Intraspecific inflorescence and palynological variations in the morphotypes of Amorphophallus paeoniifolius

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Short Report

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Abstract

The genus *Amorphophallus* Blume ex Decne. is a vast and widespread group of aroids belonging to Araceae which is taxonomically and botanically distinct due to the frequency of emergence of inflorescences and their relatively short active period of existence. *Amorphophallus paeoniifolius* var. *campanulatus* or the elephant foot yam is an important tuber crop due to its high productivity and popularity as a vegetable in various delicious cuisines and medicinal properties. Wild congenerics of the cultivated *A. paeoniifolius* known as *A. paeoniifolius* Nicolson var. *paeoniifolius* (Decne.) Sivad. possess powerful therapeutic action against piles and gastro-intestinal disorders. The wide visible morphological variation observed in wild *A. paeoniifolius* morphotypes (morphological variants) available in the Western Ghats especially in the vegetative as well as inflorescence characters led to the present investigation to analyze and appraise the inflorescence diversity in comparison with other aspects, among the wild relatives of *Amorphophallus paeoniifolius*. Variation in the surface pattern of petiole and spathe is the main difference found within the species. The shape of appendix, the colour and pattern of spathe, scale leaves (bract) subtending the spathe, the male zone, female zone, the pollen morphology as assessed by SEM and pollen fertility showed distinct variation within the species revealing six distinct morphotypes. The mitotic chromosome studies indicated structural variation which was reflected in the karyomorphology. The ISSR markers, inflorescence /floral markers and vegetative morphological descriptors also indicated clear distinction between the morphotypes in addition to the variation seen in pollen morphology

Introduction

Amorphophallus paeoniifolius Blume ex Decne. or the elephant foot yam belonging to Araceae is one of the most interesting species of the genus *Amorphophallus*. They are perennial or annual herbs, generally bearing one broad highly dissected long-petioled leaf with a rhizomatous stem called corm.

Amorphophallus paeoniifolius (Dennst.) Nicolson var. *campanulatus* (Decne.) Sivad. is the cultivated species which is rich in starch and is used as staple food. It has developed into a cash crop in India with good economic returns to farmers due to its high tuber productivity and popularity as a vegetable in various delicious cuisines and usefulness as a medicinal tuber and adorns the position as the ‘king of tubers’(Raghu et al. 1999). It is a highly nutritious staple food in several parts of Asia and Africa. The petiole and immature inflorescence are used as delicious vegetable (Anil et al. 2011). It is a rich source of carbohydrate, protein, minerals like calcium, iron, phosphorous, vitamin A, B, C, flavanoids, and fibre (Kay 1987; Shilpi et al. 2005). Important medicinal attributes of *Amorphophallus* include hepatoprotective, antioxidant and uterus stimulating activity (Laderman 1983; Singh et al. 2011). The corms are aperient, carminative and expectorant. The corm extract is applied externally as an irritant to treat acute rheumatism, administered internally in the treatment of dysentery, diarrhoea, piles, hemorrhoids and in the formulation of indigenous medicines to cure inflammatory conditions and ophthalmia (Watt 1889; Bala et al. 2007; Purwal et al. 2011). Wild congenerics of the cultivated *A. paeoniifolius* known as *A. paeoniifolius* Nicolson var. *paeoniifolius* (Decne.) Sivad. (Nicolson 1987) possess powerful therapeutic action against piles and gastro-intestinal disorders. The root extracts are used to cure abdominal colic diseases, elephantiasis, skin and blood diseases, fistula, glandular swellings in the neck, urinary diseases and dropsy and has depressant

action on central nervous system (Jayaweera 1981; Dey et al. 2011). The crushed seed relieves tooth ache and the seed paste is applied to relieve rheumatic swellings (Anonymous 1988). It is used in the folk medicine to arrest tumor growth, lung swelling, asthma, vomiting, abdominal pain, piles, hemophilic conditions, skin diseases, obesity, dyspepsia, debility and to control intestinal worms (Prajapathi et al. 2004).

Nicolson (1977) made a new combination of binomial namely *A. paeoniifolius* (Dennst.) Nicolson as applying to *A. campanulatus* (*sensu lato*). The wild and cultivated elements differ in many respects even though they resemble in general appearance and many other characteristics and hence they were treated as two varieties of *A. paeoniifolius* - *A. paeoniifolius* (Dennst.) Nicolson var. *paeoniifolius* and *A. paeoniifolius* (Dennst.) Nicolson var. *campanulatus* (Nicolson 1987).

Since a lot of variation was observed in the vegetative morphology of the wild *A. paeoniifolius*, this study aimed at observing the reproductive morphological variations and palynology and correlating it with cytological and molecular variations based on ISSR markers.

Materials And Methods

A preliminary norm for the germplasm collection was the recognition of vast range of visible morphological variation observed in wild *A. paeoniifolius* especially in the petiole pattern. Hence in the present study a random stratified sampling was made in selected biogeographic zones where *Amorphophallus* naturally occurs or cultivated to get a representative germplasm. The morphotypes of *A. paeoniifolius* available in Western Ghats, the semi-arid zones (represented by Gujarat State), Gangetic plains (represented by Bihar State) and the Deccan plateau (represented by Tamil Nadu and Andhra Pradesh) were selected for this study. This comprised of seven morphotypes of *A. paeoniifolius* (10 plants per accession) which included five morphological variants collected from wild along with cultivated elephant foot yam, cv. Gajendra (GJ), and local cultivar karunaikizhangu (T10) from Tamil Nadu (Table 1). Individual plants were maintained in earthen pots (25 cm dia) filled with a mix of sand:soil:cow manure (1:1:1 w:w:w). The corm/cormel, dipped in *Trichoderma*-cow manure (1:1 w: w) slurry, was used as planting material and planting was done during March to April. Plants were irrigated by hand daily in the morning. No fertilizer or pesticides were applied, and potted plants were maintained in an open space in the Botanical Garden of University of Kerala, Thiruvananthapuram, Kerala, India. When plants defoliated in October they were left unharvested in the soil for the emergence of inflorescence.

