6	4	5	4	5	36
7	9	7	9	9	15	14	63
8	14	8	7	7	9	9	54
9	7	5	5	4	5	5	31
10	9	7	9	9	18	18	70
11	18	17	10	10	13	13	81
12	18	13	12	11	17	17	88
	144	108	101	103	149	146	

The varieties with the highest number of copies of the RTBV endogenous fragment were Blumbungan (149 copies) followed by TN1 (146 copies), Utri Merah (144 copies), Pulut Sawijan (108 copies), Bekongan (103 copies), and Srogen Abang (101 copies) .

More in-depth analysis of the number of specific fragment copies shows integration of RTBV in the rice genome can occur at different loci between varieties. There were 57 copies of ERTBV specific fragments integrated at specific loci in Utri Merah and not found in other varieties (Table 3). The number of copies of RTBV specific fragments at the specific locus in Blumbungan was 23 copies, Pulut Sawijan 22 copies, TN1 18 copies, Srogel Abang 3 copies, and Bekongan 1 copy. The highest number of copies of ERTBV specific fragments in Utri Merah is located on chromosomes 11 and 12. Chromosomes 11 and 12 are known to have the highest number of genes related to the nature of resistance to pathogenic microorganisms (Waksman Institute, 2005).

Table 3: Number of Endogenous RTBV Fragments in Specific Locus of Test Rice Varieties

Chromosom	Local Rice Varieties						Total
	UM	PSWJ	SAB	BKG	BLBG	TN1	
1	7	4	0	0	2	1	14
2	8	1	0	0	2	2	13
3	4	2	0	0	1	0	7
4	2	2	0	0	2	2	8
5	3	1	0	0	0	0	4
6	5	1	0	0	1	1	8
7	3	1	1	1	5	4	15
8	5	1	0	0	1	1	8
9	3	2	0	0	0	0	5
10	4	1	1	0	4	2	12
11	5	4	0	0	2	2	13
12	8	2	1	0	3	3	17
	57	22	3	1	23	18	

Phylogenic Analysis

Phylogenic analysis of test rice varieties was made based on the total number of copies of the ERTBV fragment integrated in the rice genome of the Indica group. The results of the analysis show resistant variety groups (Utri Merah) and susceptible (TN1) were clearly separated in two groups (Figure 2). The first group is the Red Utri and Pulut Sawijan groups with a genetic distance between them which is 0.60 while between Utri Merah-Srogel Abang is 0.41; Utri Merah-Bekongan 0.52, Utri Merah-Blumbungan 0.39, Utri Merah-TN1 0.40. The second group consisted of the first 2 subgroups namely Srogel Abang and Bekongan with a genetic distance between the two 0.88 while Srogel Abang-Blumbungan 0.56, Srogel Abang-TN1 0.58. The second sub group was Blumbungan and TN1 where the genetic distance between them was 0.89. This group difference can be an indicator of the number of copies of the endogenous fragment of RTBV in the rice genome determines the nature of the resistance of a variety.

