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The use of effector gene based-markers to facilitate identification of *Fusarium* sp. infected shallot in Java, Indonesia

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Abstract. *Herlina L, Istiaji B. 2020. The use of effector gene based-markers to facilitate identification of Fusarium sp. infected shallot in Java, Indonesia. Biodiversitas 21: 4677-4685.* One of the most important diseases and become challenge in breeding resistant variety of shallots in Indonesia is *Fusarium* disease, caused by *Fusarium oxysporum* f.sp. *cepae* (*FOC*). To discriminate the *F. oxysporum* into forma speciales is uneasy, often managed through laborious and time-intensive disease assays., therefore molecular approaches become the most relevant choice. *FOCs* from Indonesia were examined by molecular criteria based on putative and effector genes-based markers, i.e.: *SIX*-genes, *C5*-gene, and *CRX1-2*-genes. The alignment analysis discovered some regions which sequences highly contained conserved bases, while amplification result bands vary in size, between 400 - 500 bp. The combined of 7 *SIX*-genes primers and 3 effector primers (*C5* and *CRX1-2)* used in the clustering analysis of 15 *FOC* isolates in this study showed that they succeeded in separating 15 *FOC* isolates into 4 groups through NTSys. Clustering analysis showed that those markers succeeded in grouping 15 FOC isolates into different clades (by coefficient of similarity: 0.69). Phylogenetic analysis based on *CRX* genes sequence as putative effector genes confirmed that *CRX1* and *CRX2* genes were able to classify the FOC into their forma speciales. Those effectors genes are potential to serve as marker-templates to facilitate identification of *FOC* which infested shallots in Indonesia.

Keywords: *CRX* genes, effector, *FOC*, *Fusarium*, phylogenetic analysis, *SIX* genes

Abbreviations: FOC: *Fusarium oxysporum* f.sp. *cepae*, SIX: Secreted In Xylem, CRX: C-terminal RXLR, VCG: Vegetative Compatible Group, SAHN: Sequential Agglomerative Hierarchical Nested Cluster Analysis

INTRODUCTION

Fusarium oxysporum f.sp. *cepae* (FOC), the foremost important disease of shallots, is reported as a serious threat to the assembly of shallots in our country which causes loss of up 50% to 100% (Wiyatiningsih 2003; Udiarto et al. 2005, Herlina et al. 2019). Pathogens infect the basis or basal bulb plate, and further infections usually occur at the top of the season, resulting in severe losses in postharvest (Rabiei-Motlagh et.al. 2010). Control of this disease must be administered comprehensively. Unfortunately, none of the resistant variety to the disease has been produced. The assembly of a resistant variety of shallot is seemingly constrained by the shortage of resistance germplasm resources, causing the difficulty of valid detection of FOC. Therefore, creating breakthroughs in identifying pathogenic FOCs quicker but accurate is urgently needed.

Information about the pathogenicity genes related to the *F. oxysporum* is extremely useful for better understanding the complexity of *F. oxysporum* and for further considering in engineering resistant variety. However, identification of species Fusarium still relies heavily on phenotypic characteristics, through the diagnosis of the symptomatic microscopic tissue, followed by identification of pathogens supported morphological and biochemical characters. This really requires special expertise and still produce an opportunity for error (Singha et al. 2016). Moreover, to discriminate of *F. oxysporum* forma speciales is uneasy, and sometimes managed through laborious and time-intensive disease assays, but inadequate and loaded with various limitations (Recorbet et al. 2003; Covey et al. 2014; Lievens et al. 2009). Molecular detection approaches are therefore highly desired.

The use of virulence genes to spot fungal plant pathogens has proven successful within the past for other Fusarium species (Hogg et al. 2007; Mbofung et al. 2011). Pathogenic strains of *Fusarium oxysporum* produce effectors referred to as SIX (Secreted In Xylem), they are detectable within the xylem sap of tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici* (FOL). These genes encode for little secreted proteins, most of them are cysteine-rich. The effector gene (SIX1) is required during the first stages of infection to induce root penetration and continues through the invasion of xylem vessels but is not detected upon plant necrobiosis (Maldonado et al. 2018). *SIX* genes are absent in non-pathogens strains of *Fusarium oxysporum*, and each forma specialis features a particular array of *SIX* genes. A selective pressure given by the host might promote the adoption of a specific group of secreted protein genes that gives *SIX* loci of various isolates (Maldonado et al. 2018). So far, there are 14 *SIX* genes reported (*SIX1* to *SIX14*). However, the confirmation of their role in pathogenicity has been only demonstrated for *SIX1* in *FOL* and *Fusarium oxysporum* f.sp. *conglutinans*

(FOCG) and *SIX3*, *SIX4*, *SIX5*, and *SIX6* in Fol (Maldonado et al. 2018).

