

Disease Identification and *In situ* Screening of Gumamela (*Hibiscus rosa-sinensis* Linn) Germplasm and Hybrids to Leaf Anthracnose for Disease Resistance Breeding

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Hibiscus germplasm and hybrids planted at the Crop Science Cluster - Institute of Plant Breeding (CSC-IPB) breeding blocks have been observed to be affected by a few diseases. The identification of these diseases and their causal organism plus the identification of natural sources of resistance is crucial for the breeding program. The diseases that were identified included the following: a) anthracnose, commonly known as the leaf spot disease caused by *Colletotrichum gloeosporoides* Penz., b) early wilt and die-back attributed to *Fusarium oxysporum* Schlect, c) stem rot at the base of the plant, wilting and blighting of the plant caused by *Rhizoctonia solani* Kuhn. All fungal isolates demonstrated pathogenicity to hibiscus. Out of the 36 hibiscus genotypes evaluated *in situ*, 12

showed less than 10% average of the leaf area infected for leaf anthracnose. In contrast, two *Hibiscus* species namely: *Hibiscus cooperri* and *H. schizopetalus*, two hybrids namely: *H. rosa-sinensis* ‘Perla Santos Ocampo’ x *H. rosa-sinensis* ‘Loren B. Legarda’ and *H. rosa-sinensis* ‘Gelia T. Castillo’ x *H. rosa-sinensis* ‘Betty Go-Belmonte’ including three other varieties like ‘Reddy or Not’, ‘Wilcox’ and ‘Petite Peach’ were not infected by the leafspot disease or anthracnose under field conditions, which suggests that they could have some form of resistance to this disease. This further suggests that the resistant related species especially *H. cooperri* having red and white variegated leaves could be used for hybridization with the susceptible varieties to develop a variety with a dual purpose trait, i.e. resistant to the fungus and leaves with variegations as another ornamental trait of the hybrid variety.

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Submitted: January 17, 2012

Revised: June 25, 2012

Accepted: July 22, 2012

Published: October 25, 2012

Editor-in-charge: Gisela P. Padilla-Concepcion

Reviewers:

Rex Sadaba

Thomas Edison de la Cruz

KEYWORDS

Colletotrichum gloeosporoides, die-back, *Fusarium oxysporum*, hibiscus, pathogenicity, *Rhizoctonia solani*, wilting

INTRODUCTION

The hibiscus or gumamela (*Hibiscus rosa-sinensis* L.), also known as China rose and Shoeflower, belongs to the Malvaceae family which is a native of China. Its natural beauty makes it



Figure 1. Leaf anthracnose symptom in gumamela.



Figure 2. Early wilting of whole plant (a) with stem rot showing mycelial growth near stem base (b).

one of the most widely cultivated flowers in tropical and sub-tropical places. The five petal-type hibiscus has been noted for its medicinal properties and the flowers were considered astringent. The roots contain mucilage that is soothing on the mucous membranes of the digestive and respiratory tracts (Sharma and Sultana 2004).

The flowers contain substantial quantities of flavonoids and proanthocyanidins, which are good antioxidant, antipyretic (fever-reducing), analgesic (pain-relieving), and spasmolytic (spasm-inhibiting) activities (Rummel 2005). Other compounds that have been isolated from hibiscus are: quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid. *Hibiscus* also contain acidic polysaccharides that stimulate specialized skin cells, modulates immunity and presumably promote wound healing. Furthermore, it contains a high concentration (15 to 30%) of simple organic acids such as citric and malic acids (Rummel 2005). They are essential ingredients in the manufacture of herbal shampoo and oil for the cosmetic industry and for the preparation of beverage like the hibiscus herbal tea.

The Institute of Plant Breeding (IPB) released different popular series of gumamela hybrids that are now commercially grown (Pimentel 1999, Magdalita et al. 2009). These series include the 'Centennial', 'Millenium', 'Celebrity Star', 'Oblation', 'Women in Science', 'Women in Public Service' and 'Women in the Arts' (Magdalita and Pimentel 2010, Magdalita et al. 2011). Many gumamela hybrid varieties and germplasm collections in the IPB nursery are planted in pots or directly in the soil in breeding blocks. However, most varieties in the germplasm collection were observed to be affected by several diseases that caused yellowing, defoliation and plant wilting resulting in the loss of valuable germplasm materials. Studies dealing with gumamela diseases are very limited in the Philippines, however there have been occurrences of diseases on Malvaceae or on other

ornamental plants (Bailey et al. 1996, Yang et al. 2009, Sutton 1992). Hence, this research study was conducted to: a) identify the diseases present and b) evaluate the hibiscus hybrid varieties and germplasm collections to the most predominant and damaging disease. The study on disease identification and pathogenicity tests were conducted in 2007 during the wet season until 2008, while the *in situ* evaluation on the reaction of the hibiscus hybrids and germplasm collections to prevalent diseases was done during the wet season in 2009.