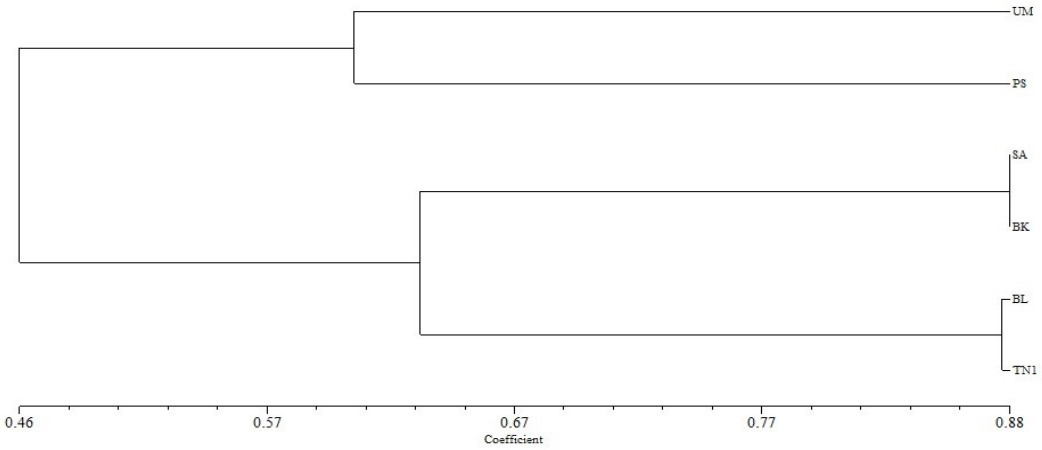

Figure 5: The closeness of the genetic relationship in tested rice varieties

Protein Motif Analysis

The results of analysis of the motifs of protein endogenous sequences of RTBV were found, among others, Group specific of Antigen (GAG) or groups of specific antigens, aspartate protease, retroviruses, retrovirus proteins 1 and 2, asparagine glycosidation, ATP-GTP A, casein kinase II, myristil, protein kinase C, isoleucine, amidation , DVF321, CAMP phosphorilation, and nebulin. The types of protein motifs from the endogenous RTBV sequence in the rice genomes of Utri Merah, Bekongan, Pulut Sawijan, Blumbungan, Srogel Abang and TN1 varieties are protein motifs used in the process of replicating viruses and proteins used for plant metabolism.

Proteins used for viral propagation are GAG asp proteases, protein retroviruses, protein 1 and 2 retroviruses, while those used for plant metabolism as a whole are aspartate proteases, asparagine glycosidation, ck2 phosphorilation, myristil, pkc phosphorilation, isoleucine, amidation, camp phosphorilation and nebulin. Isoleucine motifs are only found in Utri Merah, Bekongan, Srogel Abang, Pulut sawijan varieties. While the amidation protein motif was found in TN1 and Blumbungan varieties.

4. Discussion

Integration of RTBV into plants occurs as an evolutionary process that goes through the process of deletion, inversion, insertion and duplication. According to Dewannieux et al., (2013) and Hayward et al., (2013) endogenous viruses are remnants of infection by ancestors, because exogenous ancestor viruses at the time of infection and the integrated DNA are suitable in the genome and can spread from one species to another through cross-species transmission. Therefore, the presence of RTBV sequences in the local rice variety sequences tested was a sign of the presence of endogenous RTBV integration into the local rice genome (Kunii et al., 2004; Jacowitch et al., 1999). The occurrence of integration is indicated by several results of analysis, namely analysis PCR. Nucleotide blast analysis which shows that the nucleotides in local rice varieties are very similar to nucleotides in endogenous RTBV accessions in genbank with 99% degree of similarity; blast protein analysis which showed that the test varieties had proteins that were very similar to polyprotein P194 (66.9%) and ORFy (93.35%). According to Hull (2004) and Kunii et al., 2004 ORF III is P194 which has several components such as protein movement (MP), coat protein (CP), asparatat protease (PR), and RT / RNase H (RT / RH). Another protein motif that strengthens the sequence is Caulimovirus viroplasmin as part of RTBV. Caulimovirus viroplasmin is a form of body inclusion in the Caulimoviridae family which has viral properties whose proteins are encoded through VI gene (comparable to ORF III on RTBV) (Volovitch et al., 1990). The inclusion body also carries out DNA synthesis and produces DNA synthesis products (Wintermantel et al., 1993).

Although the endogenous sequence of RTBV was originally a sequence originating from outside the plant but when the sequence was integrated and became part of the whole rice sequence, the sequence carried out its metabolic function together with the plant (Kunii et al., 2004). The participation of endogenous RTBV sequences in the metabolic processes of rice appears from the putative retrotransposon protein Ty3-gypsy subclass (*Oryza sativa Japonica Grup*) which is a group of compounds that encode polyprotein. Retrotransposon plays a role in influencing the structural evolution of plant genomes. The effect of retrotransposon on the rate of evolution of plant genomes is determined by

population structure (how many fragments of the type of retrotransposon exist) and transposisional activity (what proportion of transposon is active and how much is transposed).

Based on the results of Gramene's analysis (http://www.gramene.org/) the number of endogenous copy number of RTBV in the rice genome varies in both the total fragment and the total specific locus. Utri Merah has the highest number of endogenous fragments, 144 followed by Pulut Sawijan (108), Srogel Abang (101), Bekongan (103), Blumbungan and TN1 fragments. In line with that the results of the NTSYSpc software analysis classify the test varieties in three separate groups based on the endogenous genetic distance of the RTBV of each variety. These groups are Utri Merah and Pulut Sawijan, Srogel Abang and Bekongan and Blumbungan and TN1. Similarly, the observation of the number of fragments at specific loci where Utri Merah has 57 specific fragments, Pulut Sawijan 22, Srogel Abang 3, Bekongan 1, Blumbungan 23 and TN1 18. These results indicate that each variety has a number of copies of its own endogenous RTBV fragments. Depending on the ability of the variety to produce the number of copies of the fragment.

