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Morphological Variability of Aerial Vegetative Characters among 20 Shatavari (*Asparagus racemosus* Willd.) Collections from Kerala, India

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Abstract

Asparagus racemosus Willd. (Shatavari) is an important medicinal plant which is well known for its galactagogue property due to the presence of phytoestrogen in tuber. Observations on 18 aerial vegetative quantitative and 7 qualitative traits were scored at full foliage stage of all the 20 accessions. Analysis of variance revealed significant ($p < 0.05$) morphological variations in all the quantitative vegetative parameters recorded.The highest coefficient of variations were accounted for length of cladode clusters, plant height, length of fresh spear and number of cladodes per whorl that signify the high degree of variability with regard to these traits. The highest genotypic and phenotypic coefficients of variation were also exhibited by the same characters with above 95% heritability. All the accessions studied for qualitative characters showed similarity only in cladode shape, cladode arrangement and stem shape. Principal Component Analysis showed 99.76% of total variance on the first four Principal Components. Significant characters accounted for variability were stem color, length of cladode clusters, plant height, stem diameter and number of cladodes per whorl respectively. The Unweighted Pair Group Method with Arithmetic Average dendrogram with 25 variables divided the accessions into two principal clusters between Euclidean distances of 0.3-1.6. The results of Principal Component Analysis and Principal Co-ordinate Analysis are concurring to the cluster analysis. High degree of phenotypic and genotypic variations indicating that genotypic component of variation was the major contributor to the total variation and the environment has little effect on the observed phenotypic variations of the traits.

Key words: *Asparagus racemosus,* genetic parameters, heritability, morphological variations, multivariate analysis, Principal Component Analysis, UPGMA.

Introduction

The genus *Asparagus* (family Asparagaceae) consists of about 300 species and is widely distributed in the world with many species in the Mediterranean region, Africa and part of Asia (Dahlgren et al., 1985). *Asparagus racemosus* Willd. is an important medicinal plant and also a minor tuber crop of tropics which has been used in herbal formulations for various ailments since ancient periods. In ayurveda, the Indian system of medicine, *A. racemosus* is considered as "queen of herbs". It promotes maternal health and noted its particular use as a galactagogue in lactating mothers due to phyto-estrogens (Ashajyothi et al., 2009), and thus recommended for healthcare of women.

In India, it is one of the 32 plant species identified as priority species for cultivation and conservation by the National Medicinal Plant Board (NMPB). With an increasing realization that hormone replacement therapy with synthetic estrogens is neither safe nor as effective as previously envisaged, the interest in plant-derived

estrogens has increased enormously, making *A. racemosus* particularly important (Bopana and Saxena, 2007). Due to its increased demand, the species has attracted the attention for its genetic improvement, conservation and cultivation.

Intraspecific variation is expected to exist in nature. These variations are of ecological and evolutionary interest with regard to the environmental influences that shape them both non-genetically in the present and genetically over an evolutionary time period. The intraspecific diversity is an integral part of biodiversity (Moritz, 2002) and is essential for sustainability, because it facilitate individual species to adapt to a changing environment and therefore ensures their long term survival (Main, 1999). The vegetative plant characterization essentially provides the extent of variability of the collected accessions and fulfills the primary steps towards elite genotype identification for the quality traits of *A. racemosus* germplasm. Therefore the present study aims to analyze the patterns of vegetative morphological variation and to know the nature and extent of genetic variability and heritability in some important traits among the *A. racemosus* accessions collected from different parts of Kerala which provide answers to the following questions: Is there any morphological diversity for *A. racemosus* in Kerala, if so then what is the extent of this variability? How much of the phenotypic differences in *A. racemosus* accessions are due to genetic and environmental factors? What are the characters contributing to their diversity? And how the diversity is distributed among the *A. racemosus* accessions in the study area? Present study therefore aimed to answer these questions.

Materials and Methods

Twenty accessions of *A. racemosus* analyzed in the present investigations were collected from different localities of Kerala state, India (Fig.1) representing ten Agro-Ecological zones of Kerala (Table 1) (ENVIS Centre). They were planted in the botanic garden of the Department of Botany, University of Kerala (8º33'03.86" N; 76º52'38.64" E; 18 m alt) under identical cultivation conditions and were used for the morphological characterization. A total of 25 characters, 18 vegetative quantitative and 7 qualitative morphological traits (Table 2) were evaluated at full foliage stage on ten randomly selected plants from each accessions with the help of descriptors developed (Table 2 and Table 3) by the National Bureau of Plant Genetic Resources (Singh et al., 2003) and e-descriptors for *A. recemosus* from Asia Medicinal Plant Database (Table 2). Yearly data were collected for the first three years and the data were analyzed.

