Evaluation of *Phytophthora colocasiae R*esistance in Taro (*Colocasia esculenta*) Using Leaf Disc Bioassay

J. Adomako
CSIR - Crops Research Institute, Plant Health Division, P.O.Box 3785, Kumasi, Ghana

Author: joeadomako@gmail.com
j.adomak0@cropsresearch.org

Abstract
Taro leaf blight disease is the most destructive disease affecting taro production worldwide. Identifying resistant genotypes is the most practical means for managing the disease. In this regard, eleven taro genotypes were screened for taro leaf blight disease resistance with four isolates of P. colocasiae (Pc7, Pc12, Pc25 and Pc35) using leaf disc assay. Leaf discs of each genotype was inoculated with approximately 1 x 10^4 zoospores of the P. colocasiae isolates which were arranged in Completely Randomised Design (CRD) with three replications in a factorial experiment. Results of the study showed varied reactions of taro genotypes to the isolates tested. Significant differences (P<0.05) in lesions size was recorded among the genotypes irrespective of isolate used. Similarly, significant genotype-isolate interactions were observed. Taro genotypes BL/SM/134 and BL/SM/10 inhibited growth of all P. colocasiae isolates. They recorded mean lesion sizes of 16.6 and 17.3 mm compared to 59.9 mm recorded for local genotype (control) at 5-days-post-inoculation. The local landrace (check) genotype was susceptible to all P. colocasiae isolates whilst 2 and 7 taro genotypes were categorized as reistant resistant and moderately resistant. It is recommended that the identified resistant genotypes (BL/SM/134 and BL/SM/10) be screened further under natural infestation to confirm results.

Key words: Disease management , Ghana, lesion size, resistance, taro leaf blight

Reaction Resistante de Genotypes De Taro (*Colocasia esculenta*) aux Isolats De Phytophthora colocasiae

Résumé
La rouille des feuilles de taro est la maladie la plus destructive qui affecte la production de taro. Identification des génotypes résistants est important pour une gestion efficace de la rouille des feuilles de taro. Onze génotypes de taro ont été dépistés contre quatre isolats (Pc7, Pc12, Pc25 et Pc35) de P. colocasiae. Le dépistage de la résistance a été effectué en utilisant la méthode de la feuille détachée. Les résultats de l'étude ont montré une réaction de résistance variée des génotypes aux différents isolats. Une différence significative (p <0,05) de la taille des lésions a été enregistrée parmi les génotypes indépendamment de l'isolat utilisé pour l'évaluation. De même, des interactions significatives ont été observées entre les génotypes et les isolats. Deux génotypes ont présenté une réaction de résistance à l'isolement de Pc1, six génotypes étaient susceptible, alors que 2 et 1 génotypes étaient modérément résistants et très sensibles à l'isolat, respectivement. Cinq, trois et deux génotypes étaient modérément résistants, susceptibles et résistants à l'isolement de Pc12 respectivement. En ce qui concerne l'isolat Pc25, les génotypes 1,

7, 2 et 1 ont exprimé respectivement des réactions de résistance, de résistance modérée, sensibles et hautement susceptibles, tandis que 5 génotypes étaient résistants à l'isolat de Pc35, les génotypes 3, 2 et 1 étant respectivement modérément résistants, sensibles et hautement susceptibles.

Mots-clés: Gestion de la maladie, Ghana, Taille de la lésion, Résistance, Susceptible, La rouille des feuilles de taro.

Introduction

Taro (*Colocasia esculenta* (L.) Schott) is an important food security crop staple in the Pacific Islands, West Africa, Asia, the Caribbean and South America (Tarla *et al*., 2016; Akwee *et al*., 2015). It is a rich source of carbohydrates, proteins, minerals and vitamins and has medicinal properties to reduce tuberculosis, ulcers, pulmonary congestion and fungal infection (Sharma *et al.,* 2008). In addition, the corms are used for the production of fructose syrup and alcohol (Misra *et al.,* 2008).

