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Karyosystematic Studies in *Amorphophallus* Blume ex Decne.

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Abstract

Cytological studies in 25 accessions revealed the occurrence of three chromosome numbers viz., 2n=28 for all the *Amorphophallus paeoniifolius* accessions, *A. dubius*, *A. smithsonianus* and *A. sylvaticus;* 2n=26 for *A. bonaccordensis*, *A. hohenackeri* and *A. commutatus;* and 2n=3x=39 for *A. bulbifer*. Zarco's asymmetry indices revealed that the accessions T2 (*A. bonaccordensis*) and T3 (*A. smithsonianus*) were the more evolved species in terms of karyotype symmetry. According to the classification of Stebbins, *A. bonaccordensis* included in 3B category was the most asymmetrical and hence considered as most evolved. *Amorphophallus paeoniifolius* var. *campanulatus* with A_1=0.40-0.43 appeared to be more evolved than *A. paeoniifolius* var. *paeoniifolius* based on Zarco's asymmetry indices. Slight differences observed in the A_1 values (0.1714-0.37) in the accessions of *A. paeoniifolius* var. *paeoniifolius* demonstrated the close relationship of the accessions. Taxa with asymmetric karyotype tend to have low total form (TF)% and accordingly cv. Gajendra (GJ) and cv. Karunaikizhangu (T10) (both classified as *A. paeoniifolius* var. *campanulatus*) having low TF% can be considered as highly evolved among the *A. paeoniifolius* accessions. UPGMA clustering based on five karyotypic parameters namely total chromosome length (TCL), average chromosome length (ACL), chromosome number, TF% and ratio of longest chromosome (LC) to shortest chromosome (SC) of the complement revealed two principal clusters at a Euclidean distance of 1.3. The two cultivars of *A. paeoniifolius* var. *campanulatus* (GJ and T10) along with *A. dubius* (A.d) were clustered in a single sub-cluster. Such clustering pattern is in tune with the morphological data which leads to make a valid assumption that *A. dubius* is a possible ancestor of the cultivars GJ and T10.

Key words: *Amorphophallus paeoniifolius*, evolution, wild species, wild relatives, asymmetry index, total form percentage

Introduction

The genus *Amorphophallus* Blume ex Decne. belonging to the family Araceae comprises about 200 species (Sedayu et al., 2010; Jaleel et al., 2011). It is a genus of perennial or annual herbs, generally bearing one broad highly dissected long-petioled leaf with a rhizomatous stem called corm. Several species have edible corms. *Amorphophallus paeoniifolius* (Dennst.) Nicolson var. *campanulatus* (Decne.) Sivad., known as elephant foot yam and *A. bulbifer* Bl. are rich in starch and are used as staple food. *Amorphophallus paeoniifolius* var. *campanulatus* is a cultivated tuber crop and popular as a vegetable in various delicious cuisines and useful as a medicinal corm (Shirly et al., 2011). Wild forms 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 (Raghu et al., 1999). Wild species of *Amorphophallus* also possess many medicinal properties which hitherto have not been explored and remain as indigenous knowledge. A concerted study of the morphology helps in distinguishing the cultivated from its wild relatives and species. Morphological descriptors have been widely and reliably used in the diversity analysis of population and species (Pereira-Lorenzo et al., 1996; Koffi et al., 2009).

Karyotypic features are systematically informative as morphological features (Stebbins, 1971; Kenton et al., 1986; Bernardello and Anderson, 1990). Karyosystematics helps to evaluate the genetic relationships among species or population and to understand the way they diverged from each other

(Guerra, 2008) as well as for supporting taxonomic studies in a genus (Pedro and Salinas, 2009).

The best known cytotaxonomic datum for almost all families and most plant genera is the chromosome number, which is the quickest, cheapest and easiest way to get any substantial information about the genome of a species. Unlike other karyotypic features, it is not influenced by external conditions, developmental phases, age etc. A karyotype describes the phenotypic aspects of the chromosome complement of a species in terms of number, size, arm ratio (or centromere position) and other landmark features of its chromosomes (Levin, 2002). Karyotypes may differ even between closely related taxa. Karyotype diversity has been a crux of plant evolution studies for several decades (Levin, 2002).

The asymmetry assessment is the most important method for karyotype analysis. Zarco (1986) opined that changes in morphological characters of the genome have been frequently related to the process of evolution in higher plants. Huziwara (1962) analyzed the karyotypes in the genus *Aster* by using the total form percent (TF%) which expressed the proportion of total length of short arms in the complement. Zarco (1986) proposed the intra-chromosomal asymmetry index (A_1) and the inter-chromosomal asymmetry index (A_2) to estimate karyotype asymmetry.

Cytological studies of *Amorphophallus* began with the work of Chandler (1943) who found out 2n=26 and 2n=36 as chromosome numbers of the species *A. titanum* and *A. bulbifer,* respectively. Marchant (1971; 1973) considered x=13 and x=14 as basic chromosome numbers of the genus based on his observations in 11 species of *Amorphophallus.* However, Chauhan and Brandham (1984) observed 2n=26, 28 and 39 in 17 species of *Amorphophallus* in addition to the previous reports. Studies on the significance of karyotypic variation in the evolution of *Amorphophallus* was carried out at the species level (Krishnan et al., 1970; Ramachandran, 1977; Chauhan and Brandham, 1984), but investigations at the intraspecific level are very meagre, except the work of Sreekumari (1992), where cultivars were studied. Shirly et al. (2011) observed the existence of morphological variation within the species. In the present report, intraspecific karyotype variations in 18 accessions of *Amorphophallus paeoniifolius* (wild and cultivated) along with the seven wild species are discussed to elucidate their phylogenetic relationships.