MATERIALS AND METHODS

Isolation and Identification of the Hibiscus Diseases

Hibiscus Leaf Spot

Hibiscus leaves with small sunken light brown to brown spots (1-2 mm diameter) having halo-like yellowing around the lesions were collected in the germplasm collection and breeding nurseries of IPB (Figure 1). Infected leaf samples were washed with distilled water and incubated overnight in Petri dishes lined with moist sterile filter paper to allow growth of the causal organism. An ordinary scotch tape was lightly pressed on the specimen and mounted on slides with a few drops of distilled water added for microscopic examination. Morphological characteristics such as size and shape of spores, fruiting bodies, presence or absence of setae and sclerotial bodies were observed. The causal organism was identified using the standard disease monographs. Ten replicates of setae sizes (l x w) and 50 replicates of conidia sizes (l x w) were recorded.

To procure pure culture of the isolate and to confirm the initial findings, the fungus was isolated from the diseased specimen by tissue planting method. This was done as follows: The diseased leaf samples were surface sterilized with 10% (v/v) sodium hypochlorite solution for three minutes and washed three times with sterile distilled water. The disinfected leaf segments were evenly spaced in Petri dishes (9 cm diameter) containing potato dextrose agar (PDA) medium. The Petri dishes were sealed using Parafilm™ and incubated at room temperature for three days. Then, the pure culture was obtained by hyphal tip isolation, that is, the fragment of fungal growth at the margin of the fungal colony was aseptically transferred to PDA plates. After seven days of incubation at room temperature (28°C) of the pure culture, cultural traits were observed. Microscopic examination was also done to confirm the initial findings that it was *Colletotrichum gloeosporioides* Penz. based on the fungal morphology and cultural characteristics.



Figure 3. Die-back symptom of the young shoot (with arrow).



Figure 4. Setae and conidia of *Colletotrichum* sp isolated from gumamela (scale bar = 25 µm) (a) and colony growth on Potato Dextrose Agar (b). Detection of *Colletotrichum gloeosporioides* using species-specific primer pair (CgInt (5'-GGCCTCCCGCCTCCGGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3')). Lane M= DNA Marker (1 kb plus ladder from Invitrogen, USA); Lane 1 and 2 = water, 3 and 4 = *Fusarium verticillioides*, 5 and 6 = *Rhizoctonia solani*, 7 = *Colletotrichum gloeosporioides* of mango, 8 and 9 = *Colletotrichum* sp from pepper, 10 and 11= *Colletotrichum* isolates from gumamela (10 has distinct band while 11 has faint band having 450 bp) (c).



Figure 5. Pinkish colony of *Fusarium oxysporum*.

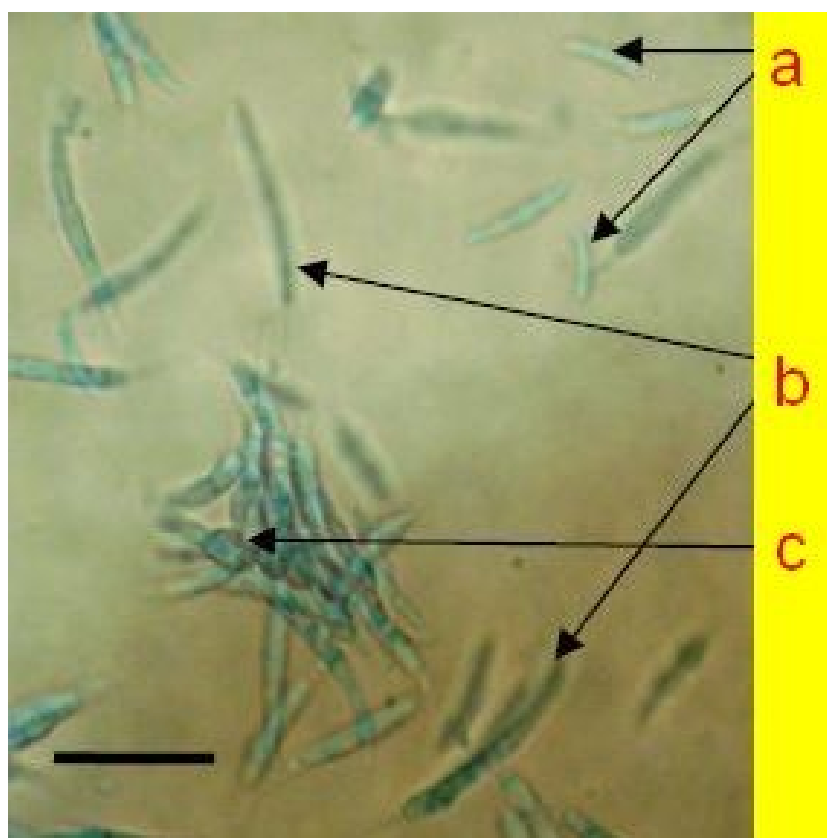


Figure 6. Microconidia (a), macroconidia (b) and chlamydospores (c) of *Fusarium oxysporum* from gumamela, (scale bar = 25 μ m).

