

OCCURENCE OF ANTHRACNOSE (*Collectotrichum gloeosporioides* PENZ.) AND RUST (*Goplana dioscoreae* CUMMINS) DISEASES OF DIOSCOREA IN SRI LANKA

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ABSTRACT

Yams (*Dioscorea* spp) are one of the most divers root crops which contain high nutrient values and medicinal properties and have the potential in commercial cultivation. Leaf spots followed by vine die-back became one of the limiting factors for *Dioscorea* cultivation in recent past. Studies were conducted at Horticultural Crops Research and Development Institute to identify causal organisms of major diseases, their field symptoms and pathogenicity. Anthracnose and rust of *Dioscorea* have been identified as two fungal diseases caused by *Collectotrichum gloeosporioides* Penz. and *Goplana dioscoreae* Cummins respectively, in Sri Lanka and their pathogenicity was established. Sixteen *Dioscorea* cultivars belonging to five *Dioscorea* spp currently grown in Sri Lanka were evaluated for their reaction to anthracnose and rust diseases under natural infections. All the cultivars of *D. alata* were highly susceptible to Anthracnose, but highly resistant to rust disease. Two out of five *Dioscorea* spp i.e, *D. rotundata*, and *D. pentaphyla* were resistant to anthracnose and rust under natural and artificial inoculation conditions. All the cultivars of *D. esculanta* were highly susceptible to rust.

Keywords: Anthracnose, *Dioscorea* spp, diseases, Resistant, Rust, Varietal resistance

INTRODUCTION

Yams (*Dioscorea* spp.) belong to the family Dioscoreaceae and to the genus *Dioscorea* consisting of over 600 species (Coursey, 1983). Yams are a major source of carbohydrates but contain about 1- 2% dietary protein, calcium, phosphorus, iron and vitamins, riboflavin, thiamin, vitamins B and C and antioxidants (Ajayi, 2012).

Out of the eight edible species existing in Sri Lanka, *D. alata*, *D. esculenta*, *D. bulbifera*, *D. hispida*, *D. opposite*, *D. trifida* and *D. pentaphylla* are present in natural ecosystems. *D. rotundata* was introduced from Africa. Two new improved *D. alata* varieties; Rajala, Kakulala and one *D. esculenta* variety kukulala (Kadira) were released by the Department of Agriculture for cultivation in Sri Lanka in year 2007 (Jayasurya, 1984; Thavabalachandran and Silva 2013). Sangakkara and Frossard (2014) reported that *D. alata* is an essential component of Sri Lankan home gardens in rural areas and has the potential to enhance food security and rural development. At present, raja ala is one of the most popular *D. alata* variety and it is grown commercially in Monaragala areas in Sri Lanka due to its export value.

Worldwide yam production is 52 million tons of which Africa produced 96%. Most of the world's production comes from West Africa representing 94%, with Nigeria alone producing 71%, equaling more than 37 million tons (IITA Annual report, 2009). Several diseases; fungi causing anthracnose, leaf spot, leaf blight, and tuber rot; and the yam mosaic virus (YMV) are known to infect *Dioscorea* throughout the World (Amusa *et al.*, 2003). In Sri Lanka, various leaf spots and vine die-back have been reported in yam growing areas and research fields as well. However, very little is known about disease incidence, severity and yield losses. Anthracnose and other leaf spots can be controlled by certain fungicides. Currently, there is no fungicide recommendation for the management of diseases in yam cultivation in Sri Lanka and farmers also not adapted to practice it. Hence, cultivation of resistant varieties is the most effective and economical means to control foliar diseases when they occurs frequently. Therefore, the objectives of this study are to identify the field symptoms, the causal organisms responsible for diseases and resistance sources from local cultivars and provide with basic information on *Dioscorea* diseases.

MATERIALS AND METHODS

Sample collection and Pathogen isolation

Disease symptoms were recorded from infected field at Horticultural Crops Research and Development institute (HORDI) and samples received from farmer fields at Ussapitiya, Ulpothagama and Matale during 2014 and 2015. Infected leaves exhibiting different symptoms such as spots, blight, dieback, sun scorch etc. were collected for pathogens isolation.

