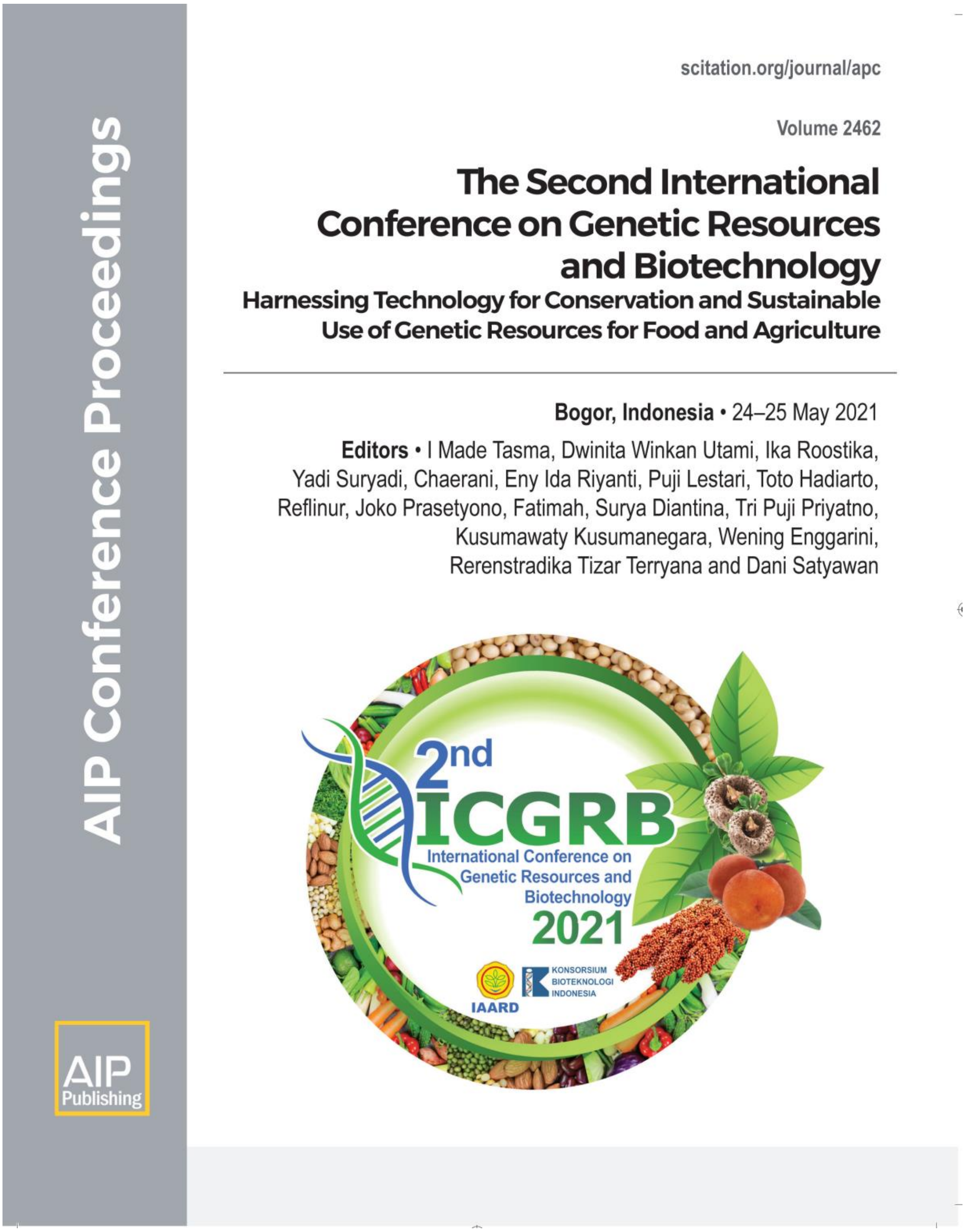

January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24^{th}-25^{th} of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Isolation and pathogenicity test of fusarium basal rot and purple blotch fungal pathogens from shallot and *Allium* spp

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Chaerani, Ragapadmi Purnamaningsih and Suci Rahayu

Isolation and Pathogenicity Test of Fusarium Basal Rot and Purple Blotch Fungal Pathogens from Shallot and *Allium* spp.