Materials and Methods

Plant material

The endemic species of *Amorphophallus* distributed in South Western Ghats (Kerala and Karnataka States) were collected. 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 included in the present study. Three accessions obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, were also included. All the accessions were maintained in the Botanic garden, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India, as pot cultures. The study material in total thus comprised 25 accessions (Table 1) (with 10 plants per accession), which included seven wild species and 18 accessions of *A. paeoniifolius*, which consisted of the morphotypes (wild relatives), cultivated elephant foot yam cv. Gajendra and local cultivar 'Karunaikizhangu' from Tamil Nadu.

Mitotic studies

For chromosome studies actively growing root tips were pre-treated in 0.002M aqueous 8-hydroxyquinoline saturated with alpha-bromo-naphthalene for 3½ to 4 hrs at 4°C, fixed in 1:3 acetic alcohol, hydrolyzed in 1N HCl for 2 min and squash preparations were made with aceto-carmine. Suitable mitotic metaphase plates were photographed using Image Analyser (Olympus BX51). Karyotype analysis was made from enlarged prints of somatic metaphase at a magnification of x 2000. At least fifty slides were prepared for each accession.

Karyomorphological studies

Karyomorphological analysis of chromosomes using numerical data such as short arm length, long arm length and total chromosome length (TCL) was carried out. 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.

Table 1.The 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 (m)
1.	V2	Vittal, DK	*A. hohenackeri*	Wild	12º.47'N/75º06'E	93.30
2.	V3	Vittal, DK	*A. bulbifer*	Wild	12º.47'N/75º06'E	93.30
3.	T2	Vithura, TVM, KL	*A. bonaccordensis*	Wild	8º678'N/77º0972'E	114.33
4.	T3	Vithura, TVM, KL	*A. smithsonianus*	Wild	8º678'N/77º0972'E	101.33
5.	A. c (TCR-135)	NBPGR	*A. commutatus*	Wild	-	0
6.	A.syl (TCR-118)	NBPGR	*A. sylvaticus*	Wild	-	0
7.	A. d (TCR-105)	NBPGR	*A. dubius*	Wild	-	0
8.	V1	Vittal, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º.47'N/75º06'E	93.33
9.	V4-P	Vittal, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º.47'N/75º06'E	93.33
10.	T1	Vithura, TVM, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	8º678'N/77º0972'E	114.3
11.	M1	Moozhiyar, PTA, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	9º27N/77ºE	75.33
12.	P1	Puttur, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º44'N/75º13'E	90.00
13.	K3-1	Thrissur, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	10º31N/76º20'E	52.33
14.	K3-2	Thrissur, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	10º31N/76º20'E	52.33
15.	K3-3	Thrissur, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	10º31N/76º20'E	52.33
16.	P17	Puttur, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º44'N/75º13'E	90.00
17.	T10	Nagercoil, TN	*A. paeoniifolius* var. *campanulatus*	Cultivated	8º30'N/77º30'E	100.00
18.	G3	Navasari, GUJ	*A. paeoniifolius* var. *paeoniifolius*	Wild	20º57N/72º9E	15.66
19.	GJ (Gajendra)	Kovvur, AP	*A. paeoniifolius* var. *campanulatus* (National variety)	Cultivated	17º01'N/81º43'E	12.00
20.	B1	Vaishali, BIH	*A. paeoniifolius* var. *paeoniifolius*	Wild	24º826'N/84º9902'E	69.00
21.	P5	Puttur, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º44'N/75º13'E	90.00
22.	K4	Thrissur, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	10º30N/76º20'E	52.33
23.	P18	Puttur, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º44'N/75º13'E	90.00
24.	P19	Puttur, DK	*A. paeoniifolius* var. *paeoniifolius*	Wild	12º44'N/75º13'E	90.00
25.	K5	Thrissur, KL	*A. paeoniifolius* var. *paeoniifolius*	Wild	10º30'N/76º20'E	52.33

*DK-Dakshina Kannada, Karnataka; PTA-Pathanamthitta; KL-Kerala; GUJ-Gujarat; AP-Andhra Pradesh; BIH-Bihar

TCL=C x 2,

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

ACL=TCL/N,

where N=total number of chromosomes in the diploid complement.

Determination of karyotype asymmetry

The karyotype asymmetry was determined by standard procedures (Stebbins, 1971; Zarco, 1986), and by considering the total form percentage (Huziwara, 1962).

The total form percentage (TF%) is the ratio of percentage of the total sum of short arm length to the total sum of chromosome length of a haploid complement.

$$TF\% = \frac{\text{Total sum of short arm length x100}}{\text{Total sum of chromosome length}}$$

Classification of chromosomes and karyotype formula

Morphological classification of chromosomes (Table 2) was made on the basis of the ratio of the long arms (l) to short arms (s) (Abraham and Prasad, 1983), which depicted the karyotype formula. Idiograms were constructed for all the species, whose karyomorphology were analyzed.

ANOVA, principal component analysis and UPGMA cluster analysis

In order to determine variations between accessions, one-way ANOVA was performed by considering five parameters like total chromosome length (TCL), average chromosome length (ACL), chromosome number, TF% and ratio of longest chromosome (LC) to shortest chromosome (SC) of the complement and means were differentiated by Duncan's Multiple Range Test.

The principal component analysis was performed to evaluate the contribution of each of the above karyotypic parameters to the ordination of species. Clustering was performed using Unweighted Pair Group Method using Arithmetic averages (UPGMA) based on Euclidean distance after calculation of cophenetic correlation coefficient (r) to examine karyotype similarity among accessions, using MVSP Version 3.1 (Kovach computing Services, Wales, UK) and SPSS 11.0 (SPSS Inc., Chicago, IL, USA) software.

