Advances in Crop Science and Technology
ISSN: 2329-8863
Research Article Open Access

Morphological and Cultural Characterization of *Phytophthora colocasiae* Isolates Collected from Taro Growing Areas of Southern Ethiopia

Zerhun Tomas[1]*, Elfinesh Shikur[2] and Alemar Said[1]

[1]Department of Plant Pathology, South Agricultural Research Institute, Areka Agricultural Research Center, Areka, Ethiopia
[2]Department of Plant and Horticultural Science, College of Agriculture, Hawassa University, Hawassa, Ethiopia

Abstract

Leaf blight of taro caused by Phytophthora colocasiae has been responsible for the serious decline in yield of taro. In Ethiopia it has contributed to the decline in taro production but still farmers have not recognized taro leaf blight as a disease associating its symptom with a maturity stage of the crop and impact of heavy rain fall. Survey was carried out to isolate the pathogen from different taro growing areas of Southern Ethiopia to study if there is variation in morphological and cultural characteristics among isolates. Totally 27 farmers' fields were surveyed and 15 P.colocasiae isolates were isolated and designated as A-O. The fifteen isolates were characterized based on morphological and cultural characteristics by observing under simple light microscope at a magnification of 40X. Studies on morphological characteristics of different isolates of Phytophthora colocasiae revealed that the mycelium was aseptate and stalked sporangia. Sporangia type for isolate A, B, D, E, G, I, L and M were Semi papillate whereas isolate C, F, H, J, K, N and O have papillate. Sporangia shape for isolate A, G, H, J, L, M and N were lemon, isolate B, C, F and O were Globose where as isolate D, E, I and K have Ovoid shaped sporangia. The color of colony is ranging from white to dull white, cottony to moderately cottony in texture and abundance of mycelium is profuse to slightly sparse growth on PDA plates. Differences were observed among the isolates with respect to growth rate per day, cardinal growth temperature response and virulence level. Growth rate of the isolates was between 5and 14 mm/day in diameter. The isolates responded well to the growth temperatures they exposed. There were significant differences among isolates in their growth temperature responses at 20°C, 28°C and 38°C.

Keywords: Taro leaf blight; Colocasia esculenta; Morphological characteristics, Cultural characteristics; Isolates

Introduction

Taro is one of the major tropical tuber crops in Ethiopia. It has been cultivated mainly and extensively in dense populated and high rainfall areas of South, Southwest and Western parts of the country. Its use as a potential crop in Ethiopians has been appreciated since 1984 famine [1]. In some areas, it used as fill seasonal food gaps when other crops are not in the field [2]. Farmers give many reasons why they cultivate taro. According to Edossa, (1996), Taro is cultivated because of produce reasonable amounts of yield when other crops hardly grow, resistant to disease and pests, ease of ecological adaptation. In Southern Ethiopia, taro grow extensively, due to the acute problems caused by enset bacterial wilt and sweet potato butter fly, which forced the farmers to replace heir staple foods enset and sweet potato by taro and maize (Simon,1992). However, this important food crop has been affected by a number of biotic and a biotic condition. Among the biotic factors leaf blight of taro caused by Phytophthora colocasiae has been responsible for the serious decline in yield of taro. According to Ooka (1994), fungal diseases of taro are the most significant ones. Because, diseases caused by fungi are aided by climatic conditions which favor the growth of taro. Leaf blight of taro also poses a serious threat to the production and biodiversity of this important food crop [3]. In India Misra et al. (2011) 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. In Ethiopia, Steward and Dagnachew (1967) reported occurrence of taro blight-like disease attributed to P. colocasiae but comprehensive details on pathogen or disease is not available. Taro leaf blight has contributed to the decline in taro production but still farmers have not recognized taro leaf blight as a disease associating its symptom with a maturity stage of the crop and impact of heavy rain fall. Survey was carried out to isolate the pathogen from different taro growing areas of Southern Ethiopia to study if there is variation in morphological and cultural characteristics among isolates.

Materials and Methods

Morphological and cultural characterization of *Phytophthora colocasiae*

Morphological and cultural characteristics of a fungus are the important basic criteria for identification of the fungus and its variability. The morphological characteristics such as mycelium type and sporangial morphology were studied for the different isolates of Phytophthora colocasiae collected from the surveyed areas. The fungal structures: mycelium type, sporangia type, sporangia shape and whether sporangia stalked or sessile were studied by observing under simple light microscope at a magnification of 40X. For sporangial morphology study, agar plugs (5-mm-diameter) containing fungal

***Corresponding author:** Zerhun Tomas, Department of Plant Pathology, South Agricultural Research Institute, Areka Agricultural Research Center, Areka, Ethiopia, E-mail: zerhuntomas1977@gmail.com

