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Research Article

Virulence Study of *Phytophthora colocasiae* Isolates Collected from Taro Growing Areas of Southern Ethiopia

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ABSTRACT

Phytophthora colocasiae is the most destructive fungal pathogen of taro (*Colocasia esculenta*), which causes taro leaf blight. Taro leaf bight symptoms differed among the same Taro cultivars grown under similar conditions. This raised the question whether *Phytophthora colocasiae* isolates vary in their virulence or not. To give responses for the question raised 27 taros growing farmers' fields were surveyed during 2017 cropping season. Out of that 15 representative *Phytophthora colocasiae* isolates were isolated in the laboratory. Then virulence test of isolates were carried out by using detached leaf disc method. All isolates were pathogenic to taro, with significantly different ($P<0.01$) lesion diameter. The most virulent isolate was Sodo Zuria isolate, followed by isolates from Damot Gale and Boloso Sore with mean lesion diameters of 46 mm, 37 mm and 35 mm, respectively. On the other hand, an isolate from Hadaro and Kachabira were considered as the least virulent with a mean lesion diameter of 17 mm. virulence of the isolates was strongly associated with altitude from which sample was collected ($r=0.55$).

Keywords: Taro leaf blight; *Colocasia esculenta*; Virulence; Lesion diameter; Altitude

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott) is one of the major starchy food plants originated from tropical swamps in China, India and so on. Taro is an important staple crop for millions in most parts of the world. Humans obtain important nutrients such as calcium, phosphorus, iron, Vitamin C, Thiamine, Riboflavin and Niacin from Taro. The corms are consumed after boiling, frying or roasting. It can also be dried and used to make flour or sliced and fried to make chips. In Ethiopia taro has been cultivated mainly and extensively in dense populated and high rainfall areas of South, Southwest and Western parts of the country. Southern part of Ethiopia especially Wolaita Zone currently grow taro extensively, due to the acute problems caused by enset bacterial wilt and sweet potato butterfly, on enset and sweet potato, respectively. In this area processed forms of taro is uncommon but it is simply boiled and consumed similar to potatoes. In some areas, taro is used to fill seasonal food gaps when other crops are not in the field. Taro production all over the world has been threatened by a lot of diseases. The most devastating among these diseases is Taro Leaf Blight (TLB). The disease was first described in Java by Marian Raciborski in 1900 [1]. It is a fungal disease of Taro caused by *Phytophthora colocasiae*. The pathogen thrives best in high humidity and high rainfall environments which aid the spread through rain splash on the free leaves. Taro has been devastated by leaf blight disease resulting 25%–60% yield loss in many countries. Trujillo and Aragaki stated that yield loss of Taro due to disease is not well known but individual fields may suffer from low yield loss to as much as 30% loss by *Phytophthora colocasiae*. The yield losses may reach 50% to 60% under severe blight conditions and susceptible taro cultivars can be destroyed completely [2].

Taro leaf blight symptoms appear as small, water soaked, round, dark brown necrotic spot with yellow halo and the disease developed yellow to red liquid drops in the middle of the spots during morning hours but when dry the liquid became solid and brown in color. Brooks described that the spots occur on the margin of the leaf and spots were circular and regularly increased in diameter. Further, yellow to red liquid drops developed in the middle of the spot in the morning but when dry the liquid became solid and brown in color. In dry weather the centre of lesions become papery and fall out producing a 'shot hole' appearance. Dead leaves often hang with their long petioles like a flag with bright orange or reddish brown plant exudates oozing from infection sites. A prominent sign of *Phytophthora colocasiae* is the white ring of sporangia around the edge of lesions [3-5].

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Bandyopadhyay, et al. observed symptoms of leaf blight caused by *Phytophthora colocasiae* in farmer's field as small, brown, coalescing lesions, sometimes with orange host exudation. White sporulation was evident on the lesion surface under wet condition. The pathogen caused rapid defoliation and killed plants. The lesions frequently form concentric zones and exude drops of yellowish liquid. Some of the diseased tissues may be covered with whitish fuzz consisting of sporangia. As the disease progresses, the lesions continue to expand and frequently coalesce. Diseased tissues disintegrate, forming holes of irregular size and shape on the affected leaves. Occasionally the pathogen may cause water-soaked lesion on the petioles. Infected leaves collapse within 20 days of unfurling, compared to 40 days for healthy leaves. The normal 6-7 leaves per plant are being reduced to 3-4 leaves per plant by severe disease incidence. In India Misra reported that severity of *Phytophthora* blight and yield losses differed among taro fields. In some taro fields, plant death was so widespread that the growers had to replant fields two or three times. In a survey of affected commercial fields, with the same taro cultivars and grown under similar conditions, incidence of leaf blight ranged from 17% to 68%, and tuber rot ranged from 4% to 45%. They also observed variation in growth and sporulation among *P. colocasiae* isolates from surveyed taro fields. For example, some isolates grew above 360°C while most of the isolates did not. In Southern Ethiopia, Tomas reported that survey carried out at taro fields with the same taro cultivars and grown under almost similar conditions, incidence of leaf blight ranged from 10% to 100% and severity ranged from 16.66% and 50%. These observations raised the question whether the isolates of *P. colocasiae* in different taro fields vary in virulence or not. This study was therefore aimed to determine if there is difference in their virulence among *Phytophthora colocasiae* isolates collected from different taro growing areas of Southern Ethiopia region [6-8].

MATERIALS AND METHODS

Description of experimental sites

From Wolaita and Kembata Tembaro Zones, 27 Taro growing farmer's fields were surveyed at 5-10 kms interval in 2017 cropping season. From them fifteen representative *P. colocasiae* isolates were isolated at Areka ARC Plant Pathology Laboratory.