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Abstract. Fusarium basal rot and purple blotch are dominant fungal diseases of *Allium* spp. As a part of mutation breeding project to develop resistant shallot varieties to the fungal agents, this study was aimed to isolate and test the pathogenicity of the isolated fungi. Fungal isolation was performed by means of standard tissue isolation technique from diseased samples collected from six shallot and scallion fields in West Java. Three isolates of *Fusarium* and four isolates of *Alternaria porri* were obtained from the respective disease symptom. Additionally, *Stemphylium* sp. was recovered from leaf blight symptom and *A. alternata* from both diseased leaf symptoms. Koch's Postulate was accomplished by pathogenicity tests and reisolation of the fungi. A modified onion bulb assay resulted in mild to extensive rot of bulbs inoculated with *Fusarium* agar plugs. Pot assay confirmed the aggressiveness of *Fusarium* isolates to shallot varieties. *Allium* spp. plants developed purple blotch after sprayed with *A. porri* spores, and leaf blight after sprayed with *Stemphylium* sp. or *A. alternata* spores. Except for *A. alternata*, all pathogens were successfully reisolated from the foliage disease symptoms, confirming their causative agents of the respective disease. For future genetic studies and species confirmation by means of DNA sequencing, single spore isolation of the pathogens should be attempted.

INTRODUCTION

Shallot (*Allium cepa* var. *aggregatum* [syn. *A. ascalonicum*]) is the second most important horticultural crop in Indonesia [1]. Because of seasonal production, current shallot production in traditional cultivation areas in ten provinces in Java, Sumatera, Sulawesi, West Nusa Tenggara, and Bali cannot meet the high demand [2]. Therefore, Indonesia's Ministry of Agriculture has issued a decree No. 472/Kpts/Rc.040/6/2018 to expand shallot planting areas to 262 regencies in 32 provinces to increase production by 7% per year to reach 1.66×10^6 t in 2021 and 2.13×10^6 t in 2024 [3, 4]. To meet this target intensive cultivation all year long under good irrigation system, off-season planting, increased provision of shallot seed, and also provision of people business credit are pursued [2]. However, off-season planting during wet season makes shallot cultivation are vulnerable to disease problems, especially to fungal diseases [1, 5]. Major fungal diseases of shallot in Indonesia are Fusarium basal rot (FBR) and purple blotch [1, 6]. Yield losses of shallot due to diseases were estimated from 24 to 100% [1].

FBR, also known as Fusarium basal plate, Fusarium rot, Fusarium wilt, damping off disease of seedlings, or die-back disease of seedlings are associated with twelve *Fusarium* species with *F. oxysporum* and *F. proliferatum* are the most prevalent [5]. In Indonesia this disease is known as "penyakit moler" or "penyakit inul". The pathogen invades the plant through roots and the basal stem plate via the soil [7]. When the pathogen is transmitted from the bulb, FBR symptom starts to appear at 5 to 10 days after planting, and when it is transmitted via the soil the symptom is visible at 3 weeks after planting [8]. At the seedling stage FBR symptoms appear as delayed emergence, damping off, and stunted growth of seedling [7]. Fusarium basal rot on mature plants appear as leaf chlorosis which leads to tip and leaf necrosis, leaf twisting, drooping, and rapid wilting of plants, leading to plant death [5, 7]. When

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uprooted, roots and stem plate are easily separated from the bulb and under high disease severity white mycelium mat can be observed on the basal portions of exterior bulb scales [7].

Purple blotch (Indonesian: "penyakit bercak ungu" or "penyakit trotol") is a common disease of *Allium* spp. under warm and humid environment [9]. Five *Alternaria* spp. have been associated with purple blotch symptom [9–11]. The disease is characterized by grey purplish spots with concentric rings on leaf, which usually appears on older leaves as white color flecks. These flecks enlarge and produce numerous small sunken purple lesions under favorable environmental conditions. When the disease progresses, lesions enlarge and produce brownish-purple necrotic lesions which kill the leaves leading to delayed bulb formation and maturation [9]. The late stage of purple blotch symptom is similar to leaf blight symptom incited by *Stemphylium* [6]. Purple blotch also affects flower stalks and bulbs which make bulbs become undersized leading to highly reduced yield [12].

