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THE USE OF RAPDS TO DETERMINE GERMPLASM COLLECTION STRATEGIES IN THE AFRICAN SPECIES *PHYTOLACCA DODECANDRA* (PHYTOLACCACEAE)

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SUMMARY

Variation in the RAPDs among African *Phytolacca dodecandra* (endod) populations and three related species was examined. The data were analyzed by principal coordinate and contour mapping of coordinate scores. RAPDs were found to be useful in detecting polymorphisms among the taxa. The RAPDs of Madagascar *Phytolacca dodecandra* were not similar to the African *P. dodecandra*, but were more closely associated with *P. icosandra* and *P. rivinoides* (South America). Four major sub-groups were discovered among *P. dodecandra* populations: Madagascar; Nigeria; Ethiopia; and southern Africa. These groupings correspond rather well with a previous study using non-polar hexane chemicals. Future germplasm collections should focus on the four regions. DNA extracted from tissue culture, seed and herbarium specimens of types 3, 17 and 44 produced polymorphic bands. The RAPDs from individual seedlings of types 3, 17 and 44 produced about 43% polymorphic bands.

INTRODUCTION

There has been a continued interest in the triterpenoid saponins of *Phytolacca dodecandra* (Phytolaccaceae), commonly called endod (Lemma et al., 1984; Parkhurst, et al., 1990). The saponins of endod possess molluscicidal, insecticidal, spermicidal, antifungal, and antiprotozoan properties. The geographical variation of *Phytolacca dodecandra* in Africa has been investigated in terms of morphology, leaf chemistry and triterpene aglycones (Adams et al., 1989, 1990; Parkhurst et al., 1990).

Analysis of total aglycone derivatives (Parkhurst et al., 1990) divided *Phytolacca* populations into a high oleanolic acid group (89%, +/-6%), and a low oleanolic acid group (53% +/- 7%). Of the three standard endod types (types 3, 17 and 44), types 3 and 44 were in the high oleanolic acid group, whereas type 17 was in the low oleanolic acid group. The molluscicidal data indicates a similar trend (Wolde-Yohannes and Kloos, 1989). It appears likely that the pathway to oleanolic acid is correlated with the production of the triterpenoid glycosides (molluscicides).

Previous analysis of the morphology (Adams et al., 1989) revealed variation both within and among regions. The first trend in the morphology was the separation of pubescent individuals from Ethiopia (E2 in Figure 1) from other populations. The second trend revealed differentiation of putative *P. dodecandra* from Madagascar (MA, Figure 1) and the third trend was the division of *P. dodecandra* into east and west African populations (Figure 1).

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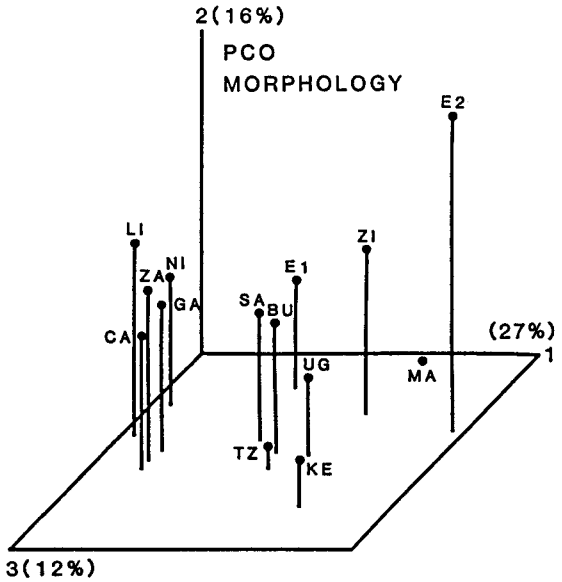


Figure 1. Principal coordinate analysis based on morphological characters of *Phytolacca dodecandra* (adapted from Adams et al., 1989). BU = Burundi and Malawi; CA = Cameroon; E1 = non- pubescent, Ethiopia; E2 = pubescent, Ethiopia; GA = Gabon; KE = Kenya; LI = Liberia, Sierra Leone & Guinea; MA = Madagascar; NI = Nigeria; SA = South Africa; TZ = Tanzania; UG = Uganda; ZA = Zaire; ZI = Zimbabwe & Zambia.