Production of the crop is however constrained by high incidence of taro leaf blight disease caused by *Phytophthora colocasiae* Racib. It is the most destructive disease infecting the crop in major producing countries (Gadre and Joshi, 2003). It is highly prevalent in Ghana (Adomako *et al.,* 2016), although its impact has not been fully established in the country. In countries such as Hawaii, the disease has been associated with heavy yield decline in taro for over 30 years (Miyasaka *et al.,* 2012) and has compelled farmers to abandon their fields or shifted to production of other staple crops. Several management strategies have been employed to manage taro leaf blight disease *viz;* crop rotation, removal of diseased leaves and use of fungicides. Although metalaxyl-based fungicides have proven effective, like most synthetic chemicals, their detrimental effect on the environment, animals and underground water bodies (Luc *et al.,* 2005) makes them inappropriate option in disease management strategies. Also high incidence of the disease renders it too expensive, as repeated applications are required to protect the crop.

Identification and use of resistant cultivar appears the most sustainable, efficient and cost effective way of managing plant diseases. Screening of taro cultivars for *P. colocasiae* resistance has traditionally been conducted in disease hot spot zones. Although this method according to Nath *et al.* (2016) remains the benchmark for evaluating taro cultivars for leaf blight resistance, it relies on the presence and evenly distribution of pathogen inoculum and conducive environmental conditions for disease development. In view of this, previous studies (Tyson and Fullerton, 2015; Brooks, 2008) successfully screened and identified taro cultivars resistance to *P. colocasiae* using leaf disc bioassay. This method curtails the limitations of uneven inoculum distribution and inconducive environmental conditions. It ensures standard inoculum pressure and uniform disease development on the test material (Nath *et al.,* 2016). Leaf disc bioassay also allows the use of multiple isolates to screen host materials to determine host-isolate interaction over a short period of time. In the present study, 11 taro genotypes were evaluated for their response to four *P. colocasiae* isolates infection using leaf disc assay.

Materials and Methods

Preparation of *P. colocasiae* inoculum

Diseased taro leaves were collected from taro

farms in Nkawkaw (Pc1), Asukese (Pc35), Dumasua (Pc25) and Kwamo (Pc12) in the semi-deciduous forest zone of Ghana and brought to the Plant Pathology Laboratory of CSIR - Crops Research Institute, Kumasi where isolations were made on V8 juice ajar medium following standard isolation techniques. *Phytophthora colocasiae* isolates were maintained on V8 juice agar medium amended with 20 mgL^{-1} Nystatin and 250 mg L^{-1} Ampicillin for 21 days at 26±2 ^{o}C in the incubator. Sporangia suspensions were produced by adding 10ml distilled water to the plate of each isolate. The surface of their mycelia were gently scrapped with the edge of a sterilised glass rod to dislodge sporangia. The mycelia-sporangia suspension was filtered through double layered cheesecloth to remove mycelia fragments before chilling at 4^{o}C for 2h to induce zoospore release (Fontem *et al.,* 2005).

Sources of taro genotypes

Ten taro genotypes *viz;* CE/IND/16, CE/MAL/32, BL/SM/132, BL/SM/116, BL/SM/134, BL/SM/10, BL/SM/16, BL/SM/115, BL/SM/80 and KAO 022, were obtained from the CSIR-Plant Genetic Resources Research Institute (CSIR-PGRRI), Bunso whilst the check, a local landrace was obtained from a farmer at Kwamo in the Ejisu-Juaben Muncipal of the Ashanti Region of Ghana.

Evaluation of taro genotypes for resistance to *P. colocasiae*

Screening of taro leaves against *P. colocasiae* was done by detached leaf method (Nath *et al.,* 2016; Tyson and Fullerton, 2015). Leaves of the 11 taro genotypes were detached from 12 week old plants and thoroughly washed under running water. Sampled leaf of each genotype was cut into leaf disc of 80 mm and surface sterilized with 70% ethanol for 60 sec. Ethanol-sterlized leaves were rinsed separately in sterile distilled water to wash off the ethanol. Single leaf disc of each genotype was placed on adaxial side in a different Petri dish lined with moistened Whatman No 9 filter paper. A single drop (0.5 ml) of inoculum of each *P. colocasiae* isolate containing approximately 10,000 zoospores was placed at the centre of the leaf disc of each genotype in a Petri dish. The set up was incubated at 26±2 °C for 5 days. Leaf discs inoculated with 0.5 ml distilled water without zoospores served as control for the experiment. The study was a factorial experiment mounted on a Complete Randomized Design (CRD) with three replications. The factors consisted of 11 taro genotypes and four *P. colocasiae* isolates. Leaf discs were visually examined daily for Taro leaf blight disease symptoms.