Received: 01-Aug-2023, Manuscript No. acst-23-108509; **Editor assigned:** 03-Aug-2023, PreQC No. acst-23-108509 (PQ); **Reviewed:** 19-Aug-2023, QC No. acst-23-108509; **Revised:** 24-Aug-2023, Manuscript No. acst-23-108509 (R); **Published:** 31-Aug-2023, DOI: 10.4172/2329-8863.1000607

Citation: Tomas Z, Shikur E, Said A (2023) Morphological and Cultural Characterization of *Phytophthora colocasiae* Isolates Collected from Taro Growing Areas of Southern Ethiopia. Adv Crop Sci Tech 11: 607.

mycelium was taken from the colony periphery or advancing margin of 7-days old culture by using cork borer. Five plugs were placed and floated on 8 ml of 1.5% non- sterile soil extract in 90-mm diameter Petri dishes then the plates were incubated for 2-3 days at temperature of 20-25°C. Once sporangia were formed, plugs immersed in a full strength (85%) lactic acid solution over night. The acid dissolves the agar and fix mycelium then after mycelium were mounted in a lactophenol cotton blue solution and at last Sporangia type (papillate, semipapillate, or nonpapillate), sporangia shape, whether sporangia is stalked/sessile were observed under simple light microscope and characterized morphologically by comparing with key for the identification of species of the genus Phytophthora [4,5]. Cultural characteristics such as colony morphology, cardinal growth temperature responses were studied for the different isolates of Phytophthora colocasiae obtained from the surveyed areas. For colony morphology study, agar plug (1 cm diameter) containing fungal mycelium was taken from the edge of 7-day-old culture of P. colocasiae by using cork borer and placed on to the centre of Petri dish containing potato dextrose agar (PDA), and the plates were incubated in darkness at a temperature of 28^{0}C [7,8]. After 7 days, the cultures were visually examined for colony texture and appearance. For temperature/growth responses, plug (1cm diameter) containing fungal mycelium was taken from the edge of 7-day-old culture of P. colocasiae by using cork borer and placed on to the centre of Petri dish containing PDA. Inoculated plates were incubated at temperatures of 20, 28 and 38°C arranged in a completely randomized design (CRD) [9]. Each isolate was replicated three times. Colony diameter was measured after 3, 5 and 7 days of incubation and the data were converted into radial growth in millimeters per day. The experiment was repeated to confirm the results.

Results and Discussion

Morphological and cultural characteristics of *P .colocasiae* isolates

The morphological characteristics such as mycelium type (septate, aseptate) and sporangial morphology (shape and type of Sporangia) were studied among the isolates of Phytophthora colocasiae [10].

Sporangial characteristics and Mycelium type of *P. colocasiae* isolates

Based on the observation under microscope all the 15 isolates had aseptate mycelium and stalked sporangia. Sporangia type for isolate A, B, D, E, G, I, L and M where Semi papillate where as isolate C, F, H, J, K, N and O had papillate type of sporangia. Sporangia shape for isolate A, G, H, J, L, M and N were lemon, isolate B, C, F and O were Globose and where as isolate D, E, I and K have ovoid shaped sporangia (Table 1) [11]. Our finding was similar to the research findings of Omane et al. (2012). Padmaja (2013) also studied morphological characteristics of different isolates of P. colocasiae and the result revealed that the mycelium of P. colocasiae isolates were aseptate, stalked sporangia, 1/4 and 3/4 of isolates had semi papillate and papillate sporangia type and 1/4 and 3/4 were ovoid and Globose in shape, respectively. The above mentioned two research results are in agreement with the present findings which indicated the mycelium of P. colocasiae isolates were aseptate, stalked sporangia, 8/15 and 7/15 of isolates were semi papillate and papillate sporangia type and 7/15, 4/15 and 4/15 were lemon, ovoid and Globose sporangia shape, respectively (Table 1).

Table 1: Morphological characteristics of sporangia and mycelium of *P. colocasiae* isolates.

Isolate	Mycelium type	Sporangia type	Sporangia shape	Stalked/sessile
A	Aseptate	Semi papillate	Lemon shaped	Stalked
B	Aseptate	Semi papillate	Globose	Stalked
C	Aseptate	Papillate	Globose	Stalked
D	Aseptate	Semi papillate	Ovoid	Stalked
E	Aseptate	Semi papillate	Ovoid	Stalked
F	Aseptate	Papillate	Globose	Stalked
G	Aseptate	Semi papillate	Lemon shaped	Stalked
H	Aseptate	Papillate	Lemon shaped	Stalked
I	Aseptate	Semi papillate	Ovoid	Stalked
J	Aseptate	Papillate	Lemon shaped	Stalked
K	Aseptate	Papillate	Ovoid	Stalked
L	Aseptate	Semi papillate	Lemon shaped	Stalked
M	Aseptate	Semi papillate	Lemon shaped	Stalked
N	Aseptate	Papillate	Lemon shaped	Stalked
O	Aseptate	Papillate	Globose	Stalked

Cultural characteristics

Colony morphology

The colony morphology of P. colocasiae isolates such as: colony color, colony shape, colony margin, colony texture and abundance of mycelium were presented in table 2.

