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Resilience response to yellow leaf curl disease and identification of resistance gene analogs (RGA) in pepper (*Capsicum annuum*)

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Abstract. *Ayu DK, Maharijaya A, Syukur M, Hidayat SH. 2021. Resilience response to yellow leaf curl disease and identification of resistance gene analogs (RGA) in pepper (*Capsicum annuum*). Biodiversitas 22: 4731-4739.* Pepper yellow leaf curl disease (PYLCD) caused by infection of Begomovirus is a serious threat to pepper production worldwide. Identification of the resistance gene analogs (RGA) and resilience response of pepper against PYLCD is needed especially for selection resistance genotype. Evaluation of resistance response involving 28 pepper genotypes was carried out through transmission of Begomovirus using whitefly (*Bemisia tabaci*) as vector. The result showed that IPB C12 and F4-012328-6-3 were potential resistance genotypes. A total of 15 R gene analogs (CaRGA) containing NBS motif, namely CARGA1 to CARGA15, were identified by degenerated PCR amplification and database mining. The alignment of deduced amino acid sequence revealed conservation of subdomains Ploop (GKTT), kinase2 (LVVLDDV), RNBSB/kinase3 (IILTTR) and GLPL. BLASTp analysis indicated that 15 RGA showed high homology at deduced amino acid level with R gene identified such as *whitefly resistance protein Mi-1.2*, *Pvr 9 gene for potyvirus*, *Begomovirus resistance protein*, *TRGA15* and *RGA 13* for putative late blight resistance. Phylogenetic analysis exhibited that isolated sequences distinguished into CNL-NBS groups. These pepper RGA could be considered as candidate sequences of resistance genes.

Keywords: Begomovirus, degenerate primer, pepper, phylogenetic analysis, whitefly

INTRODUCTION

Pepper (*Capsicum*) is one of the most important vegetables in the world (Olatunji et al. 2018; Tripodi et al. 2019). The main constraint of pepper productivity is pests and diseases. Pepper yellow leaf curl disease (PYLCD) caused by Begomovirus is an important disease of pepper that has been reported in several countries (Koeda et al. 2018; Fadhila et al. 2020). Begomovirus was transmitted by the whitefly vector has led to the development and spread of PYLCD epidemics (Islam et al. 2018). It has been reported to occur widely in Asia (Kenyon et al. 2014), Australia, China, Europe, Middle Eastern Countries (Alshihi 2019), Africa (Ouattara et al. 2020) and the Americas (Barboza et al. 2018). The incidence of PYLCD in Indonesia was first reported between 1994 and 1999 (De Barro et al. 2008). In Indonesia, the yield loss due to PYLCD ranges from 20 to 100% (Annisaa et al. 2021).

The disease is transmitted by vector, i.e., whitefly *Bemisia tabaci* Genn (Firdaus et al. 2013; Wei et al. 2017; Pandey et al. 2021) in a persistent and circulative way. *B. tabaci* is a species of phloem-feeding insect belonging to the Aleyrodidae (Ellango et al. 2015; Milenovic et al. 2019). During feeding the virus is taken up by the whitefly, which takes about 24 hrs, and during a latency period that takes 4- 24 hrs, it is transferred to the salivary gland and then during feeding (>24 hrs.) excreted with the saliva to infect new plants (Ghanim 2014). Management of begomoviruses has become a challenge due to the continuous evolution and the emergence of new strains. Currently, PYLCD problems are dealt with by combating its vector. However, *B. tabaci* has relatively high reproduction rate and a short life cycle, it has a rapid dispersal ability, high survival rate to climatic changes, and quickly develops resistance to pesticides that make it difficult to control (Gilbertson et al. 2015). Various attempts have been made to control this disease, including protecting plants using net screen, planting border crops, application of insecticides (Horowitz et al. 2011), and biological control (Calvo et al. 2009; Soesanto et al. 2020). However, this strategy has not succeeded in preventing the spread of the disease. The use of resistant cultivars is always recommended, but commercial pepper cultivars carrying resistance to PYLCD are not available yet.

Plants have resistant genes which have the potential to detect the onset of infection and further activate resistance mechanisms against pathogenic infections (Yang et al. 2021). Structurally, resistant genes have special characteristics indicated by presence of conserved amino acid motifs. The conserved amino acid motif allows the identification of resistant gene fragments in genome of plant through amplification using degenerate primers.

These primers are designed based on sequences of DNA resistance genes from other plants, known as resistance gene analogs (RGA) (Naresh et al. 2017). One of the largest RGA classes that have been characterized is the Nucleotide Binding Site. The NBS region is important for ATP binding and hydrolysis, so it is believed to be involved in signal transduction triggered by pathogen infection. NBS receptor protein recognizes pathogen effector protein that produces transduction signals to stimulate the expression of defense against pathogens (Caplan et al. 2008).