Table 1
Different accessions of *Amorphophallus* used in the study and its assigned code

Sl. No	Accession Code	Place of collection*/ origin	Genetic group/Species	Status:wild or cultivated	Latitude/ Longitude	Altitude (ft)
1. 18.	V1	Vittal, DK	*A. paeoniifolius* var. *paeoniifolius*	wild	12º.47'N/75 º06'E	280ft
2. 19.	V4	Vittal, DK	*A. paeoniifolius* var. *paeoniifolius*	wild	12º.47'N/75 º06'E	280ft
3. 10.	T1	Vithura, TVM, KL	*A. paeoniifolius* var. *paeoniifolius*	wild	8º678'N/77 º0972'E	343ft
4. 14.	K3-2	Thrissur, KL	*A. paeoniifolius* var. *paeoniifolius*	wild	10º31N/76º20E	157ft
5. 17.	T10 (karunaikizhangu)	Nagercoil, TN	*A. paeoniifolius* var. *campanulatus*	cultivated	8º30'N/77º30'E	300ft
6. 19.	GJ (Gajendra)	Released variety	*A. paeoniifolius* var. *campanulatus* (National variety)	cultivated	17º01'N/81º43'E	36ft
7. 24.	P19	Puttur, DK	*A. paeoniifolius* var. *paeoniifolius*	wild	12º44'N/75 º13'E	270ft
*DK-Dakshina Kannada, Karnataka;, KL-Kerala; TN-Tamil Nadu						

Observations on 33 vegetative qualitative traits were scored at full foliage stage based on descriptors developed by Anil et al (2011, 2019) based on NBPGR for elephant foot yam (Abraham et al., 2006) with a few additional characters as appropriate to wild collections based on descriptions of Hetterscheid and Ittenbach (1996).

Amorphophallus is a single-leaved plant with an umbrella-shaped lamina (the canopy). The inflorescence is a spadix covered by a spathe (Fig. 2). The inflorescence descriptors were developed based on Hetterschied and Ittenbach (1996) and modified based on the observations obtained during this study (Table 2).

Table 2. List of inflorescence /floral descriptors evaluated for systematics of *A.paeoniifolius*

Sl. No.	Character description	Descriptor state
1	Length of peduncle	1-short,2-long,3-very short/sessile
2	peduncle pattern	1-sculptured as petiole,2-patterned as petiole,3-not as petiole
3	Spathe shape	1-elongate-triangular,2-triangular,3-ovate,4-broadly ovate,5-variously shaped,6-cymbriform (boat shaped)7-campanulate,8-funnel-shaped
4	Spathe colour outside	1-brownish purple with purple blotches, 2-shades of brownish purple with dark green/black dots,3-pale purplish with innumerable dark green dots along the entire surface which gives a blackish appearance towards the limb, 4-Shades of greenish purple with white blotches and dark green dots,5-Bright green in kettle with purplish limb with white blothches,6-pale purplish with dark green dots along the entire surface limb
5	Spathe colour inside	1-paler colour,2-intermediate,3- reddish purple
6	Spathe base shape	1-convolute,2-open,3-connate (edges united)
7	Spathe connection with limb	1-not separated fromlimb,2-clearly separated from the limb by a constriction,3-rounded,4-urceolate,5-funnel-shaped
8	Spathe inside nature	1-smooth,2-clothed with ridges,3-clothed with warts,4-clothed with large warts,5-clothed with hairs
9	Limb shape	1-rim-shaped,2-broadly triangular,3-elongate triangular,4-erect,5-spreading,6-oblique,7-fornicate
10	Limb margin	1-entire,2-lobed,3-flat or undulate,4-plicate(folded as a fan)
11	Limb apex	1-acute,2-acuminate,3-caudate,4-obtuse
12	Limb colour	1-pale,2-intermediate,3- reddish purple
13	Spadix nature	1-sessile,2-shortly stipitate
14	Spadix length	1-shorter than spathe,2-equalling spathe,3-longer than spathe
15	Female zone shape	1-cylindric,2-fusiform,3-conic,4-obconic
16	Female zone –male zone connection	1-contiguous,2-separated by a sterile zone
17	Female flower	1-congested,2-slighly congested 3-distant,4-surrounded by staminodes
18	Sterile zone	1-staminodes,2-mixed with pistillodes(rare),3-partly naked,4-entirely naked,5-absent
19	Male zone	1-cylindric,2-fusiform,3-conic,4-obconic,5-very small continuous with the lower part of appendix

20	Male flowers	1-congested,2-distant,3-variously fused in the upper part of male zone,4-sometimes fused into vertical rows,5-fused helically,6-verticillate(whorls),6-mixed with staminodes
21	Appendix	1-absent,2-contiguous with male zone ,3-separated by a constriction,4-separated by a short stipe
22	Appendix outline	1-conic,2-fusiform,3-triangular,4-myosuroid,5-ovate,6-globose,7-subglobose,8-large longitudinal folds,9-deep crack
23	Appendix surface	1.smooth ,2-rugulose,3-with distinctly shaped staminodes often only at the base
24	Exudation from appendix	1-warm during anthesis and spreading strong smell,2-emitting droplets of a clear fluid
25	Appendix apex	1-acute,2-obtuse
26	Appendix nature	1-erect,2-horizontal,3-arching,4-nodding,5-pendulous
27	Appendix wall	1-thin,2-massive
28	Appendix inside	1-narrow canal,2-a large cavity
29	Female flower-ovary nature	1-sessile,2-short stipitate
30	Female flower-ovary shape	1-globose,2-sub globose,3-depressed,4-ovate,5-rounded,6-angulate
31	Female flower-ovary locule	1-one,2-two,3-three,4-four
32	Female flower-ovary attachment	1-basifixed,2-axillary
33	Style presence	1-present,2-absent
34	Style shape	1-cylindric,2-slightly conic,3-obconic
35	Style length	1-equalling ovary,2-shorter than ovary,3-longer than ovary,4-very long
36	Stigma	1-indistinct,2-large
37	Stigma position	1-terminal,2-sub terminal
38	Stigma shape	1-globose,2-hemisheric,3-concave,4-flattened,5-entire,6- 2-3 lobed,7- 3-4 lobed
39	Male flower-no.of stamens	1-1-3,2-6-8
40	Stamen shape	1-depessed,2-elongate
41	Filaments	1-present,2-nearly absent