The fungal pathogens were isolated from lesions of affected leaves. Small pieces (2 x 2mm) of infected leaf tissues were surface sterilized with 70% alcohol for 1-2 min and placed aseptically on PDA plates containing streptomycin 0.1/W/V. The plates were incubated for 1- 2 days at room temperature (28 – 30°C). The isolates were sub cultured and incubated for 5 days at RT for sporulation. The isolates were purified using the single-spore isolation method with slight modifications (Choi *et al.*, 1999). *Pure cultures were stored at 4°C on PDA*

The mycelium growth and conidia development on PDA of purified isolates were observed. The isolated pathogens were identified based on morphological characteristics such as colony characters as well as shape and size of conidia under light microscope (Kumar, 2014).

Spores of Rust disease were collected from pustules of the infected leaves and observed under light microscope to identify the pathogen of rust disease (Chung *et al.*, 2009).

Pathogenicity test of Anthracnose and Rust diseases

Ten cultivars belonging to five species of *Dioscorea* were selected and Pathogenicity of both pathogens were separately tested under green house condition. Test plants were raised in sterilized soil in plastic pots and inoculation was made at 5 -6 leaf stage of growth.

Inoculum for anthracnose pathogen was prepared from one purified isolate (H1-Kiriwelwila) of *D. alata* (Figure 3). Conidia of H1 isolate were suspended in sterilized

ANTHRACNOSE AND RUST IN DIOSCOREA

distilled water and the concentration of conidia in the suspension was adjusted by hemocytometer to about 10^5 conidia/1ml. The spore suspension was sprayed using a hand sprayer into the air above the test plants and droplets were allowed to settle on them. The control plants were sprayed with sterilized distilled water. Inoculated plants were kept in the green house and maintained (humidity nearly 100%) at room temperature for 2 days. Plants were observed daily for development of anthracnose symptoms. When the disease symptoms appeared the fungus was re-isolated from the inoculated plants to fulfill the Koch's postulates.

For the Rust pathogen, same varieties were inoculated with rust spores by dispersing uredinospores (1mg/ml) in a dilute solution of surfactant (2 drops of Tween 20 per 100/ml of distilled water), and spreading it on to leaf surface to run off and inoculated plants were maintained 100% RH at room temperature (28-30°C). The control plants were sprayed with sterilized distilled water.

Varietal screening for anthracnose and rust diseases under field conditions

Dioscorea cultivars established at research field, HORDI for conservation, multiplication and characterization purposes were used to test their reaction of anthracnose and rust diseases during 2015. Sixteen cultivars (including three recommended varieties) belonging to 5 *Dioscorea* species were selected (Table 2). Crop was established in May 12, 2015 in three 5m long, 3 rows at 1m x 1m spacing between rows (15 plants/ plot). All agronomic practices followed based on the recommendation of the DOA. The disease incidence and severity (Table 1) were recorded at weekly intervals until harvesting stage.

Table 1. Disease severity scores

Scale	Level of infection	Description
0	No infection	Highly Resistant (HR)
1	1-5% Leaf area affected	Resistance (R)
2	6-25% Leaf area affected	Moderately Resistant (MR)
3	26-50% Leaf area affected	Susceptible (S)
4	>50% Leaf area affected	Highly Susceptible (HS)

$$\text{Disease incidence} = \frac{\text{No of affected plants}}{\text{Total No of plant}} \times 100$$

RESULTS AND DISCUSSION

Disease symptoms observed in the field

Different symptoms of anthracnose such as dark brown or black lesions on “Raja ala” variety collected from Ulpathagama area (Figure 1f) or large gray lesions bordered by an olive green patch starting from lamina edge extending inwards on leaves of “Kiriwelwila” cultivar were observed (Figure 1c). Blighted appearance with large purple blotch or dark brown burnt leaf surface, sometimes on petioles, stems and tubers observed in highly susceptible cultivars, i.e. Raja ala, Monaragala Raja ala etc (Figure 1a & 1b). Some lesions were often surrounded by a chlorotic halo and enlarged and coalesced on leaves of “Hingurala” and “Angili ala” (Figure 1d), resulting in extensive necrosis of the leaves followed by die-back of the stem in highly susceptible cultivars. The withered leaves and stem die-back gave the plant a scorched appearance. The aservuli were found as the dark concentric circle on the infected leaves. Anthracnose reduces the effective photosynthetic area of yam leaves, thus resulting in yield loss. The commencement of the epidemic before or during tuber formation resulted in about 80 – 90% yield loss (Adefoyeke *et al.*, 2008).