Results and Discussion

Karyomorphological studies

Karyomorphological details and the somatic chromosome numbers of the accessions of *A. paeoniifolius* and the wild species of *Amorphophallus* are detailed in Table 2. The somatic chromosome number recorded was 2n=28 in wild species like *A. smithsonianus, A. sylvaticus* and *A. dubius*, whereas *in A. hohenackeri*, *A. bonaccordensis* and *A. commutatus* the somatic chromosome number was 2n=26 and in *A. bulbifer* the chromosome number was 2n=3x=39 (Fig.1). All the accessions of *A. paeoniifolius* showed 28 chromosomes at metaphase stage (Figs. 2 and 3). The karyotype formula, ACL, TCL, the chromosome length range, karyotype category, the ratio of longest chromosome to shortest chromosome (LC/SC) and TF% of all the accessions were determined (Table 2).

The TCL ranged from 90.73 μm (K3-2) to 303.64 μm (V3). Among the accessions of *A. paeoniifolius,* which formed the major group, the TCL varied from 90.73 μm (K3-2) to 175.83 μm (P5). The ACL varied from 3.77 μm (K3-2) to 10.48 μm (A. sy). Among the accessions of *A. paeoniifolius,* ACL varied from 3.77μm (K3-2) to 6.28 μm (P5). The LC/SC varied from 1.73 (T1) to 3.22 (A. sy). The ratio varied between 1.73 (T1) to 2.92 (T10) among the different accessions of *A. paeoniifolius.*

TCL and TF%

The range of TF% was from 33.73% (T2) to 43.6% (K3-1) (Table 2). Variations in the total chromosome length (TCL) in *A. paeoniifolius* accessions ranging from 90.73 μm (K3-2) to 175.83 μm (P5) suggested that changes in genome size was non-random and the variation in the amount of DNA was equally distributed among all the chromosomes of the complements.

The increase in TF% implies that DNA was added on the short arm of the chromosome whereas decrease in TF% indicated a reverse pathway (Gao et al., 2012). Variation in the TF% in the entire germplasm from 33.73 (T2) to 43.6 (K3-1) observed in the present study may be due to the increase or decrease in the amount of DNA content in the chromosomal arms. This distribution difference of nucleic acid results in the variation in arm ratio and symmetry leading to the evolution of karyotype. Among the different *A.paeoniifolius* accessions, TF%

