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Research Article

Assessment of Variability of Different *Phytophthora colocasiae* Populations in Western Kenya

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Abstract

Characterization of the fungus *Phytophthora colocasiae* and variability in the DNA of 14 of its isolates was assessed in this study. The occurrence of the Taro leaf blight disease which was assessed using severity and incidence showed that areas with high rainfall of about 2000 mm per annum, high temperatures of about 31°C had higher disease incidences of about 89% whereas the higher altitude areas with lower temperatures and high rainfall showed lower incidences (about 74%) but higher severity scores or a total leaf loss on the plant. Variability among *P. colocasiae* isolates collected from different fields was assessed using RAPD markers, 99% were polymorphic. There was apparent correlation of the genetic variability of the fungus to the agro ecological conditions in the region of origin of the fungus. The results of this study displayed a high degree of genetic variation among the isolates from the various agro ecological regions further illustrating that the different environmental conditions and human influence have an effect on the genetic constitution of the fungus in different regions growing taro in western Kenya.

Keywords: *Phytophthora colocasiae;* Assessment; Variability; RAPD marker; Western Kenya.

Introduction

Taro (*Colocasia esculenta*), the root of the Arum lily known across Africa as the cocoyam and in Kenya as arrow root forms a basic starch staple food in the diet of many groups [1]. The Nduma, as it is called across the many Kenyan regions is thought to be one of the oldest cultivated plants. Its natural habitat is the edge of water courses and is cultivated along river banks, by ponds and dams and in marshy areas where few other crops would succeed [2]. The root acts as the storage organ for the plant and is available for harvest approximately six months after planting. However, the valuable starches and sugars are not available until the root is cooked, by boiling or roasting for at least four hours, otherwise the root remains indigestible to humans [3]. Once cooked, it is sweet and is often ground to make the flour used for porridge or a pollenta-like cake which forms the major starch content of a meal. It can also be used as an ingredient for sweet cakes.

In South East Asia, cocoyam leaves are consumed as green or dry vegetable and the stem is either cooked and eaten on its own or together with other dietary staples or pound into flour. The leaves are consumed because they are rich in protein and vitamins while the root is rich in carbohydrates and minerals [4]. Contrastingly, cocoyams in Cameroon are prepared, processed and consumed in a variety of ways including eating with a vegetable sauce, as ekwen (tying grated, peeled corms with younger leaves together with palm oil, fish, crayfish, salt and pepper), belb (a sauce), kokhi-beans (together with beans and plantain), kokhi-corn (together with corn), or as akwacoco (porridge) [5].

In East Africa, cocoyams have traditionally been steamed and eaten as a snack alongside tea or a beverage. For many communities in central Kenya, such as the Kikuyu, the arrowroot is a central part of their cuisine. It is grown on the many streams and rivers emanating from Mount Kenya and the Aberdares [6]. Not only does the root provide starch and dietary fibre, the leaves can also be used as a green vegetable, a source of vitamins like A, B1, proteins and minerals. The arrow root plays a key role in food security of the cultivating communities due to its high nutritional value, production yields and storage properties. Its corms, cormels, stalks and inflorescence are all utilized for human consumption [7].

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Cocoyams have also been established to be a low maintenance crop that will maintain ground cover in the field to reduce soil erosion. Therefore, with increasing land degradation as evidenced by declining soil fertility, decreasing vegetation cover and water availability, wetlands are providing alternative centres for agricultural productivity [8] including a large part of the East African region and cocoyam is among the major crops grown in these wetlands.

One way to address the problems posed by climate change and decreasing food security is to promote production of crops that are adapted to diseases and pests, earlier or late sowing, or to drought [9]. However, taro leaf blight, caused by *Phytophthora colocasiae* is one of the most destructive diseases affecting cocoyam production in Sub-Saharan Africa. There is as such a profound need to develop taro leaf blight resistant germplasm for improved productivity and livelihoods [10]. This study will therefore, contribute to food security by bringing quick innovations in the agricultural cocoyam production systems. It was aimed at characterizing the isolates of *Phytophthora colocasiae* and assessing its variability in western Kenya. This work will provide a basis for further studies on the population structure of the taro leaf blight pathogen and the proper management of the taro leaf blight disease.