C5, *CRX1*, and *CRX2* genes are novel effectors which first reported by Taylor et al. (2016). *C5* has no homology to any sequenced gene, which can be *FOC* specific. *CRX1* and *CRX2* contained RxLR domains, which are shown to facilitate entry into host cells (Kale 2012). Putative RxLR effectors have also been reported in *FOL* (Ma et al. 2013). *CRX2* only had a partial association with pathogenicity in FOC, but had very close homologs in *F. oxysporum* f.sp. *radicis-lycopersici*, *F. proliferatum*, and *F. redolens* (Taylor et al. 2016).

Based on *SIX*, *C5*, and *CRX* genes sequences obtained from the NCBI database, we developed primers to spot the existence of these effector genes partial sequences from 15 *FOC* isolates collected from Java island and conducted a phylogenetic analysis to reveal the clustering of *FOC* isolates during this study.

MATERIALS AND METHODS

Study area

The study was conducted at the greenhouse and microbiology laboratory of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor, Indonesia (6º34'31.58"S; 106º47'07.37"T, 221 m above sea level), in 2017.

Genomic DNA extraction

In order to obtain DNA from each of the identified *FOC*, single-spore from fifteen isolates of selected *FOC* (Table 1) were grown for five days at 25°C in Potato Dextrose Agar (PDA) (Difco). Mycelium (~0.1-0.2 g) was collected using sterile scalpel from PDA media and placed in Eppendorf tubes. Cultured cells were opened by adding 500 µL of CTAB extraction buffer (100 mM Tris HCl (pH 8), 2% (wt/v) CTAB, 50 mM EDTA, 0.7 M NaCl, 0.17% (v/v) β-mercaptoethanol and 1% (w/v) PVP), pre-warmed to 65°C, two glass beads added and the mixture placed in miller at a frequency of 30 sec for 5 min. Samples were incubated at 65°C for 30 min in a water bath, then extracted with phenol/chloroform/ isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). DNA was then precipitated by adding two volumes of absolute ethanol and pelleted by centrifugation for 15 min at 15,000 g. The pellet was washed with 70% ethanol, air-dried, and resuspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). RNA was degraded by treatment with RNase A (50 mg.ml^{-1}) for 30 min at 37°C. DNA concentration and purity were measured using NanoDrop machine.

Amplification of genomic DNA using specific primers

PCR amplification was carried out for 15 *Fusarium oxysporum* isolates using published-primers (Table 2). These primers were chosen because they have been confirmed to be used as markers that able to differentiate *Fusarium oxysporum* isolates into their forma speciales. The amplification reactions were performed in 25 µL volumes in thin-walled PCR tubes after optimization in a PTC-100 (Programmable Thermal Controller), programmed for an initial cycle of 1 min at 95°C, 5 min at 95°C, annealing at 58°C and extension 1 min at 72°C, followed by 34 cycles of 5 min at 95°C, annealing at 58°C and extension 1 min at 72°C. There was a final extension step of 5 min at 72°C followed by cooling to 4°C until samples were recovered. Amplified products were analyzed on a 1.5% agarose gel in 1X TAE buffer (40 mM Tris-acetate and 1.0 mM EDTA) and documented using Bio-Rad documentation system. PCR results were confirmed as 'positive' (having an effector gene from *FOC*) if specific DNA bands were obtained according to the markers size. Phylogenetic analysis was carried out using the Neighbour Joining method (using NTSys. 2.02) based on the genotyping data.

Sequencing the CRX2 gene and phylogenetic analysis

For sequencing, three selected PCR amplicons sized of 400-1000 bp generating from amplification of isolate *FOC-1, FOC-2,* and *FOC-3* with *CRX2* and *CRX1* primers were extracted and purified using a QIA quick PCR Purification Kit (Qiagen). The PCR products were sequenced for the DNA region coding for the *CRX2* and *CRX1* genes using the BigDye terminator Cycle Sequencer (ABI, Foster City, CA). Custal W method (Thompson et al. 1994) was used for aligning sequences and the result was performed using MView 1.63. The sequences were compared with closest matches in GenBank database through BLAST (www.ncbi.nlm.nih.gov/BLAST) to confirm the identification of effector genes of *CRX*. Phylogenetic tree was build using MEGA version 7 (Tamura et al. 2007) from the partial sequences of the *CRX2* and *CRX1* genes from this study and other sequences of *CRX* genes as reference from NCBI database. Clustering analysis carried on using UPGMB clustering method with Kmer4_6 distance method, running on MUSCLE version 3.8.31. Bootstrap value was set at 1000 replicates.