Research has been done to identify NBS in various plants such as wheat, barley, and tomatoes (Kushwaha et al. 2015; Yamaguchi et al. 2018). However, the identification of NBS is far behind in pepper against PYLCD. The objective of this research is to identify the NBS gene in pepper related to PLYCD resistance, which might be further developed toward PYLCD resistance breeding in pepper.

MATERIALS AND METHODS

Study area

The pepper genotypes used in this experiment were obtained from a collection of Plant Breeding Laboratory, Department of Agronomy and Horticulture, IPB University (Table 1). All genotypes belonged to *Capsicum annuum*. Isolate of Pepper yellow leaf curl virus (PYLCV) was used as the initial inoculum and whiteflies (*B. tabaci*) colony was provided by Plant Virology Laboratory, Department of Plant Protection, IPB University. The isolates were maintained in pepper in a restricted screen house; whereas whiteflies were reared on cotton plants inside insect cages.

Procedure

Resistance test of 28 pepper genotypes to PYLCD

Virus transmission for resilience test was carried out by individual transmission method, using 10 whiteflies for each plant. Whiteflies were given 24 hrs acquisition feeding period on PYLCV-infected plants and then transferred to test plants for 48 hrs inoculation feeding period. The age of test plants at the time of transmission was 10 days after transplanting.

The experiment was designed using a randomized block design with genotype as the treatment. Each treatment was performed thrice, and each consisted of 30 plants. The following parameters were observed in infected plants: type of symptoms, incubation period, incidence, and severity of the disease. Symptoms were categorized on a relative scale of 0 to 4. Then the resilience response was classified based on the disease severity (DS) i.e., 0-20%, >20-40%, >40-60%, and >60% as resistant, moderately resistant, susceptible, and very susceptible, respectively. Data were analyzed by ANOVA using SPSS version 15.0 and a further test was conducted using the Duncan test (alpha 0.05). Confirmation of Begomovirus was carried out by polymerase chain reaction (PCR) using two degenerate primers SPG1 (5'-CCCCK-GTGCGWRAATCCAT-3') and SPG2 primer (5'-ATCCVAAYWTYCAGGGAGCT-3') with a target amplicon of 912 bp. DNA amplicons were sent to First Base company (Malaysia) for sequencing; then the sequence was analyzed using the Seq Trace 0.9.0 and Geneious software version 8.1.6 (Biomatters, USA).

Isolation of Nucleotide Binding Site (NBS)

A pair of degenerate primers, previously designed based on conserved domains (P-loop and GLPL regions) among known NBS-LRR R genes from other plant (Zhang et al. 2008), were used (Table 2). The amplification reaction was carried out in the PCR machine with the following program: pre-denaturation at 95 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 45 sec, primer annealing at 55 °C for 1 min, and elongation of DNA at 72 °C for 1 min. In the last stage, the PCR process was carried out at a final elongation at 72 °C for 10 min. The amplification product was electrophoresed on 1.2% agarose gel, followed by staining with EtBr (0.5 mgL^{-1}) and visualization using UV Transilluminator. The presence of NBS in the pepper sample was indicated by the amplification of the 500 bp DNA fragment. The targeted NBS DNA fragments were sent for sequencing.

Table 2. Degenerate primers used for amplification of nucleotide binding site fragment in pepper

Degenerate Primers	Primer Sequence (5'-3')	Reference
Ploop-F2	TGSSRGGHWYRGGBAAAACTAC	Zhang et al. (2008)
GLPL-R2	HRCWARAGGVARCCCTYBACA	Zhang et al. (2008)

Table 1. List of pepper genotypes used in resistance evaluation against yellow leaf curl disease

Name of genotypes			
IPBC2	Ayesha	F4-012328-1AB-2	F7-145174-9-3-1-5-3
IPBC5	Seloka	F4-012328-6-3	F7-145291-14-9-3-12-1
IPBC12	Yuni	F3-74 x Hot Chilli	F7-145291-10-7-1-1-1-1
Gada	SSP	F3-Elegance x 74-2	F5-145291-14-9-3
Bara	Anies	F3-74 x Biola	F7-1200005-141-16-2-9-4
Genie	Ungara	F7-160291-4-13-9-8-1	F7-1200005-120-7-1-7-8
Giant	Seroja	F7-145293-19-8-3-113-1	F7-1200005-141-16-3-5-4

Data analysis

DNA sequences were analyzed using the Seq Trace 0.9.0 software. The sequences were then translated into amino acids using the Transeq tool from the EMBOSS software suite (http://www.ebi.ac.uk/Tools/st/emboss_transeq/). Peptide sequences with uninterrupted ORF were selected and used for further analysis in this study. Identity and similarity searches of nucleotide and amino acid sequences were performed using BLAST at the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/BLAST/). The deduced amino acid sequences of the RGA isolated from the pepper, with other related plant RGAs were aligned with *Multiple Sequence Allignment* (MUSCLE) method and used for phylogenetic tree construction using MEGA (Molecular Evolutionary Genetic Analysis).