The number of fragment copies is related to the nature of plant resistance to disease. In line with that, Kunii et al. (2004) stated that rice species that had a low number of endogenous fragments of RTBV were known to be very susceptible to RTBV. Based on this statement, TN1 and Blumbungan varieties, which have a large number of fragment copies, are also categorized as resistant varieties, but both of these varieties are categorized as vulnerable. This condition is thought to be related to the degree of mutation and mutation of proteins as the expression of genes produced. Severe mutations are related to the number of fragment copies produced by a variety. Kunii et al., (2004) stated that due to the presence of rice sequences with the number of endogenous fragments RTBV has many tendency to occur heavy mutations in these sequences, mutations that occur have an impact on the expression of genes produced and can be seen in the motifs of the protein produced.

Mutations that occur have an effect on gene expression products in the form of protein motifs that have the potential to be associated with plant resistance properties. In the Utri Merah, Pulut Sawijan, Bekongan and Srogel Abang varieties there are fundamental differences regarding the motives of proteins produced by Blumbungan and TN1. The results of data analysis using MyHits software (http://myhits.isb-sib.ch/) prove that Utri Merah, Pulut Sawijan, Bekongan and Srogel Abang have ile-rich motifs, while Blumbungan and TN1 varieties have motifs of protein Amidation. An ile-rich motif is a sequence that encodes the formation of the amino acid Isoleucine. Isoleucine amino acid is processed by crossing Aspartate Protease (Asp) (Galili, 2011) which is then incorporated in Lys biosynthesis / catabolism and through cross salicylic acid (SA) together with Methionine, Isoleucine, Threonin, and Glysin to produce a compound known as pipecolic (Pip) name through Aberrant Growth and Death2 (AGD2) pathway -like defense response protein1 (ALDI1) -dependent pipecolate pathway (Yang and Ludewig, 2014). Pipecolic acid has the potential to control plant diseases, especially tungro rice disease. Protein motives in Blumbungan and TN1 varieties are Amidation which are peptides that will produce hormones for plant growth (Wang et al., 2006; Vickery and Pucher 1939).

5. Conclusion

The results of the study concluded that (1) integrated endogenous RTBV is in a different chromosome and locus arrangement in local rice, (2) There are more number of copies of specific endogenous fragments of RTBV in the Utri Merah variety compared to other test varieties, and (3) Number of copies of endogenous fragments of RTBV associated with the characteristics of local varieties resistance to tungro rice disease.

6. Acknowledgements

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References

[1] **Agrios G.N.,** 2005. Plant Pathology, 5th edn. Academic Press. Tokyo.
[2] **Bhat A.I., T. Hohn, R. Selvarajan.** 2016. Badnavirus: The Current Global Scenario. Viruses, **8(177):**1-29.
[3] **Castagnone, P Sereno. 2002.** Genetic variability in phartenogenesis root knot nematode, *Meloydogyne* spp., and their ability to overcome plant resistance genes. *Nematology*, **4**:605-608.
[4] **Chen S., R. Liu, K.O. Koyanagi and Y. Kishima,** 2014. Rice genom recorded ancient pararetrovirus activities: Virus genealogy and multiple origins of endogenization during rice speciation. Virology **471-473**(141-152).
[5] **Cruz F.C., R. Hull, and O. Azzam. 2003**. Change in level of virus accumulation and incidence of infection are critical in the characterization of rice tungro bacilliform virus (RTBV) resistance in rice. Arch Virol **148**:1465-1483
[6] **Dewannieux M., and T. Heidmann**. 2013. Endogenous retrovirus: acquisition, amplication and taming of gene invaders. Virology **3(6)** 646-656.
[7] **Feschotte C and C. Gilbert**. 2012. Endogenous viruses: insight into viral evolution and impact on host biology. Nature review genetics **13**(283-296).
[8] **Galili G. The aspartate family pathway of plants**. 2011. Journal plant signaling and behavior **6(2):** 192-195.
[9] **Havecker E.R., X. Gao, and D.F. Voytas**. 2004. The diversity of LTR retrotransposon. Genome Biology, **5(6):**1-6.
[10] **Hayward A., C.K. Cornwallis, P. Jern**. 2015. Pan-vertebrate comparative genomics unmasks retrovirus macroevolution. PNAS, **112(2):**464-469.
[11] **Hibino H, M. Roechan, S. Sudarisman**. 1978. Association of two types of virus particles with penyakit

Habang (Tungro Diseases) of Rice in Indonesia. Phytopathology **68**:1412-1416.