42	Filament nature	1-massive,2-thin,3-separate,4-partly fused within one flower
43	Anther shape	1-short,2-elongate,3-subglobose,4-globose,5-truncate,6-rounded,7-depressed
44	Connective	1-indistinct,2-massive with one or more projections
45	Pores	1-apical,2-lateral
46	Shape of connective	1-rounded,2-reniform,3-elongate
46	Staminode shape(in sterile zone)	1- sheild –like,2-globose,3-hairlike
47	Staminode shape on the appendix	1- sheild –like,2-globose,3-hairlike,4-conic,5-warts,6-echinae
48	Infructescence nature	1-long peduncled,2-short peduncled
49	Fruiting part shape	1-globose,2-elongate
50	Colour of fruiting part	1-red,2-orange-red
51	Seeds	1-globose,2-subglobose,3-ovate,4-elliptic

Natural seed setting was recorded in most of the wild morphotypes and seed setting was obtained in cultivated type by artificial cross pollination. For pollen viability studies fresh pollen (or anther loculi or germinated grains) was dispersed on a microscope slide. Two to three drop of 1% acetocarmine stain was placed on slide followed by covering with coverslip and pollen viability was recorded after 5–10 min. The dark red colored grains were counted as viable pollens. Pollen Viability (%) = Number of stained pollen grains/ Total number of pollen grains on slide × 100.

The pollen grains were subjected to Scanning Electron Microscope (SEM) analysis at the National Institute for Interdisciplinary Science and Technology (NIIST), Thiruvananthapuram. For SEM studies the fresh pollen grains were stored in 70% ethanol. Since flowering was absent in T10 (cv. Karunaikizhangu), only six accessions were used for constructing dendrogram based on inflorescence/floral descriptors.

The vegetative morphological data as well as the inflorescence/floral descriptors were subjected to clustering using Unweighted Pair Group Method using Arithmetic averages (UPGMA) based on Euclidean distance using MVSP Version 3.1 (Kovach computing Services, Wales, UK) and SPSS 11.0 (SPSS Inc., Chicago, IL, USA) software, and then compared to cytological and ISSR marker data for seven accessions.

For chromosome studies actively growing root tips were pretreated in 0.002 M aqueous 8-hydroxyquinoline saturated with alpha-bromo-naphthalene for 3½ to 4 h at 4°C, fixed in 1:3 acetic alcohol, hydrolyzed in 1 N HCl for 2 min and squash preparations were made with aceto-carmine. Based on the mitotic metaphase

plates (Anil et al 2013) photographed using Image Analyser (Olympus BX51), karyomorphological analysis of chromosomes were made and numerical data such as short arm length, long arm length and total chromosome length (TCL) were measured. Average values of long arm(l),short arm(s) and absolute chromosome length (c) of the chromosome were determined in microns. Homologous chromosomes were categorized on the basis of this data. Arm ratio (r = l/s) and total length of the diploid chromosome complement (TCL) and average chromosome length (ACL) were calculated.

TCL = Cx2, where C = total length of all the chromosomes in the haploid complement.

ACL = TCL/N, where N = total no. of chromosomes in the diploid complement.

Morphological classification of chromosomes was made on the basis of the ratio of the long arms (l) to short arms (s) (Abraham and Prasad, 1983), the frequency of M (Median-l/s = 1), nm(nearly median-l/s = 1.01–1.63), nsm-(nearly sub median-l/s = 1.63–2.99), SM(sub-median-l/s = 3), nsm+ (nearly sub median-l/s = 3.01–4.26), were depicted for the seven accessions, in a bar diagram, which depicted the karyotype formula (Anil et al 2013). Based on the intra-chromosomal asymmetry index (A_1) and inter chromosomal asymmetry index (A_2) described by Zarco (1986), the scatter diagram was constructed. A1 values will be low when chromosomes are metacentric.

$$\mathrm{A1} = \frac{1 - \sum_{i=1}^{n} \frac{\mathrm{bi}}{\mathrm{Bi}}}{\mathrm{n}}$$

where A_1 = intra-chromosomal asymmetry index,

n = no. of homologous pairs or groups,

bi = average length for short arms in every homologous pair or group,

Bi = average length for long arms in every homologous chromosome pair or group.

Interchromosomal asymmetry (A_2) is the ratio between the standard deviation (s) and the mean of chromosome length (x) for each accession.

$$\mathrm{A2} = \mathrm{s}/\overline{\mathrm{x}}$$

17 ISSR primers (University of British Columbia, Canada) (Table 3) that exhibited amplification and polymorphism were used for molecular characterisation. This included 5 anchored (3) and 3 anchored (11) and non-anchored primers (3). The PCR amplification was empirically determined by testing concentrations of genomic DNA and primer. The PCR amplification was performed by using 25 µL reaction mixture using the same protocol a described by Anil et al (2019). The PCR reaction was carried out in a gradient PCR machine (Mastercycler, Eppendorff, Hamburg, Germany) with the following protocol: an initial denaturation at 95°C for 5 min, followed by 45 cycles with a denaturation step at 94°C for 30 s, annealing step at the temperature (Ta) specific to the primer (obtained from initial standardization experiments) for 1 min and an extension step at 72°C for 2 min, followed by a final extension step at 72°C for 7 min. Amplified

products were resolved by electrophoresis on horizontal agarose gel (1.8%). The molecular size of amplicons was determined with reference to the DNA ladders, 100 bp (GE Healthcare, Chicago, IL) and 1 kb ladder (Fermentas). The PCR reactions were repeated at least twice. The binary data (matrix) prepared was used for calculating Jaccard's coefficient of genetic similarity (Sneath and Sokal, 1973) between all possible pairs of accessions based on which a dendrogram (cluster diagram) was constructed by applying UPGMA and principal coordinate analysis (PCoA) using NTSYS-pc (ver. 2.02; Rohlf, 1998).