Application of fungicide as seed treatment for dealing with FBR and as regular sprays to manage purple blotch is required to lower disease incidence and severity [8]. Fungicide is expensive when repeated application during rainy season has to be implemented. Use of resistant varieties is a low cost effective method to minimize disease incidence and progress in the field. When combined with fungicide application and other control methods it can provide maximum protection to the crop. Unfortunately, there are few varieties with resistance to the fungal pathogens available locally. Among eleven national released varieties, only two varieties are moderately resistant and resistant to *F. oxysporum* f. sp. *cepae* (*Foc*), and one variety is described as moderately resistant to *A. porri* [13]. With the goal to increase the number of resistant shallot varieties to these pathogens, our research group has initiated resistance mutation breeding project. As a part of the project, this study was aimed to isolate and confirm the pathogenicity of both fungal pathogens and other significant fungi on shallot and *Allium* spp.

MATERIALS AND METHODS

Fungal Isolation

Visits to farmers's fields in Majalengka and trial plots of Indonesian Vegetable Research Institute (IVEGRI), Lembang, West Bandung, Pacet Experimental Station of Indonesian Center for Agricultural Biotechnologi and Genetic Resources and Development (ICABIOGRAD), Cianjur, and Indonesian Center for Horticultural Research and Development, Bogor were done in October 2020 until Januari 2021. Purple blotched leaves of shallot and scallion (*A. fistulosum*), and wilted shallot plants were sampled and brought to laboratory for fungal isolation. The pathogens were isolated from infected samples by the standard tissue isolation technique [14]. Leaves of shallot and scallion plants showing leaf spot symptoms and bulbs of shallot plants showing yellowing and twisted leaves were washed under running tap water, cut into 1–2 cm segments, surface-sterilized with 0.5% sodium hypochlorite for 1 min, and rinsed in sterile distilled water three times. After blotted dry on sterile filter paper, the leaf and bulb segments were transferred to plated 1.5% water agar (Hi-Media). Plates were incubated at room temperature under natural sunlight by the window. After 7–10 days colonies showing typical characteristics of the respective fungal species were transferred to potato dextrose agar (PDA) in Petri plates supplemented with 0.05% streptomycin sulfate and 0.05% chloramphenicol. Plates were incubated under the same condition as described above. Cultures were purified several times on PDA plates.

Pathogenicity Tests of *Fusarium*

In vitro and pot assays were performed to test the pathogenicity of *Fusarium* isolates. A modification of onion (*A. cepa*) bulb assay from Kalman *et al*. [15] was employed for *in vitro* pathogenicity assay. After removal of the outer scales, bulbs were surface sterilized with 70% ethanol, blotted dry, and perforated using a scalpel on four sites. An agar section (7 mm × 7 mm) taken from the edge of 7-day-old fungal colony was placed on each hole, covered with the scale, and wrapped with cling film. Each bulb was inoculated with one isolate and each hole was considered as replicates. Bulbs inoculated with *Foc* isolate IVEGRI M20.0127 obtained from IVEGRI and bulbs received PDA medium were used as control. Bulbs were incubated in the dark at room temperature. Disease symptoms were observed daily beginning at 3 days after inoculation (DAI) and rated qualitatively.