Colony color

Colony color of the isolates varied from dull white to white. Out of the 15 isolates studied, the isolates A, B, C, D, E, F, H, J, L and O had white colony color where as the colony color of isolates G, I, K M and N was dull white. Similarly, Padmaja (2013) studied variation in colony colors of P. colocasiae isolates and the result revealed that colony color varied from dull white to white. In his studies about half of isolates have white and the remaining half have dull white colony color on carrot agar medium. Another finding of the same author have found some similarity in the colony color of isolates when used PDA and Carrot agar.

Colony shape and margin

From the 15 P. colocasiae isolates tested six, seven and two isolates has circular, irregular and filamentous colony shape, respectively. On the other hand, six, four and five isolates had entire, filiform and irregular colony margin, respectively. These characteristics are not reported in another literature and in the identification keys; they might be used as important morphological feature for identification of P. colocasiae because variation in colony shape or margin was observed among the isolates (personal observation).

Colony texture

Colony texture of the 15 isolates varied from cottony to moderately cottony aerial mycelium. Out of the 15 isolates tested, the isolates A, B, C, D, E, F, H, J, L and O had cottony aerial mycelium where as isolates G, I, K, M and N had moderately cottony mycelium. Similar observations were made by Padmaja (2013) and Tsopmbeng et al. (2012), who reported cottony to slightly cottony texture of P. colocasiae isolates on carrot agar medium. The research results confirmed with the present findings that the colony texture of isolates varied from cottony to moderately cottony aerial mycelium even if there is difference in culture media used.

Abundance of mycelium

The P. colocasiae isolates had profuse and slightly sparse mycelium

abundance. The isolates A, B, D, E, F, G, J, L and O had profuse mycelium abundance, where as isolates C, H, I, K, M and N had slightly sparse mycelial growth on PDA medium. Similarly Padmaja (2013) reported that 3/4 and 1/4 of P. colocasiae isolates had profuse and slightly sparse mycelium growth on carrot agar medium, respectively (Table 2).

Cardinal growth temperature

a) Growth rate

All the 15 P. colocasiae isolates differed in their colony growth rate (mm/day) at 28^{0}C temperature. The growth rate ranged from 14 to 5 mm/day. There was significant difference among isolates in their colony growth rate. The isolate A, recorded mean maximum growth rate of 14 mm/day whereas minimum growth rate of 5 mm/day was recorded in isolate O (Table 3). Similar observations were made by Padmaja (2013); he studied variation in colony growth rate (mm/day) of P. colocasiae isolates. All the isolates differed in their colony growth rate which ranged from 10.3 to 12.6 mm/day in carrot agar. The slight difference in growth rate of the two researches might be attributed to the difference in types of culture media used.

b) Cardinal growth temperature responses

The isolates responded well to the growth temperatures they exposed. Almost all isolates grew at temperatures of 20, 28 and 38^{0}C. Some of the isolates A, B, E, G, H, L, N and O did not grow at 38^{0}C. Only isolates C, D, F, I, J, K and M grew at 38^{0}C (approximately 3.5 mm/day). There were significant differences among isolates in their growth temperature responses at 20^{0}C, 28^{0}C and 38^{0}C. Optimum temperature for growth of isolates varied from 20 to 28^{0}C (Table 3). Misra et al. (2011) also studied cardinal growth temperature responses of P. colocasiae isolates and their result revealed that most isolates grew at temperatures of 10, 15, 20, 25, 28, 30, 32 and 35^{o}C. However, only few isolates grew at 40^{0}C (approximately 0.4 mm/day). In their finding the optimum temperature for growth among the isolates varied from 20 to 28^{0}C. Mean colony growth (mm). Means with the same letter in a column are not significantly different according to Duncan multiple range test (DMRT) at (p<0.01) (Table 3).

Table 2: Colony morphology of *P. colocasiae* isolates on PDA medium.

Isolate	Colony color	Colony shape	Colony margin	Colony texture	Abundance of mycelium
A	white	circular	entire	cottony	profuse
B	white	filamentous	filiform	cottony	profuse
C	white	irregular	irregular	cottony	slightly sparse
D	white	circular	entire	cottony	profuse
E	white	circular	entire	cottony	profuse
F	white	irregular	filiform	cottony	profuse
G	dull white	irregular	irregular	moderately cottony	profuse
H	white	irregular	filiform	cottony	slightly sparse
I	dull white	irregular	irregular	moderately cottony	slightly sparse
J	white	circular	entire	cottony	profuse
K	dull white	irregular	irregular	moderately cottony	slightly sparse
L	white	circular	entire	cottony	Profuse
M	dull white	irregular	irregular	moderately cottony	slightly sparse
N	dull white	circular	entire	moderately cottony	slightly sparse
O	white	filamentous	filiform	cottony	profuse

Table 3: Mean cardinal growth temperature response and mean growth rate of *P. colocasiae* isolates at 7 days incubation period (mm).