Materials and methods

Randomly amplified polymorphic DNA pattern markers were used to study the variation existing at the DNA level between 14 different *P. colocasiae* isolates [3]. These isolates were selected based on their geographical origin. Isolates were grown on nutrient agar containing yeast extract (2 g mL^{-1}), glucose (10 g mL^{-1}), KNO_3 (3 g mL^{-1}), KH_2PO_4 (2 g mL^{-1}), adjusted to pH 6·0 using NaOH 5 m, sterilized at 120°C for 20 min and antibiotics added (rifampicin 10 mg mL^{-1}, piramicin 10 mg mL^{-1}, ampicillin 250 mg mL^{-1}). DNA extraction was conducted using New Glucanex (Novo Nordisk Ferment Ltd, Bern, Switzerland – 18 mL H_2O, 42 mL NaCL 1 m, 1·8 g New Glucanex adjusted to pH 6·0). Digestion of cell walls was conducted in Eppendorf tubes (2 mL) for 2 hours at room temperature, and precipitation was done using isopropanol and centrifugation for 7 min. Precipitates were washed with 70% ethanol. RNase treatment was done in 100 μL of TE with 1 μg/mL of RNASE incubated at 37°C for 60 min. DNA concentration was evaluated on 0·8% agarose gels (1 μL DNA, 1 μL of 1% methyl blue, and 9 μL sterile H_2O).

DNA extracts were diluted to 1 ng mL^{-1}. The solution for PCR amplification was composed of 5 ng of DNA extract, 2·5 μL of buffer (tris-HCl, pH 8·8, 67 mm NH_4, 16·6 nm $2SO_4$), 2 μL DNTP, 1 μL $MgCl_2$ (50 mm), 5 μL of primer, 9·2 μL H_2O, 0·3 μL H_2O, 0·3 μL of TAQ polymerase (Eurobio, Montpellier, France). The PCR programme consisted of 45 cycles of denaturation (95°C, 1 min), hybridization (35°C, 1 min) and elongation (72°C, 8 min). Amplified extracts were taken through electrophoresis on agarose gels (1·4% in TBE 1X) using constant voltage (100 V) for 3 hours. Coloration was done by soaking the gel in an ethidium bromide solution for 115 minutes, followed by rinsing in water for10 min and observation and photography done under UV. Four primers were used for the analysis. All clearly detectable bands were scored for their presence with (1) or absence with (0) by visual observation. All observable bands scored for either being polymorphic or monomorphic by comparing with the other bands. All this information was included in the analysis. Bands were assumed to be independent and those that were of identical size were assumed to have identical sequences [7].

Result and discussion

Farming systems and productivity

One of the survey objectives was to investigate the most practiced farming system. From figure 1, it was found that out of the 27 farmers interviewed, 68% of farmers practiced mixed farming. The taro crop was intercropped with maize, bananas or sugarcane. 32% of farmers planted taro as a sole crop. In reference to the farmers' response, mixed farming was practiced to maximize on the arable land available land to ensure at least two crops would be available when the planting season was over. 90% of the farmers intercropped maize and taro (maize being the predominant food crop in the region). Close to 70% of the farmers who practiced mixed farming intercropped taro with mostly maize, bananas and sometimes vegetables [6]. From table 1, young sugar cane, in sugarcane growing areas was also observed on a few farms.