RESULTS AND DISCUSSION

Symptoms of disease in pepper plant

Symptoms of the disease that appear on all genotypes of pepper plants observed in the greenhouse, included yellowing, curling, mosaics, and bending upward or downward (Figure 1), but the dwarf symptom was not found. Out of 28 genotypes, 22 genotypes, namely IPB C2, IPB C5, Bara, SSP, Gada, Seloka, Yuni, Ungara, Seroja, F4-012328-1AB-2, F4-012328-6-3, F3-74 x Hot Chilli, F3-Elegance x 74-2, F3-74 x Biola, F7-145293-19-8-3-113-1, F7-145174-9-3-1-5-3, F7-145291-14-9-3-12-1, F5-145291-14-9-3, F7-1200005-141-16-3-5-4, F7-1200005-141-16-2-9-4, and F7-1200005-120-7-1-7-8 showed severe symptoms as described earlier; while 6 other genotypes, namely IPB C12, Genie, Giant, Ayesha, F7-160291-4-13-9-8-1, and F7-145291-10-7-1-1-1-1 exhibited mild symptoms including only yellowing and curling of leaves.

Confirmation of PYLCV in symptomatic plants

DNA fragments of 912 bp were successfully amplified from 28 symptomatic leaf samples using SPG1/SPG2 primers. Results of sequencing analysis confirmed that the virus infecting tested plants in the greenhouse had 100% homology with the virus inoculum source. Furthermore, the viral isolates used as inoculum showed 94.23% and 96.67% homology with the Pepper yellow leaf curl Indonesian virus [Ageratum] DNA, DNA-A segment (AB267838), and Pepper yellow leaf curl Indonesia Talibeng virus 1 AC1 gene (LC381258), respectively.

Genotype response to yellow leaf curl disease in pepper

Symptoms of disease in all plants appeared between 5-28 days after inoculation (dai). The longest i.e.12-28 dai incubation period occurred in IPB C12; while the shortest incubation period i.e. 5-18 dai occurred in IPB C5 (Table 3). The results showed that there was high variability in the level of resistance to PYLCD among the genotypes tested. The large variations among genotypes indicated the possibility of selection for resistance to this disease. Statistical analysis showed that genotype treatment significantly affected the incidence and severity of yellow leaf curl disease (Table 3). Disease incidence ranged from 20.00% to 93.33%, while disease severity ranged from 18.33% to 72.78%. The observational results showed that the greater the percentage of disease incidence and the symptom score, the greater the magnitude of disease severity. Low incidence and severity of disease were found in IPB C12 and F4-012328-6-3.

Isolation and sequence analysis of NBS fragments

The degenerate primers were designed to amplify the area between the P-loop motif and GLPL from the NBS area. DNA fragments of 500 bp were successfully amplified and nucleotide sequences were obtained from 15 DNA fragments. Results of nucleotide (501 bp to 528 bp in length) and deduced amino acid (166 to 177 amino acid) analysis revealed that 15 fragments namely, CARGA1, CARGA2, CARGA3, CARGA4, CARGA5, CARGA6, CARGA7, CARGA8, CARGA9, CARGA10, CARGA11, CARGA12, CARGA13, CARGA14, and CARGA15 were containing NBS regions.

CARGA1 showed the similar identity of 72.41% with *whitefly resistance protein Mi-1.2* [*Solanum peruvianum*]; CARGA2, CARGA6, CARGA7, CARGA8, CARGA13, CARGA14, CARGA15 had a similarity of 81.71%, 83.53%, 87.57%, 79.44%, 76.74%, 92.05%, and 86.93%, respectively with *Pvr9-like protein 2* [*Capsicum annuum*]; CARGA3 and CARGA4 fragments had similar identity of 89.88% and 89.82% with *TRGA15* [*Capsicum annuum*], while CARGA5, CARGA 9, CARGA10, CARGA11, and CARGA12 had 85.12%, 70.83%, 76.65%, 70.00% and 74.42% similarities with resistance protein *RGA13*, respectively [*Capsicum annuum*] (Table 4).

Sequence homology analysis among 15 RGA fragments showed that the fragments are having varied amino acid similarities ranged from 44.4% to 87.5% (Table 5).