To confirm the species of *Colletotrichum gloeosporioides* isolated, PCR assay was conducted using a species-specific primer to *C. gloeosporioides*, the primer pair CgInt/ITS4 from *pe/B* gene (Medeiros et al. 2010 and Freeman et al. 2000).

DNA Extraction

The DNA extraction procedure was based on the method of Pascual et al. (2000) with some modifications. Pure culture of the isolate was grown on Potato Dextrose Broth for three days and the mycelial mat was harvested by vacuum filtration and blotted dry. The dry mycelial mat was homogenized in sterile mortar and pestle added with 500 μ l of extraction buffer (0.1 M NaCl, 0.5 M Tris-HCl, and 5% SDS). When thoroughly homogenized, the solution was transferred to 1.5 ml microcentrifuge tube and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a new 1.5 ml tube and added with equal volume of Phenol:Chloroform:Isoamyl (24:1:1) alcohol (Invitrogen, USA). Tubes were gently inverted several times until thoroughly mixed and centrifuged for 5 min at 10,000 rpm. The clear aqueous phase was transferred to a new 1.5 ml microcentrifuge tube and the DNA extract was precipitated by adding 2.5x volume of isopropanol alcohol and inverted several times. Tubes were incubated at 4°C for 1 hour. After incubation, tubes were centrifuged at 10,000 rpm for 10 min. Supernatant were discarded and pellets were washed twice with 70% EtOH, air-dried, and resuspended in TE buffer.

PCR analysis using species-specific primer CgInt/ITS4

A 15 μ l volume of PCR reaction mixture containing 10.73 μ l DEPC water, 1.5 μ l 1x PCR buffer, 0.75 μ l MgCl₂ (Invitrogen, 2.5 mM), 0.30 μ l dNTPs (0.20M), 0.30 μ l each of primer pair
 Primer CgInt 5'
 'GGCCTCCCGCCTCCGGGCGG-3'
 and Primer ITS4 5'-
 TCCTCCGCTTATTGATATGC-3' (0.20 μ M), 0.12 μ l and 1 μ l DNA template (from isolate) was prepared for amplification in automated PCR machine

(PC 960 Thermal Cycler, CR Gobett Research). The PCR conditions were as follows: initial denaturation at 94°C for 3 min (1 cycle), 35 cycles of 94°C denaturation for 1 min, 50°C annealing for 1 min, 72°C of extension for 2 min, and a final extension of 72°C for 5 minutes.

Stem Rot and Wilting of Whole Plant

Basal stem rot with white fungal growth (Figure 2b) and characterized by wilting of whole gumamela plant (Figure 2a) was collected. Infected stem taken from tissue with healthy and infected part was cut into 5 mm sections, and the pure culture was isolated by tissue planting as described previously. After seven days of incubation at 28°C, morphological characteristics of mycelia and other structures like sclerotia and spores were evaluated. Since there was no spore detected, the culture was allowed to grow until sclerotial body formed. Cultural characteristics were also observed. Results were compared to disease monographs and literatures, i.e., *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control (Sneh et al. 1996).

Die-back and Wilting Disease in Potted Hibiscus

Diseased stem tissues of *Hibiscus* collected from the breeding blocks that exhibited early symptoms of wilting and dieback (Figure 3) were cut into 5 mm sections and processed into pure culture by tissue planting method similar to the protocol used in basal stem rot diagnosis. After 10 d incubation at 28°C, the morphological characteristics of mycelia and spores or conidia of the isolates were examined under the microscope (Lietz Biomed). Furthermore, the cultural characteristics were examined daily during incubation on PDA. Results were compared to disease monographs and literatures, i.e., Bailey and Jeger (1992).