Table 3
The ISSR primers and their melting and annealing temperatures

Primer:UBC code	Primer sequence 5' -3'	Tm	Ta
888	BDB(CA)7	52.4	50
807	(AG)8T	47.0	49
810	(GA)8T	45.4	48
814	(CT)8TG	47.7	49
821	(GT)8T	49.9	63
823	(TC)8C	48.1	50
826	(AC)8C	52.8	54
834	(AG)8YT	49.2	52
835	(AG)8YC	50.2	52
844	(CT)8RC	48.6	50
845	(CT)8RG	48.1	50
852	(CT)8RA	47.1	52
866	(CTC)6	60.0	55
872	(GATA)4	28.9	42
873	(GACA)4	45.0	55
890	VHV(GT)7	52.2	54
891	HVH(TG)7	50.3	52

Results And Discussion

The accessions of wild variety/wild relative of *A. paeoniifolius* var. *paeoniifolius* showed wide variations in the vegetative morphological characters like petiole surface pattern, corm flesh colour, leaf sheath colour,

corm size, canopy and leaflet characters resulting in the intraclusteral distance in them. Accession T1 was distinct from the other accessions of this group due to the presence of light yellowish green corm flesh colour (Shirly et al. 2011). The cultivated form cultivar 'Gajendra' (GJ) *A. paeoniifolius* var. *campanulatus* have bright green smooth petiole pattern with white blotches and massive habit with large corm with few cormels as against all other dark green combinations and patterns in the other wild types. The dendrogram constructed based on Euclidean distance using 33 qualitative vegetative characters indicated P19, GJ and T10 as placed separately in the dendrogram (Fig. 1).

Six inflorescence morphotypes were identified within *A. paeoniifolius.* Morphotypes were classified based on the differences in the spathe pattern, bract, appendix shape, length of style and filament, length of male zone and female zone and pollen morphology. Inflorescence was solitary in all morphotypes. In all accessions, the inflorescence emerged before leaf development. The peduncle was short. The outer spathe surface pattern in all morphotypes resembled respective petiole surface pattern and varied from shades of brownish purple with purple blotches (K3-2), shades of brownish purple with dark green/black dots (V1), pale purplish with innumerable dark green dots along the entire surface which gives a blackish appearance towards the limb (P19), Shades of greenish purple with white blotches and dark green dots (V4),Bright green in kettle with purplish limb with white blothches (GJ), pale purplish with dark green dots along the entire surface limb (T1). The bract surface pattern was similar to the respective spathe surface pattern in all morphotypes. Inner limb surface pattern of the various morphotypes also varied. In V1, V4 and K3-2 it is pale colour, whereas in P19, T1 it is intermediate reddish purple and in cultivated type (GJ) it is dark reddish purple. The kettle portion is pale green in V1 whereas in GJ it is reddish purple. Clear female and male zones were observed in all the morphotypes. The male and female zones ranged between 2–5 cm in all infloresences depending on the size of the inflorescence which in turn is dependent on the size of the corm (tuber). But the length of male and female zones were equal. In P19 though the female zone was clear and measured 4–5 cm - the male zone was less than 1 cm and mostly fused with the lower part of the appendix (Fig. 2). Flowering did not occur in T10 accession, i.e., karunaikizhangu cultivar in this study and the studies of previous workers (personal communication, Dr. K. C. Velayudhan, Anil et al (2011, 2019). The dendrogram constructed based on inflorescence/floral descriptors excluding the non-flowering T10 (cv.Karunaikizhangu), placed all the six accessions distantly (Fig. 3)

Natural seed setting was observed in all the wild morphotypes by natural cross pollination and by artificial pollination in cultivated type except P19. All the morphotypes showed 90–95% pollen viability except P19 where pollen grains were very few with less than 10% viability. Pollen grains were heteromorphic in P19. The exine ornamentation as observed by SEM revealed clear difference between the pollen grains of the morphotypes (Fig. 4). P19 showed heteromorphic pollen with shriveled macropollen (Fig. 4F). Exine ornamentation also showed considerable difference between the morphotypes.

The chromosome number of all morphotypes of *A. paeoniifolius* is reported as 2n = 28 (Anil et al 2014). The bar diagram drawn based on the frequency of sub-median (SM) chromosomes stated that the morphotypes/accessions P19, K3-2, T10 and GJ can be considered more asymmetrical among the selected accessions (Fig. 5). The complete absence of median chromosomes (M) and presence of nsm+ (nealy sub

median) in GJ (cv.Gajendra) raised it to a highly evolved status (Anil et al 2014), which is again clear in the comparison of A1 and A2 symmetry analysis (Fig. 6)

The mean polymorphism of 83.19% observed with ISSR markers indicates existence of high level of genetic variation within the accessions of *A. paeoniifolius*. The similarity coefficient based on Jaccards ranged from 0.291 (between K3-2 and T10) to 0.692 (betweenV4 and T1) within the *A. paeoniifolius* accessions. The dendrogram constructed using Jaccards distance based on 17 ISSR markers placed T10 and P19 as distinct outliers (Fig. 7). The above comparisons with different markers indicates the uniqueness of T10 (cv.karunaikizhangu) as a separate entity (Anil et al., 2019).

The appearance of different morphotypes with difference in the petiole pattern, spathe surface pattern, pollen morphology, the difference in the length of the female and male zones, the pollen viability and seed setting indicates that *A.paeoniifolius* is still evolving. The morphotypes may be the result of intraspecific crosspollination, phenotypic plasticity or a genetic change.

If the variation for a particular attribute patterns into discrete classes then this indicates that there may be a barrier to gene flow between the morphotypes as observed in *Acacia* sp.(Miller et al., 2002). However, if the variation is continuous it may indicate that hybridisation occurs among the morphotypes. In our studies different patterns exist in the petiole surface pattern, spathe colour, shape of appendix and pollen morphology and mostly continuous. Developmental, genetic or environmental factors (or a combination of these) may be responsible for the observed patterns of character variation within populations.

If the two morphotypes had different genetic signatures it would indicate that they may be of local origin, and that the similar morphology may be due to parallel evolution or separate hybridisation events. Only molecular marker techniques can address such events.