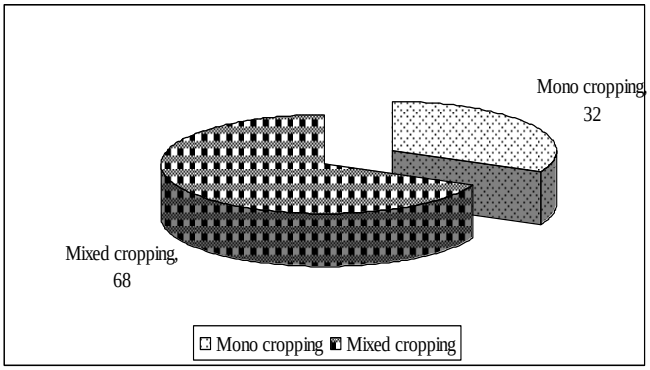

Figure 1. Taro farming systems practiced in the three study regions of western province

Table 1. Effect of type of farming system on yield per acre

	Sum of squares	Degrees of freedom	Mean square	F-. value	Signifi-cance
Between groups	0.369	1	0.369	1.689	0.197[a]
Within groups	21.391	25	0.218		
Total	21.760	26			

Preferred taro trait

Each farmer rated the varieties grown according to the agronomic suitability and consumption traits based on the experiences they had with the variety. The farmers who had some excess to sell preferred a high yielding variety but those who used it mainly for subsistence preferred the palatability and tastiness in the variety. Farmer trait preference differed from one area to another because of differences in economical, social physical and environmental factors. No single variety was rated highly in all attributes, consistent with the common thinking that no single variety can meet all farm household and market needs. 80% of the farmers interviewed preferred a high yielding variety. High yield is considered in terms of number and size of corms. Texture, shape and taste influenced the popularity of a given variety in a homestead. The less fibrous high sugar content taro was the most preferred. From the women farmers interviewed, the less watery corms make the food appealing whether when used with stews or as a snack. Traits of little preference which led to the rejection of the seed included its being highly fibrous and watery. Farmers did not like planting taro on the dry land because they chose to use that land for more 'profitable' crops like maize and beans. This resulted in poorer quality corms and higher susceptibility to diseases when taro is grown in the swampy areas. There was minimal direct contact between the farmer and public extension officers with regard to access to new information. 9 % had had direct contact with public extension officers, while 91% had not had any direct contact. 79 % of farmers had not been exposed to any new technologies in taro breeding. Only 21% of farmers reported having been exposed to new information on taro farming. However, access to new technologies or improved technologies by the farmers was high despite the low direct contact with public extension officers.

Pathogen isolation and identification

The results of the culturing experiments for the isolation of *P.colocasiae* are shown in the table 2. From table 2, out of the 24 plates, 11 were considered uniquely different and therefore used for sub culturing to obtain pure cultures.

The sub cultures comprised new sub groups are given in table 3. From table 3 for the sub culturing, it was clear that the samples from the region marked 1-8 and 18-24 did not grow a lot of fungi in the artificial conditions. The region labeled 9-16 had the highest growth numbers of colonies. The second region marked 8-16 also had the earliest growing fungi at day 17.The region marked 1-8 had the lowest numbers of colonies growing out of the three regions. After sub culturing, identification was done by isolating a single spore, staining with lactophenol and observing under a dissecting microscope. The mycelia observed had hyphae which were septate. Because most of them showed the presence of spores, it was possible to observe the motile spores. One conidium observed was ellipsoid and with a stalk (caducous). Fourteen distinctively different isolates were identified based on the colony characteristics, sporangial morphology and conidia type and shape. The pure culture was stored at -20°C and used for DNA extraction.

From figure 2, the Genomic DNA dilution was done at the ratio of 3:1:1 where 3 refers to the molecular water, 1 the mother DNA and1 the loading dye. The total volume is 5.The total DNA loaded on the gel well was 4ng. The clear banding showed above indicates the presence of genomic DNA in the samples of the fungal species of the three regions 1 – 8 (Kakamega), 9-16 (Butere) and 17-24 (Bungoma).