Pathogenicity Test

Leaf Spot of Hibiscus

Ten ml sterile distilled water with a drop of Tween 20 was poured in each PDA plate containing seven-day-old pure

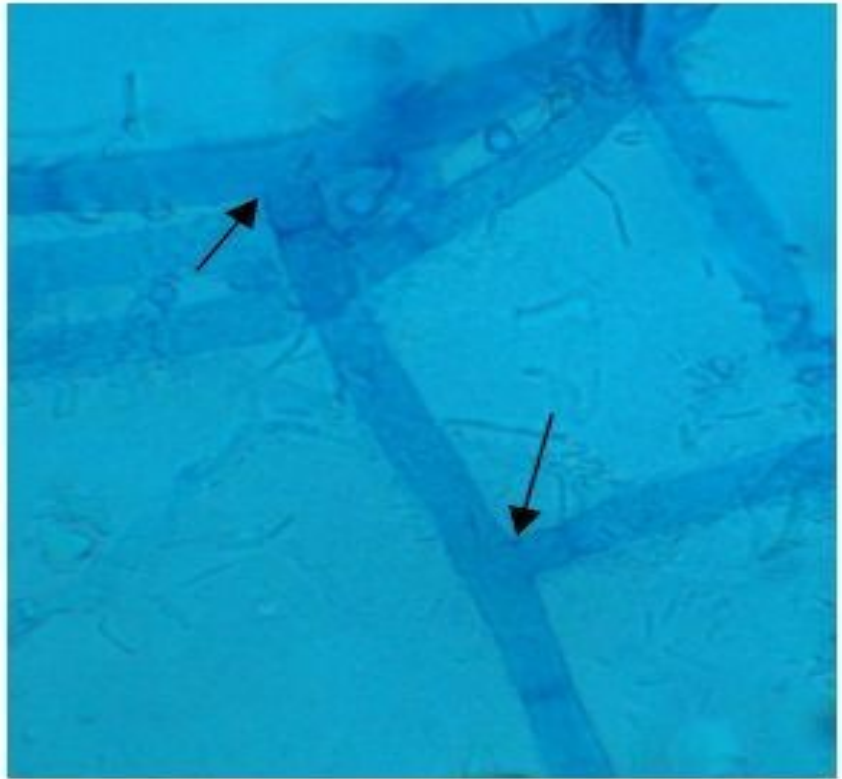


Figure 7. Right angle branching arrow) of *Rhizoctonia solani* hyphae (1,000x in phase contrast microscope).

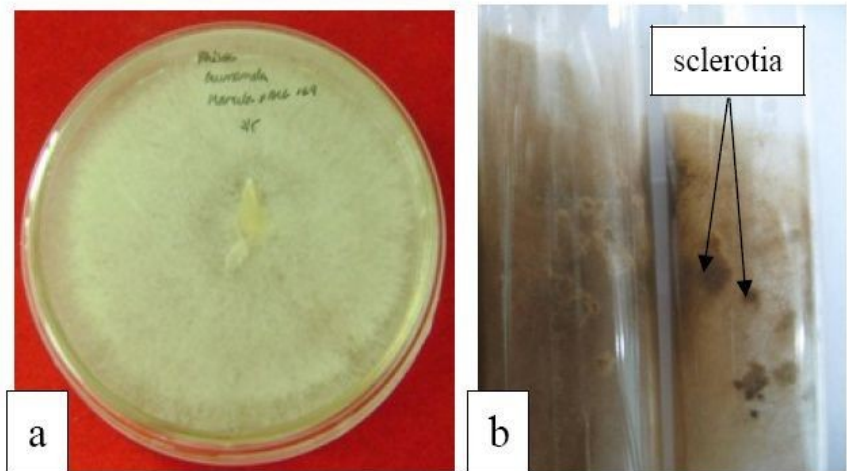


Figure 8. Colony growth of *Rhizoctonia solani* on PDA at 5 days of incubation (a) and 2-month culture (b).

culture of the causal organism. The concentration of conidia in the suspension was determined using a hemacytometer. The conidial suspension was adjusted to a concentration of 1×10^5 conidia per ml water. Subsequently, the suspension was inoculated on healthy two-month-old potted hibiscus plants grown from cuttings by deposition of 0.1 ml of conidial suspension at 4 points/leaf in 5 leaves for each potted plant. Five plants were inoculated with the conidial suspension while 3 plants were uninoculated serving as control. The experimental plants were placed inside a plastic chamber in the greenhouse, where there is a high relative humidity being maintained by placing the potted plants on several layers of moist cheese cloth. The expression of symptom was monitored on daily basis. Six days after inoculation, symptom development was assessed then

the organism was re-isolated to confirm whether the reproduced culture was the same as the original purified culture.

Two Diseases Exhibiting Die-back with Wilting and Stem Rot with Wilting

Mycelial plugs (10 mm diameter) were prepared from seven-day-old culture on PDA of the two fungal isolates causing die back and stem rot and were inoculated on healthy potted hibiscus plants.

For stem rot pathogen, inoculation was done by placing the mycelial plug on the pricked basal part of the stem of five healthy plants. The inoculated plant parts were wrapped with plastic film to hold the inoculum in place on the inoculated site.