Adams et al., (1990) found the major trend in the non-polar hexane extractables to be the separation of putative *P. dodecandra* from Madagascar (MA, Figure 2) from all the African populations. The second trend found was separation of Nigeria (NI), Zambia (ZB) and Zimbabwe (ZI) populations from other African populations (Figure 2). The third trend was the differentiation of the Ghanan (GH) population (Figure 2). Aside from the separation of putative *P. dodecandra* from Madagascar, the morphology and non-polar hexane extractables did not show much correlation (cf. Figures 1 and 2).

However, taken together, the chemical and morphological data did suggest four areas for germplasm collection: north east Africa (Ethiopia, Kenya); Madagascar; southern Africa (Zambia, Zimbabwe); and east Africa (Nigeria, Ghana).

Recently, RAPDs (Random Amplified Polymorphic DNAs) have been used as molecular markers to detect genetic polymorphisms among different taxa (Williams, *et al.*, 1990). RAPDs which are generated by amplification of genomic DNA with a single primer of arbitrary nucleotide sequence produce polymorphisms that can be used as genetic markers (Caetano-Anolles et al., 1991; Demeke et al., 1992; Hu and Quiros, 1991; Williams et al., 1990).

In this work we report on DNA polymorphisms among African *Phytolacca* and related species using RAPDs. The results are compared with the morphological, chemical, and terpenoid data already available. Three other species of *Phytolacca* were included in this study. Individual endod plants of types 3, 17, 44 and from Zimbabwe were also studied to determine the extent of variability among seeds of this dioecious species.

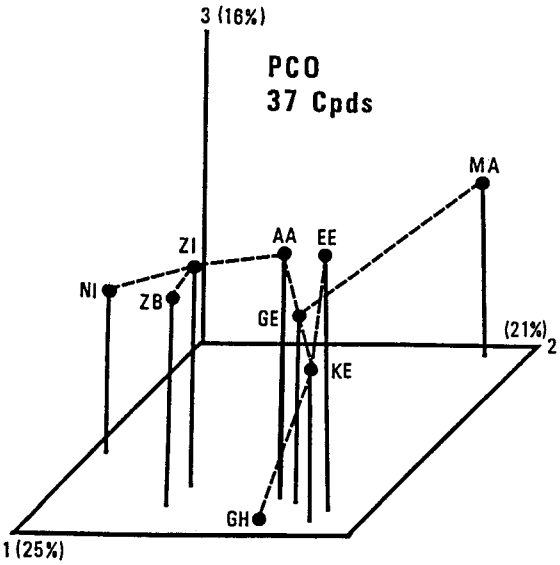


Figure 2. Principal coordinate analysis based on thirty-seven leaf chemicals for nine populations of *Phytolacca dodecandra* with a minimum spanning network superimposed (Adams et al., 1990). AA = Addis Ababa, Ethiopia; EE = Entoto Mtn., Ethiopia; GE = Guder, Ethiopia; KE = Kenya; NI = Nigeria; GH = Ghana; MA = Madagascar; ZI = Zimbabwe; ZB = Zambia.

MATERIALS AND METHODS

DNA was extracted from the plant materials (Table 1) by a modification of the SDS protocol (Dellaporta et al., 1983). PVP(0.18%) was added to the extraction buffer. DNA was also extracted from five individual seedlings of types 3, 17, 44 and from three seedlings of Zimbabwe endod to examine the polymorphisms among individuals.

Recently, RAPDs (random amplified polymorphic DNAs) have been used as molecular markers to detect genetic polymorphisms among different taxa (Williams et al., 1990). RAPDs, which are generated by amplification of genomic DNA with a single primer of arbitrary nucleotide sequence, produce polymorphisms that can be used as plants. PCR was performed in a volume of 25 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 1.5 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.5 ng genomic DNA and 1 unit of TAQ DNA polymerase. A control PCR tube containing all components but without genomic DNA was run with each primer to check for contamination. Seventeen 10-mer primers (Table 2) that gave several bright bands and did not have any false bands (in the controls) were used.

DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle used was 94°C (1.5 min) for initial strand separation, then 40 cycles of 37°C (2 min), 72°C (2 min), 94°C (1 min). Two additional steps were used: 37°C (2 min) and 72°C (5 min) for final extension. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667. pGEM

Table 1. Sources of plant materials used in this study.

Sources of plant materials	Symbols	Sources of DNA
Phytolacca dodecandra		
Type 3, IPB (Ethiopia)	E1	seed
Type 3, tissue culture	E2	leaves
Type 17, IPB (Ethiopia)	E3	seed
Type 17, tissue culture	E4	leaves
Type 17, Adams 5290	E5	leaves
Type 44, IPB (Ethiopia)	E6	seed
Type 44, tissue culture	E7	leaves
Type 44, Adams 5289	E8	leaves
Kenya, Adams 5324	KE	leaves
Madagascar, Adams 5624	MA	seed
Zambia	ZB	seed
Zimbabwe, Adams 5595	ZI	leaves
Nigeria	NI	seed
Ghana, Adams 5559	GH	leaves
P. americana	AM	seed
P. rivinoides, Zanoni 5679	RV	seed
P. icosandra, Zanoni 5680	IC	seed

Table 2. List of primers used in this study. The RC42 was synthesized at the Plant Biotechnology Institute, NRC, Canada. The other primers (116- 247) were purchased from the University of British Colombia.

Code	Sequence (5'- 3')	Code	Sequence (5'- 3')
116	TAC GAT GAC G	218	CTC AGC CCA G
123	GTC TTT CAG G	227	CTA GAG GTC C
131	GAA ACA GCG T	232	CGG TGA CAT C
134	AAC ACA CGA G	237	CGA CCA GAG C
143	TCG CAG AAC G	239	CTG AAG CGG A
153	GAG TCA CGA G	244	CAG CCA ACC G
184	CAA ACG GAC C	247	TAC CGA CGG A
204	TTC GGG CCG T	RC42	GCA AGT AGC T
212	GCT GCG TGA C		

DNA (Promega) was used as a molecular size marker. The size of the bands and their intensity was scored as: 0= no band; 1= very, very faint; 2= very faint; 3= faint; 4= medium; 5= bright; 6= very bright band. Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum value for that character over all taxa (= Gower metric; Gower, 1971; Adams, 1975). Principal coordinate analysis (PCO) follows Gower (1966).

RESULTS AND DISCUSSION

Principal coordinate analysis (PCO) of RAPDs for *P. dodecandra* populations plus three other *Phytolacca* species removed 61% of the variation on the first five eigenroots (25, 12, 9, 8, 7%). Ordination of the first three axes reveals three major groups (Figure 3): complete separation of *P. americana;* a group containing *P. icosandra* (Dom. Rep.), *P. rivinoides* (Dom. Rep.) and putative *P. dodecandra* from Madagascar; a group of *P. dodecandra* populations from Africa.

The clustering of putative *P. dodecandra* (Madagascar) with the two *Phytolacca* species from the Dominican Republic raises an interesting question. This clustering may be due to non-homologous band scoring. Although Adams and Demeke (1993) successfully applied RAPDs at the section level in *Juniperus,* these *Phytolacca* species may be too distantly related for homologous band scoring.

In addition to the aforementioned three groups, it is interesting to note the clustering of the Ethiopian *P. dodecandra* (E1 - E8, Figure 3). These plants do not cluster by type. However, these types (3, 17, and 44) were based on various leaf types and growth habits. It should be noted that the types are not inbred lines but merely plants identified in the field and in test plots that have certain leaf shapes and habits.