Pot assay under a glasshouse environment was performed by inoculating autoclaved soil and compost (2:1, v/v) in 12 cm-diameter pots with 14-day-old *Fusarium* cultures in autoclaved sorghum grains [16]. The top soil in each pot was mixed with ±7 g of sorghum culture containing about 1.9×10^9 spores, thereafter planted with 1% NaOCl surface sterilized bulbs (30 min) [17] of six shallot varieties (Bima Brebes, Kuning, Sumenep, Trisula, Violetta

Agrihorti 1, and Violetta Agrihorti 2). The aggressiveness of the isolated *Fusarium* was compared to that of *Foc* isolate IVEGRI M20.0127. Pots received autoclaved sorghum grains were used as control. For each combination of isolate and variety there was one pot with three bulbs per pots. Disease incubation period was observed daily. Leaf disease severity was rated at 30 day after planting (DAP) on a modified scale of 0 to 5: 0 = none of the leaf yellowing, 1 = 1–10% leaf yellow or dry, 2 = 11–30%, 3 = 31–50%, 4 = 51–75%, and 5 = >75% of total foliage yellow or dry [17, 18]. The percentage of disease index (PDI) was calculated using the formula:

$$PDI = \Sigma(n \times v)/(N \times V) \times 100\%$$

where n is the observed sample, v is the disease score, N is the number of samples observed, and V is the highest disease score [18]. Cultivars resistance were categorized as immune (PDI = 0%), resistant (PDI = 1–20%), moderately resistant (PDI = 21–40%), moderately susceptible (PDI = 41–60%), susceptible (PDI = 61–80%), and highly susceptible (PDI >80%) using modified resistance interval classes [18, 19].

Pathogenicity Test of Foliar Pathogens

The pathogenicity of foliar pathogens was tested by spray inoculation of spores on *Allium* spp. plants. The pathogens were cultured on PDA in Petri plates for 7–10 days and incubated at room temperature under diurnal light from the sun. Spores of the representative isolate of each fungal species were prepared by pouring 10 ml distilled water on 10–14-day-old PDA culture in Petri plates and rubbing the colony surface using a microscope glass slide. After filtration through two layers of cheesecloth, the spore suspension volume was adjusted to 20 ml, and added with one drop of Tween 20. Spores were sprayed using a 30-ml atomizer on one to two pots of 30 to 40-day-old plants of shallot (cv. Bima Brebes), scallion, and onion in pots until run-off. Inoculation was done at 5 PM. Shortly after inoculated, plants were incubated in a closed transparent plastic tunnel located outdoor under a roof and given periodic misting supplied from a mini ultrasonic mist maker connected to a timer. Two experiments were done. In the first experiment periodic misting was supplied for 45 min/h every night (12 h) during 7 days of experiment. The tunnel was opened every morning and closed again in the night. In the second experiment continuous misting was given during the first 2 h of incubation in the night followed by 45 min/h misting during the next 46 h. In the next 5 days the plastic tunnel was opened every morning and closed again during the 12-night incubation under 45 min/h mist treatment. Disease symptoms were observed daily beginning at 3 DAI.

The Koch's postulate [14] was accomplished by reisolation of the causative agents from diseased bulbs or foliage by the standard tissue isolation technique as described previously on PDA. Microscopic examination was performed on fungal structures.

RESULTS AND DISCUSSIONS

Fungal Isolation

Field samples of shallot plant showing yellowing, chlorosis and drooping leaves, and wilting symptoms were collected (Fig 1). Three *Fusarium* isolates were successfully isolated from roots and bulbs of plants with these disease symptoms (Table 1).

(a) (b)

FIGURE 1. Shallot plants with leaf necrosis and stunted growth in a farmer's field in Cijurey Kidul, Majalengka (a) and leaf chlorosis and wilting symptoms in a shallot display plot in Cikeumeuh, Bogor (b), which indicated Fusarium basal rot disease.

Fusarium isolates on PDA had colonies with varying color, from white pinkish to purplish with white aerial hyphae on the surface, which resembles the colony of *Foc* isolate IVEGRI M20.127 (Fig. 2). On older culture the purple color, especially the culture of isolate F-CKM, intensified. Microconidia were hyaline, aseptate, varied from oval to kidney-shaped, and produced in cluster at the tips of hyaline conidiophores (Fig. 2). Microconidia were more commonly produced than macroconidia.