For the fungal isolate with die back symptom, the pathogenicity test was conducted on five healthy plants by placing 5 mm disk of agar with fungus on the basal portion of the stem on the soil line and wrapped with scotch tape. Three non-inoculated plants served as control. The inoculated plants were maintained in a moist chamber at ambient temperature in the laboratory for 24 h in the dark and later transferred to the greenhouse. Watering was done every day to keep the soil moist. Symptom development was assessed daily for 15 days. The causal organism for each wilting disease was re-isolated to confirm its identity.

In situ Screening of Hibiscus Hybrids and Germplasm Collections Against Leaf Spot Disease

During the wet season in 2009 from July to December, the hibiscus hybrids and germplasm collections planted in the hibiscus breeding blocks were screened against the leaf spot caused by *C. gloeosporoides*. A total of 36 hibiscus hybrids and germplasm collections were evaluated for their reaction to the leaf spot under natural conditions. Each test plant was evaluated visually for the occurrence of the disease. Three to five plants were evaluated per hybrid/germplasm



Figure 9. *Hibiscus cooperri*, red form (left) and white form (right), a related species of hibiscus showing resistance



Figure 10. *Hibiscus rosa-sinensis* 'Reddy or Not' (left) and 'Wilcox' (center), varieties of hibiscus exhibiting resistance to leaf anthracnose caused by *Colletotrichum gloeosporoides*, and *Hibiscus rosa-sinensis* hybrid 'Loren B. Legarda' x 'Lilia B. de Lima' (right), which is susceptible to the disease showing yellowing of the leaves.

collection. The average per cent disease severity per plant was recorded.

RESULTS AND DISCUSSION

Morphological and Cultural Examinations of Hibiscus Leaf Spot

The causal fungus was identified initially from the spot lesions of *Hibiscus* leaves after one day incubation in humid condition on sterile plate lined with moist ordinary paper towel. Microscopic examination of the fungus from incubated diseased specimen showed that morphologically, it has typical cylindrical and hyaline conidia and black setae, typical characters of *C. gloeosporioides* (Figure 4a).

The colony on PDA was off-white with blackish dots concentrated at the center with few orange conidial masses (Figure 4b). The conidia that formed measured 16-21 $\mu\text{m} \times 7-8.5 \mu\text{m}$ in size (similar to Figure 4a). Blackish brown setae were observed in acervuli on PDA and reached up to 120 μm long. The mycelial growth rate of the isolate was optimal at 28°C and elongated up to 9 mm per day on PDA. The morphological characteristics of the pure culture corresponded to the descriptions of *Colletotrichum* sp. described by Sutton (1992) and Bailey and Jeger (1992).

To confirm the identity of the isolated fungus more conclusively at the species level, DNA of *Colletotrichum* sp isolate from gumamela was purified and PCR reaction with the species specific primer CgInt 5

'GGCCTCCCGCCTCCGGGCGG-3' and Primer ITS4 5'-TCCTCCGCTTATTGATATGC-3' showed product that generated reproducible band fragment of approximately 450 bp (Figure 4c). The distinct and faint bands of the two isolate cultures from gumamela were similar to *C. gloeosporioides* from mango (isolate from IPB collections) and negative for isolate of *Colletotrichum* sp. from pepper (isolated from pepper anthracnose previously diagnosed at IPB), *Fusarium oxysporum* Schlect and *Rhizoctonia solani* Kuhn. The results were

Table 1. Result of screening hibiscus germplasm and hybrids to leaf anthracnose.

Entry	Average leaf area infected per plant (%)
Hybrids	
<i>Hibiscus rosa-sinensis</i> 'Helen L. Valmayor'	5
<i>H. rosa-sinensis</i> 'Clare R. Baltazar'	15
<i>H. rosa-sinensis</i> 'Nova Star'	2
<i>H. rosa-sinensis</i> 'Perla Santos-Ocampo'	40
<i>H. rosa-sinensis</i> 'Estrella F. Alabastro'	2
<i>H. rosa-sinensis</i> 'Kristie Kenney'	40
<i>H. rosa-sinensis</i> 'Rosa Rosal'	5
<i>H. rosa-sinensis</i> 'Lediviña V. Cariño'	25
<i>H. rosa-sinensis</i> 'Lolay' x <i>H. rosa-sinensis</i> 'Gelia T. Castillo'	3
<i>H. rosa-sinensis</i> 'Perla Santos Ocampo' x <i>H. rosa-sinensis</i> 'Loren B. Legarda'	0
<i>H. rosa-sinensis</i> 'Kristie Kenney' x <i>H. rosa-sinensis</i> 'Gelia T. Castillo'	25
Tahitian Tau x <i>H. rosa-sinensis</i> 'Nelia T. Gonzales'	10
<i>H. rosa-sinensis</i> 'Loren B. Legarda' x <i>H. rosa-sinensis</i> 'Connie S. Angeles'	3
Accession No. 25 x <i>H. rosa-sinensis</i> 'Dolores A. Ramirez'	3
Accession No. 3 x <i>H. rosa-sinensis</i> 'Gelia T. Castillo'	60
<i>H. rosa-sinensis</i> 'Estrella F. Alabastro' x <i>H. rosa-sinensis</i> 'Emerlinda R. Roman'	10
<i>H. rosa-sinensis</i> 'Millenia' x <i>H. rosa-sinensis</i> 'Loren B. Legarda'	10
<i>H. rosa-sinensis</i> 'Gelia T. Castillo' x <i>H. rosa-sinensis</i> 'Betty Go-Belmonte'	0
Bangkok Tangerine	30