Rust symptoms appeared after 4 months of planting. Rust symptoms appeared initially as small pale spots which become yellow with a small dark center on leaves of *Dioscorea esculenta* species (Figure 2). These spots enlarged and forming pustules (‘sori’ or ‘uredia’). The pustules are brown or brownish red color, circular or oval shape and 2.0 - 2.3 mm in diameter and appeared first and more abundantly on under surface of the leaves and ruptured to expose masses of urediniospores. Severely infected leaves became necrotic and dry but tended to remain attached to the plant.

ANTHRACNOSE AND RUST IN DIOSCOREA

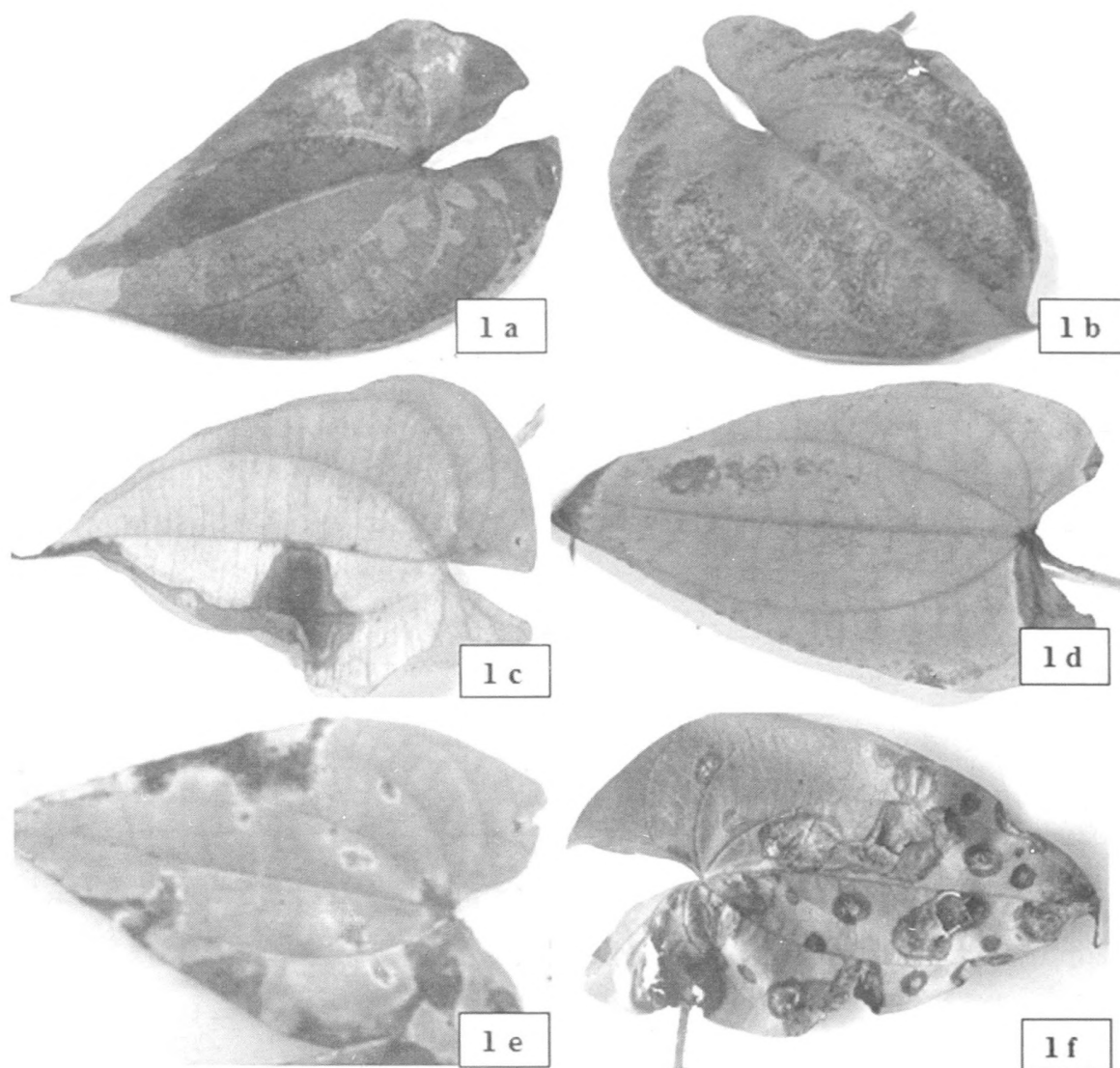


Figure 1. Different leaf symptoms of Dioscorea Anthracnose; 1a-Light to dark brown burnt leaf surface, 1b-Large purple blotch, 1c-Large gray lesion bordered by an olive green patch starting from lamina edge extending inwards, 1d-Large brown lesion at lamina edge bordered by a yellow line, 1e-Large irregular brown spot with yellow halo, 1f- Light brown spot surrounded by a dark brown ring.