RESULTS AND DISCUSSION

CRX genes as putative effector in the *FOC*

Fusarium sequences obtained from amplification of *CRX* genes-primer were compared with *CRX* genes sequences from National Center for Biotechnology Information (NCBI) database using BLAST 2.0 (http://www.ncbi.nlm.nih.gov/BLAST) and the result as showed in Table 3. Based on the results of the blast, of the 100 accessions nominated in the analysis results, only five accessions were taken with percent identity in the range of 68 to 70% to be reported, and will be used in phylogenetic analysis. In this case, the interesting point is of the five accessions, four of them constitute the *CRX2* gene sequence while the *CRX1* sequence has only one accession, namely KP965011.1. Those *CRX* gene sequences were then used in sequence analysis. The percentage of identity that does not reach the 90% level is most likely due to the limited number of sequences that can be explored as a reference in the database against this consideration, so the

percent identity that is close to 70 is considered sufficient to insert the tested sequence into the *CRX* gene identity.

The *CRX* sequence of *FOC* isolates was aligned with the consensus region using to CLUSTAL W Omega Program, with 1000 bootstrap replicates were performed (Figure 1). The results of sequence alignment showed that consensus percentages were in the range of 70 to 100%, wherein the regions of 801 - 880 bp, 1041-1120 bp, and 1121 - 1200 bp all the sequences analyzed have the highest number of conserved bases compared to other regions (indicated by blue color box).

The genetic distance between *CRX1* and *CRX2* genes

Phylogenetic analysis applied to the sequence *CRX* genes from *FOC* isolates combined with 10 sequences of *CRX* genes from other *Fusarium* species taken from NCBI database were formed three clusters (Figure 2.), suggested the existence of variability in *CRX* genes of *Fusarium* species. Genetic distance is indicated by numbers that follow the name of the sequence listed in the phylogenetic tree. Based on these cladograms, it can be seen that F.ox_fsp_cepae-3_*CRX1* and F.ox_fsp_cepae-2_*CRX1* are in separate clades separated from the *CRX1* or *CRX2* gene sequences from Fusarium of other species used as a comparison. And interestingly, only Fox.fsp_cepae-1_*CRX2* is in the same clade as the *CRX2* gene from other *Fusarium* species. Thus, the *CRX1* gene in both the F.ox_fsp_cepae-3 and F.ox_fsp_cepae-2 isolates may be specific effector for this *FOC* strain (Figure 2).

Table 1. List of isolate of *Fusarium* spp. collected in this study

Species	Isolate code	Isolate originated (material or part of plant)	Host originated (scientific name)	Host variety name/site of collection
Fusarium oxysporum f.sp. *cepae*	F.sp.-1*	Culture collection	*Allium cepa*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-2*	Culture collection	*Allium cepa*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-3*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-4*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-5*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-6*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-7*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-8*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-9*	Shallot bulb	*Allium cepa*	Demak/ Central Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-10*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-11*	Shallot bulb	*Allium cepa*	Demak/ Central Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-12*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-13*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-14*	Scallion leave	*Allium sativum*	Unknown/Bogor, West Java
Fusarium oxysporum f.sp. *cepae*	F.sp.-15*	Shallot bulb	*Allium cepa*	Biru lancor/East Java

Note: Isolates which code followed by marked (*) are subject to use in molecular analysis

Table 2. Primers of *SIX* genes, *CRX1*, *CRX2* and *C5* genes

Primers	Sequence 5' – 3' (forward primer to reverse primer)	Annealing temp. (°C)	Reference
SIX3	CCAGCCAGAAGGCCAGTTT/GGCAATTAACCACTCTGCC	51.1	Taylor et al. (2016); this study
SIX5	ACACGCTCTACTACTCTTCA/GAAAACCTCAACGCGGCCAAA	49.65	Taylor et al. (2016); this study
SIX7	CACTTTTTCGCCGACTTGGT/CTTAGCACCCTTGAGTAACT	48.65	Taylor et al. (2016); this study
SIX9	GGCCCAGCCCTAGTCTAACTCC/AACTTAACATGCTGGCCGTCAATCG	53.3	Taylor et al. (2016); this study
SIX10	GTTAGCAACTGCGAGACACTAGAA/AGCAACTTCCTTCCTCTTACTAGC	51.5	Taylor et al. (2016); this study
SIX12	CTAACGAAGTGAAAAGAAGTCCTC/GCCTCGCTGGCAAGTATTTGTT	50.9	Taylor et al. (2016); this study
SIX14	ACAACACCGCGACGCTAAAAAT/GCACACTCAGTGCGACAAGTTC	55.65	Taylor et al. (2016); this study
C5	AGAGTGTGAAGTGAGGACGAGGGA/CTACGTTCGCCTCACTCATTGCCT	56.5	Taylor et al. (2016); this study
CRX1	CACCATCTGTCTACATAAGGCCGCCC/AAAGTTCAAGGACCGGACCGCCG	58.35	Taylor et al. (2016); this study
CRX2	TTAGTCGCACATCTACCATCACTG/GGAGTCGATCTAACTTCAGG	49.15	Taylor et al. (2016); this study

