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MORPHOLOGICAL, ANATOMICAL AND CHEMICAL ANALYSES OF AMORPHOPHALLUS PAEONIIFOLIUS AND RELATED TAXA

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ABSTRACT

A study on **the** affinity of undescribed taxa of *Amorphophallus* with *A. paeoniifolius* and its related species was undertaken using numerical analyses of data derived from preserved and living specimens. The results obtained were compared with the system of classification produced by classical taxonomic methods. The Javanese wild and cultivated forms *of Amorphophallus paeoniifolius* respectively known locally as *"Amorphophallus campanulatus* f. *hortensis"* and *"Amorphophallus campanulatus* f. *sylvestris"* were confirmed as two distinct but closely related taxa.

ABSTRAK

Penelitian hubungan takson-takson *Amorphophallus* yang baru untuk ilmu pengetahuan dengan *Amorphophallus paeoniifolius* dan jenis kerabatnya dilakukan dengan menggunakan analisis numeris yang datanya diambil dari spesimen hidup dan mati. Hasil yang diperoleh diperbandingkan dengan sistem klasifikasi taksonomi klasik. Bentuk liar dan kultivar *A. paeoniifolius* yang berturut-turut dikenal sebagai *A. campanulatus* f. *hortensis* dan *A. campanulatus* f. *sylvestris* dalam kepustakaan setempat dinyatakan sebagai dua takson yang berbeda tapi berkerabat dekat.

INTRODUCTION

Amorphophallus Bl. is one of several important genera in the family Araceae grown for human consumption as well as for animal feed, medicine and other industrial purposes. The elephant yam *(Amorphophallus paeoniifolius* (Denns.) Nicolson), devil's tongue *(A. konjac* C. Koch) and *A. oncophyllus* Prain ex Hook.f. are among the approximately one hundred species

which make up this genus, which is widely spread throughout the tropics of Asia, Africa, the Pacific Islands and some other areas in the subtropics. The main characteristics of the genus is that the tuber produces an evil-smelling inflorescence with a terminal sterile spongy phallic appendage enclosed in a spathe. Male flowers are on the upper part of the sterile zone of the spadix and female ones below. It has a single petiole and a compound leaf which dies down before the plant flowers.

The discovery of the recently described *Amorphophallus lambii* Mayo & Widjaja (1983) as well as an undescribed cultivar from Timor (hereafter referred to as *Amorphophallus* EAW 1177) and two other taxa from Thailand *(Amorphophallus* NG 2 and *Amorphophallus* RBG 1) which in some respects resembled *Amorphophallus paeoniifolius,* made it necessary to study their taxonomic relationships to other described species of *Amorphophallus.* Since not all of these undescribed taxa produced inflorescences, all available data which might be useful as taxonomic evidences have been employed. Pending their formal descriptions as new taxa, the present study was designed to investigate the affinity of these undescribed taxa with *A. paeoniifolius, A. oncophyllus, A. bulbifer* (Roxb.) Bl. and *A. variabilis* Bl. using data derived from vegetative morphology, anatomy and chemotaxonomy.

MATERIALS AND METHODS

Both living plants and herbarium specimens were used in this study. A total of 30 accessions of living plants were grown, including four accessions of cultivated *A. paeoniifolius,* six of wild A, *paeoniifolius,* seven of *A. variabilis,* eight of *A. oncophyllus* and one accessions each *of A. bulbifer, A. lambii, Amorphophallus* EAW 1177, *Amorphophallus* NG 2 and *Amorphophallus* RBG 1 (Widjaja 1981).

Twenty-three of these living accessions had been collected in various parts of Indonesia. Living materials were brought to Birmingham (England) in August 1980 and the tubers were planted in John Innes No. 2 compost in pots and grown in a glass house at about 25°C. Automatic watering was used and mercury vapour provided supplementary lighting, especially during the winter period. It was difficult to obtain leaf material under standard conditions and impossible to obtain replicate leaves for any one accessions, since only one tuber was available of most accessions and the leaves were produced singly and erratically, independent of any season. One accession of cultivated *A. paeoniifolius* was bought from an Indian food shop in Smethick near Birmingham.

Herbarium specimens, living collections and paintings were studied at the Royal Botanic Gardens, Kew. These were particularly valuable for pro-

viding data on inflorescence characters since only *A. variabilis* flowered in Birmingham.