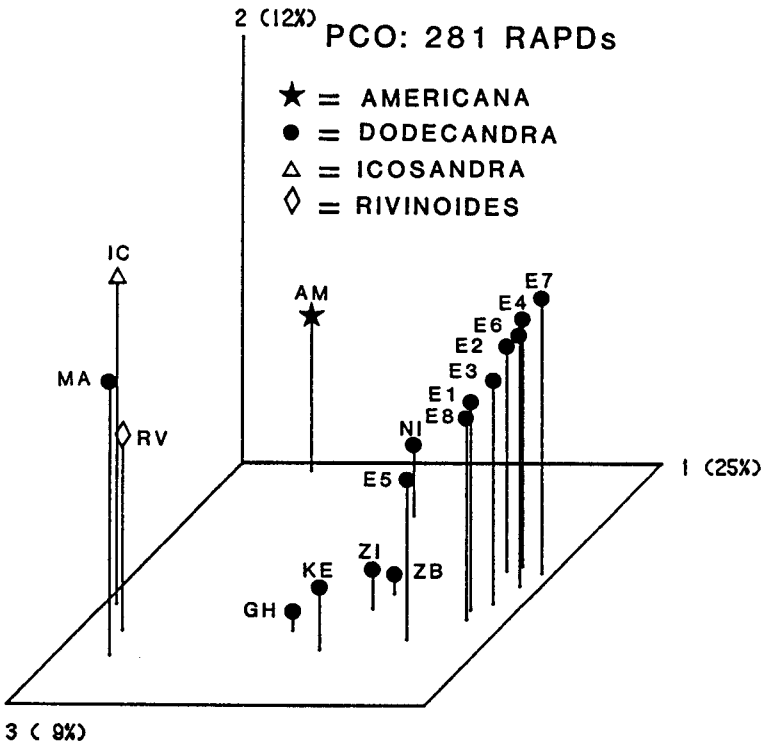


Figure 3. Principal coordinate analysis of 281 RAPDs of *Phytolacca americana, P. dodecandra, P. icosandra* and *P. rivinoides.* Note the four cluster groups (Ethiopian endod; Kenyan, South African and West African endod; *P. americana; P. icosandra, P. dodecandra* from Madagascar and *P. rivinoides.* See Table 1 for acronyms.

Because *P. dodecandra* is dioecious, one would expect to find lots of genetic diversity in these 'types' and the RAPDs show that. Controlled breeding crosses would

be needed if isogenic lines were desired. Figure 3 also shows the divergence of the three types of *P. dodecandra* (3, 17 and 44) from Ethiopia. DNA extracted from tissue culture, seed and herbarium specimens revealed several polymorphisms. The herbarium specimens were from the original clonal materials of types 17 and 44, whereas the tissue culture plants were raised from seeds collected from open pollinated plants of types 3, 17 and 44.

In order to more readily examine the trends within *P. dodecandra*, PCO was performed using just the populations of *P. dodecandra*. This resulted in four eigenroots (23.5, 19.2, 16.8, 11.4, % of variance; total = 79.4%) before the eigenroots began to asymptote. The first principal coordinate separates the Madagascar putative *P. dodecandra* (MA, Figure 4) and the second coordinate separates the Ethiopian plants E1, E3, E6, Figure 4) from other African mainland populations. The third axis shows