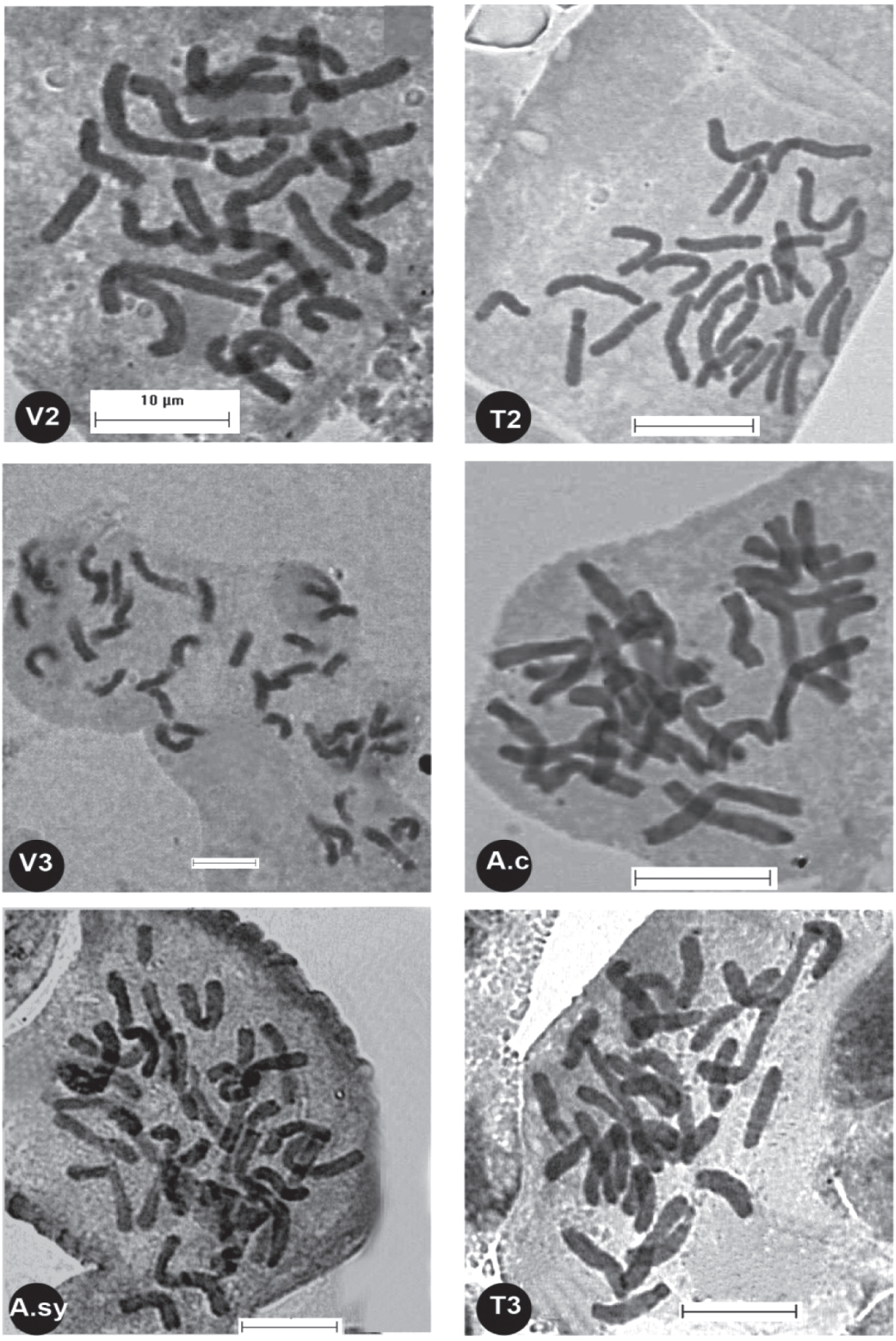

Fig.1. Mitosis in root tip cells of different species of *Amorphophallus*. **V2**. *A. hohenackeri (2n=26);* **T2**. *A. bonaccordensis (2n=26);* **V3.** *A. bulbifer (2n=3x=39);* **A.c**. *A. commutatus (2n=26);* **A.sy.** *A. sylvaticus (2n=28);* **T3**. *A. smithsonianus (2n=28)* (scale=10 μm)

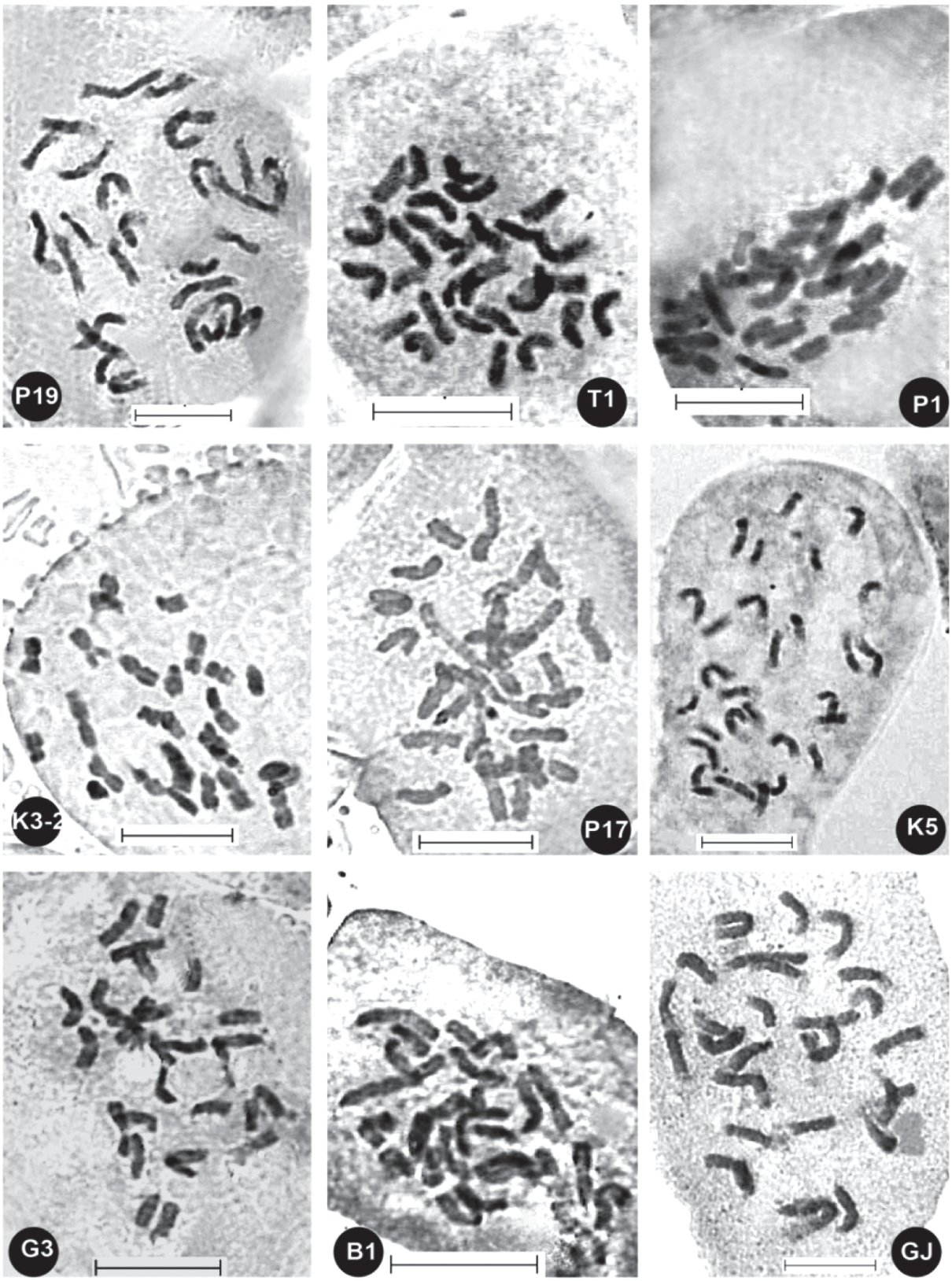

Fig.2. Mitosis in root tip cells of different species of *Amorphophallus*. **P19**. **T1, P1**, **K3-2, P17, K5, G3** and **B1** *A. paeoniifolius var. paeoniifolius* accessions; **GJ**. *A. paeoniifolius* var. campanulatus cv. Gajendra; somatic chromosome number in all the accessions 2n=28 (scale=10 μm)