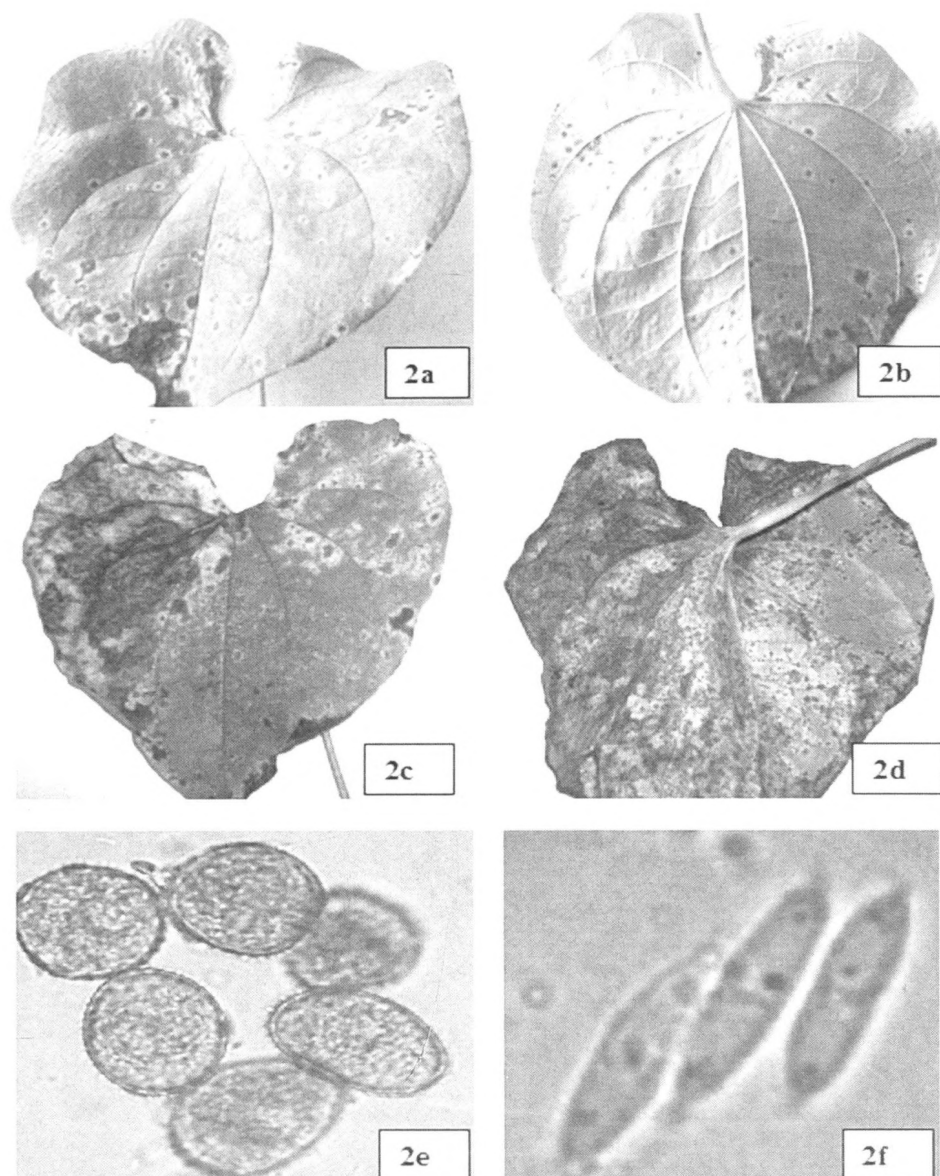


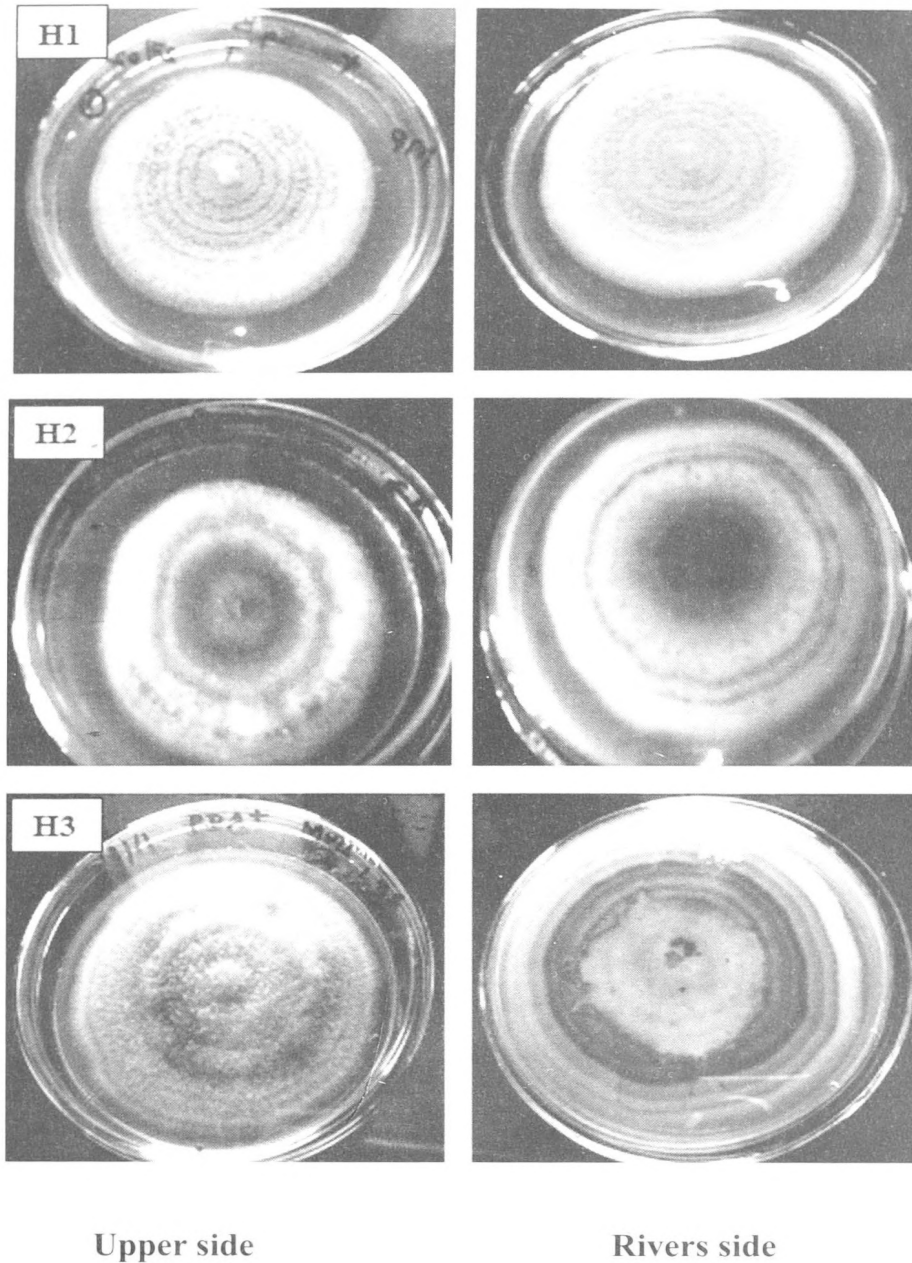
Figure 2. Different leaf symptoms of *Dioscorea* Rust; 2a, 2b-They had tan to brown, textured centers and irregular margins, and ranged in diameter from 1-2 mm to 2.5cm. Individual lesions were surrounded by chlorotic (yellow) halos, 2c, 2d- Brown spots rapidly expanding lesions often coalesced into extensive, 2e- Urediniospore(18-28 μ m length x 16 -23 μ m width) of *Goplana dioscoreae*, 2f-Teliospores of *Goplana dioscoreae*