Table 2. Details of *Phytophthora colocasiae* isolates observed after the initial culturing

Isolate code	Day when colony first seen after inoculation	Colony colour	Colony diameter(cm)	Nature/ pattern of colony margin
KAK 1	No colony observed			
KAK 2	23	white	7	Even
KAK 3	20	pink	6.2	uneven
KAK 4	No colony observed			
KAK 5	19	grey	7.6	uneven
KAK 6	No colony observed			
KAK 7	23	white	6	Even
KAK 8	24	white	6.5	Even
BUT 9	14	white	8.3	uneven
BUT10	12	white	7.9	Even
BUT 11	17	Pink	1.8	Even
BUT 12	18	white	8.1	Uneven
BUT 13	No colony observed			
BUT 14	18	white	7.3	uneven
BUT 15	15	white	6.9	uneven
BUT 16	22	white	7.6	Even
BUN 17	No colony observed			
BUN 18	No colony observed			
BUN 19	No colony observed			
BUN 20	No colony observed			
BUN 21	25	white	6.2	Even
BUN 22	No colony observed			
BUN 23	26	Light pink	7.5	Even
BUN 24	No colony observed			

Figure 2. Gel image of DNA quantification of the isolates obtained from the three study regions marked 1-24. L is the Ladder at 50 ng/ul which represented 50 ng

Table 3. Subculturing group

Plate identity	Rate of growth	Mycelia colour	Edge shape	Colony shape
11c, 12c, 8b 16c	very fast	clear white	even	round
17b, 16c	fast	white	uneven	round
10a, 9a, 9b	fast	White	uneven	round
16a	slow	white	uneven	round

The RAPDs analysis performed produced 14 markers which were polymorphic as shown in figure 3.

The ecological factors of temperature, rainfall, altitude and the global positioning (GPRS), of the three regions were compared one by one for each factor. Region one (KAK1-8) had comparatively low severity of 1.0 compared to 1.5 in region 2 (BUN9-16), relatively small sized lesions and the highest number of lesions. It had moderately lower temperatures and altitude compared to the two other regions studied. The incidence is high 34% compared to

11% and 5% in the other two regions. This shows that the high rainfall, low altitude and moderate temperatures favour the reproduction and dissemination of the *P. colocasiae*.

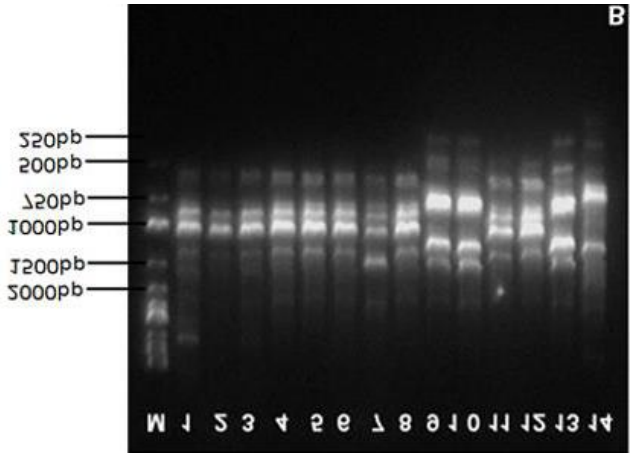

Figure 3. Gel image of DNA quantification of the isolates obtained from the three study regions marked 1-14

Low rainfall, low altitude and low temperature do not favour the growth, multiplication and dissemination of this fungus. Region two (BUN) had the highest severity score of 1.5 compared to 1.2 and 1.0 in the other two regions, with moderate numbers of small sized lesions. This region has the lowest temperature among the regions studied indicating that the severity scores increase in areas with low temperature, high altitude and high rainfall. Region 3(BUT17-24) has high severity and with the largest sizes of lesions, the lesions being moderately numbered. The region has temperatures and rainfall figures in between the other two studied regions. Inter cropping taro with other plants increases competition for light and nutrients reducing the rate of photosynthesis and growth of the crop respectively. Where the striga weed is endemic and therefore affects maize, intercropping of maize and taro has been useful in suppressing the emergence of *Striga hermonthica* and weeds like *Amaranthus retroflexus* due to the shading effect of the taro leaves [9].