[12] **Hibino H.**, 1983. Relation of rice tungro bacilliform and rice tungro spherical viruses with their vector, Nephotettix virescens. Ann Phytopathol soc Jap 49:545-553.

[13] Hibino H., 1996. Biology and epidemiology of rice viruses. *An annual review of Phytopathology* **34**: 249-274.

[14] **Hull R**. 1996. Molecular biology of rice tungro viruses. Annu. Rev. Phytopathol, **34**:275-279.

[15] **Hull R and Matthews**, 2004. Plant Virology, 4th edn. Academic Press. London. pp 174-180.

[16] **Hwang J.N, S.O. Chang, B.C. Kan**. 2013. Translation elongation faktor 1B (eEF1B) is an essential host factor for Tobacco Mosaic Virus infection in plants. J Virology.**439**:105-114

[17] **INGER [International Network for Genetic Evaluation of Rice].** 1996. Standard evaluation system for rice. 4th ed. International Rice Recearch Institute, Los Banos Philipina. pp 22-23.

[18] **Yang H and U. Ludewig**. 2014. Lysine catabolism, amino acid transport, and systemic acquired resistance. What is the link? Plant signaling and Behaviour, 9:e28933.1-e28933.3. Institute of Crop Science. Germany.

[19] **Koenning S.R., K.R. Barker and D.T. Bowman**. 2001. Resistance as Tactic for management of Meloydogyne incognita on cotton in North Carolina. Journal of Nematology **33**:126-131

[20] **Kunii M., M. Kanda, H. Nagano, I. Uyeda, Y. Kishima and Y. Sano**. 2004. Reconstruction of putative DNA virus from endogenous rice tungro bacilliform virus-like sequences in the rice genome: implications for integration and evolution. *BMC Genomic* **5(80):**1-14.

[21] **Kush G.S**. 1980. Breeding rice for multiple diseases and insect resistance in : Rice improvement in China and other Asian countries. pp 219-238 IRRI Los Banos Philipines

[22] **Ou S.H**. 1984. Exploring tropical rice disease: A reminiscence. Ann.Rev.Phytopathol. 22:1-10

[23] **Pakki S, A. Bastian, dan Suharti**. 2010. Sebaran penyakit tungro di sentra produksi padi di Indonesia Timur. Prosiding seminar ilmiah dan pertemuan PEI dan PFI XX komisariat Daerah Sulawesi Selatan.

[24] **Sitaremi T., R.H. Wening, A.T. Rakhmi, N. Yunani, U. Susanto**. 2013. Pemanfaatan plasma nutfah padi varietas lokal dalam perakitan varietas unggul. Iptek Tanaman Pangan, **8(1):**22-30.

[25] **Vickery H.B and G.W. Puscher.** 1939. The Metabolism of Amides in Green Plants. Journal Biol Chem, **128**: 703–713.

[26] **Volovitch M., N. Modjtahedi, Y. Chouikh, P Yot.** 1990. DNA sequence of gen VI of Caulimoflower MOSAIC Virus Strain PV147. Virology, **18**:5297-5297.

[27] **Waksman Institute**. 2005. The sequence of rice chromosomes 11 and 12, rich in disease resistance genes and recent gene duplications. BMC Biology, **3(20):**1-18

[28] **Wang G., X. Ding, M. Yuan, D. Qiu, X. Li,C. Xu, S. Wang,** 2006. Dual function of rice OsDR8 gene in disease resistance and thiamine accumulation. Plant Mol.Biol **60(3):** 437-449.

[29] **Wintermantel W.M., G.J. Anderson, J.E. Schoelz**. 1993. Identification of domain within gene VI of Caulimoflower Mosaic Virus that influence systemic infection of Nicotiana bigelovii in a light dependend manner. Virology, **196**:789-798.

[30] **Yang H and U Ludewig**. 2014. Lysine catabolism, amino acid transport, and systemic acquired resistance. What is the link? Plant signaling and Behaviour, 9:e28933.1-e28933.3. Institute of Crop Science. Germany

[31] **Zeigler R.S and S. Savary**. 2010. Plant diseases and the world's dependent on rice in the role of plant pathology in food safety and food security editor Strange R.N and M.L Gullino. p3-8. Springer UK