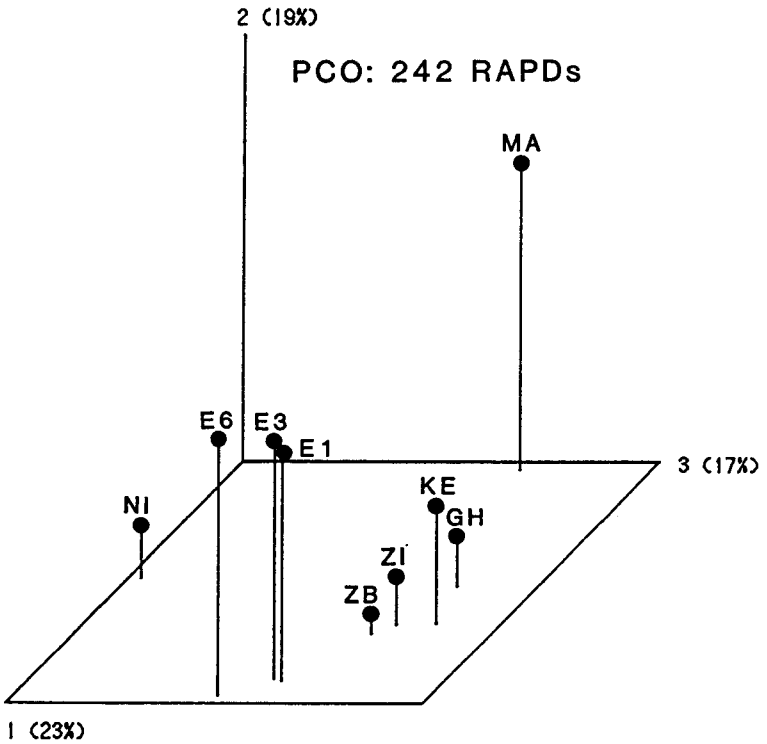


Figure 4. Principal coordinate analysis of 242 RAPDs of *Phytolacca dodecandra* from Africa. Note the separation of Madagascar *Phytolacca* from the rest of African *P. dodecandra*. See Table 1 for acronyms.

the divergence of the Nigerian plants as well as central and southern African plants. The fourth coordinate (11.4%, not shown) reveals differentiation of the Kenyan plants and the fifth axis (8.5%, not shown) separates the Ghana plants from all others.

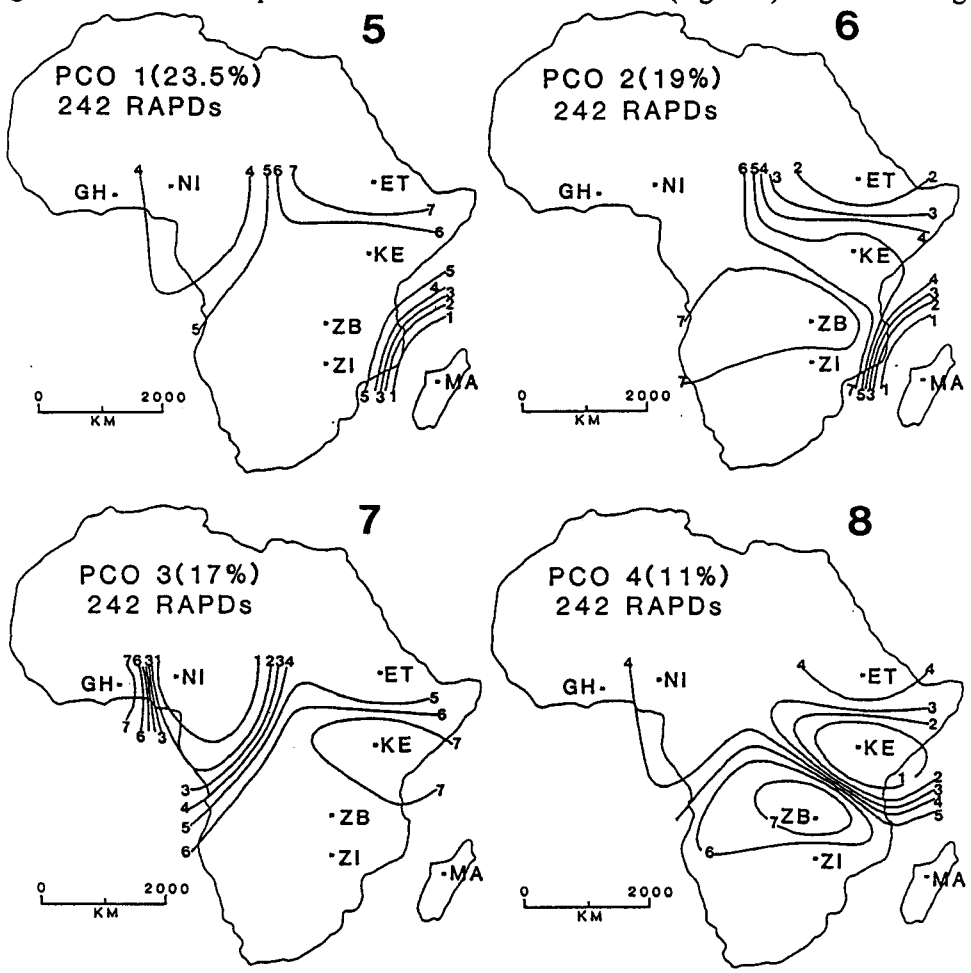
Both the previous morphological and chemical data analyses (Figures 1, 2) also separated the Madagascar *Phytolacca* from the rest of the African *Phytolacca* (Adams et al., 1989, 1990). The recognition of *P. goudotii* Briquet from Madagascar (Walter, 1909) appears to warrant possible consideration. In fact, in the previous morphological study (Adams et al., 1989) *Phytolacca* (putative *dodecandra*) from Madagascar and *P.*