A practical taro population though, has to be managed to ensure effective management of weeds and soil fertility. In areas where taro populations have been maintained as sole crops, the yields are always higher. This confirms the dependence of yield on the farming system as per the results of [1]. It is evident that over 90% of the farmers interviewed harvested less than 2bags of taro at the end of a growing season, with only 2% of the farmers interviewed harvesting over 4 bags of taro at the end of a growing season. Taro production was not practiced on a large scale and the type of farming system practiced by the farmer had a significant effect on the yields obtained. Farmers who practiced mono cropping had better yields compared to farmers who practiced mixed cropping. Farmers in the large scale sugarcane growing areas of (Butere, Bungoma and Kakamega) reported a reduced production as an effect of the prolonged use of di-ammonium phosphate (DAP), a fertilizer applied to the sugarcane. DAP accumulation in the soil interferes with the pH levels, negatively affecting the growth of the taro crop. The yields per acre were low across the three districts averaging 500kg/ha. This can be attributed to different environmental stresses like drought, pests and diseases, low farm inputs and land shortage [5]. With the current increase in population, the pressure on land is high. Poor farming methods are adopted by farmers in an effort of maximizing on the limited land space available to increase food security [2]. Continuous intensive cultivation on the same piece of land speeds up its' rate of mineral depletion. Taro farming is mainly practiced with little or no farm inputs and this has a negative impact on the yields. High population increase and shrinking farm sizes has resulted in taro farming being relegated to only wetlands which has resulted in diminishing yields and quality. Effective taro breeding technology depends on farmers' access to new information. There exists diverse methods of access to new information but direct contact with extension officers, mass media and social networks are important among farmers. Majority of the farmers interviewed had no access to new information concerning taro farming, on average over the past two years. Direct contact with public extension is not common in Kenya, where only 9% of the interviewed population received information from extension officers through direct contact. This finding concurs with that of [8].

There is a possibility of the farmers not attending announced field days, therefore limited exposure to new information. Majority of the farmers interviewed are poor with low levels of education, therefore efficient methods of information dissemination is required to make an

effective change in the farming methods. Adoption of new information is expected to be low where there is low access to it. Taro is generally long season crop giving 1harvest per year in most countries. Most biotic and abiotic constraints to taro production have not been managed through breeding. Climate change is anticipated to cause a new outbreak of diseases and pests that is expected to further negatively affect taro production. There has been a sharp decline in taro production in Kenya [10].

The decline is attributed to vulnerability of the commonly grown varieties to various constraints like taro leaf blight, low soil fertility and drought. Farmers lack the ability to rapidly adopt taro varieties to the changing climate and the increasing biotic and abiotic constraints. As a result, food insecurity is rising with the current population increase in Kenya. Analyses on polymorphism proportion done using Genstat software version 12 showed that the pathogen has a high level of variability.

This kind of information regarding the current *P. colocasiae* population and its evolutionary potential is useful for making informed choices on disease-control strategies to mitigate the Taro leaf blight disease. In the present investigation, RAPDs technique was employed to assess the variability of *P. colocasiae* obtained from different fields. The results demonstrated the use of RAPDs markers to assess variability among the isolates. The four primers used showed clear bands. The high proportion of polymorphic loci found in the isolates revealed high levels of variability. Variability such as was found in the present study have previously been described in *P. colocasiae* populations from Southeast Asia and Pacific region [4] as well as from India [9]. Even fungi isolated from the same region showed distinct differences. Fungi from different fields on the other hand showed genetic closeness. This is an indicator of possible mutation due to exposure to various mutagens that may be found in fungicides or other agrochemicals.

The dendogram reconstituted based on the genetic similarity coefficient summarizes the interrelationship among *P. colocasiae* isolates from the three different geographical locations. The majority ($66^0/_0$) of the isolates, irrespective of the geographical origin were clustered together, meaning the genetic distance is not directly correlated with geographical distance. Some isolates depicted close relatedness with each other.