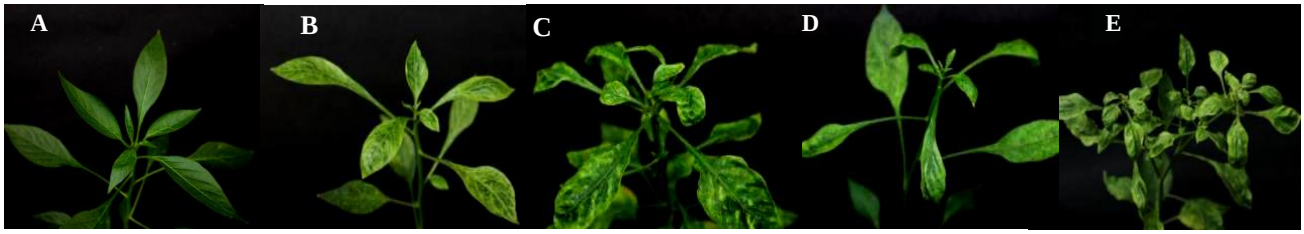

Figure 1. Symptoms appear on pepper plants: A. Healthy plant, B. Yellow leaves C. Yellow and curly leaves, D. Yellowing and bending upwards or downwards, E. Yellowing, curling, and bending upwards and/or downwards

Identification and analysis of the motifs of RGA pepper protein and other plant-R genes

The results of consensus analysis of amino acid prediction of the 15 fragments with NBS-LRR pepper plant protein and other plant R genes that had been deposited in Gene bank showed that NB-ARC had four conservative structures that were characteristic of the NBS region. The four conservative structures of amino acids were the P-loop/kinase-1a (GKTT) motif, kinase-2 (LVVLDDV), RNBS-B/kinase-3a (IILTTR), and hydrophobic amino acid (GLPL) (Figure 2). The amino acid length including the P-loop region to GLPL was 167 amino acids. The results of phylogenetic analysis of amino acids predicted sequences showed that R protein and the pepper NBS-LRR protein were divided into two groups, namely TNL-NBS and CNL-NBS. All fragments obtained from this study along with proteins in other *Solanaceae* families and monocotyledons plants were as wheat, barley, rice, corn belonged to the CNL-NBS class, while the dicotyledons plants were included in the CNL-NBS class and TNL-NBS class (Figure 3).

Grouping resistance responses

The grouping of resistance responses based on DS value in 28 tested genotypes showed an even distribution from resistant to very susceptible group (Figure 4). Based on the disease severity (DS) value, the two genotypes namely, IPB C12 and F4-012328-6-3 could be classified as resistant (0 < DS < 20%) had 18.33% and 19.44% disease severity, respectively. There were 13 resistant genotypes (20 <DS <40%), five susceptible genotypes (40 <DS <60%), and eight very susceptible genotypes (DS> 60%).

Table 3. Genotype influence on incubation time, disease incident (DI) and disease severity (DS)

Genotypes	Incubation period (day)	DI (%)	DS (%)	Genotypes	Incubation period (day)	DI (%)	DS (%)
IPBC2	9-19	93.33 a**)	62.50 abc	F4-012328-1AB-2	11-28	40.00 cde	27.77 d
IPBC5	5-18	86.67 a	62.77 abc	F4-012328-6-3	11-28	33.33 de	19.44 d
IPBC12	12-28	20.00 e	18.33 d	F3-74 x Hot Chilli	9-19	73.33 ab	58.89 abc
Gada	10-26	56.67 bc	32.78 d	F3-Elegance x 74-2	9-26	46.67 cd	26.66 d
Bara	6-22	90.00 a	54.16 bc	F3-74 x Biola	11-28	40.00 cde	28.89 d
Genie	10-27	43.33 cd	31.66 d	F7-160291-4-13-9-8-1	11-25	36.67 cde	35.00 d
Giant	10-27	43.33 cd	31.66 d	F7-145293-19-8-3-113-1	10-24	40.00 cde	28.89 d
Ayesha	11-25	46.67 cd	28.33 d	F7-145174-9-3-1-5-3	10-28	36.67 cde	28.33 d
Seloka	10-27	50.00 cd	31.11 d	F7-145291-14-9-3-12-1	8-20	86.67 a	63.89 abc
Yuni	7-19	73.33 ab	62.22 abc	F7-145291-10-7-1-1-1-1	8-18	73.33 ab	51.66 c
SSP	6-16	83.33 a	57.50 abc	F5-145291-14-9-3	7-20	80.00 a	53.89 bc
Anies	11-26	50.00 cd	29.16 d	F7-1200005-141-16-2-9-4	6-19	90.00 a	72.78 a
Ungara	10-25	46.67 cd	33.33 d	F7-1200005-120-7-1-7-8	6-15	83.33 a	61.66 abc
Seroja	10-20	90.00 a	71.11 ab	F7-1200005-141-16-3-5-4	6-14	86.67 a	60.00 abc

Note: **) Different numbers followed by the letter reveals significant differences (Duncan test with the level of 5 %).