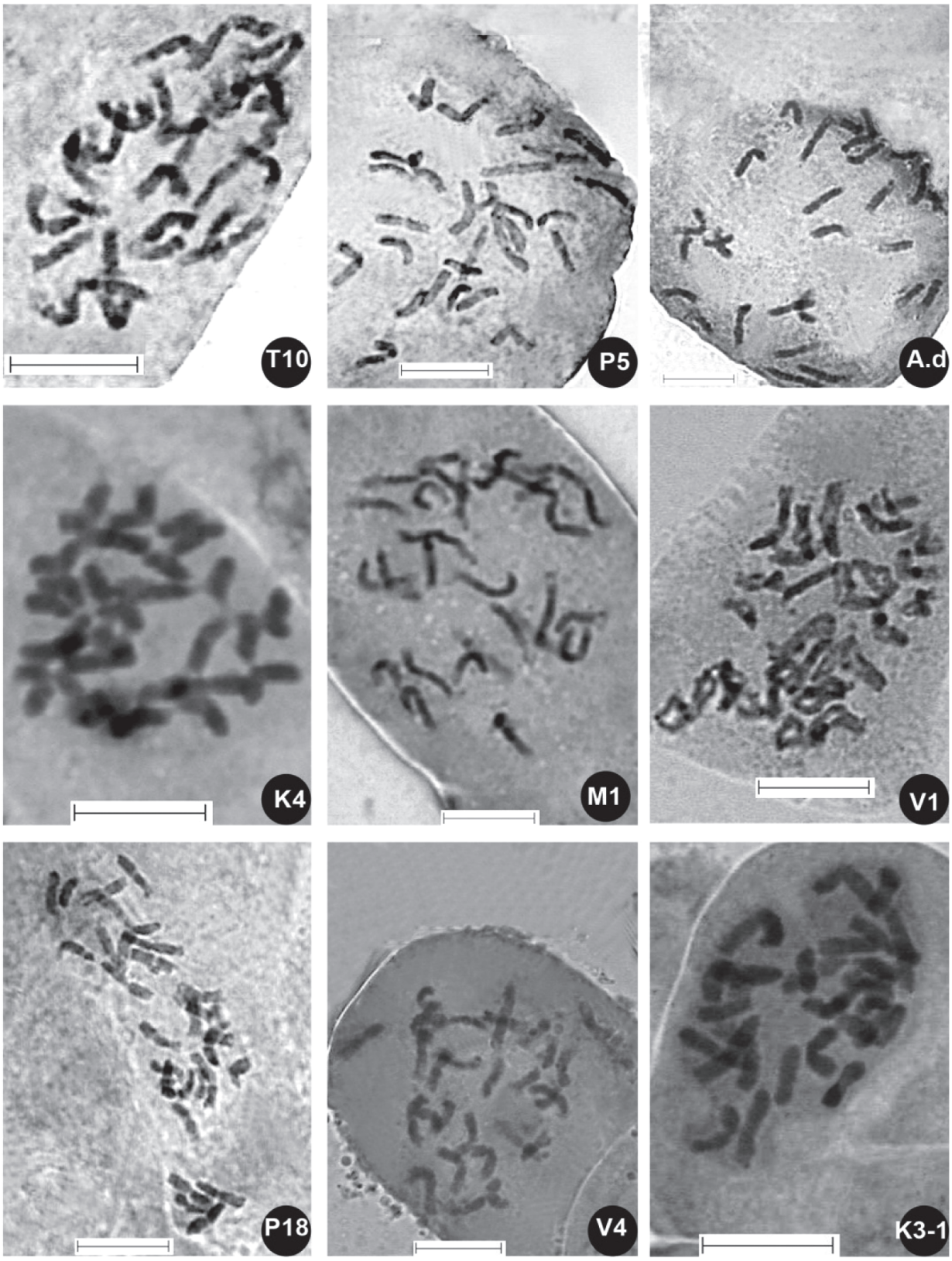

Fig.3. Mitosis in root tip cells of different accessions of *A. paeoniifolius var. paeoniifolius* **P5**. **K4, M1**, **V1, P18, V4, K3-1; T10.** *A.paeoniifolius var. campanulatus cv. Karunaikizhangu;* **A.d.** *A. dubius;* somatic chromosome number in all the accessions 2n=28 (scale=10 μm)

varied between 35.34% (GJ) to 43.6% (K3-1), with TF% of 37.64% in T10. Huziwara (1962) opined that low TF% is associated with asymmetric karyotypes. Accordingly cv. Gajendra (GJ) and T10 (both *A. paeoniifolius* var. *campanulatus*) having low TF% (Table 2) can be considered as highly evolved among the *A. paeoniifolius* accessions.

Karyotype asymmetry and evolution

The *nm* chromosomes were the most commonly occurring type (49%) in all the accessions studied. Percentage of *nsm*-chromosomes, *M* chromosomes, *SM* chromosomes, *nsm+* chromosomes were 29.5%,14%, 2.3% and 4% respectively. The *nst*- chromosomes were rare (1%). Karyotypes of most of the accessions of *A. paeoniifolius* var. *paeoniifolius* were the most symmetrical. But karyotypes of *A. bonaccordensis and A. commutatus* with 3 and 1 *nst*-chromosomes respectively and *A.smithsonianus* with 6*nsm+* chromosomes were comparatively asymmetrical.