goudotii were closely ordinated showing their close similarity. It seems likely that our sample of Phytolacca from Madagascar is *P. goudotii.*

The suggestion of Nowicke (1969) that the Madagascar and Ethiopian *Phytolacca* are merely ecotypic forms is not supported by our present data. Of course, it is possible that both *P. goudotii* and *P. dodecandra* co-occur on Madagascar, and we have inadvertently sampled *P. goudotii.* This is an area that deserves additional field work and analyses.

To visualize the regional trends in variation within *P. dodecandra,* the coordinate scores for each population were used as z values and the geographic locations were used as x, y values to produce contour maps for each coordinate. Figure 5 shows the divergence of the Madagascar population as well as some differentiation of the Nigeria population.

The second coordinate (Figure 6) indicates a co-differentiation of the Ethiopia and Madagascar populations. The Kenya population appears to be somewhat intermediate between Zambia and Ethiopia on this coordinate (Figure 6). The divergence of the Nigerian *P. dodecandra* plants is seen on the third coordinate (Figure 7). It is interesting



Figures 5- 8. Contour maps of the four principal coordinates based on 242 RAPDs. See text for discussion and Table 1 for acronyms.

to note the sharp difference between Nigeria (NI) and Ghana (GH) plants. The divergence of Nigeria and Ghana endod plants has also been shown by chemical analysis (Figure 2). Figure 8 shows the differences between Kenya plants and those from Zambia and Zimbabwe.

In addition, the RAPDs from five seedlings of types 3, 17 and 44 were examined and considerable variability among these plants and among the types were found (data not shown). The percentage of polymorphic bands among 5 plants of types 3, 17 and 44 was 45, 42 and 41%, respectively. *Phytolacca dodecandra* is a dioecious plant and, thus, variability among individual plants is expected. It is apparent that RAPDs will be very useful for the taxonomic evaluation of plants derived from dioecious plants. The RAPDs from the three seedlings of Zimbabwe gave only 16% polymorphic bands, indicating a closer similarity. The percentage of polymorphic bands between the three types were as follows: 3 & 17 = 57.1%; 3 & 44 = 60.3%; 17 & 44 = 55.4%; 3, 17 & 44 = 61.3%; 3, 17, 44 & Zimbabwe = 67.5%. Thus, there is a higher level of polymorphism among the types than among individual plants within a type.

Molecular markers such as RFLP and isozymes have been widely used in DNA fingerprinting and gene mapping. RFLP is very popular but it is costly, time consuming and laborious. RAPDs are much cheaper, less labor intensive and do not use radioactive chemicals and are, thus, desirable for DNA fingerprinting work. Germplasm screening and taxonomic studies are one of the major activities in germplasm centers. Morphological analysis coupled with RAPDs will be very useful for these research activities. Genetic analysis with RAPDs will be especially applicable to developing countries because of the low cost and less training needed. Conventional breeding programs will also be aided by genetic analysis using RAPDs.

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