Data derived from observations of chromosomes can identify barriers to reproduction, since plants with different numbers of chromosomes are generally incompatible. In this study, all the morphotypes of A.paeoniifolius are having same chromosome number 2n = 28, hence may be genetically compatible. Hence hybridisation among morphotypes, leading to the creation of new morphotypes, can be considered a major factor in diversification in *A. paeoniifolius* (Davies 1976; Randell 1992; Pedley 2001). The intermediate morphotypes with character like pollen surface pattern in *A. paeoniifolius* suggests that the intermediate form may be a hybrid derived from the local parental plants. However, hybrids do not always express the intermediate character states of the parents, as some contain novel or transgressive morphological characters (McDade 1990; Riesberg and Ellstrand 1993). Sometimes morphotypes may hybridise to create unique progeny but these may be infertile as seen in P19 which is infertile with heteromorphic pollen. The presence of unique bands P19 and T10 using ISSR markers (Anil et al 2019) also indicates the identity of these morphtypes. Both these genotypes are infertile as far as seedsetting is concerened in P19 and complete absence of flowering in T10. In these cases the hybrids may increase the amount of variation observed within a population but, being infertile, do not contribute to future gene pools. Locally derived hybrids appear to dominate some populations (Miller et al 2002). However in *Amorphophallus* the propagation happens through corm and hence the created variability is maintained.

Sporadic hybridisation occurs both within morphotypes and among plants differing in morphological characteristics. These plants are maintained in the populations due to apomictic reproduction. Recurring hybridisation among morphotypes and their subsequent maintenance by apomictic reproduction creates the highly variable populations. (Miller et al 2002). Though apomixes is not observed in *A. paeoniifolius*, it is a common phenomenon in related species *A. bulbifer*. The polyploidy and apomixis act as barriers to gene flow and allow the maintenance of these hybrids.

More surveys should be made in least visited areas. These collections will amplify the knowledge of variation and may uncover diploid members of the complex that could be putative progenitors. DNA markers should be used at the population level to address the issues of hybridisation. These markers also should be used to test the relationships of morphotypes both within and among populations. The different markers/descriptors studied indicates that the accessions P19, T10 (cv. Karunaikizhangu) and cultivated elephant foot yam can be considered as separate botanical varieties or at least convarieties which may be further confirmed using advanced markers or barcoding.