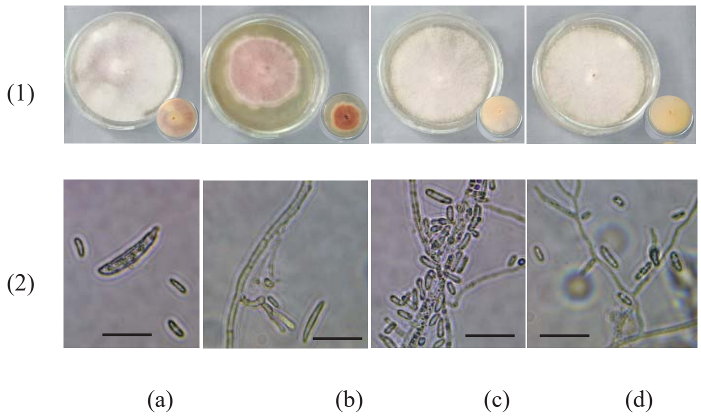

FIGURE 2. Variability of 7-day-old cultures on potato dextrose agar (1) and conidia (2) of *Fusarium oxysporum* f. sp. *cepae* isolate IVEGRI M20.0127 (a), *Fusarium* isolate F-CKM (b), F-MJL1 (c), and F-MJL2 (d). Bars = 10 µm.

A. porri could be isolated from field samples of shallot and scallion showing typical purple blotch symptom (Fig. 3), *Stemphylium* sp. was obtained from shallot leaf blight, and *A. alternata* was recovered from both purple blotch and leaf blight symptoms. In total, four *A. porri*, one *Stemphylium*, and three *A. alternate* isolates were obtained (Table 1). *A. porri* and *Stemphylium* producted similar symptoms at the later stage of disease, and both fungi can coexist on the same lesions [6].

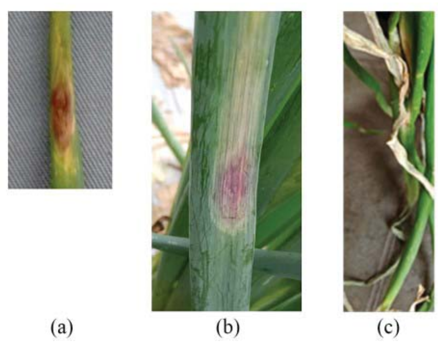

FIGURE 3. Purple blotch on shallot (a) in a trial plot of Pacet Experimental Station, Cianjur, purple blotch (b), and leaf blight (c) on scallion in a trial plot of Indonesian Vegetable Research Institute, Lembang, West Bandung.

Colonies of *A. porri* on PDA were yellowish-beige color to smoky grey with brownish tinge and typically produced diffusible brown pigments in the agar medium beneath the mycelia mat (Fig. 4a.1). *Stemphylium* colonies appeared dark olive-grey (Fig. 4b.1), whereas those of *A. alternata* were smoky grey (Fig 4c.1). The three fungi have dark-colored mycelia and spores, muriform spores and velvety or cottony-textured colonies. Spores of *A. alternata* and *Stemphylium* are profusely produced after 7 days of culturing, whereas those of *A. porri* are hard to produce unless special treatments are given [20]. In our case, placing *A. porri* cultures by the window under diffuse sunlight could induce sporulation although spores were scantily produced. Spores of *A. porri* are large, ellipsoid or obclavate, have single or branched long beaks, and are produced solitary on conidiophores (Fig. 4a.2). *A. alternata* spores are smaller than those of *A. porri*, have short and non-branching beaks, and were produced in chains (Fig. 4b.2). Spores of *Stemphylium* are cubical-shaped and produced singly on conidiophores (Fig. 4c.2).

TABLE 1. Fungal pathogens isolated from diseased shallot (*Allium cepa* var. *aggregatum*) and scallion (*A. fistulosum*) fields in West Java.