The A_1 values among all the accessions varied from 0.1714 (K4) to 0.501 (T3). The range of A_1 values among *A. paeoniifolius* accessions was 0.1714 (K4) - 0.368 (M1). A_2 values varied from 0.0 (P17) to 0.053 (T10). Karyomorphological data of *Amorphophallus* based on symmetry type of Stebbins (1971) is shown in Table 2 and asymmetry indices of Zarco (1986) are represented in Fig.4. Zarco's asymmetry indices of A_1 and A_2 will help to determine the most asymmetrical type of karyotype among population. Intra-chromosomal asymmetry index (A_1) expresses the arm ratio of each pair of homologous chromosomes. The inter-chromosomal asymmetry index (A_2) corresponding to Pearson's coefficient of dispersion gave an idea of asymmetry caused by the different lengths of the chromosomes. The high A_1 values indicate the asymmetry of the karyotype. Higher values of A_1 observed in the species of *Amorphophallus* other than *A. paeoniifolius* var. *paeoniifolius* in the present investigation suggest the asymmetry of the karyotype indicating the advanced nature of those species. The high A_1 values observed in the accessions of cultivated species, *A. paeoniifolius* var. *campanulatus* represented by cv. Gajendra (GJ) (0.43) and 'Karunaikizhangu' (T10) (0.409) suggest their advanced status in the evolutionary process. Slight differences observed in the A_1 values (0.171-0.37) in the accessions of *A. paeoniifolius* var. *paeoniifolius* (Fig.4) demonstrate the close relationship of the accessions in spite of the morphological variability observed. Zarco's asymmetry indices revealed that the accessions T2 (*A. bonaccordensis*) and T3 (*A. smithsonianus*) are the most evolved species in terms of karyotype symmetry.

Overall, karyotypes were quite symmetrical and most of

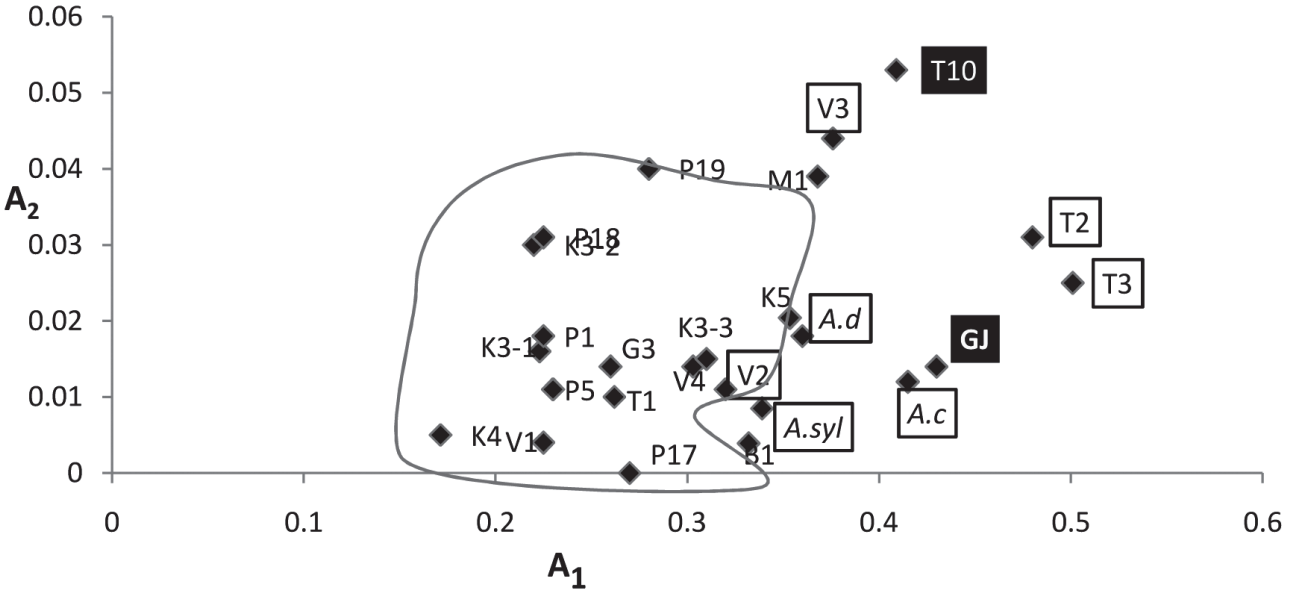

Fig. 4. Scatter diagram showing karyotype asymmetry A_1 (asymmetry due to ratio between arm length) against A_2(assymmetry due to variation between chromosome total length). The unfilled blocks indicate species other than *A. paeoniifolius;* the black blocks indicate *A. paeoniifolius* var. *campanulatus* and the remaining all represent accessions of *A. paeoniifolius* var. *paeoniifolius*

Table 2. Karyomorphological data scored in different accessions of *Amorphophallus*