Table 4. Sequence identity among predicted amino acid sequence on pepper sample and resistance protein deposited from GenBank

NBS fragment	Genotype origin	Accession on GenBank				
		Pvr9-like protein 2 [*C. annuum*]	Begomovirus resistance protein [*S. lycopersicum*]	White fly resistance protein Mi-1,2 [*S. peruvianum*]	Resistance protein RGA13 [*C. annuum*]	TRGA15 [*C. annuum*]
CARGA1	IPB C12	60.34%	58.19%	72.41%	65.66%	64.88%
CARGA2	Gada	81.71%	53.30%	67.43%	69.28%	69.64%
CARGA3	Genie	67.61%	52.51%	71.02%	87.43%	89.88%
CARGA4	Giant	66.48%	53.04%	72.73%	87.43%	89.82%
CARGA5	Anies	66.47%	51.18%	71.86%	85.12%	83.33%
CARGA6	Ayesha	83.53%	50.00%	66.47%	63.86%	65.06%
CARGA7	Seloka	87.57%	47.51%	71.19%	71.08%	72.29%
CARGA8	Ungara	79.44%	50.81%	72.22%	77.11%	77.11%
CARGA9	F4-012328-1AB-2	61.90%	61.18%	60.12%	70.83%	69.64%
CARGA10	F4-012328-6-3	61.40%	60.11%	68.05%	76.65%	74.40%
CARGA11	F3-Elegance x 74-2	55.03%	56.40%	58.58%	70.00%	67.65%
CARGA12	F3-74 x Biola	58.43%	59.02%	64.20%	74.42%	73.84%
CARGA13	F7-160291-4-13-9-8-1	76.74%	66.27%	63.37%	65.06%	66.27%
CARGA14	F7-145293-19-8-3-113-1	92.05%	41.77%	71.59%	73.49%	74.70%
CARGA15	F7-145174-9-3-1-5-3	86.93%	33.79%	72.73%	73.49%	73.81%

Table 5. Homology (%) among the predicted amino acid sequence of pepper samples

CARGA1	100													
CARGA2	68.0	100												
CARGA3	69.5	70.9	100											
CARGA4	65.3	69.5	87.5	100										
CARGA5	73.7	64.7	80.4	82.2										
CARGA6	61.1	82.1	65.9	63.7	100									
CARGA7	61.7	72.0	64.2	65.7	72.3	100								
CARGA8	78.5	78.3	71.3	71.4	69.8	78.5	100							
CARGA9	59.9	60.9	61.9	68.9	50.0	60.0	71.3	100						
CARGA10	60.4	63.4	65.8	71.9	53.7	60.4	68.9	74.4	100					
CARGA11	57.7	52.7	62.9	60.0	44.4	57.7	67.3	70.2	63.5	100				
CARGA12	63.4	69.7	75.6	78.6	60.8	63.4	64.2	56.5	59.5	57.6	100			
CARGA13	80.8	69.4	63.9	60.5	64.1	80.8	74.7	51.2	62.6	60.4	66.7	100		
CARGA14	84.2	80.0	69.0	69.7	77.4	84.2	79.2	60.7	61.9	57.7	61.3	76.7	100	
CARGA15	84.1	79.4	69.7	68.2	73.8	84.1	81.1	56.5	65.3	56.8	64.4	82.6	85.1	100