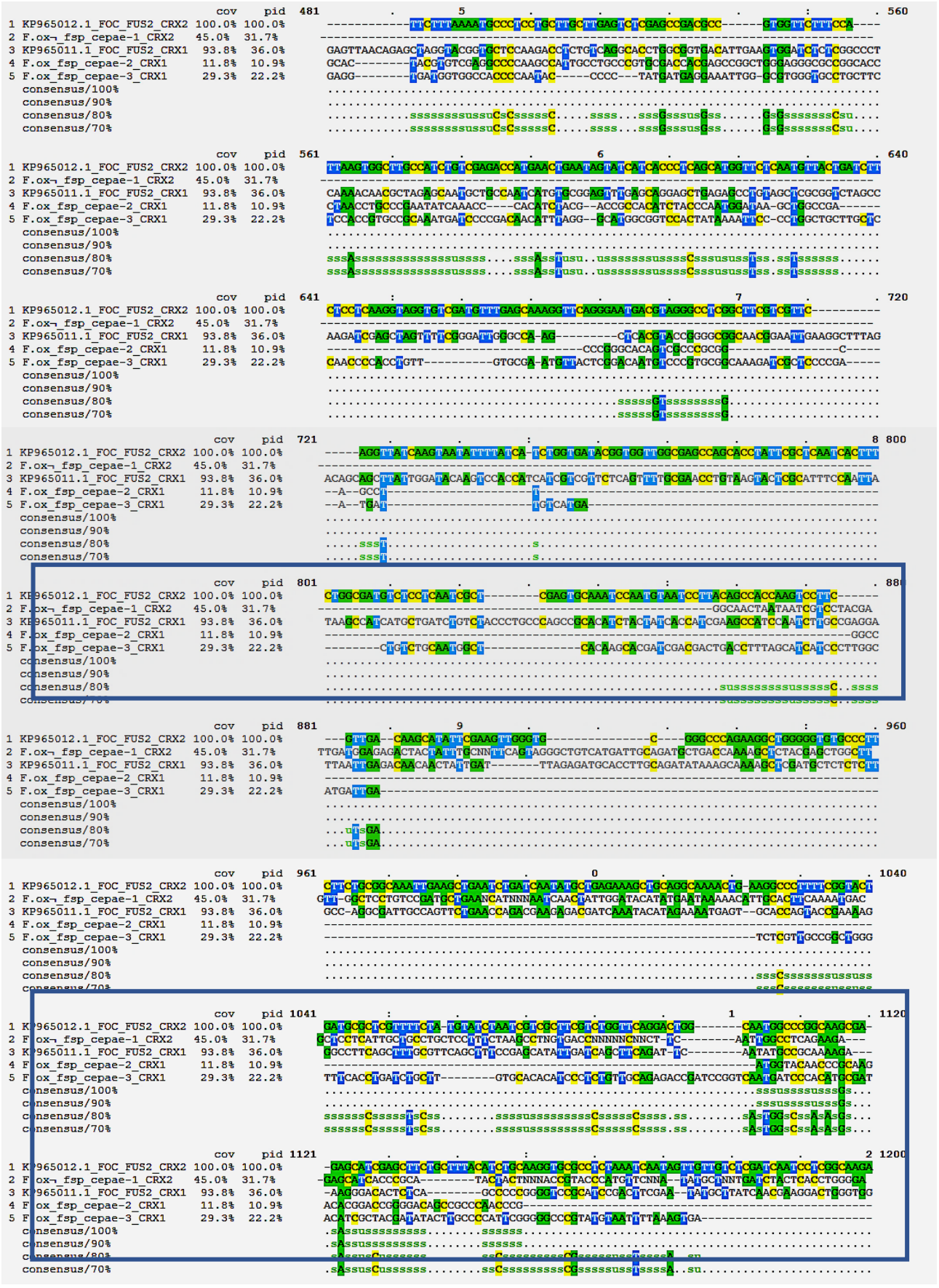

Figure 1. Part of sequence alignment of CRX gene of FOC isolates using MView 1.63

Table 3. Description of blast'n' result from sequence of DNA of *FOC* isolates which amplified with *CRX* primers

Accession	Description	Max score	Total score	Query Cover	E value	Per. ident
KP965012.1	*Fusarium oxysporum* f.sp. *cepae* isolate FUS2 putative pathogenicity protein *CRX2* gene, complete cds	176	231	60%	2e-42	70.32%
KP965015.1	*Fusarium oxysporum* isolate G12 putative pathogenicity protein *CRX2* gene, partial cds	168	214	60%	9e-40	69.50%
KP965014.1	*Fusarium oxysporum* isolate A28 putative pathogenicity protein *CRX2* gene, partial cds	167	222	60%	9e-40	69.83%
KP965016.1	*Fusarium oxysporum* isolate PG putative pathogenicity protein *CRX2* gene, partial cds	138	181	54%	5e-31	68.25%
KP965017.1	*Fusarium oxysporum* f.sp. *pisi* isolate FOP2 putative pathogenicity protein *CRX2* gene, partial cds	114	114	38%	5e-24	69.37%
KP965011.1	*Fusarium oxysporum* f.sp. *cepae* isolate FUS2 putative pathogenicity protein *CRX1* gene, complete cds	77.0	77.0	30%	1e-12	68.70%