No.	Isolate	Genus/species	Origin[a]	Crop	Disease symptom	Date of collection
1	F-MJL1	*Fusarium*	1	Shallot[b]	Leaf necrosis, stunted growth	Nov 11th, 2020
2	F-MJL2	*Fusarium*	1	Shallot[b]	Leaf necrosis, stunted growth	Nov 11th, 2020
3	F-CKM	*Fusarium*	2	Shallot[b]	Yellowing, wilt or droopy, and stems were easily separated from rotten bulbs	Nov 20th, 2020
4	Alt-PCT1	*Alternaria porri*	3	Shallot[b]	Purple blotch	Oct 22th, 2020
5	Alt-PSC	*A. porri*	4	Scallion	Purple blotch	Oct 22th, 2020
6	Alt-LBG1	*A. porri*	5	Shallot[c]	Purple blotch	Jan 27th, 2021
7	Alt-LBG2	*A. porri*	5	Scallion	Purple blotch	Jan 27th, 2021
8	S-LBG1	*Stemphylium*	5	Shallot[d]	Leaf blight	Oct 6th, 2020
9	Aa-LBG	*A. alternata*	5	Shallot[d]	Leaf blight	Oct 6th, 2020
10	Aa-PCT1	*A. alternata*	3	Scallion	Purple blotch	Oct 22th, 2020
11	Aa-PSC	*A. alternata*	4	Scallion	Purple blotch	Oct 22th, 2020

[a]1 = farmer's field in Cijurey Kidul, Majalengka, 2 = display plot of Indonesian Center for Horticultural Research and Development, 3 = Pacet Experimental Station of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, 4 = farmer's field in Pasir Cina, Cianjur, 5 = Lembang Experimental Station of Indonesian Vegetable Research Institute.

[b]cv. Bima Brebes.

[c]cv. Bali Karet.

[d]mixed cultivars: Violetta 1 Agrihorti, Violetta 2 Agrihorti, Violetta 3 Agrihorti, Bima Brebes, Sumenep, and Kuning.

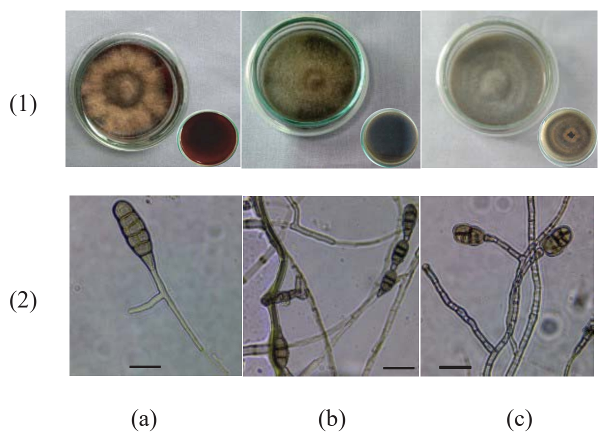

FIGURE 4. Appearance of 7 to 14-day-old cultures on potato dextrose agar (1) and spores (2) of *A. porri* (a) isolated from purple blotch symptom of shallot and scallion, *A. alternata* (b), and *Stemphylium* (c) isolated from leaf blight symptom of shallot. Bars = 10 µm.

Pathogenicity Tests of *Fusarium* Isolates

Water soaked areas surrounding wounded onion bulbs inoculated with *Fusarium* isolates were observed on 3 DAI. This area continued to grow until 7 DAI forming sunken, rotten area with white mycelia mat, a sign of successful fungal growth (Figs. 5b, 5d, 5e, and 5f). Bulbs received PDA plugs remained free of rot areas and no indication of white mycelia growth (Figs. 5a and 5c). By qualitative assessment of the sunken area, isolate F-MJL1 andF-CKM was categorized as the most aggressive, followed by *Foc* isolate IVEGRI M20.0127 and *Fusarium* isolate F-MJL2. Fungal reisolation on PDA media recovered white colonies with pinkish, reddish or purplish tinge color on the reverse side of Petri dish, which are characteristics of *Fusarium* colony.

(a) (b) (c) (d) (e) (f)

FIGURE 5. Onion bulbs showing rot symptom and white mycelia growth at 7 days after inoculation with potato dextrose agar (PDA) plugs of *Fusarium oxysporum* f. sp. *cepae* isolate IVEGRI M20.0127 (b), *Fusarium* isolate F-MJL1 (d), F-MJL2 (e), and F-CKM (f). Bulbs inoculated with PDA remained free of rot symptom and pathogen growth (a and c).