Morphological observation

Thirty-five morphological characters of tubers, petioles and leaves were recorded for 15 accessions of living plants of four species. Twenty-five characters were recorded for all 15 accessions but some data were unobtainable for some accessions of *A. oncophyllus* and in this case the average value of the appropriate characters from the other accessions was used (Widjaja 1981).

Chemotaxonomic study

Chromatography of phenolic compounds

Fully mature top folioles were collected from 25 plants and dried in a forced ventilation oven at 45°C for 96 hours. Half a gram of dried leaves were ground and extracted with sufficient 3 ml 0.5% v/v hydrochloric acid in methanol kept overnight in darkness. Leaf extracts were applied to a 1.5 cm diameter circle 4.5 cm from the corner of Whatman No. 1 filter paper. The papers were clipped into a Shandon Universal Holder and run in 250 ml of the first solvent (butan-1-ol : acetic acid : water = 3:1:1) for about 12 hours; the papers were dried then run in the second solvent (acetic acid : water = 15% v/v) for 4 hours. After drying, the papers were analysed under an ultra violet lamp. Any spots and their colours were recorded and their distribution was examined.

Electrophoresis of proteins

Electrophoresis of proteins was done by polyacrylamide gel methods following the method of Davis (1964). About 10 grams of good fresh tubers and 7.5 grams of fresh leaves were sampled from each of 14 accessions. After washing with tap water and cleaning from soil, tubers and leaves were kept in polythene bags in the deep freeze for at least 4 hours. Tubers and leaves were thawed to ± —1°C and then crushed in the polythene bag by hand at room temperatures. The juices were collected, spun in a centrifuge for about 6 minutes at 15,000 g and then kept in the freeze at ± —3°C. Samples of 0.1 — 0.2 ml of protein extract were analysed and after electrophoresis the gels were stained with 0.1% w/v naphthalene black in 7% v/v acetic acid for 1 — 2 days and stored in the dark. Examinations were made over fluorescent light and the distribution of bands of proteins from each sample were recorded.

Anatomical observations

Only the forms of raphide were investigated during the course of this anatomical study. Raphides were prepared by crushing the tubers in a mortar with some distilled water and allowed to settle or filtered by filter paper and dried. A pinch of sample was taken and placed on a stub and coated with gold. Examinations were conducted under a light microscope and a scanning electron microscope (Cambridge Stereoscan 600).

Data analyses

Data were analysed numerically using Principal Components Analysis, and clustering techniques by Euclidean Distance and Ward's method using Clustan l.C programmes. The chromatographic and electrophoresis data were also analysed by Jaccard's Coefficient and UPGMA using Clustan l.C programme (Wishart 1978).

RESULT AND DISCUSSIONS

MORPHOLOGY

Cluster analyses based on all 35 morphological characters of tubers, petioles and leaves of all 15 accessions indicated that A. *oncophyllus* was very distinct and clearly separated from the other species in Principal Components Analysis by the first factor. This distinction was maintained even when only 25 characters recorded from all four accessions of *A. oncophyllus* were used.

Because *A. oncophyllus* was so distinct, further analyses were made of the remaining 11 accessions using all 35 characters. Principal Components Analysis of four accessions of the wild *A. paeoniifolius* showed that they formed a very distinct group. However, one accession was shown to be distinct from the other accessions of the wild *A. paeoniifolius* and rather similar to the undescribed *Amorphophallus* EAW 1177 and three accessions of the cultivated A. *paeoniifolius* (Fig. 1).. The cluster analysis also showed that the two accessions of *A. variabilis* were similar to each other.

CHEMOTAXONOMY

Chromatography

Chromatographically speaking *A. oncophyllus* appeared as a distinct group, with two spots appeared only in this species. The chromatographj also showed that *A. bulbifer* and *Amorphophallus* RBG 1 were separatee from each other as well as from the other species.