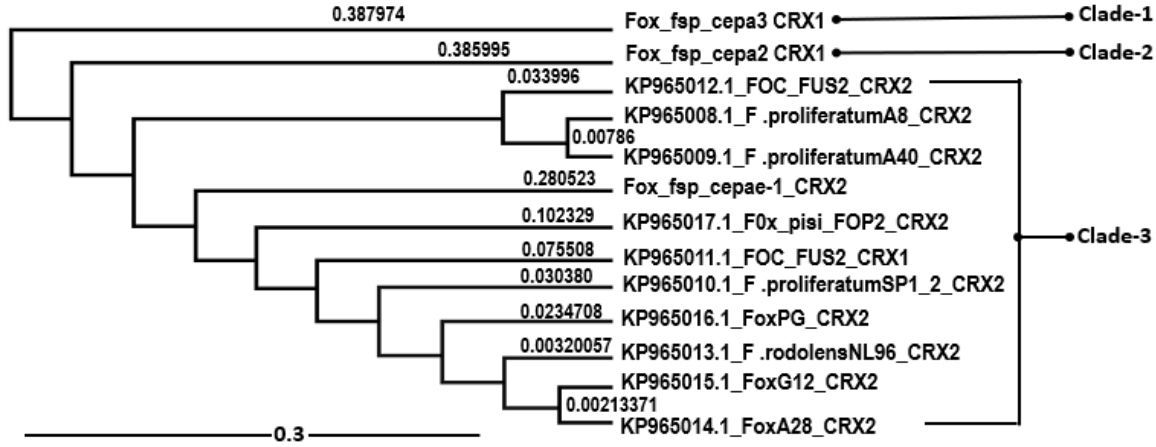

Figure 2. Phylogenetic tree constructed from unambiguously aligned DNA sequences *of CRX1 and CRX2 genes* as produced by NJ

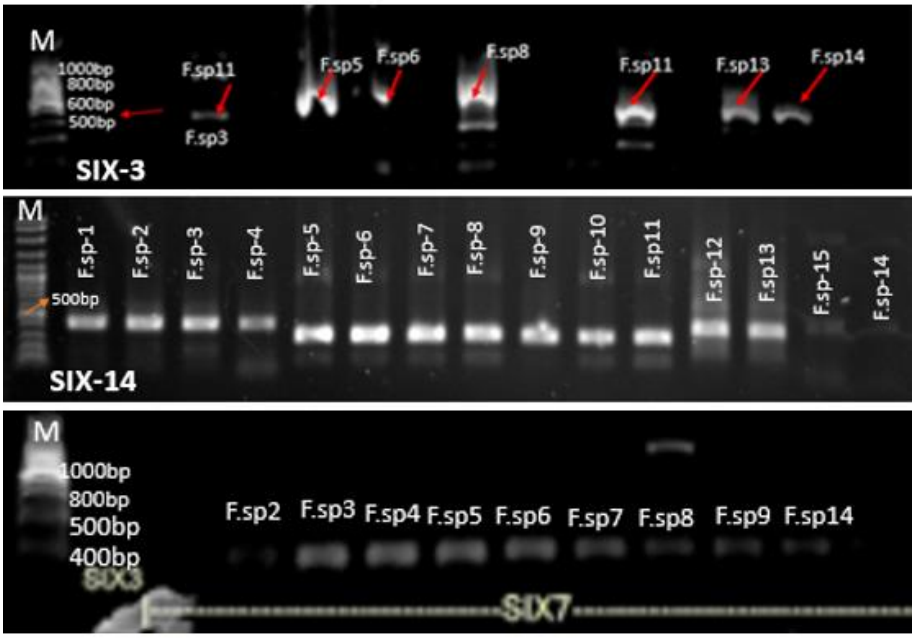

Figure 3. Amplification of genomic DNA of several *FOC* isolates using *SIX3, SIX14,* and *SIX7* genes based markers