Further pathogenicity test of *Fusarium* isolates under pot assay obtained dry, necrotic leaf symptoms (Fig. 6) which were started to visible on 11 DAI. These symptoms were more pronounced on 30 DAI, except on cv. Sumenep (Table 2). Among four isolates, F-CKM was the most aggressive by inciting on average of 51% (0–100%) disease index across shallot varieties. This was 1.5 folds than the PDI caused by the standard isolate IVEGRI M20.0127 (33%, range 0–80%). The remaining two isolates turned out to be less aggressive (PDI = 21–24%). The average PDI across *Fusarium* isolates indicated that cv. Sumenep was resistant; followed by Trisula, Violetta 2 Agrohorti (resistant), Kuning, Violetta 1 Agrihorti (moderately resistant), and Bima (susceptible).

(a) (b) (c) (d) (e)

FIGURE 6. Thirty-day-old shallot plants of cv. Bima Brebes showing dry, necrotic, and twisted leaves after inoculation with sorghum grain culture of *Fusarium oxysporum* f. sp. *cepae* isolate IVEGRI M20.0127 (b) and *Fusarium* isolate F-CKM (c). Milder disease severity developed on pots inoculated with isolate F-MJL1 (d) or F-MJL2 (e). Disease symptoms did not develop on pots infested with autoclaved sorghum grains only (a).

TABLE 2. Percentage of disease index (%) incited by *Fusarium* isolates on six shallot varieties under glasshouse environment at 30 days after planting[a].

Cultivar	M20.0127[b]	F-CKM	F-MJL1	F-MJL2	Cultivar average[c]
Bima Brebes	80.0	100.0	50.0	40.0	67.5 (S)
Kuning	46.7	66.7	20.0	20.0	38.3 (MR)
Sumenep	0.0	0.0	6.7	0.0	1.7 (R)
Trisula	20.0	26.7	13.3	33.3	23.3 (MR)
Violetta 1 Agrihorti	33.3	70.0	20.0	33.3	39.2 (MR)
Violetta 2 Agrihorti	20.0	40.0	13.3	20.0	23.3 (MR)
Isolate average	33.3	50.6	20.6	24.4	

[a]Steam-sterilized potting media were inoculated with ±7 g of 14-day-old *Fusarium* cultures in autoclaved sorghum grains and planted with 1% NaOCl surface-sterilized shallot bulbs.
[b]*F. oxysporum* f. sp. *cepae* obtained from Indonesian Vegetable Research Institute.
[c]Resistance category was based on the percentage of disease index modified from [18, 19] as follows: 0 = immune, 1–20% = resistant (R), 21–40% = moderately resistant (MR), 41–60% = moderately susceptible (MS), 61–80% = susceptible (S), >80% = highly susceptible (HS).

The identification of *Fusarium* isolates obtained in this study has not been attempted. Extensive survey in shallot production centers in Java and South Sulawesi revealed that *F. oxysporum* causes bulb rot symptom, while *F. solani* and *F. acutatum* are also the causal agents of three diseases syndromes, i.e. bulb rot, wilting symptom, and twisted disease [21]. Other species that have been implicated in shallot bulb rot disease in Java are *F. proliferatum*, *F. verticillioides*, and *F. pallidiosoreum* [19]. These species can be distinguished molecularly by PCR amplification of the small subunit ribosomal RNA gene, internal transcribed spacer (ITS), the *T12* beta-tubulin gene, the translation elongation factor-1 alpha (*TEF1*) gene, the *Foc* secreted in xylem genes 3 (*SIX3*), and the *F. proliferatum* partial calmodulin gene (*CLPRO*) [15].