All five accessions of the wild A. *paeoniifolius* clustered together. *Amorphophallus* EAW 1177 was included in this cluster and two accessions *of A.*

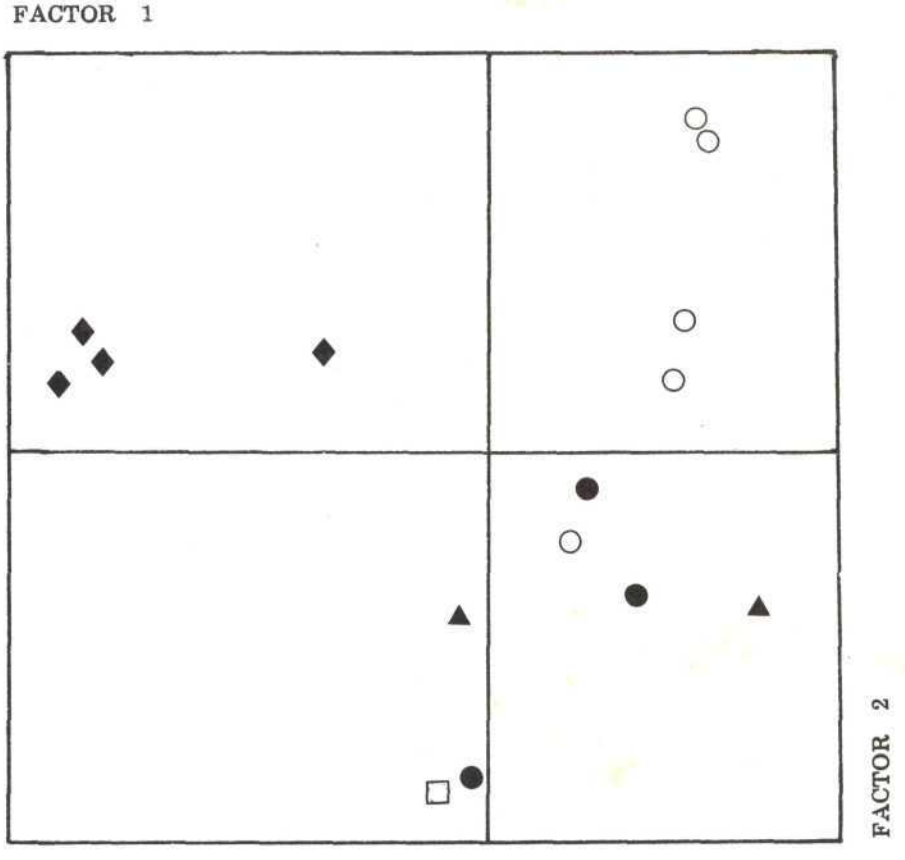

Fig. 1. Principal Components Analysis on morphology of *Amorphophallus* :

- ◆ *A. oncophyllus*
- ▲ *A. variabilis*
- ○ Wild *A. paeoniifolius*
- ● Cultivated *A. paeoniifolius*
- □ *Amorphophallus sp.* EAW 1177

variabilis were close to it. However, the remaining accessions of *A. variabilis* clustered together outside these groups.

The three accessions of the cultivated *A. paeoniifolius* occured in two clusters together with accessions of *A. lambii* and *Amorphophallus* NG 2. These last two taxa biochemically are rather similar to the cultivated *A. paeoniifolius* although the latter has more prickles on their petioles.

Based on the above data, it seemed that the wild *A. paeoniifolius* is related to the cultivated one and both taxa are closer to *A. variabilis* than to *A. oncophyllus*. *Amorphophallus* RBG 1 and *A. bulbifer* are clearly distinct from *A. paeoniifolius* and *A. variabilis*.

Electrophoresis

Leaf proteins

From the electrophoresis of leaf proteins a total of 25 protein bands were recognised, of which as many as 11 were detected in one accession but as few as three in another. There were bands which were observed in many taxa (e.g. band 22) but others could be detected only in one species. Consequently band 12 was found in *A. paeoniifolius* but not in any other species, so that it can be used as a diagnostic character for this species. Band no. 4, 14 and 18 may be considered as diagnostic characteristic for the cultivated *A. paeoniifolius* and band 17 for the wild one. Both dendrograms derived from Jaccard or Euclidean approaches agreed in placing all four accessions of the wild *A. paeoniifolius* in a single cluster.

A. oncophyllus, *A. lambii* and *Amorphophallus* NG 2 were fairly similar to each other, but *Amorphophallus* EAW 1177 showed little similarity to any of them either by Jaccard's or Euclidean Coefficient.

Tuber proteins

The result of tuber electrophoresis of 23 accessions *of Amorphophallus* showed a total of 18 protein bands, with as many as 11 bands in one accession but as few as one in another. The most universal protein was band 15 which appeared in all accessions of *Amorphophallus*. Band 2 and 3 were found in *A. variabilis* but not in other species.