Data collected and analysis

Lesion size was recorded five days post inoculation (dpi). Lesion size was measured using a leaf area meter. Relative lesion size was determined as a proportion of damaged area of each leaf disc and rated on a 0-5 rating scale (Table 1) to determine disease severity. Based on the disease severity, taro genotypes were categorised as immune, highly resistant, resistant, moderately resistant or susceptible. Disease incidence was determined as the number of taro leaflets infected by an isolate compared to total number of leaflets inoculated. Data collected on lesion size was subjected to Analysis of variance (ANOVA) using the Genstat statistical package version 12. Means were separated using Tukey's Honest significant difference test (HSD) at $P<0.05$.

Results

Lesions were found on *P. colocasiae* inoculated sites on all taro leaf discs (Plate 1A) compared to control (Plate 1B) which showed no lesions.

At five days post inoculation, lesion size

Table 1: Disease rating scale of Taro leaf blight disease using leaf disc essays

Rating Scale	Leaf Area Damage	Host Reaction
0	0.00	Immune
1	0.01-10	Highly Resistant
2	10.01-25	Resistant
3	25.01-40	Moderately Resistant
4	40.01-60	Susceptible
5	>60.01	Highly susceptible

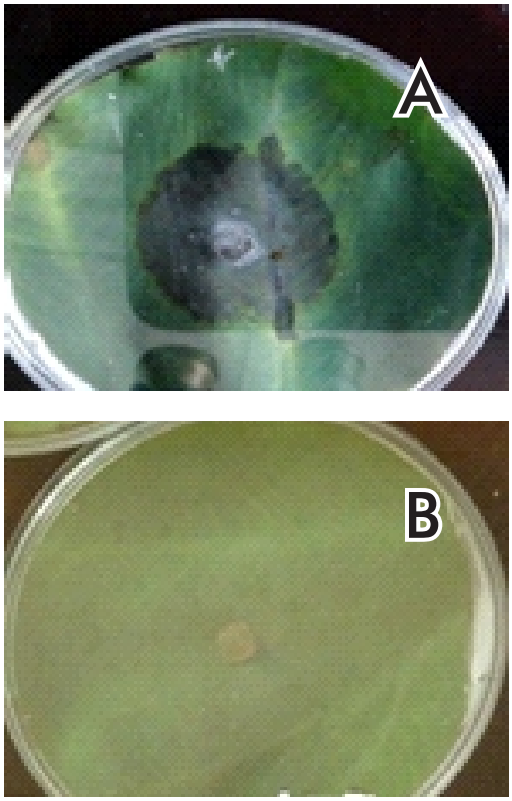

Plate 1. Lesion symptoms (A) and assymptomatic (B) taro leaflets following *P. colocasiae* infection

on leaf discs, was significantly different ($P < 0.05$) among taro genotypes irrespective of *P. colocasiae* isolate used (Table 2). For taro genotypes screened with isolate Pc1, mean lesion size ranged from 13.4 mm in genotype BL/SM/10 to 60.4 mm in the local material (check). Similarly, the local taro genotype (check) recorded the highest mean lesion size of 55.2 mm compared to 10.4 mm recorded in BL/SM/134 when the taro genotypes were screened with isolate Pc12 . The local taro genotype (check) again recorded the highest mean lesion size of 62.2 mm and 61.9 mm when taro genotypes were screened with isolates Pc 25 and Pc35 respectively . For the same isolates mean lesion size of 19.4 mm and 18.3 mm were recorded for genotypes CE/IND/16 and BL/SM/115 respectively. Genotype x isolate interaction was significantly different ($P<0.05$) indicating that taro genotypes responded differently to *P. colocasiae* isolate used (Table 2).