Name of taxa/accession	Accession code	Chromosome no.	TCL (μm)	ACL (μm)	Chr. length (Range)(μm)	Karyotype details	Karyotype category (Stebbins)	LC/SC	TF%
A. paeoniifolius var.	V1	28	153.25	5.47	3.75-8.50	1M+10nm+3nsm-	2B	2.26	43.40
paeoniifolius	V4-P	28	160.00	5.70	3.89-7.78	2M+7nm+5nsm-	2B	2.00	40.72
	P1	28	121.00	4.29	3.18-5.90	2M+10nm+2nsm-	2A	1.86	43.60
	P5	28	175.83	6.28	4.44-8.89	1M+10nm+3nsm-	2A	1.77	43.12
	K4	28	125.71	4.48	3.09-6.40	5M+7nm+1nsm-+1nsm+	2B	2.07	45.26
	P17	28	133.06	4.75	3.06-6.70	2M+7nm+4nsm-+1nsm+	2B	2.20	41.87
	P18	28	148.30	5.29	3.22-7.09	4M+8nm+2nsm-	2B	2.20	43.26
	P19	28	168.72	6.02	4.35-8.97	3M+6nm+4nsm-+1SM	2B	2.06	41.49
	T1	28	133.65	4.77	3.65-6.34	2M+9nm+3nsm-	2A	1.73	42.15
	K3-1	28	120.20	4.29	3.18-5.90	2M+10nm+2nsm-	2A	1.86	43.60
	K3-2	28	90.73	3.77	1.95-4.87	5M+6nm+2nsm-+1SM	2B	2.50	43.45
	K3-3	28	161.67	5.77	3.89-7.78	2M+7nm+5nsm-	2B	2.00	40.72
	K5	28	121.56	4.34	3.12-5.62	6nm+7nsm-+1SM	2A	1.80	39.07
	G3	28	99.350	3.55	2.39-4.56	3M+7nm+4nsm-	1A	1.91	42.23
	M1	28	117.56	4.21	2.68-6.34	3M+4nm+6nsm-+1SM	2B	2.36	38.17
	B1	28	108.80	3.88	2.80-6.00	1M+8nm+3nsm-+1SM+1nsm^{+}	2B	2.14	39.34
A. paeoniifolius var.	GJ	28	166.05	5.93	4.21-8.94	6nm+6nsm-+1SM+1nsm^{+}	2B	2.13	35.34
campanulatus	T10	28	154.70	5.41	3.52-10.29	3M+4nm+6nsm-+1SM	2B	2.92	37.64
A. dubius	A. d	28	164.69	5.88	3.12-7.80	2M+6nm+6nsm-	2B	2.50	39.09
A. hohenackeri	V2	26	239.50	8.70	6.20-12.96	2M+6nm+3nsm^{-}+2nsm^{+}	2B	2.09	40.45
A. bulbifer	V3	39	303.64	7.76	5.00-11.25	6nm+5nsm-+1SM+1nsm+	2B	2.25	36.36
A. bonaccordensis	T2	26	234.34	8.70	5.81-13.64	5nm+3nsm^{-}+2nsm^{+} +3nst^{-}	3B	2.35	33.73
A. smithsonianus	T3	28	254.24	9.08	5.75-14.84	6nm+2nsm-+6nsm+	2B	2.58	33.84
A. commutatus	A. c	26	251.74	9.68	7.39-13.9	7nm+5nsm-+1nst-	2A	1.88	37.65
A. sylvaticus	A. syl	28	293.50	10.48	4.50-14.50	2M+2nm+10nsm-	2B	3.22	39.01

the species were placed into 2B category based on Stebbins'(1971) system of classification (Table 2). However, a wide range of asymmetry indices i.e., A_1 = 0.17–0.501 and A_2 = 0.0–0.06 was observed following Zarco's (1986) system. Stebbins classification of karyotypic asymmetry also implies the asymmetrical nature of *A. bonaccordensis* since it belongs to 3B category, which suggests the highly evolved nature of the species. *A. paeoniifolius* var. *campanulatus* (A_1=0.40-0.43) appeared to be more evolved than *A. paeoniifolius* var. *paeoniifolius* based on Zarco's asymmetry indices.

Analysis of variance of karyotypic features

A statistical comparison using five karyotypic parameters TCL, ACL, TF%, LC/SC and chromosome number demonstrates significant differences among the accessions for all the traits (P>0.01) as shown by ANOVA test. This indicates that significant quantitative change has occurred in the amount of chromatin in species diversification process of *Amorphophallus.*

Variation in chromosome structure is possible either by the shift of centromere positions or by the addition of supernumerary DNA on the long arms of chromosomes (Hong, 1990; Peruzzi et al., 2009). In relation to the genome size variation, the ratio between the length of the longest and the shortest complements varied from 1.7 (T1) to 3.22 (A.sy) (Table 2). These differences among complement length of diploid species are in accordance with that of *Lathyrus* (Rees and Hazarika, 1969; Narayan, 1983; Narayan and Durrant, 1983). Variation in genome size may cause striking changes that have occurred during the divergence and evolution of the chromosome complements.

Principal component analysis

Principal component analysis (PCA) based on the five parameters, TCL, ACL, TF%, LC/SC and chromosome number showed that the first two principal components accounted for 73% of the total variance.The first principal component (53.47%) with the highest value for eigen coefficient, emphasized on TCL, ACL, TF% and LC/SC whereas the second principal component (20.29%) emphasized on chromosome number.

Clustering of accessions

UPGMA clustering based on the above parameters (Fig.5) revealed two principal clusters at a Euclidean distance of 1.3. The correlation coefficient (r) between the original distance matrix and the cophenetic matrix of the UPGMA method was 0.9332, which indicated a good data fit. The first principal cluster consisted of two small clusters with *A. bulbifer* (V3) as a single sub cluster and all the other species viz., *A. bonaccordensis* (T2), *A. smithsonianus* (T3), *A. hohenackeri* (V2), *A. commutatus* (A.c) and *A. sylvaticus* (A.sy) grouped together in the second sub cluster. All the accessions in this principal cluster were characterized by large chromosomes and high TCL.

The second principal cluster with *A.dubius* (A.d) and all the accessions of *A.paeoniifolius* constituted two sub clusters. The two varieties of the cultivated species of *A. paeoniifolius* var. *campanulatus* (GJ and T10) clustered with *A.dubius* (A.d) and six *A.paeoniifolius* accessions in a single subcluster (Fig.5). It suggests a common ancestry of the two species. Nicolson (1987) reported the grouping of *A. paeoniifolius* var. *paeoniifolius*, *A. paeoniifolius* var. *campanulatus* and *A. dubius* based on long styled flowers of the species. The intraclusteral differences observed between the different accessions of *A. paeoniifolius* is indicative of the minor differences in karyotype which might have occurred during the course of evolution.