All accessions of the wild *A. paeoniifolius* formed a single cluster. Similarly all accessions of *A. variabilis* also formed a single tight cluster. *A. oncophyllus* and the cultivated *A. paeoniifolius* formed two distinct but related clusters by Euclidean Distance, but were more intermingled by Jaccard's Coefficient. *Amorphophallus* EAW 1177 was similar to *A. paeoniifolius* accession 7D. The difference between accession 7D and 7Y is noteworthy since the former was sampled from the main tuber and the latter was sampled from a young tuber which grew from the main tuber (Fig. 2).

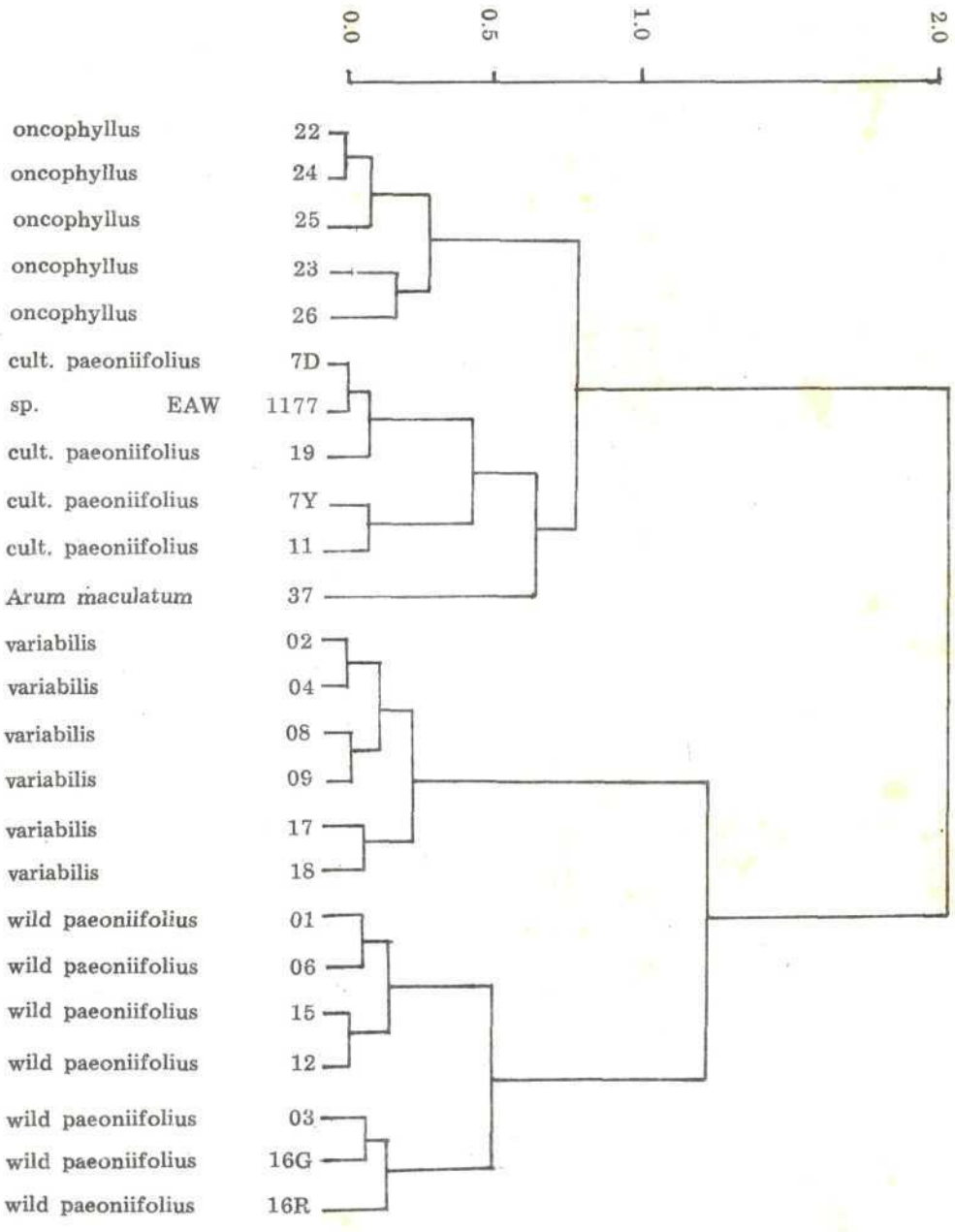

Fig. 2. Cluster analysis of *Amorphophallus* tuber electrophoresis.