None of the taro genotypes screened was found to be immuned or highly resistant to any of the *P. colocasiae* isolates used (Table 3). Taro genotypes BL/SM/134 and BL/SM/10 showed resistant reaction to three isolates-Pc1, Pc12 and Pc35 (Table 3). Seven taro genotypes (BL/SM/132, BL/SM/116, BL/SM/80, BL/SM/16, CE/MAL/32, BL/SM/115 and CE/IND/16), showed moderately resistant reaction to isolate Pc12 with two genotypes (KAO 022 and the local taro genotype) being

Table 2: Reaction of taro genotypes to four *P. colocasiae* isolates

Taro Genotypes	Isolates				
	Pc1	Pc12	Pc25	Pc35	Mean (Genotypes)
BL/SM/10	13.4	12.5	31.7	11.7	17.3
BL/SM/115	28.3	38.1	30.0	18.3	28.7
BL/SM/116	36.6	32.0	33.0	30.0	32.9
BL/SM/132	32.4	32.2	25.5	32.3	30.6
BL/SM/134	13.7	10.4	25.7	16.6	16.6
BL/SM/16	37.3	31.2	37.9	16.6	30.8
BL/SM/80	37.9	38.9	38.6	17.7	33.3
CE/IND/16	27.4	33.5	19.4	32.7	28.3
CE/MAL/32	38.4	37.7	27.3	27.5	32.7
KAO 022	42.8	52.7	50.2	46.4	48.0
CHECK	60.4	55.2	62.2	61.9	59.9
Mean (Isolates)	33.5	34.0	34.7	28.3	
HSD (P<0.05)					
HSD (Isolates)			1.0		
HSD Genotypes)			1.7		
HSD (GxI)			3.5		

Data are means of three replications

susceptible and highly susceptible respectively (Table 3). Seven taro genotypes (BL/SM/134, BL/SM/10, BL/SM/16, BL/SM/80, BL/SM/115,CE/MAL/32 BL/SM/132) and one taro genotype (CE/IND/16) were moderately resistant and resistant to isolate Pc25 (Table 3). Taro genotype KAO 022 and the local genotype were susceptible to isolate Pc 25. With respect to *P. colocasiae* isolate Pc35, taro genotypes, BL/SM/10, BL/SM/134, BL/SM/16, BL/SM/80 and BL/SM/115 were found to be resistant (Table 3).

Discussion

The objective of the study was to screen for resistant taro genotypes with different *P. colocasiae* isolates. It was observed that taro genotypes reacted differently to *P. colocasiae* isolates used. The observed variations in genotypes confirm the need to use numerous pathogen isolates to screen for resistance as single isolate may not adequately confirm the susceptibility or resistance of a host genotype. The significant interaction observed between *P. colocasiae* isolates and taro genotypes demonstrates the existence of physiological races in *P. colocasiae*. Identification of resistant taro genotypes to different isolates of

Table 3: Reaction level of taro genotypes to different *P. colocasiae* isolates

Taro Genotypes	Isolates			
	Pc1	Pc12	Pc25	Pc35
BL/SM/10	R	R	MR	R
BL/SM/115	MR	MR	MR	R
BL/SM/116	MR	MR	MR	MR
BL/SM/132	MR	MR	MR	MR
BL/SM/134	R	R	MR	R
BL/SM/16	MR	MR	MR	R
BL/SM/80	MR	MR	MR	R
CE/IND/16	MR	MR	R	MR
CE/MAL/32	MR	MR	MR	MR
KAO 022	S	S	S	S
CHECK	HS	S	HS	HS

R - Resistant, MR - Moderately resistant,
S - Susceptible, HS - Highly susceptible