comparable to the findings of Medeiros et al. (2010) demonstrating that the amplification using primers CgInt/ITS4 was positive for all isolates of *C. gloeosporioides*, generating fragments of approximately 450 bp and negative for isolates of other *Colletotrichum* species. The isolates of *C. gloeosporioides* were confirmed by the use of species-specific primers.

The Cause of Early Wilting and Die-back in Gumamela

Colonies were fast growing, having a cottony aerial

Table 1. continued.

Entry	Average leaf area infected per plant (%)
Germplasm Collections	
Madeline	85
Karamello	80
Peach double	5
Tarantella	10
Petite Orange (H-89)	5
Kristy My Darling (H-029)	2
Accession No. 2	3
Accession No. 24	40
Accession No. 27	50
Accession No. 113	10
CBA	5
Ph 2	20
<i>Hibiscus cooperri</i>	0
<i>Hibiscus schizopetalus</i>	0
Reddy or Not	0
Wilcox	0
Petite Peach	0

mycelium which was whitish at first. After 3 days, fresh cultures of the isolates appeared pink with tinge of purplish color (Figure 5) which is similar to the descriptions in published reports (Nelson et al. 1981) on *F. oxysporum* on PDA as being pink to salmon pink or purplish. Fungal isolates that morphologically resembled the *Fusarium oxysporum* complex (FOC) were found to be associated with the diseased-plants showing die-back symptom. Three types of spores were produced including: microconidia, macroconidia, and chlamydoconidia (Figure 6). Microconidia were elongated, slightly curved, unicellular, hyaline, with conidial size of 5-15 x 3-5 µm. Macroconidia were slightly crescent, usually 3-5 septate, 24-35 µm x 3-5 µm. Chlamydoconidia were intercalary or terminal, spherical, occurring singly or in groups of two to three. These morphological characteristics of the fungus are in accordance with those of *F. oxysporum* amend. Snyder & Hans (Nelson et al. 1981).

Fusarium oxysporum is an imperfect fungus belonging to the ascomycetes or sac fungi. It spreads by means of asexual spores (microconidia, macroconidia, or chlamydoconidia). Chlamydoconidia can survive in the soil and the mycelium can survive and incubate in the waste from infected plants. The fungus is cosmopolitan and once it becomes established in an area, it is very difficult or maybe impossible to eliminate.

Stem rot and Wilting Caused by *Rhizoctonia solani*

The third pathogen from hibiscus plant causing stem rot (Figure 2) and wilted or blighted symptoms, was easily identified microscopically by the characteristic right-angle branching of the fungal hyphae (Figure 7). No spore or conidium was observed which is typical of the morphological characteristic of *R. solani* according to the published descriptions of Parmeter and Whitney (1970) and by Pascual et al. (2000). The fungal colony was whitish which grew very fast covering the 90 mm PDA plate just

in 5 days (Figure 8a). The colony produced few ~1 mm whitish sclerotia which turned brown and aggregated as the fungus aged (Figure 8b). The mycelia also became brown with time.

The identification and classification of *Rhizoctonia solani* are primarily based on anastomosis behaviour (Ogoshi 1972). In recent years, 14 anastomosis groups (AGs) have been recognized (Carling et al. 2002, El-Samawaty et al. 2008). Isolates of *R. solani* from different AGs generally do not anastomose with each other (Carling et al. 1996). Many of these AGs have been subdivided on the basis of host range, cultural morphology and biochemical or molecular characteristics (Ogoshi 1987).

Rhizoctonia solani isolate described in this study caused basal stem rot. Verma (1991) indicated that AG4 isolates mainly attack adult plants that can cause basal stem rot. To accurately determine the anastomosis group of this *R. solani* isolate from hibiscus, pairing the isolate with tester strains and observing the hyphae for fusion with the tester should be done.