Based on the tuber electrophoresis it can be concluded that the wild *A. paeoniifolius* was slightly distinct from the cultivated one and was distantly related to *A. variabilis*. On the.other hand the cultivated A *paeoniifolius* was somewhat related to *A. oncophyllus*.

ANATOMY

Two kinds of raphides, one small and the other one much larger, were observed in the wild and cultivated *A. paeoniifolius, A. oncophyllus, A. variabilis* and *Amorphophallus* EAW 1177. The length of the large raphides of *A. variabilis* was 0.0112 —0.0128 mm and the small ones was± 0.0004 mm. In *Amorphophallus* EAW 1177 the large raphides measured ± 0.0136 mm and the small one ± 0.0032 mm in length. The large raphides of *A. paeoniifolius* may reach 0.0144 mm long and the small one ± 0.0004 mm. It is difficult to distinguish the two forms of this species by the length of their large raphides but under a light microscope it could be seen that the small raphides in the wild *A. paeoniifolius* were more abundant than in the cultivated one. This result is contrary to Kirtikar's (1954) findings who reported that there were two kinds of raphides in the wild variety while the cultivated one showed only one kind.

Besides the size, the raphides of the species investigated could also be distinguished by their grooves and their surface ornaments. The tip ends of the large raphides of *A. oncophyllus* were acute and tapering. There are canaliculate grooves on the side, of this large raphide and its surface is sparsely ornamented with thin but broad protuberances. The large raphides of *A. variabilis* were also provided with tapering and acute ends and canaliculately grooved, but unlike those of *A. oncophyllus* the surface is ornamented with sharp barb-like protuberances. In both the wild and cultivated forms of *A. paeoniifolius* the large raphides appear to have interrupted canaliculate grooves. In this species the barbs occur on the tip ends, whereas in *Amorphophallus* EAW 1177 they could not be found at the tip ends of the large raphides. The sharpness and the position of the barbs on the surface of the raphides as well as the abundance of the small raphides might be used as characteristics to distinguish the forms of *A. paeoniifolius* although it was difficult to see.

CONCLUSIONS

As a general conclusion of this study it can be stated that within the genus *Amorphophallus, A. oncophyllus* is very distinct on morphological, chromatographic as well as tuber electrophoretic grounds. *A. bulbifer* is also distinct, although it clustered with *A. oncophyllus* in the chromatographic studies. The results of leaf electrophoresis, however, showed *A. bulbifer*

to be different from *A. oncophyllus.* In Engler's (1911) classification *A. bulbifer* and *A. oncophyllfis* were placed as neighboring species in section *Conophallus,* a conclusion which is supported by the present study. It appears that *A. variabilis* is closer to *A. paeoniifolius* than to *A. oncophyllus;* this is contrary to Engler's contention who placed *A. variabilis* in the section *Conophallus* as well.

The wild *A., paeoniifolius* stands out as a distinct taxon based on morphological, electrophoretic as well as the chromatographic studies. It is linked with the cultivated *A. paeoniifolius* and *A. variabilis* rather than with *A. oncophyllus.* The smooth petioled *Amorphophallus* EAW 1177 which was collected in Timor Island as a cultivated plant in the kitchen garden is very similar to the cultivated *A. paeoniifolius* in morphological and tuber electrophoretic characteristics. Its leaf chromatography, however, points to a relationship with the wild *A. paeoniifolius.* In Javanese literature the wild and cultivated forms of *A. paeoniifolius* have been known respectively as *A. campanulatus* f. *sylvestris* Backer and *A. campanulatus* f. *hortensis* Backer (Backer 1920, Backer & Bakhuizen van den Brink f. 1968, Sastrapradja *et al.* 1977). Their nomenclature is being worked out in the light of the present study.

On vegetative morphological grounds *Amorphophallus* NG 2 and *Amorphophallus* RBG 1 appear related also to these last complex of taxa but in the absence of the inflorescence their true relationships cannot as yet be ascertained. Electrophoretically they are related to *A. oncophyllus* and *A. bulbifer,* as is the case with *A. lambii.*

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