P. colocasiae using leaf disc assay agrees with Padmaja (2013) and Brooks (2008) who effectively evaluated and identified resistant taro genotypes using the leaf disc method. Taro genotypes resistant to *P. colocasiae* have been reported in previous studies by Bassey *et al*. (2016) and Singh *et al.* (2006) in Nigeria and Papua New Guinea respectively indicating the presence of resistant genotypes in taro populations. Also, the identification of moderately resistant genotypes identified in this study corroborates findings of similar studies by (Amadi *et al.*, 2015; Singh and Okpul, 2000) who identified moderate resistance of improved genotypes to isolates of *P. colocasaie* in Nigeria, and Papua New Guinea respectively. The high susceptibility of the local genotype to local *P. colocasiae* agrees with Ackah *et al*., (2014) and Amadi *et al.,* (2 0 1 5) who observed that landrace genotypes of taro were highly susceptibility to local pathogen compared to improved genotypes. Genotypes BL/SM/134 and BL/SM/10 exhibited resistance to multiple *P. colocasiae* isolates. This confirms Singh *et al*, (2012) assertion that resistance in taro is controlled by horizontal or partial resistant traits. Horizontal resistance is polygenic and controlled by several genes. It is considered to be more stable and according to Agrois (2005) horizontal resistance is relatively difficult for a pathogen of interest to overcome numerous genes which individually may provide only minor effect against the pathogen. The practical method to manage taro leaf blight disease is to identify and use resistant cultivars which is the most sustainable and cost effective way of managing plant diseases. Screening for resistance have traditionally been in field trials. However, the success of it depends on conducive environmental conditions, inoculum pressure and evenly distribution of the pathogen inoculum on the field (Iramu *et al.,* 2004). Selecting resistant genotypes based on the leaf disc approach is faster and overcomes the challenges associated with field evaluations. In conclusion, the study observed varied resistant reactions among the genotypes. Two genotypes, BL/SM/134 and BL/SM/10 were found to be resistant to three isolates of *P. colocasiae* used in the study. Eight of the genotypes were moderately resistant to the pathogen whilst the local landrace was highly susceptible to all isolates of the pathogen. The promising genotypes, BL/SM/134 and BL/SM/10 should be screened further under field conditions to validate the laboratory results.

Acknowledgements
The author appreciate the financial support of the West Africa Agricultural Productivity Programme (WAAPP). Author is also thankful to Ms. Afia Nyarko for providing the local taro landrace and field to carry out this work. The assistance of Mr. Francis Dadzie of

the Plant Pathology section, CSIR-Crops Research Institute is very much appreciated.

References

Ackah, F. K., Puije, G.C. van der & Moses, E. 2014. First evaluation of taro (*Colocasia esculenta*) genotypes against leaf blight (*Phytophthora cololosiae*) in Ghana, *HortFlora Research. Spectrum*, 3(4): 390-391.

Adomako, J., Kwoseh, C.K., Moses, E. & Larbi-Koranteng, S. 2016. Prevalence of *Phytophthora* leaf blight of taro (*Colocasia esculenta* (L.) Schott) in the Semi deciduous Forest Zone of Ghana. *American Journal of Experimental Agriculture,* 11(4): 1-7.

Agrios, G. N. (2005). Plant Pathology. Academic Press, Burlington, MA. Pp. 922.

Akwee, P.E, Netondo, G., Kataka, J.A., & Palapala, V.A. 2015. A critical review of the role of taro *Colocasia esculenta* L. (Schott) to food security: A comparative analysis of Kenya and Pacific Island taro germplasm. *Scientia Agriculturae*, 9 (2): 101-108.

Amadi, C. O., Onyeka, J., Chukwu, G. O., Okoye, B. C., Ezeji, L. and Ezigbo, E.C. (2015). Evaluation of exotic genotypes of taro (*Colocasia Esculenta*) in Nigeria. *Nigeria Agricultural Journal*, 46(1): 36-42.

Bassey, E.E., Umoh, G.S., Ndaeyo, N. U., Nneke N. E. and Akpan G.U. 2016. Investigations into taro [*Colocasia esculenta* (L.) Schott] leaf blight outbreak and identification of resistant cultivars in Akwa Ibom State, Nigeria. *International Journal of Current Research in Biosciences and Plant Biology*, 3(5): 137-143.