Other reports indicated that because *Rhizoctonia* species do not produce spores, these fungi are identified by the characteristics of their hyphae. *Rhizoctonia* hyphae produce branches at right angles to the main hyphae (Parmeter and Whitney 1970), which jibed with the result of this study. Aggregates of the mycelia clumped together to form irregularly round sclerotial bodies (1-2 mm diameter). These sclerotial bodies are resistant to extreme environmental conditions, thus allowing the fungus to survive under adverse conditions. *R. solani* is a fungus with a very broad host range (Chase 1998).

Pathogenicity Tests

Leaf Spot of *Hibiscus*

Pathogenicity test for the disease showing small leaf spots was conducted by inoculating healthy leaves of hibiscus with the conidial suspension (10^6 spores/ml). Within seven days, disease symptoms such as small sunken light brown to brown spots (1-2 mm diameter) having halo-like yellowing around the lesions, similar to those observed on naturally infected leaves of hibiscus developed. Leaves inoculated with distilled water serving as control did not produce any symptom. Results indicate that the isolate used was the cause of leaf spot in hibiscus at IPB nursery.

Colletotrichum sp. was consistently isolated from diseased plant tissue which was identified as *Colletotrichum gloeosporoides* based on morphological and cultural characteristics. This identification was based on the description of Sutton (1992). It has cylindrical conidial shape with an average conidial size of $18.2 \mu\text{m} \times 8 \mu\text{m}$ (similar to Figure 4). The colonies had sparse, pale grey to blackish mycelium, and setae were also present.

Dieback and wilt disease

Initial symptoms caused by the isolated wilt pathogen

occurred 7 days after inoculation in the greenhouse which was characterized by brown dieback lesions, similar to the originally collected specimen. They produced similar symptoms to naturally infected plants. No symptoms occurred on plants inoculated with water only which served as control plants.

The fungus was re-isolated from the lesions on the inoculated plants. The causal fungus was identified as *F. oxysporum* based on the pathogenicity and morphological characteristics. Similar to the report of Nelson et al. (1981), the first sign of *Fusarium* vascular wilt was often a clearing of the outer edges of young leaves followed by drooping. It was explained that infection was already well established as the fungus invades the plant through the roots where it travels through the xylem of the plant that conducts water and nutrients from the root to the crown of the plant. As the mycelium ramified throughout the plant, the xylem became obstructed and the plant wilted and eventually died.

Wilt disease can kill the entire plant in one or two weeks or sometimes even more quickly under natural or field condition. Mature plants may survive despite the presence of the fungus as observed in some plants in the nursery. If just a tip or branch is wilted, then the plant has dieback disease, which can be cured by pruning away the damaged branch. The *Fusarium* wilt fungus can survive in soil for many years. Hence, it is extremely important that sanitation procedures be practiced diligently to prevent the entrance of *Fusarium* wilt into stock production areas.

Stem rot and wilt disease

The isolated pathogen causing stem rot and wilt was pathogenic to the inoculated hibiscus potted plants grown in autoclaved soil. At first it induced wilting of the plant then followed by blighting of the foliage and rotting of the base of the stem two weeks after inoculation. Similarly, foliage blight is the typical symptom caused by *R. solani* in other crops grown in the field (Kim et al. 1994, Pascual et al. 2000). The typical *R. solani* is similar to the originally collected isolate and to the re-isolated fungus taken from the inoculated hibiscus test plants. No stem rot symptom was observed in the control plants.

In situ Evaluation of Hibiscus Hybrids and Germplasm to Prevalent Disease

Generally, resistance is the most effective and economical method of disease control. Hence, evaluation of resistance to the predominant disease of hibiscus in the nursery was initiated during the wet season of 2009 from July to December. Hybrids and germplasm collections in the gumamela nursery were evaluated *in situ* for leaf spot caused by *Colletotrichum* leaf spot. Out of the 38 entries evaluated, 14 showed less than 10% average leaf area infected for leaf anthracnose (Table 1). Seven other entries consisting of two *Hibiscus* species, ie. *H. cooperri* and *H. schizopetalus* (Figure 10-left & center), two hybrids ie. *H. rosa-sinensis* 'Perla Santos-Ocampo' x *H. rosa-sinensis* 'Loren B. Legarda' and *H. rosas-sinensis* 'Gelia T. Castillo' x *H.*

rosa-sinensis ‘Betty Go-Belmonte’ and three other varieties, i.e. ‘Reddy or Not’, ‘Wilcox’ (Figure 9) and ‘Petite Peach’ were not infected by the *Colletotrichum* leaf spot. However, *Hibiscus rosa-sinensis* ‘Loren B. Legarda x *H. rosa-sinensis* ‘Lilia B. de Lima’ is susceptible to the disease (Figure 10-right). This result indicates that these genotypes could have some form of resistance against the *Colletotrichum* leaf spot disease. This further suggests that the resistant related species especially *H. cooperri* having red and white variegated leaves (Figure 9) could be used for hybridization with the susceptible varieties to develop a variety with a dual trait, i.e. resistant to the fungus and leaves with variegations as another ornamental trait of the hybrid variety.