Pathogenicity Test of Foliar Pathogens

One isolate from the respective foliar fungal pathogen species, i.e. *A. porri* Alt-PCT1, *Stemphylium* S-LBG, and *A. alternata* Aa-LBG, was chosen for pathogenicity tests on *Allium* spp. In the first experiment disease symptoms failed to develop. Improvement of misting period in the second experiment resulted in disease symptoms on few plants. The typical purple blotch symptom in the field could be reproduced on scallion sprayed with by *A. porri* spores (Fig. 7a). Brown lesions surrounded by a chlorotic halo and leaf blight developed on onion sprayed with *Stemphylium* spores (Fig. 7b). Purple blotch and *Stemphylium* blight symptoms appeared first on older leaves as tiny, white, circular or irregular lesions, which gradually increase in size and become sunken, oval- or irregular-shaped and pale yellow-colored, and produced concentric rings. Inoculation of shallot plants with *A. alternata* spores resulted in blighted foliage with dark olive green areas in the middle (Fig. 7c).

(a) (b) (c)

FIGURE 7. Purple blotch symptom developed on scallion plant sprayed with *Alternaria porri* spores (a), leaf spots (white arrow), and leaf blight (red arrow) on onion plant sprayed with *Stemphylium* spores (b), and leaf blight on shallot plant sprayed with *A. alternate* spores (c). Photographs were taken at 7 days after spray inoculation.

Pathogen reisolation successfully obtained identical colonies and spores as compared to that of *A. porri* and *Stemphylium*, confirming the pathogenic ability of the two fungi on *Allium* spp. Meanwhile, pathogen reisolation from blighted shallot foliage failed to recover *A. alternata*, but instead *Curvularia* sp. was obtained. Because single spore isolation was not applied, it is likely that *A. alternata* colonies were still contaminated with *Curvularia*, which has similar colony appearance to that of *A. alternata.*

Our result with *Stemphylium* agreed with that of Halhuly *et al*. [6], who firstly identified *Stemphylium* as the causative of shallot leaf blight in Indonesia based on PCR amplification with *Alternaria*-specific primers. The pathogenicity role of *A. alternata* and *Curvularia* on shallot and *Allium* spp. should be retested because *A. alternata* has been identified as the causal agent of onion leaf blight in South Africa [22] and *C. lunata* has been identified as seed borne fungus on onion bulb [23].

Under a humidity chamber, duration of leaf wetness is the key to successful development of disease symptoms caused by *Alternaria* spp. on various crops [9, 24–26]. Germination of *A. porri* spores requires at least 9 h dew duration and infection takes place with increasing leaf wetness period to 10–16 h [25]. Prolonged leaf wetness duration until 24 h increased *A. porri* infection on onion leaves at 5° to 25°C temperature range [9]. Our results indicate that post inoculation condition was conducive for development of purple blotch and leaf blight symptom on onion and scallion, but not on shallot. The variety used in the spray inoculation (cv. Bima) may not susceptible to infection by *A. porri*, despite the isolate used was originated from this variety. To obtain purple blotch symptoms, a range of shallot varieties should be tested.

A. porri belong to the large-spored *Alternaria* group which have wide morphological variation [27], and therefore misidentification is highly likely. Among five *Alternaria* spp. that have been associated with purple blotch symptom or dark brown lesion with a chlorotic halo on onion, *A. porri* and *A. vanuatuensis* are highly similar in colony morphology, but the latter has spores with significantly shorter beaks and less aggressive than *A. porri* [10]. *A. porri* can be distinguished from the other species based on eight nucleotide sequence of *RPB2* gene [10, 27]. Comprehensive morphological characterization and phylogenetic analysis of the large-spored *Alternaria* from purple blotch and dark brown spot of Alliaceae should use a large number of isolates and DNA sequence analysis.

All fungal pathogens in this study were obtained by means of diseased tissue isolation technique with several purifications steps by mass transfer until seemingly uniform colonies were resulted. Such method will not yield uniform culture, which is a prerequisite for genetic studies including aggressiveness test, resistance screening, as well as molecular identification. Therefore, single spore isolation must be pursued.

CONCLUSION

Three *Fusarium* isolates from shallot plants showing chlorotic, wilting, and droopy leaves; four *Alternaria porri* isolates were recovered from purple blotch symptom of shallot and scallion; one *Stemphylium* sp. from leaf blighted shallot. Three *A. alternata* isolates were recovered from both purple blotch and leaf blight symptoms. Pathogenicity assays confirmed that *Fusarium*, *A. porri*, and *Stemphylium* were the causative agents of the respective disease.

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