Declarations

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Figures

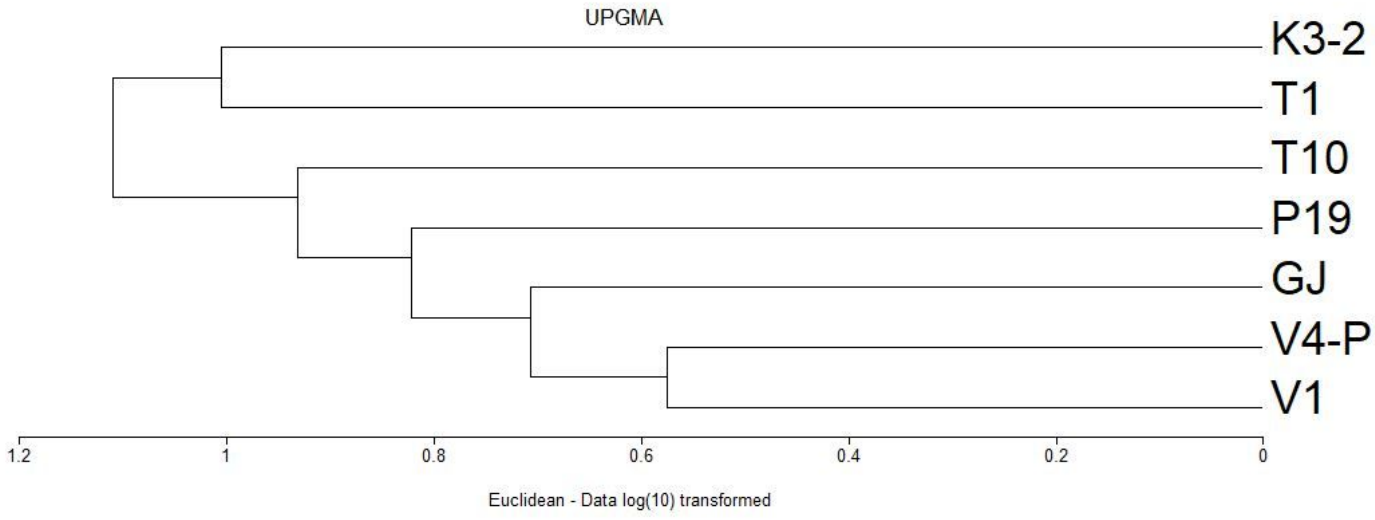

Figure 1

Dendrogram based on qualitative vegetative descriptors

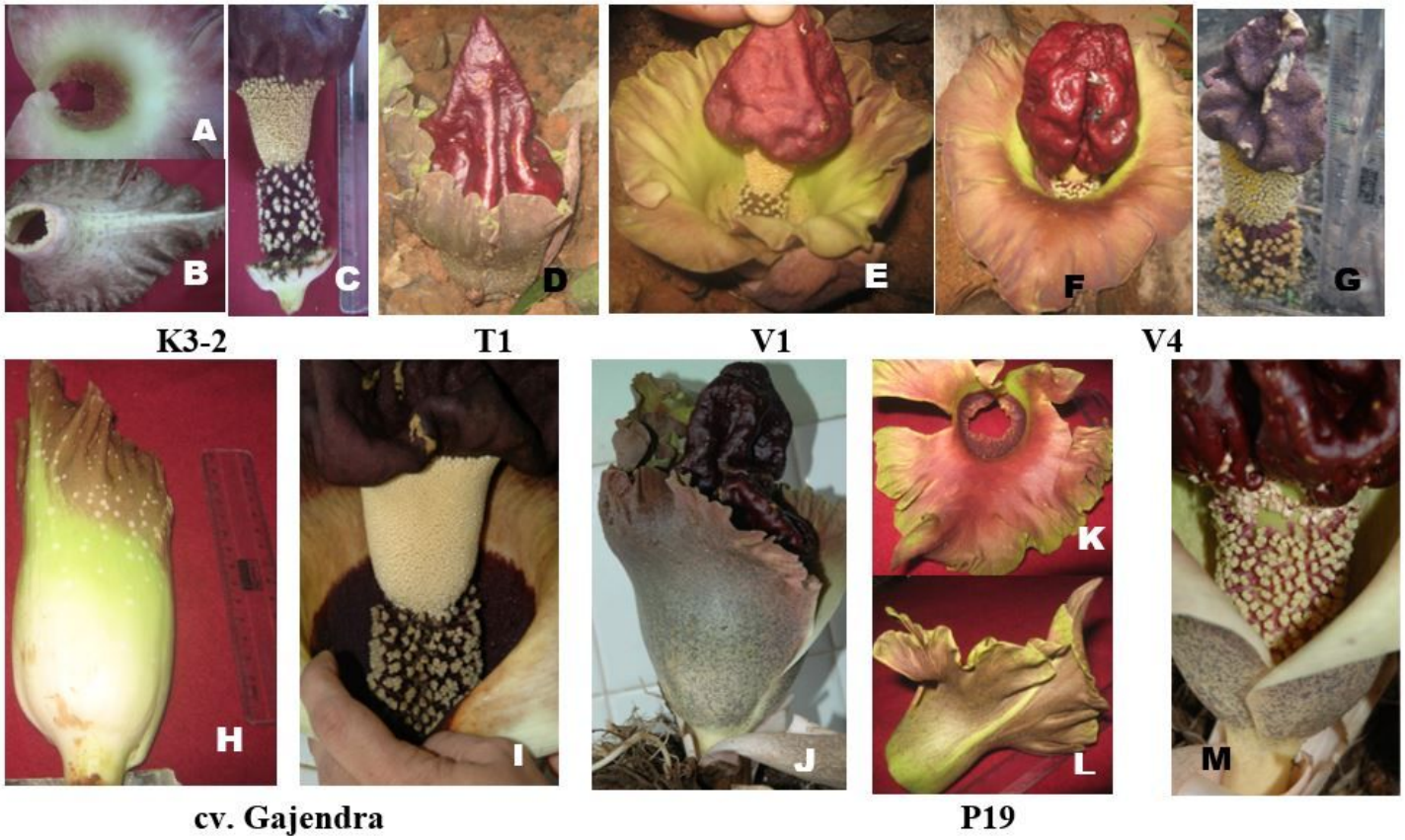

Figure 2

Description of the observed Inflorescence variability **A**-K3-2-spathe inner view; **B**-K3-2 outer view of spathe ,C-K3-2 inflorescence with lower female zone, upper male zone and appendix; **D**- T1-inflorescence with spathe ,note the surface pattern of spathe ;E-V1-Inflorescence with spathe note the inner side of spathe ; **F**-V4- note the appendix shape and spathe ;G-V4 Inflorescence ; **H**-cv,Gajendra- the outer surface pattern of spathe; I- cv,Gajendra -Inner surface pattern of spathe and the female and upper male zones; J- P19-inflorescence with spathe (note the outer surface pattern of spathe and appendix ; K- P19-inner surface pattern of spathe; L-P19-outer surface pattern of spathe; M-P19-note the lower female zone and a very small male zone with very few male flowers mostly attached to the lower part of the appendix.

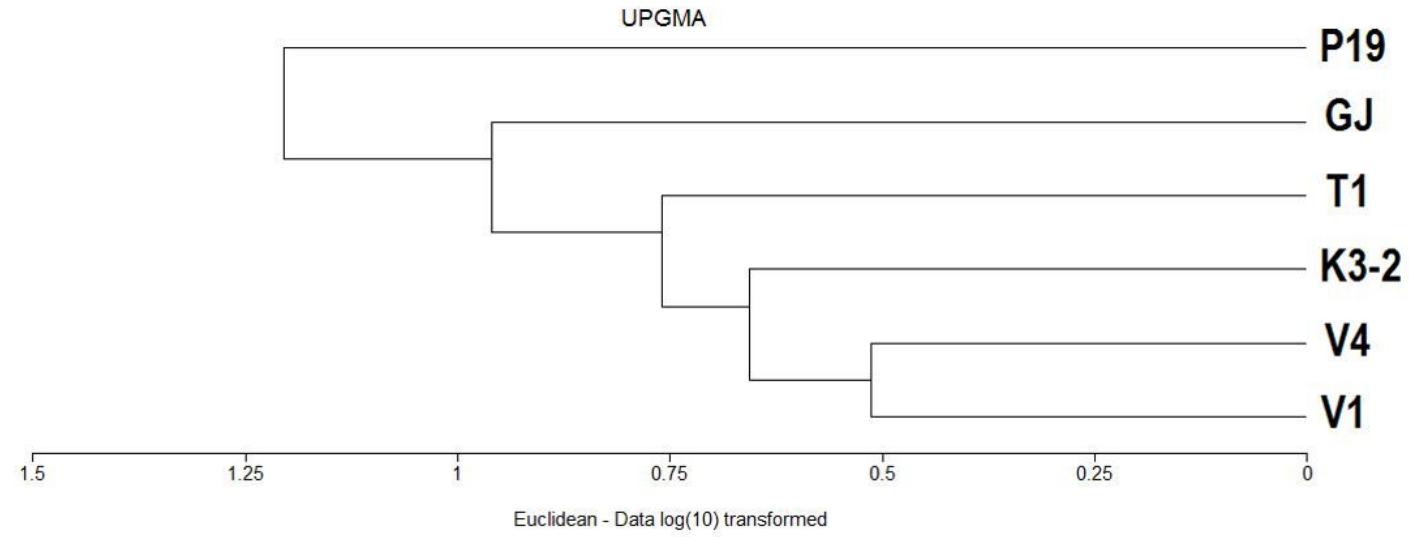

Figure 3

Dendrogram based on inflorescence/floral descriptors (T10 not included as it is non-flowering)