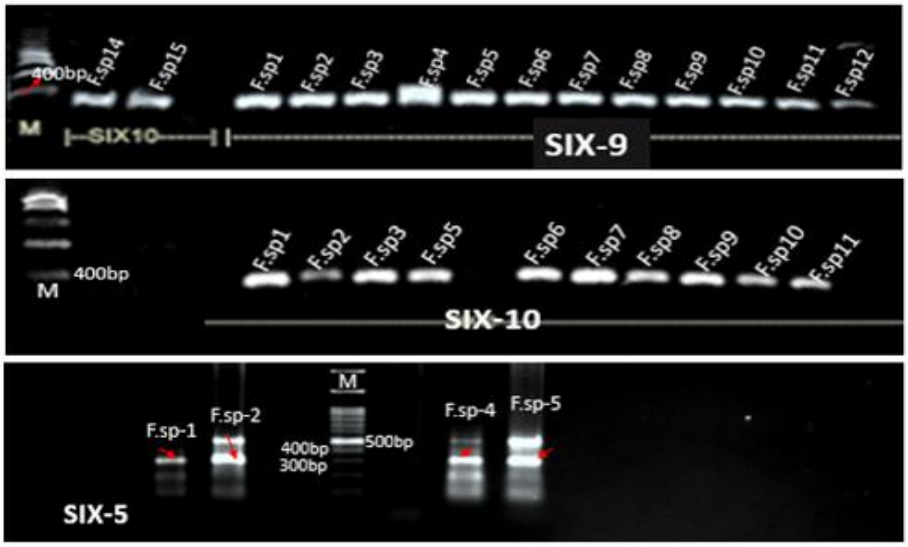

Figure 4. Amplification of genomic DNA of several *FOC* isolates using *SIX9, SIX10,* and *SIX5* genes based markers

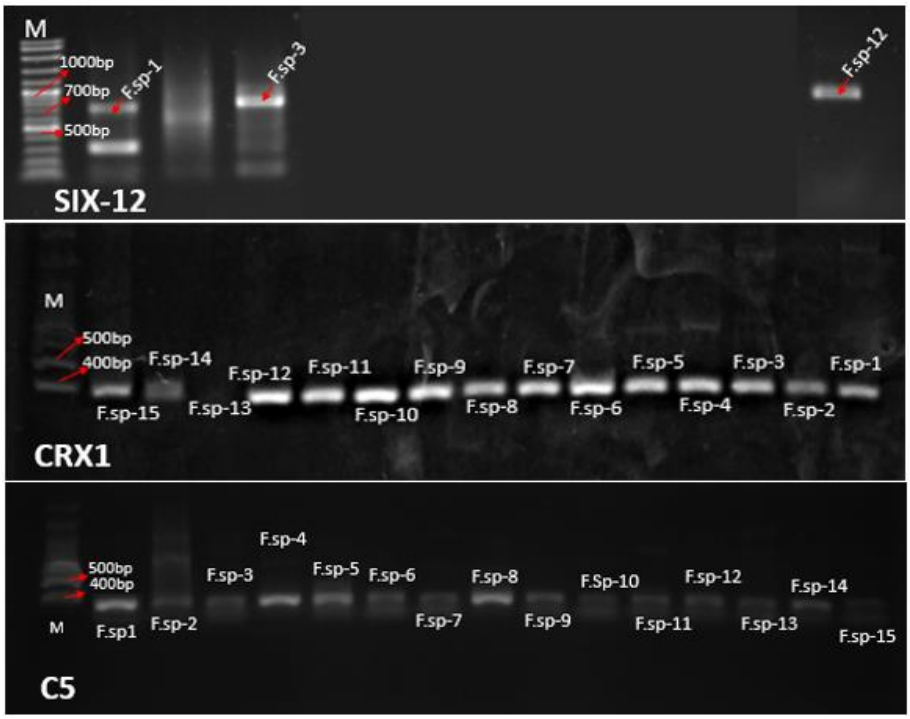

Figure 5. Amplification of genomic DNA of several *FOC* isolates using *SIX12, CRX1, CRX2, and C5* genes based markers

Variability of SIX genes and effector genes of FOC

A total of 10 effector genes (7 *SIX* genes, 2 *CRX* genes, and *CR* gene) based primers were used for amplified the genomic DNA of 15 *Fusarium oxysporum* isolates from infected shallots in -Java. The amplification results bands which vary in size, between 400 - 500 bp. (Figures 3, 4 and 5). This shows the high variability of *SIX* genes and effector genes in the *Fusarium* isolates tested, moreover the result was supported with genotyping analysis based on the *SIX* genes and *CRX* genes-based markers (Table 4). The combined of 7 *SIX*-genes primers and 3 effector primers (*C5* and *CRX1-2)* used in the clustering analysis of 15 *FOC* isolates in this study showed that they succeeded in separating 15 *FOC* isolates into 4 groups through NTSys using UPGMA method by coefficient value 0.69. It was interesting that the isolate of *FOC* derived from scallion was separated in different clade, i.e., Clade-2, and Clade-3 (Figure 6).