ANTHRACNOSE AND RUST IN DIOSCOREA

Identification of pathogen of anthracnose

Visual and microscopic studies showed that isolates taken from different varieties (isolates; H1- Kriwelwila var., H2-Angiliala var. and H3-Raja ala var.) belonging to *D. alata* produced different growth patterns, colony colour and other morphological characters in cultures (Figure 3). Some isolates were fast growing while others were slow growing indicating that growth rate also differed among the isolates. Mycelial color types varied from white, grey to, dark brown and some isolates with salmon pink spore masses (Figure 3H1) on the upper surface and grey to black, white to pink on the lower surface while growth pattern ranged from concentric growth with distinct rings to less pronounced rings. The fungus produced abundant and visible fruiting bodies with submerged acervuli in the old cultures.

Spore dimension and shape of three *Colletotricum* isolates (H1- H3) when the cultures were grown on PDA are presented in Table 4. The size of conidia varied between isolates, but all were within the published range, (*Kumar, 2014*) Size of the spore of isolate H2 was smaller than H1 and H3 isolates. The conidia shape varied from, cylindrical to fusiform with obtuse to pointed ends, hyaline, aseptate and differed with different isolates. All isolates studied possessed key features in cultures, which Mordue, 1971 describe as typical for *C.gloeosporioides*.



Upper side

Rivers side

Figure 3. Different isolates *Colletotricum* morphological characters on PDA; H1- Pronounced concentric growth with orange pink rings viewed from upper and rivers side, orange acervuli, H2- Grayish dark brown growth with an outer light brown growth ring. Black growth surrounded by a light brown /gray concentric ring on the underside, H3- Dark gray fluffy mycelia growth, less pronounced growth rings, light/dark gray concentric rings growth on the reverse side

ANTHRACNOSE AND RUST IN DIOSCOREA

Table 2. Reaction of *Dioscorea* cultivars to anthracnose pathogen, *C. gloeosporioides* during 2015.

Species /Variety	Disease Incidence (%)			Disease Severity Score	Disease reactions**
	2 MAP*	4 MAP	6 MAP		
<i>D.alata</i>					
Raja ala	86.66	100	100	4	HS
Monaragala raja ala	0	40	100	4	HS
Jaffna rasawalli	26.66	60	100	4	HS
Kakulala	0	26.66	46.66	3	S
Suta Hingurala	0	0	20	1	R
Maha Hingurala	0	0	20	1	R
Dandila	0	33.33	60	4	HS
Angiliala	0	33.33	60	1	R
Kiriwelwila	0	0	60	1	R
Ethpada	6.66	26.66	60	3	S
<i>D.esculanta</i>					
Nattala	0	0	0	0	HR
Mahakukulala	0	13.33	33.33	1	R
Sutakukulala	0	0	20	1	R
<i>D.bulbifera</i>					
Udala	0	20	20	2	MR
<i>D.rotundata</i>					
Eshuma	0	0	0	0	HR
<i>D.pentaphyla</i>					
Katuala	0	0	0	0	HR