Isolation and identification of fungi

From 27 farmer fields surveyed 15 representative taro leaf blight symptoms collected were surface-sterilized with 0.5% sodium hypochlorite solution for 60 sec and rinsed three times in sterile distilled water. Surface-sterilized leaf fragments were dried on sterile filter paper in a laminar flow hood and for each sample four leaf fragments were transferred into sterilized Petri dishes containing solidified cool Potato Dextrose Agar (PDA) medium amended with antibiotics (Penicillin, Rifampicin and Nystatin). Then, the Petri dishes were labeled and placed in an incubator at temperature of 22°C-26°C. After 2-3 days, the culture was sub cultured in to new Petri dishes to obtain pure culture of isolates and designated as A-O. Then isolated fungi were identified as *Phytophthora colocasiae* based on its mycelia and sporangial characters using standard mycological keys [9].

Virulence test

Virulence tests of *P. colocasiae* isolates were done by using detached leaf disc method. One 60 mm diameter leaf disc was taken from youngest, fully expanded and disease free, local taro cultivar (Molia) and placed into 90 mm diameter Petri dishes containing water amended with 150 µg/liter kinetin. Each leaf disc was inoculated in the center with each isolate of 6 mm diameter agar plug taken from 2 days old cultures of *P. colocasiae*. The inoculated leaf discs were incubated for 4 days at 25°C in the dark. Then mean lesion diameter was measured to identify virulent isolate. The treatments were arranged in CRD and replicated three times. The experiment was repeated to confirm the results [10-13].

Data collection and analysis

Data was collected from mean lesion diameters produced by *P. colocasiae* isolates on 60 mm diameter leaf disc and analyzed by using SAS computer software program and significant means were compared using Duncan Multiple Range Test (DMRT) at 99% level of probability [14-17].

RESULTS AND DISCUSSION

Virulence test

From the *P. colocasiae* isolates inoculated on the detached leaf disk of taro plant, isolate A, collected from Sodo Zuria produced extensive necrotic lesions on the leaf and the pathogen was found to be more virulent when compared with the other fourteen isolates, followed by isolates from Damot Gale and Bolosso Sore with mean lesion diameter of 46 mm, 37 mm and 35 mm, respectively. On the other hand, an isolates from Hadaro and Kachabira were considered as the least virulent with a mean lesion diameter of 17 mm. There were significant differences among isolates in the degree of necrotic lesions development (Table 1). Similarly Misra reported that there are significant differences among *P. colocasiae* isolates in their virulence on taro leaves. Isolates necrotic lesion development on the taro leaf disc was strongly associated with altitude, from which the sample was collected. For this experiment, scattered diagram and simple correlation analysis was used for studying the association between altitude and mean lesion diameter. Determined Pearson correlation coefficients (r) were used as indices for strength of the association. The correlation between MLD of the isolates and altitude is statistically significant ($P<0.05$) and positively associated with correlation coefficient values of r=0.55 (Figure 1) [18].

Table 1: Mean lesion diameters (mm) of *P. colocasiae* isolates after 4 days of incubation on the detached leaf disk.

Isolate	Altitude	Mean lesion diameter
A	2221	46
B	1977	21
C	2002	37
D	1994	29
E	1704	28
F	2116	21
G	1935	29
H	2051	20
I	1647	19
J	1974	35
K	1614	17

L	1554	20
M	1988	18
N	2081	29
O	1778	17
CV (%)		3.33

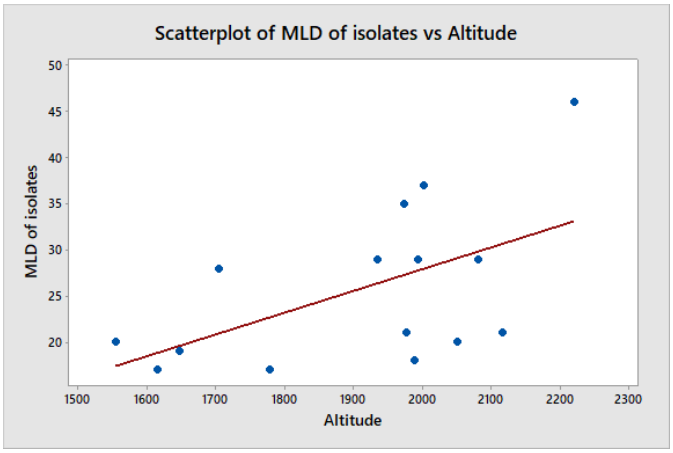

Figure 1: Diagram showing the relationship between MLD of the isolates and altitude (m) with Pearson correlation coefficients (r) =0.5. **Note:** MLD = Mean Lesion Diameter.

CONCLUSION AND RECOMMENDATIONS

The results obtained on virulence test of *Phythophthora colocasiae* on detached leaf disk revealed that all the 15 isolates showed variable necrotic lesions development. They caused lesions, on inoculated leaves. Isolate "A" showed extensive necrotic lesions on the leaf. There was variability in virulence among *Phythophthora colocasiae* isolates based on the small lesion lengths produced on detached leaf disk of Taro plant, isolate "A" produced extensive necrotic lesions on the leaf and followed by isolates from "C" and "J" with mean lesion diameter of 46 mm, 37 mm and 35 mm, respectively. On the other hand, an isolates from "K" and "O" were considered showed a mean lesion diameter of 17 mm. There was a significant difference (P=0.01) in mean lesion diameter on exposure to the different fungal isolates. Future research should be directed towards surveying more agro ecologies and epidemiological studies of the pathogen are needed to establish the correlation between the disease, virulence level of pathogen and weather factors.

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