Discussion

There is no single method that worked flawlessly in recognizing species, and molecular data have largely supported previously inferred relationships that were based on the other characters (Geiser et al. 2007). Since its rapid implementation, PCR has also opened up opportunities as an alternative tool for the detection and identification of pathogenic fungi, especially plant-borne pathogens (Kageyama et al. 2003; Vincelli and Tisserat 2008). There are large amounts of information that have been accumulated in databases for several parts of the nuclear ribosomal rRNA repeat (in particular the ITS and 28S) (Singha et al. 2016) but still lack information with the use of effector genes. The effector's role in relation to pathogen studies is becoming increasingly important, along with supporting genomic-based characterization. The effector can stimulate the activation of the immune system contained in its host, among others by changing the structure and function of the host cell, accumulating mutations that stimulate the formation of new effectors by the ability to outwit the R protein system and trigger the host defense mechanisms (Urban et al. 2015; De Wit et al. 2009).

It has recently been shown that host specificity is associated with a series of effector genes contained in the genome strain *F. oxysporum* (van Dam et al. 2016). The presence of polymorphism and the type of effector gene sequence of individuals can be predicted for the range of strain hosts. Therefore this gene forms the strongest basis for discrimination formae speciales in the *F. oxysporum* species complex *(FOSC*) (Lievens and Thomma 2005; Lievens et al. 2009). However, certain effectors may also have no role in fungal-plant pathogen interactions if they share homology with other Fusarium species. The real effector generally does not have a homolog on a species that is very close kinship. Here we confidently applied effector genes from *SIX* genes and putative effector gene from *CRX* genes to provide alternative tools for deeply recognizing *FOC* in particular for their forma specialis.

Several reports indicate that the use of virulence genes to identify pathogenic fungal plants has been shown to be successful in the past for other *Fusarium* species (Hogg et al. 2007). In FOSC, this approach has been applied to distinguish *Fusarium oxysporum* f.sp. tropical race 4 cubense targeting target effector genes (Aguayo et al. 2017). In addition, *Fusarium oxysporum* f.sp. *lycoperici* and *F. oxysporum* f.sp. *cubense* can be distinguished from other formae speciales through the use of PCR primers designed to detect specific gene sequences Secreted In the Xylem (SIX) effector gene sequence (van der Does et al. 2008; Lievens et al. 2009). In this study, no comparative studies were carried out for the *SIX* genes but instead tested the opportunities of *CRX2* genes and *CRX1* genes as effectors, which could be used as a specific species identifier of *FOC*.

This study showed that *CRX1* and *CRX2* genes sequence from the *FOC* isolates tested had a high percentage of consensus when aligned with other *CRX* genes, and also all sequences together shared a high number of conserved bases in some parts of their region (Figure 1). This suggested that the sequences isolating from the *FOC* DNA genome in this study were the *CRX* genes. Based on the phylogenetic analysis of these genes, the *CRX1* gene from KP965011.1FPC_FUS2 grouped on the same cluster with the *CRX2* gene of Fusarium species used in this study. However, F.ox_fsp_cepae-3_CRX1 and F.ox_fsp_cepae-2_CRX1 were in separate clades and also placed in a separate clade from the *CRX1* or *CRX2* gene sequences from other Fusarium species. (Figure 2). Only Fox.fsp_cepae-1_CRX2 is in the same clade as the *CRX2* gene from other Fusarium species. According to van der Does et al. (2008), genes related to virulence tend to be identical among members who have the same polyphyletic forma from *F. oxysporum.* For this reason, they have a predictive value for the host strain range. Moreover, forma specialis marker is basically the smallest set of effector genes that all strains of *forma specialis* may have and do not exist or differ in sequence (at least one set) in all other strains (van Dam et al. 2016). We suggested that the *CRX1* genes in both the F.ox fsp_cepae-3 and F.ox_fsp_cepae-2 isolates may be specific for this *FOC* strain.

Clustering based on *SIX* genes, *CRX* genes, and *C* gene polymorphism classified *Fusarium* isolates tested into 4 clades (Figure 5). It is proven that the *CRX* gene can be used in combination with the *SIX* genes as a marker to classify *Fusarium* species. In study's results, it turns out that the effector-based gene markers (*SIX*, *C5*, and *CRX*), which were refrained on 15 *Fusarium* isolates originating from Java could not classify *FOC* isolates according to province origin. However, these markers can classify isolates based on their host plant species, as shown in Figure 5. Isolates derived from scallions can be grouped separately from shallots. With very limited examples of host plant species, it does indeed make it rather difficult to see the ability of markers to classify isolates based on their host species. However, based on these results, it shows that the *CRX* gene has the potential to be used as a marker for classifying *Fusarium oxysporum* according to its special form. Further confirmation through further research is strongly recommended, including by adding other host plant species, so that validation of this potential effector gene can be proven.