*MAP-Months After Planting ** 0 HR -Highly Resistant (No infection), 1R- Resistant (1-5% leaf area affected), 2 MR Moderately Resistant (6-25% leaf area affected), 3 Susceptible (26-50% leaf area affected),4 Highly Susceptible (>50% leaf area affected)

Table 3. Reaction of *Dioscorea* cultivars to rust pathogen, *G. dioscoreae* during 2015.

SPP. /Variety	Disease Incidence (%)		Disease Severity Score	Disease reactions**
	4 MAP*	6 MAP		
<i>D.alata</i>				
Raja ala	0	0	0	HR
Monaragala raja ala	0	0	0	HR
Jaffna rasawalli	0	0	0	HR
Kakulala	0	0	0	HR
Suta Hingurala	0	0	0	HR
Maha Hingurala	0	0	0	HR
Dandila	0	0	0	HR
Angiliala	0	0	0	HR
Kiriwelwila	0	0	0	HR
Ethpada	0	0	0	HR
<i>D.esculanta</i>				
Nattala	100	100	4	HS
Mahakukulala	100	100	4	HS
Sutakukulala	100	100	4	HS
<i>D.bulbifera</i>				
Udala	0	0	0	HR
<i>D.rotundata</i>				
Eshuma	0	0	0	HR
<i>D.pentaphyla</i>				
Katuala	0	0	0	HR

*MAP-Months After Planting

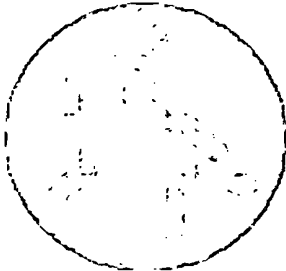
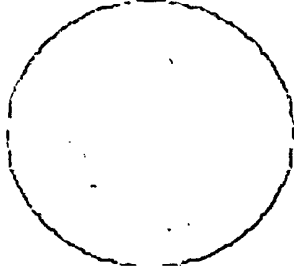
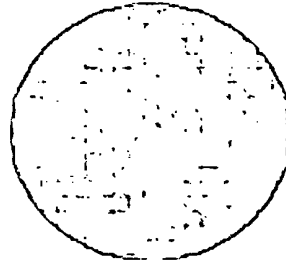
** 0 HR -Highly Resistant (No infection), 1R- Resistant (1-5% leaf area affected), 2 MR Moderately Resistant (6-25% leaf area affected),3 Susceptible (26-50% leaf area affected),4 Highly Susceptible (>50% leaf area affected)

ANTHRACNOSE AND RUST IN DIOSCOREA

Identification of pathogen of rust disease

The microscopic observation of urediniospores are globose, subglobose, ellipsoid, hyaline and pale yellow to chestnut brown and mean sounding 18-28 μm length x 16 -23 μm width. Telia are large, cylindrical, and colorless; with round to acute apex, septate, hyaline 30.8 μm length and 5.7-10.4 μm width (Figure 2e). These urediniospores and teliospores are taxonomically very similar to *Goplana dioscoreae* Cummins. (Cummins,1941). All key features are within the published rang on typical for *Goplana dioscoreae* (Chung *et al.*, 2009).

Table 4. Groups of *Colletotrichum* isolates based on spore size and shapes.

Isolates	Mean Length (μm)	Mean Width (μm)	Shape of spores	
H1	15.42	5.32	Cylindrical shaped. One end is pointed and other end is rounded. Hyaline. Aseptate	
H2	14.72	6.23	Very small in size. Fusiform. Acute at both ends. The center is swollen. Hyaline. Aseptate	
H3	17.39	5.34	Cylindrical shaped. Both ends are rounded. Hyaline. Aseptate	

Pathogenicity test

Symptoms typical to anthracnose were observed on test plants seven days after inoculation. The conidia produced on inoculated leaves, cultured and their morphological features of the *C.gloeosporioides*, re isolated from diseased leaves were the same as the initial inoculums. The control plants had no symptoms of infection.