Migration events are quite common in the population of *P. colocasiae*. A similar observation was reported in previous studies where authors failed to identify geographic grouping in *P. colocasiae* isolates revealed by RAPD [7]. Even isolates obtained from the same geographical area have different RAPD patterns and were grouped differently, indicating that many populations of these oomycetes are made up of more than one genet and that some could be derived clonally. Molecular studies have shown that fungi assumed to be exclusively clonal actually are capable of recombination in nature [8], and this appears to be the case with *P. colocasiae* as well. The presence of larger than expected RAPD variation in isolates of *P. colocasiae* suggests that genetic recombination (or less likely hybridization) is at least possible in these oomycetes.

Sexually reproducing populations make management of disease more difficult due to the constant appearance of new genotypes that increase the variability of features like fungicide resistance, higher aggressiveness, and better fitness in the population. However, there are several lines of evidence that indicate the absence or rare occurrence of sexual reproduction in *P. colocasiae* as compatible mating types (A1 and A2) are seldom found in the same field [5]. A high level of recombination is suggested by the low index of association and it is possible that mitotic recombination events greatly contribute to the variation in *P. colocasiae* populations. Alternatively, other mechanisms, such as mutation, translocations, chromosomal deletions and duplications are common in Phytophthora species [2], which may also contribute to genetic variation observed in the *P. colocasiae* populations. Mitotic gene conversion was observed to occur at remarkably high frequencies in *Phytophthora colocasiae* documenting the potential for rapid generation of variation [6].

In general, populations with large effective sizes tend to have higher genetic diversity, as more alleles can emerge through mutation and fewer alleles will be lost due to random genetic drift [8]. Variability in the

pathogen population also could be elucidated by the fact that the isolates were collected from different climatic classifications, although, the limited number of isolates used in the study would not allow for a robust inference to be made about the influence of climate in the variability in the pathogen population. For breeding programs aimed at reducing the negative effects of fungal pathogens, the evolutionary potential of populations is of prime importance.

Fungal populations are considered to have high evolutionary potential when they have a mixed reproductive system, moderate gene or genotype flow, and large effective population size. This variability shows that the *P. colocasiae* populations could respond rapidly to selection exerted by newly introduced host resistance genes or fungicides, underlining the importance of relying on integrated disease management. The ten arbitrary primers chosen for the present analysis revealed 123 polymorphic bands that were consistently and unambiguously scorable in fourteen isolates of *P. colocasiae* studied. These bands were treated as genetic loci. All of the 123 RAPD loci were polymorphic. Maximum numbers of bands were produced by primer OPA-10. Evaluation of RAPD-PCR data produced infrahost genetic distance values ranging from 0.113 to 0.658. The RAPD data matrix of band presence and absence was transformed into an allelic frequency table.

Mean number of allele per locus (AE) was 2.000. Observed mean hetrozygosity (HO) and expected mean hetrozygosity was (HE) 0.3380 and 0.3706 respectively. Value of observed mean hetrozygosity was less than those of expected mean hetrozygosity. Mean fixation indices (F) were 0.0879, indicating an overall conformance to Hardy-Weinberg equilibriums. F value was significantly greater than zero and positive, indicating excess of homozygotes. Outcrossing rates (t) based on fixation indices was 0.8348. Both Isozyme and RAPD yielded almost similar genetic variability estimates.

The dendrograms constructed through the method UPGMA is presented. Considerable variation was observed among the isolates. Cluster analysis showed a distinct separation of the isolates and formed two major groups. Isolates collected from northern part of India formed a separate group and clustered together, while isolates collected from southern part of India form separate group and separated alone except isolate 98-111, indicating the possibility for the isolate to evolve from southern part of India. Genetic Genotyping of *P. colocasiae* 69. Differentiation between the mating types isolated from the same location (Chelavoor, Calicut, Kerala) was not significantly high, indicating that hybridization occurred between A1 (98-35a) and A2 (98-35b) mating types in that region.

Conclusions

There is some variability between populations of *P. Colocasiae* that are from closely located or far fields and populations of *P. Colocasiae* from fields that are wide geographical distances apart. This therefore implies that the variability of the pathogen is influenced by the prevailing weather conditions and human practices within the region.

Conflict of interest

Authors have declared no conflict of interests.

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