Isolate	Mean cardinal growth temperature responses (mm)			Mean growth rate mm/day 28^{0}C
	20^{0}C	28^{0}C	38^{0}C	
A	92^a	98^a	0^d	14^a
B	40^c	43^e	0^d	6.2^e
C	70^b	85^{cd}	16^c	12.2^{cd}
D	86^a	94^{ab}	13^c	13.4^{ab}
E	88^a	88^{bc}	0^d	12.6^{bc}
F	41^c	39^e	15^c	5.6^e
G	69^b	85^{cd}	0^d	12.2^{cd}
H	30^d	40^e	0^d	5.7^e
I	82^{ab}	80^d	15.5^c	11.4^d
J	91^a	96^a	28^b	13.7^a
K	83^{ab}	85^{cd}	42^{ab}	12.2^{cd}
L	91^a	87^{bc}	0^d	12.4^{bc}
M	37^{cd}	44^e	52^a	6.3^e
N	84^a	94^{ab}	0^d	13.5^{ab}
O	40^c	40^e	0^d	5^e
CV (%)	**5.51**	**2.51**	**32.54**	**2.478**

Conclusion and Recommendation

Studies on morphological characteristics of different isolates of Phytophthora colocasiae revealed that the mycelium was aseptate and stalked sporangia. Sporangia type for isolate A, B, D, E, G, I, L and M were Semi papillate whereas isolate C, F, H, J, K, N and O have papillate. Sporangia shape for isolate A, G, H, J, L, M and N were lemon, isolate B, C, F and O were Globose whereas isolate D, E, I and K have Ovoid shaped sporangia.. The color of colony is ranging from white to dull white, cottony to moderately cottony in texture and abundance of mycelium is profuse to slightly sparse growth on PDA plates. Differences were observed among the isolates with respect to growth rate per day, cardinal growth temperature response and virulence level. Growth rate of the isolates was between 5and 14 mm/day in diameter. The isolates responded well to the growth temperatures they exposed. There were significant differences among isolates in their growth temperature responses at 20^{0}C, 28^{0}C and 38^{0}C. The study shows that there is variability among isolates on morphological and cultural features. Future research should be directed towards surveying more agro ecologies and molecular characterization of the pathogen in addition to morphological characterization is needed to confirm the results.

References

1. Bandyopadhya R., Sharma K, Onyeka TJ, Aregbesola A, Kumar PL (2011) First report of taro Colocasiae esculenta leaf blight caused by Phytophthora colocasiae in Nigeria. Plant Disease 95(5): 618.
2. Edossa Etissa (1996) Root and Tuber Crops: Potential as food crops in the humid areas of Ethiopia. In IAR Newsletter of Agric Res 2 (1).
3. Misra RS, Mishra AK, Sharma K, Jeeva ML, Hegde V (2011) Characterization of Phytophthora colocasiae isolates associated with leaf blight of taro in India. Achieves of phytopathology and plant protection 44 (6): 581-59.
4. Omane E. Oduro KA, Cornelius EW, Opoku IY, Akrofi AY, et al. (2012) First report of leaf blight of taro Colocasiae esculenta caused by Phytophthora colocasiae in Ghana. Plant Disease 96 (2): 292-293.
5. Ooka JJ (1994) Taro diseases A guide for field identification HITAHR Research Extension 148.
6. Padmaja G (2013) Studies on Phytophthora leaf blight of taro colocasiae esculenta L Schott PhD Thesis. Acharya NG Ranga Agricultural University India. 134.

7. Ristaino JB (2012) A lucid key to the common species of Phytophthora. Plant Disease 96 (6): 897-903.

8. Simon Adebo (1992) Taro roots in North Omo Farm Africa. AA Ethiopia FPR TPP 2.

9. Stewart B, Dagnachew Y (1967). Index of plant diseases in Ethiopia. Haile Selassie I University College of Agriculture Debre Zeit 95.

10. Tewodros M (2012) Diversity analysis of taro Colocasiae esculenta in Ethiopia. Lambert Academic Publishing 72.

11. Tsopmbeng GR, Fontem DA, Yamde KF (2012) Evaluation of culture media for growth and sporulation of Phytophthora colocasiae Racib Causal agent of taro leaf blight. International Journal of Biological Chemistry Sciences 6 (4): 1566-1573.