Microscopic observation and the pathogenicity test revealed that the cause of the anthracnose in Dioscorea is the fungus *Colletotrichum gloeosporioides* Penz. *D. esculanta*, *D. rotundata*, *D. pentaphyla* free of infection after artificial inoculation of anthracnose pathogen indicating that these 3 species were resistant to anthracnose disease.

Typical symptoms of the rust lesion were observed after 4 weeks of inoculation in inoculated plants of *D. esculenta* which were identical to those observed in the research field and urediospores of the rust pathogen collected from inoculated plants. However, control plants remained healthy.

According to the disease symptoms, microscopic observation of urediospores and teliospores and the pathogenicity test, the causal agent of rust disease was identified as fungus *Goplana dioscoreae* Cummins.

Varietal resistant to anthracnose and rust diseases under field conditions

The results of field screening of yam cultivars against anthracnose and rust disease are given in Table 2 and 3. Anthracnose symptoms first appeared in *D. alata* varieties of “Raja ala” (86%) and “Jaffna Rasawalli” (26%) after 2 months of planting. Disease incidence reached 100% in *D.alata* species var. “Raja ala”, “Monaragala raja ala” and “Jaffna rasawalli” after 6 months of planting and disease severity rated score 4 (>50% leaf area affected) recorded as highly susceptible to anthracnose. Disease incidence of rest of the varieties belonging to *D. alata* species ranged from 20 - 60 % at 6 months of planting. However, “Suta” and “Maha Hingurala”, “Angiliala” and “Kiriwelwila” showed low disease severity (DS score 1) rate showing resistance to anthracnose. “Udala” (*D.bulbifera*) showed anthracnose symptoms after 4 months but the incidence was low (20%), severity score is 2 (25% leaf area affected) which was rated Moderately Resistant.

ANTHRACNOSE AND RUST IN DIOSCOREA

Anthracnose symptoms vary according to the varieties, age of the leaf and the weather condition prevailed during the cropping period. Adefoyeke *et al.*, (2008) reported that *D. alata* known as water yam (greater yam) is the most susceptible *Dioscorea* species to anthracnose in other countries. Two out of 5 species *D. rotundata* and *D. pentaphyla* and “Nattala” (*D. esculanta*) which were free of infection of anthracnose under natural infection and artificial inoculation as well, were recorded as highly resistant to anthracnose.

All varieties of *D. esculenta* showed 100% disease incidence for rust disease and disease severity score was 4 (>50% leaf area affected) and recoded as highly susceptible to rust disease. Rest of the cultivars belonging to other species (*D. alata*, *D. bulbifera*, *D. rotundata* and *D. pentaphyla*) were free of infection of rust under natural conditions and disease severity score was 0 (No infection). They were recorded as highly resistant to rust disease.

Therefore it can be suggested that cultural management including removal and destruction of disease leaves at early stage of crops and use of resistant cultivars and crop rotation are the most important strategies to control anthracnose and rust diseases of susceptible varieties of *Dioscorea*.

CONCLUSIONS

Anthracnose and rust diseases of *Dioscorea* have been identified as two fungal diseases, caused by *Colletotrichum gloeosporioides* Penz. and *Goplana dioscorea* Cummins, respectively in Sri Lanka. All the varieties of *D. alata* were highly susceptible and *D. bulbifera* moderately resistance to Anthracnose. Both species of *D. rotundata* and *D. pentaphyla* were resistant to anthracnose and rust disease. All varieties belonging to *D. esculanta*, except “Nattala” were resistant to anthracnose. Cultivars; “Nattala”, “Mahakukulala” and “Sutakukulala” belonging *D. esculanta* were highly susceptible to rust disease and the rest of the *Dioscorea* species were resistant to rust. Variation of spore and colony morphology of different isolates of *Collectotrichum* was observed. Further research on molecular identification of *Collectotrichum* isolates and studies on post-harvest losses due to pathogens need to be carried out.

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