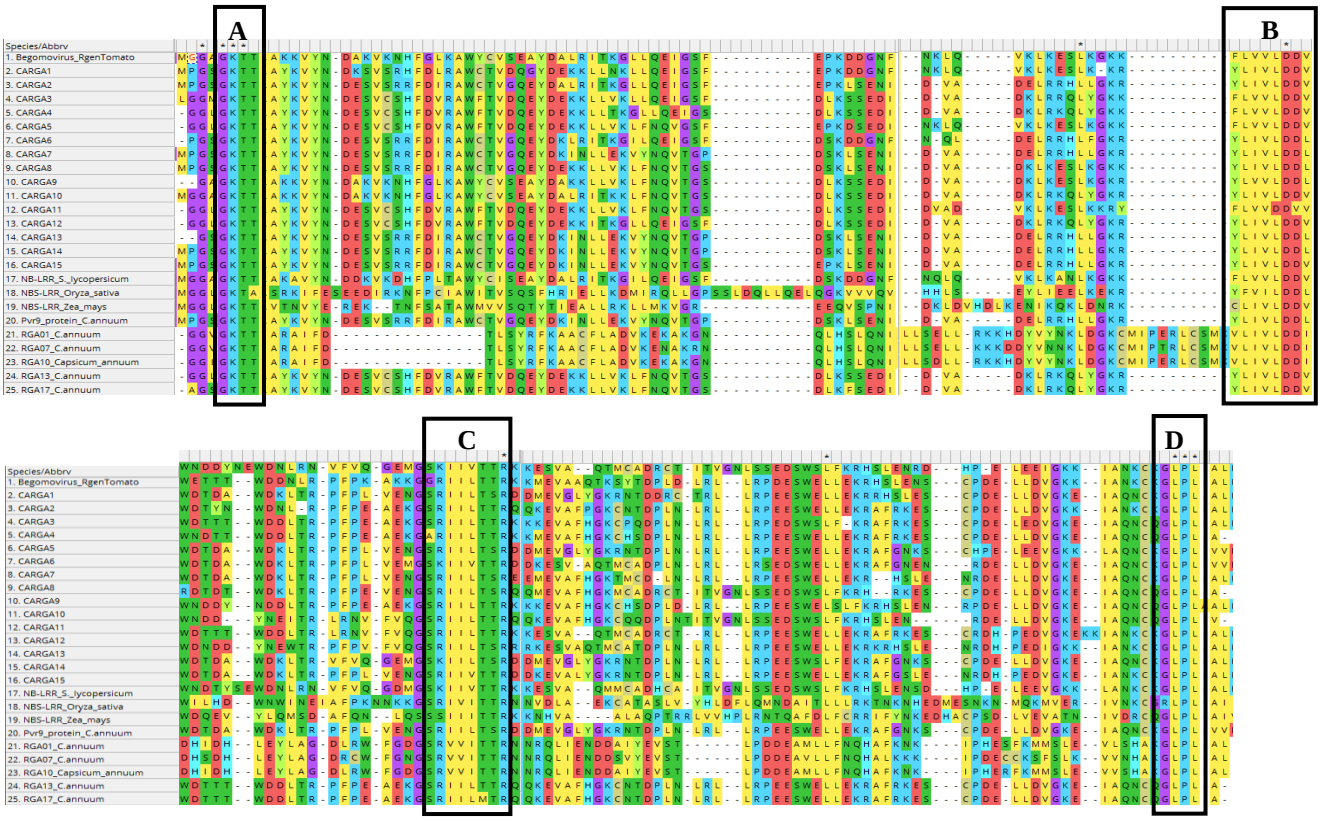

Figure 2. Alignment analysis of predicted amino acid (CARGA1-CARGA15) with several Solanaceae proteins on NBS site and R gene of other plans deposited on Genbank NCBI by MUSCLE method. Conserved domain areas are marked with bold line upon the sequence (A= Ploop or kinase-1, B=kinase-2, C=RNBSB/Kinase-3a, and D= hydrophobic domain or GLPL).

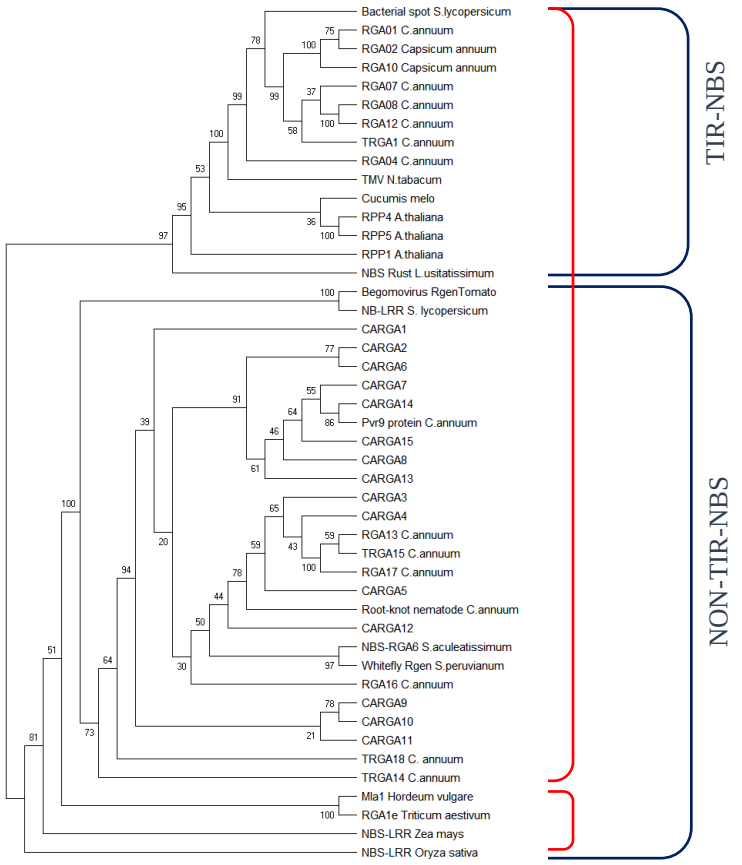

Figure 3. Phylogenetic tree of predicted amino acid sequences from pepper RGA (CARGA1-CARGA15) and several proteins from other plants based on MEGA X analysis and clustering due to NJ method by bootstrap 1000. M, Monocotyledons; D, Dicotyledons