Unlike the *SIX* genes, which have been known to have homologs in the form of other pathogens from *F. oxysporum* (van Dam et al. 2016), there is a lack of information about *CRX2* and *CRX1* genes. For this, the specificity of the marker cannot be evaluated before, and cross-reaction with special formae nontarget is might find (Lievens et al. 2009). The majority of the function of effector genes in pathogenicity and virulence has been investigated through the mechanism of gene disorders, gene knockdown, complement genes, and expression tests. However, in the case of the *CRX* gene, there are no reports about its specific functionality.

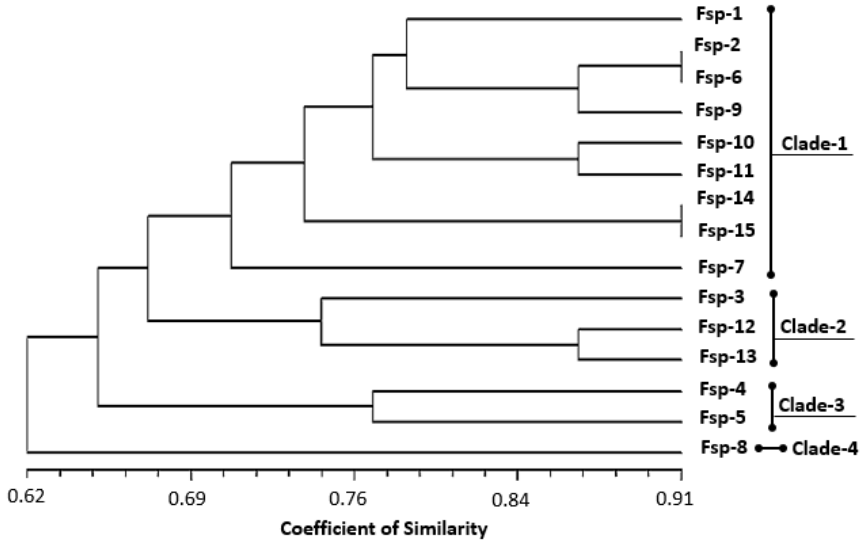

Figure 6. Clustering analysis of *Fusarium oxysporum* isolates based on polymorphisms of *SIX* genes and *CRX* genes sequences based on 71% similarity

Table 4. Distribution of effector gene (*SIX* genes, *C5* and *CRX* genes) in *FOX* isolates

Fusarium species	Host origin	Isolate code	Pathogenicity (+ / -)	*SIX* genes							*C5*	*CRX1*	*CRX2*
				3	5	7	9	10	12	14			
Fusarium oxysporum	Shallot	F.sp.-1	+	-	-	-	+	+	+	+	+	+	+
Fusarium oxysporum	Shallot	F.sp.-2	+	-	-	+	+	+	-	+	+	+	+
Fusarium oxysporum	Scallion	F.sp.-3	+	+	-	+	+	+	+	+	+	+	+
Fusarium oxysporum	Scallion	F.sp.-4	+	-	+	+	+	-	-	+	+	+	+
Fusarium oxysporum	Scallion	F.sp.-5	+	+	+	+	+	+	-	+	+	+	+
Fusarium oxysporum	Shallot	F.sp.-6	+	+	-	+	+	+	-	+	+	+	+
Fusarium oxysporum	Shallot	F.sp.-7	+	-	+	+	+	+	-	+	+	+	+
Fusarium oxysporum	Shallot	F.sp.-8	+	+	+	+	+	+	-	+	+	+	-
Fusarium oxysporum	Shallot	F.sp.-9	+	-	-	+	+	+	-	+	+	+	+
Fusarium oxysporum	Shallot	F.sp.-10	+	-	-	-	+	+	-	+	+	+	-
Fusarium oxysporum	Shallot	F.sp.-11	+	+	-	-	+	+	-	+	+	+	-
Fusarium oxysporum	Shallot	F.sp.-12	+	-	-	-	+	-	+	+	+	+	-
Fusarium oxysporum	Shallot	F.sp.-13	+	+	-	-	+	-	+	+	+	-	-
Fusarium oxysporum	Shallot	F.sp.-14	+	+	-	+	-	+	-	-	+	+	-
Fusarium oxysporum	Shallot	F.sp.-15	+	-	-	-	+	+	-	+	+	+	-

So far, research on how abundant effector genes found in *Fusarium* species is still very rare. Some of the known *Fusarium* species whose effectors are F.ox f.sp. *cubense*, F.ox f.sp. *musae*, and F.ox f.sp. *lycopersici* (An et al. 2019; Chang et al. 2020). As for the *FOC*, knowledge about the types of effector genes is currently unknown. Therefore research into this subject is very important to do. These results were to confirm that the *CRX1* and *CRX2* as putative effector genes are very potential to use as markers for classifying the *FOC* according to their forma speciales. Finally, this research is also expected to be an inspiration to elaborate other effector genes contained in *FOC* as material to assemble markers based on other effector genes. Thus, more complete markers can be developed so that in the future, it will be easier to identify the *FOC* in more detail and validity.

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