SUMMARY, CONCLUSION AND RECOMMENDATION

The different hibiscus genotypes and hybrids at the CSC-IPB breeding blocks have been attacked by a few diseases. The etiology of these diseases, their causal organism including the identification of the natural sources of resistance is crucial for the *Hibiscus* breeding programme. This is the first report dealing with three diseases of *Hibiscus* occurring in the breeding nursery in the Philippines. Leaf spot disease of *Hibiscus* caused by *C. gloesporoides*, was isolated from leaf spot lesions of infected plants. The organism has typical morphological and cultural characteristics of *C. gloesporoides*. The species identity was confirmed by PCR using species-specific primer pair CgInt/ITS4. Pathogenicity showed that the disease can be reproduced in healthy hibiscus leaves within seven days after inoculation.

Another disease causing wilt and die-back of hibiscus was found to be caused by *Fusarium oxysporum*. *F. oxysporum* produces three types of spores including: microconidia, macroconidia, and chlamydospores which were all found in the isolated pathogen. The dieback and wilt symptoms caused by *Fusarium* wilt was also observed after pathogenicity test which proved the identity of the causal organism.

Fusarium wilt occurring in the nursery can be controlled by drenching a mixture of 1 pint of household bleach with 2 qt of warm water into the base of the affected plant until all the potting mix is saturated. This will kill off the fungi in the mix but will not affect the fungi already in the plant. Alternatively, fungicides can be used at recommended dosage. While the infected plant is recovering, keep it in the shade and do not over water it.

Rhizoctonia solani is another fungal pathogen that was identified that caused stem rotting of the base of the plant, wilting and blighting of hibiscus plants. Characteristically, the fungal hyphae has a right angle branching and it has no spore or conidium, a typical morphological characteristic of the fungus.

The *R. solani* isolate was pathogenic to the inoculated hibiscus potted plants grown in autoclaved soil. At first, it induced wilting of the plant followed by blighting of the foliage and then rotting of the base of the stem two weeks after inoculation.

Out of the 38 *Hibiscus* genotypes evaluated for resistance to leaf anthracnose, 14 showed less than 10% average leaf area infected for leaf anthracnose. In contrast, two *Hibiscus* species namely: *H. cooperri* and *H. schizopetalus*, 2 hybrids *H. rosa-sinensis* ‘Perla Santos-Ocampo’ x *H. rosa-sinensis* ‘Loren B. Legarda’ and *H. rosas-sinensis* ‘Gelia Castillo’ x *H. rosa-sinensis* ‘Betty Go-Belmonte’, including 3 other varieties like ‘Reddy or Not’, ‘Wilcox’ and ‘Petite Peach’ were not infected by *Colletotrichum* leaf spot or leaf anthracnose under field conditions, suggesting that they could have some form of resistance to *Colletotrichum* leaf spot disease and could be used by the breeder in improving the hibiscus variety.

These findings are the first report of the three gumamela diseases in the Philippines. This can be due to the fact that research study on gumamela diseases was very limited in the country. However, since this ornamental plant is becoming popular in recent years, attention on its diseases and pests should be given importance.

ACKNOWLEDGMENTS

The authors acknowledge the Crop Science Cluster-Institute of Plant Breeding, College of Agriculture, University of the Philippines Los Baños (UPLB) and the Las Piñas City Government thru the Villar Foundation for the resources used in this study. The assistance rendered to the authors by Ms. Ana Kristine S. Barcos, Mr. Mark Angelo O. Balendres, Ms. Amalia R. Ilagan, Ms. Rizalina L. Tiongco, Ms. Maria Fe H. Cayaban, Mr. Marcelino T. Gregorio and Mr. Jessie V. Silverio is also acknowledged.

CONFLICTS OF INTEREST

This study has no conflicts of interest with other researchers. To our knowledge there are no other research works on diseases of ornamental *Hibiscus* in the Philippines.

CONTRIBUTION OF INDIVIDUAL AUTHORS

Dr. Cecilia B. Pascual is the plant pathologist who identified the diseases reported in this study, while Dr. Pablito M. Magdalita is the plant breeder who is in charge of the development of the hibiscus varieties used in the experiments. He is also the project leader of the Hibiscus Breeding Project at the Crop Science Cluster and Institute of Plant Breeding, College of Agriculture, University of the Philippines Los Baños, College, Laguna.

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