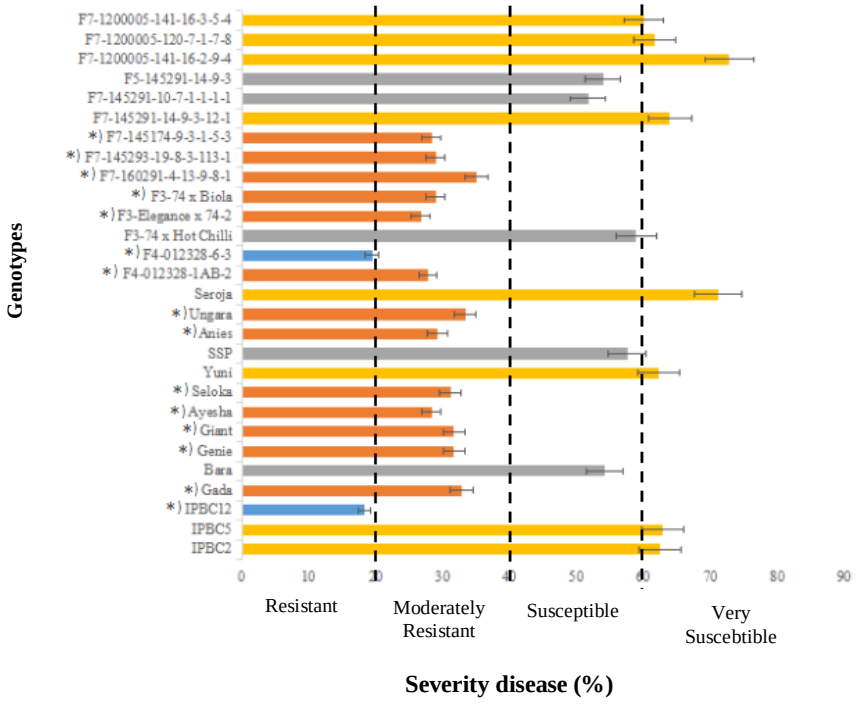

Figure 4. Grouping of resistance response to yellow leaf curl disease on 28 genotypes of pepper. The "*" marks the genotype that has the RGA fragment

Discussion

The symptoms of pepper yellow leaf curl disease found in greenhouses are varied. Several factors that influence the variation of symptoms are pepper genotype, viral virulence, and environmental conditions, especially light intensity (Kenyon et al. 2014). All these environmental factors were also included in the present study. Thus, the varied results indicate high variability in the level of resistance to yellow leaf curl disease among the genotypes tested. The large variation among genotypes indicates the possibility of selection for resistance to this disease. Plants have succeeded in developing mechanisms for the recognition and protection of pathogens. Interactions between host and pathogen will trigger local and systemic responses including hypersensitive response (Balint-Kurti 2019). The NBS and LRR domains are generally associated with regulating the activity of the R-protein (Kourelis and Van der Hoorn 2018; Wang et al. 2015) which is the first defense of plant against attacks from pathogens.

In this study, 19 specific DNA fragments were successfully amplified from 28 samples of pepper DNA. From the 19 fragments, there were 15 fragments encoded by CARGA1-CARGA15 containing NBS areas with uninterrupted ORF. The results showed that there were 4 conservative structures of the NBS region. The four conservative structures of amino acids were the P-loop/kinase-1a (GKTT), kinase-2 (LVVLDDV), RNBS-B/kinase-3a (IILTTR), and hydrophobic amino acids (GLPL) (Tan et al. 2012). Nevertheless, not all motifs are required for function, such as rice Pb1 CNL protein, which lacks P-loop (Hayashi et al. 2010). The LRR domain protein works as detector against invading pathogens, either by direct interaction with the effectors released from pathogens, or by monitoring the status of host proteins targeted by the effectors. Due to recognition, the NBS domain would undergo conformational shift, ADP-bound state to an open ATP-bound state with exposed N-terminal domains to trigger hypersensitive reactions, finally causing apoptosis of infected cells to suppress the spread and proliferation of pathogen (Andersen et al. 2018).