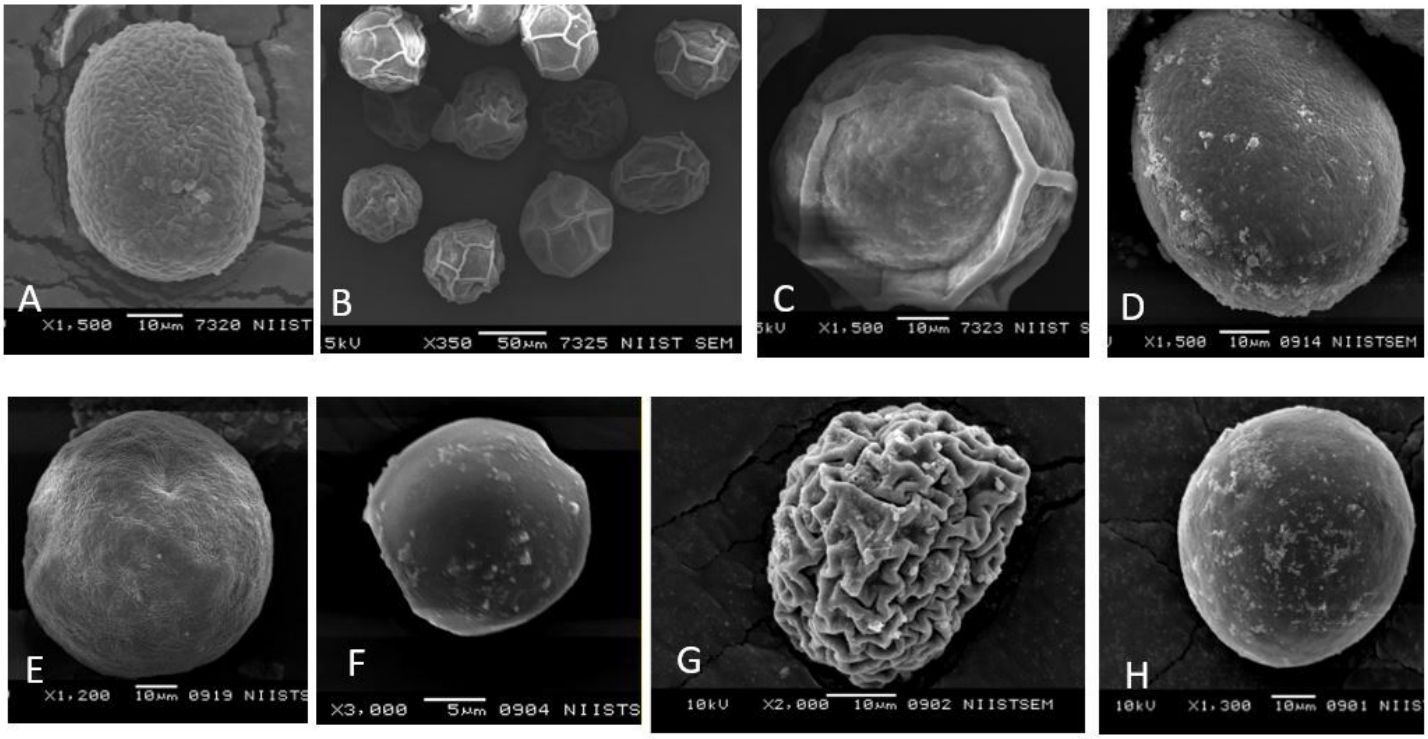

Figure 4

Description of Pollen Grain Surface Pattern of the morphotypes **A**-K3-2-Inaperturate,vermiculite striate(matted reticulate); **B**-T1-pollen grains ; **C**-T1-megareticulate; **D**- V1-striate matted reticulate; **E**- V4-matted reticulate with diagonal bridge like structures with coir-like formations; **F&G**- P19- heteromorphic

pollen (micro pollen and macro pollen) ,matted reticulate with granulate clumps; **H-** cv- surface with granulate clumps on nearly levigate base and granular to verrucate elevations.