The subgroup of the second subcluster comprising of GJ *(A. paeoniifolius* var. *campanulatus)* positioned as separate entity along with *A. paeoniifolius* var. *paeoniifolius* accessions indicates the evolution of *A. paeoniifolius* var.*campanulatus* from *A. paeoniifolius* var. *paeoniifolius* by selection process. The close relationship of the accessions T10 and A. din the second subcluster and separately from GJ suggests that the cultivated accession T10 (cv. Karunaikizhangu) may be derived from the accession *A. dubius.* Thus T10 and GJ follow separate evolutionary pathways leading to their cultivation. On the basis of morphological observations it was suggested that *A. dubius* is a possible ancestor of cultivated accession T10 of *A. paeoniifolius* var. *campanulatus* (Shirly et al., 2011). Isozyme studies also showed a separate evolutionary pathway for T10 and GJ (Shirly et al., 2013).

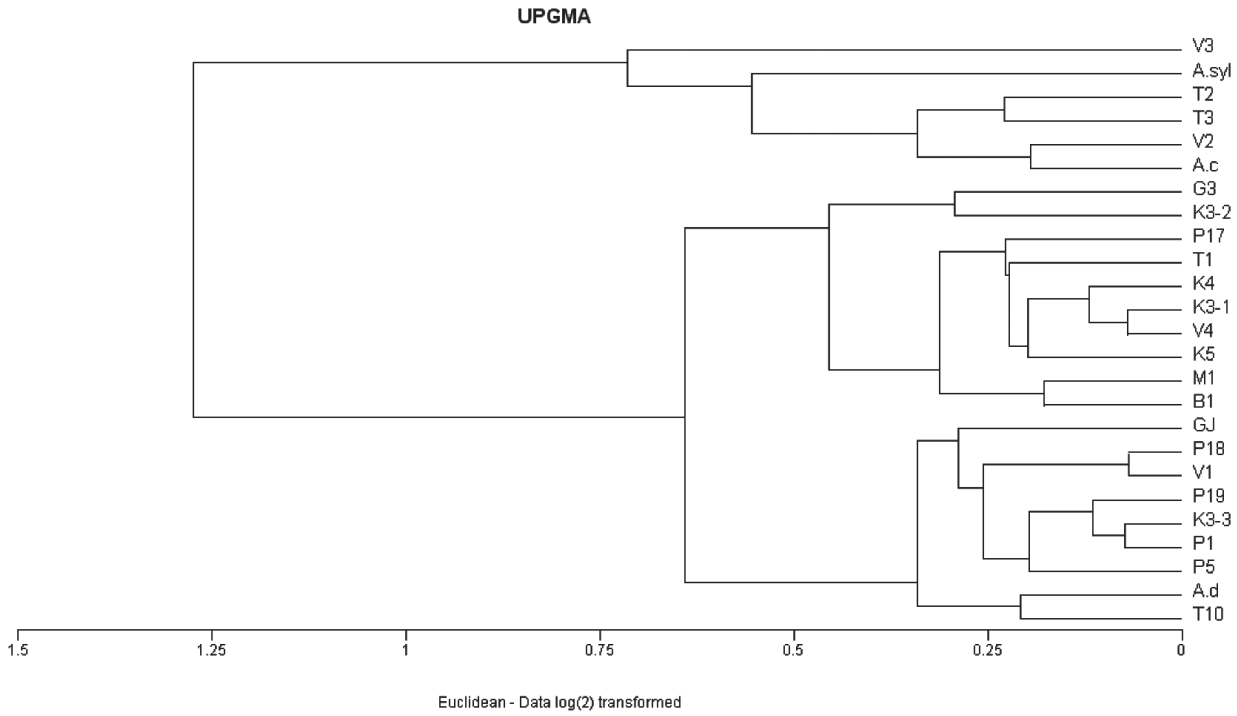

Fig. 5. Dendrogram of 25 accessions of *Amorphophallus* spp. based on five karyotypic parameters using UPGMA cluster analysis

Slight variations in karyotype formulae and asymmetry indices found among various accessions suggest that structural changes may have contributed to the diversification of the genus. On the other hand, grouping of accessions that share major karyotype characteristics may indicate that the mechanisms of speciation within each group involved chromosome rearrangements which were not large structural mutations, but small or cryptic changes. Alternatively, if speciation has occurred as a consequence of large chromosome modifications such as paracentric inversions or reciprocal translocations, these may not reflect in the karyotype (Seijo and Fernandez, 2003). Interestingly it is added that *A. bonaccordensis* (T2) resembles *A. hohenackeri* (V2) in most of its morphological characteristics with slight difference in habitat, fruit set, corm flesh colour and increased endemism.

The difference in karyotype evidenced by the presence of secondary constrictions, satellites and the nearly subtelocentric chromosomes in *A.bonaccordensis* (Fig. 1) suggest that it may be more evolved. Absence of secondary constrictions and satellites in other accessions may be due to the technical imprecision. Secondary constrictions and intercalary satellites were associated with the evolution pattern as reported in Liliaceae (Peruzzi et al., 2009). However, these structures did not cause any change in the karyotype asymmetry. Subtelocentric/telocentric chromosomes were recorded in *A. commutatus* (1nst-) and *A. bonaccordensis* (3nst-). But these chromosomes did not alter overall symmetry of the karyotypes as they are present in very few numbers.

Chromosome variation definitely played an important role in the evolution of *Amorphophallus* which might have resulted in changes in the chromosome fine structure (Gao et al., 2012) which may be evident from molecular level analysis. Advanced technologies like chromosome banding or fluorescent *in situ* hybridization (FISH) along with DNA markers can help in understanding karyotype evolution and speciation in *Amorphophallus.*

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