The motif of kinase-1a/Ploop (GKTT), formed by several glycine residues (G) and a fixed residue of lysine (K), is involved in the phosphate binding of nucleotides. These motifs play a role in binding ATP to I-2 and M1 proteins from potato plants (Tameling et al. 2002). P-loop is also responsible in ATP-binding proteins involved with an ATP synthase ras protein, β subunit, adenylate kinase with molecular switches and ribosomal elongation factor (Bahramnejad 2014). The same thing might also happen to the NBS protein. Motif kinase-2 (LVVLDDV) contains a fixed residue of aspartate (D) which coordinates the Mg^{2+} ions needed for the phosphotransferase reaction. Kinase-3a/RNBSB (IILTTR) generally contains tyrosine (Y) or arginine (R) which interacts with purine bases from ATP, while internal hydrophobic domains that have conserved motifs for GLPL has unknown function. However, seeing that its existence is highly conserved in the NBS-LRR class of the R-gene sequence, it is assumed that the domain has an important function related to the activity of the gene concerned (Jones et al. 2006). NBS-LRR are divided into two main subclasses, as CNL and TNL, and the distribution of NBS-LRR into these two subgroups is not comparable across different plant species (Jupe et al. 2012; Kang et al. 2012; Wan et al. 2013). CNL and TNL proteins are responsible for the recognition of specific pathogen (Collier et al. 2011). All fragments obtained from this study belong to the CNL. The absence of TNL not only occurs in pepper, but also has been reported in other monocotyledons plants like rice, wheat, maize, sorghum, and sugarcane (Jiang et al. 2020; Xu et al. 2018; Zhang et al. 2011). CARGA1 - CARGA15 fragments have high identical similarities with 5 types of proteins available in Genebank, namely *whitefly resistance protein Mi-1,2* (*Solanum peruvianum*), *Begomovirus resistance protein* (*Solanum lycopersicum*), *Pvr9-like protein 2* (*Capsicum annuum*), *TRGA15* (*Capsicum annuum*), and *Resistance protein RGA13* (*Capsicum annuum*) (Table 4). The five proteins encode successive resistance to whiteflies, begomovirus, potyvirus, late blight, and root-node nematodes. R proteins form a subclass of the signal transduction ATPases with numerous domains super family, class of molecular switch that are involved in various mechanisms, including immunity, apoptosis, and transcriptional regulation (Takken et al. 2012). This indicates that the 15 isolated NBS fragments may act as a resistance gene against some pests and diseases.

Information on grouping resistance responses is very important in plant breeding programs to determine the inheritance pattern of a trait. One of the assumptions that must be fulfilled to determine the inheritance pattern of a trait is that genes are more evenly spread among elders. 15 genotypes that have RGA fragments belong to the resistant and moderately resistant group, although they have a sequence identity for *Begomovirus resistance protein* (*Solanum lycopersicum*) ranging from only 33.79% - 66.27%, and they also have a sequence similarity of 58.58% - 72.73% against *whitefly resistance protein Mi-1,2* (*Solanum peruvianum*) (Figure 4). It can be expected that whitefly and virus resistance interaction is highly linked (Zaidi et al. 2017).

Mi-1,2 protein provides resistance against phloem-feeding organisms such as whiteflies, aphids, psyllids and root-knot nematodes (Pallipparambil et al. 2014; Chen et al. 2015). Several possibilities have been hypothesized for the way *Mi-1,2 protein* confers resistance in tomato, but the actual mechanisms remain unclear. *Mi-1,2* transcripts are present before insect attacks and resistance factors in *Mi-1,2* are detected by *B. tabaci* early during the stylet penetration pathway, indicating that those factors might be present in the leaf epidermis, mesophyll layers, or both (Jiang et al. 2001). Those factors prevent the whiteflies from reaching phloem sieve elements. Nevertheless, once the stylets reach a sieve element, whiteflies behavior did not diverge between plants with or without the gene, thus, phloem appears to be equally acceptable to the whiteflies.

The interaction of *Begomovirus resistance protein* and *whitefly resistance protein Mi-1,2* is thought to activate the mechanism of plant resistance to yellow leaf curl infection in pepper, thus providing greater resistance compared to genotypes which do not have RGA. Based on the results of

the present study, it is known that genotypes, namely IPB C12 and F4-012328-6-3 that contain RGA fragments have the potential to be resistant to PYLCD. Exploration related to RGA especially from various types of pepper genotypes in Indonesia, needs to be further developed. Several previous studies have succeeded in amplifying some RGA from pepper and their relatives (Wan et al. 2012; Naresh et al. 2017; Acquardo et al. 2020). The number of RGA with NBS-LRR from the identified pepper would be able to give an idea of the magnitude of the NBS family in pepper plants that can be exploited further in breeding programs.

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