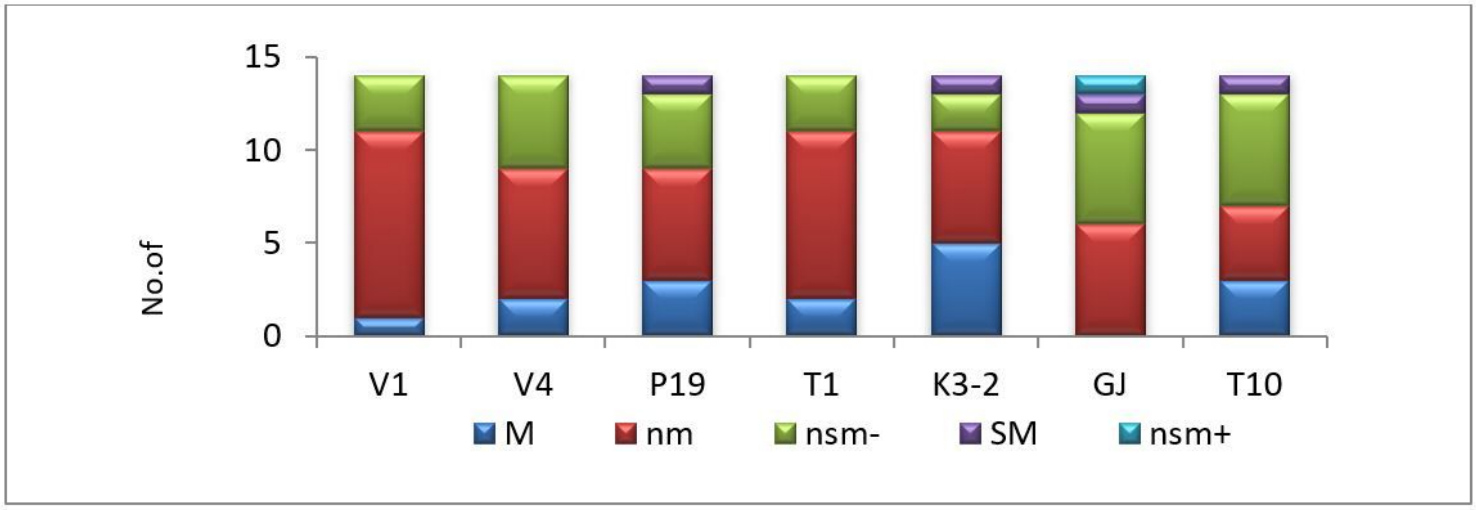

Figure 5

Frequency of different chromosome types in the different morphotypes of

A. paeoniifolius

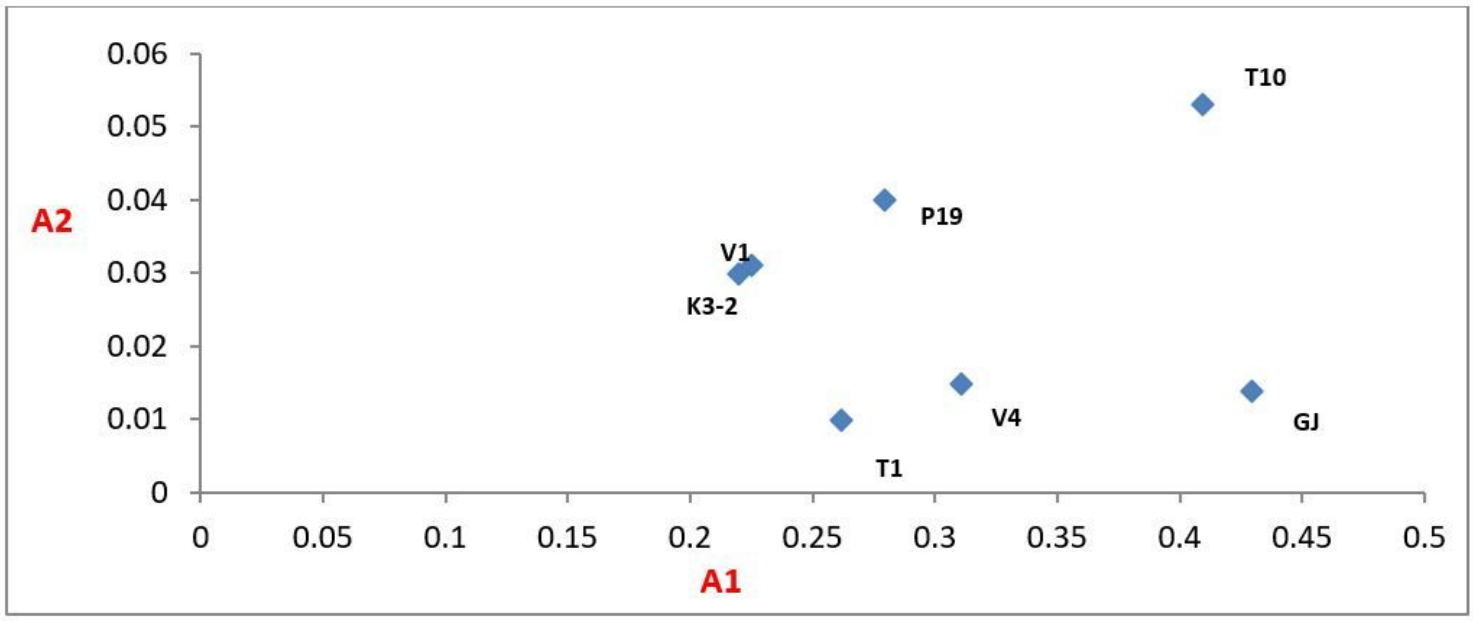

Figure 6

Scatter diagram showing karyotype asymmetry due to ratio between arm length (A_1) against that due to variation between chromosome total length (A_2).

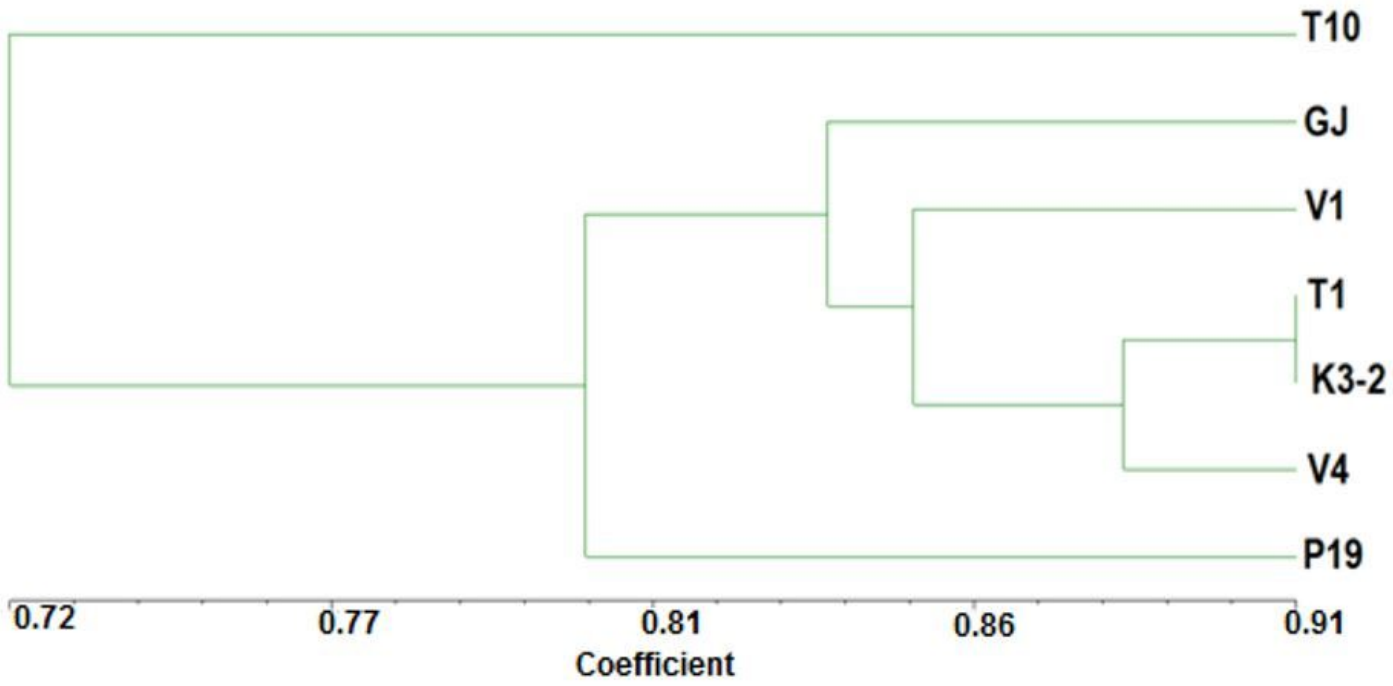

Figure 7

Dendrogram based on 17 ISSR markers