Brooks, F.E. 2008. Detached-leaf bioassay for evaluating taro resistance to *Phytophthora colocasiae*. *Plant Disease*, 92: 126–131, doi:10.1094/PDIS-92-1-0126.

Fontem, D.A., Olanya, O.M., Tsopmbeng, G.R. and Owona, M.A.P. (2005). Pathogenicity and metalaxyl sensitivity of *Phytophthora infestans* isolates obtained from garden huckleberry, potato and tomato in Cameroon. *Crop Protection*, 24:449-456.

Gadre, U.A. & Joshi, M.S. 2003 Influence of weather factors on the incidence of leaf blight of *Colocasia*. *Annals of Plant Protection Science,* 11: 168-170.

Iramu, E.T., Akanda, S, Wagih, M.E. Singh, D & Fullerton, R.A. 2004. Evaluation of methods for screening taro (*Colocasiae esculenta*) genotypes for resistance to leaf blight caused by *Phytophthora colocasiae.* Papua New Guinea Journal of Agriculture, Forestry and Fisheries, (47) 1&2:37-44.

Luc, M, Bridge, J. & Sikora, R.A. 2005. Reflections on Nematology in subtropical and tropical Agriculture. In: Plant parasitic nematodes in subtropical and tropical agriculture. Luc M, Sikora RA, Bridge. (Eds.) 2nd edition, Wallingford, UK, CABI Publishing.pp. 259-318.

Misra, R.S, Sharma, K. & Mishra, A.K. 2008. *Phytophthora* leaf blight of taro (*Colocasia esculenta)*—A review. *Asian Australasian Journal of Plant Science and Biotechnology,* 2:55–63.

Miyasaka, S.C., McCulloch, C.E & Nelson, S.C. 2012. Taro Germplasm Evaluated for Resistance to Taro Leaf Blight. *HortTechnology*, (22)6:838-849.

Nath, V. S., Basheer, S., Jeeva M. L., Hegde, V. M., Devi, A, Misra, R.S, Veena, S.S. & Raj, M. 2016. A rapid and efficient method for *in vitro* screening of taro for leaf blight disease caused by *Phytophthora colocasiae. Journal of Phytopathology*, 1-8 doi: 10.1111/jph. 12477.

Padmaja, G. 2013. Studies on *Phytophthora* leaf blight of taro. Master of Science

thesis submitted to Acharya N.G. Ranga Agricultural University, India.

Sharma, K., Mishra, A.K., & Mishra, R.S. 2008. The genetic structure of *Colocasiae esculenta*: A comparison of RAPD and isozyme markers. *Plant Biotechnology Report*, 2:191–198.

Singh, D. & Okpul, T. 2000. Evaluation of 12 taro (*Colocasia esculenta* (L) Schott) leaf blight resistant clones for yield and eating quality in Papua New Guinea. *SABRAO Journal of Breeding and Genetics,* 32: 39-45.

Singh, D, Guaf, J., Okpul, T. Wiles, G. & Hunter, D. 2006. Taro (*Colocasia esculenta*) variety release recommendations for Papua New Guinea based on multi location trials. *New Zealand Journal of Crop and Horticultural Science*, 34:2, 163-171.

Singh, D., Jackson, G., Hunter, D., Fullerton, R., Lebot, V., Taylor, M., Iosefa, T., Okpul, T. & Tyson, J. 2012. Taro Leaf Blight—A Threat to Food Security. *Agriculture,* 2:182-203; doi: 10.3390/agriculture2030182.

Tarla, D.N., Bikomo, M.R., Takumbo, E.N., Voufo, G. & Fontem, D.A. 2014. Climate change and sustainable management of taro (*Colocasia esculenta* (L.) Schott.) leaf blight in Western Highlands of Cameroon. *Revue Scientifique et Technique Forêt et Environnement du Bassin du Congo*, 6: 10-19.

Tyson, J.L. & Fullerton, R.A. 2015. A leaf disc assay for determining resistance of taro to *Phytophthora colocasia. New Zealand Plant Protection* 68: 415-419.