The collected data were subjected to statistical analysis using the statistical software SPSS package version 22 (IBM Crop, 2013). One way analysis of variance (ANOVA) was conducted using quantitative data to test the null hypothesis that there is no difference in morphological characters

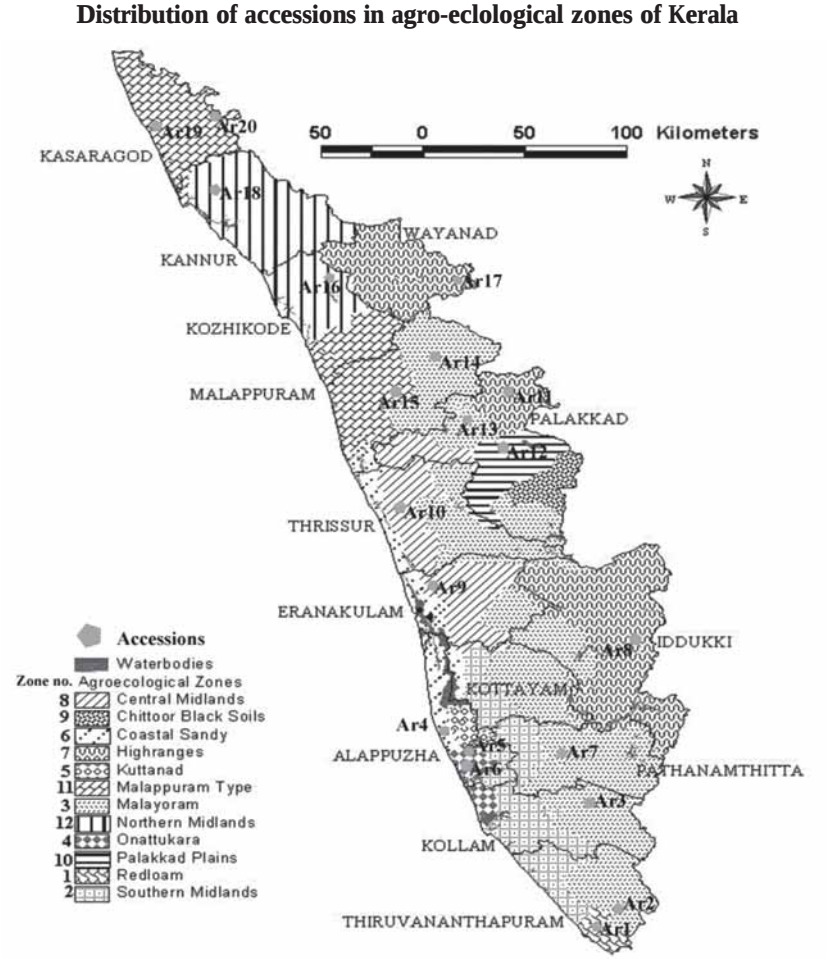

Fig.1. Distribution of accessions of *Asparagus raemosus* in the ten agro-ecological zones of Kerala (Source: KAU 2011)

Table 1. Accession of Asparagus racemosus and geographic details of their collection sites in Kerala state, India

Accession code	Place of collection	Revenue district	Agro-ecological zone	Zone No.	Latitude	Longitude	Altitude (m)	Altitude type[a]	Rainfall pattern[b]	Soil type
Ar1	Nellimoodu	Trivandrum	Red loam	1	8°22'51.81" N	77°02'31.39"E	55	I	I	Laterite without B horizone
Ar2	Kattakada	Trivandrum	Malayoram	3	8°30'11.71"N	77°05'06.81"E	79	I	I	Laterite without B horizone
Ar3	Punalur	Kollam	Malayoram	3	9°01'12.34"N	76°55'51.14"E	47	I	I	Laterite without B horizone
Ar4	Arattupuzha	Alappuzha	Costal sandy	6	9°12'53.38"N	76°25'52.65"E	08	I	I	Sandy loam
Ar5	Kareelakulangara	Alappuzha	Onattukara	4	9°11'36.38"N	76°29'06.82"E	09	I	I	Sandy loam
Ar6	Chingoli	Alappuzha	Onattukara	4	9°15'01.24"N	76°27'07.09"E	10	I	I	Sandy loam
Ar7	Kozhencherry	Pathanamthitta	Southern midlands	2	9°19'58.97"N	76°42'20.88"E	17	I	I	Laterite without B horizone
Ar8	Vandiperiyar	Idukki	Highranges	7	9°34'19.60"N	77°14'45.77"E	809	II	I$II	Red loam
Ar9	Olanad	Ernakulam	Central midlands	8	10°05'30.55"N	76°16'31.54"E	13	I	I$II	Laterite
Ar10	Vellanikkara	Thrissur	Central midlands	8	10°32'42.77"N	76°16'26.33"E	35	I	I$II	Laterite
Ar11	Silent Valley	Palakkad	Highranges	7	11°04'06.31"N	76°31'09.80"E	657	II	I$II	Red loam
Ar12	Mundur	Palakkad	Palakkad plains	10	10°57'27.44"N	76°30'19.67"E	84	I	II	Red loam
Ar13	Mannarkkad	Palakkad	Malayoram	3	10°59'35.65"N	76°27'39.59"E	85	I	I	Laterite without B horizone
Ar14	Wandoor	Malappuram	Malayoram	3	11°11'43.83"N	76°14'09.74"E	80	I	II	Laterite without B horizone
Ar15	Pandikkad	Malappuram	Malappuram type	11	11°05'33.20"N	76°13'23.84"E	40	I	II	Laterite
Ar16	Janakikkadu	Kozhikode	Northern midlands	12	11°37'47.93"N	75°47'09.09"E	49	I	II	Laterite
Ar17	Muthanga	Wayanad	Highranges	7	11°38'43.53"N	76°22'32.64"E	862	II	I$II	Red loam
Ar18	Thaliparamba	Kannur	Northern midlands	12	12°02'17.77"N	75°22'02.83"E	57	I	II	Laterite
Ar19	Kanhangad	Kasargode	Malappuram type	11	12°19'56.90"N	75°05'46.16"E	49	I	II	Laterite
Ar20	Ranipuaram	Kasargode	Malappuram type	11	12°25'09.41"N	75°21'03.45"E	1019	II	II	Laterite

[a]Altitude: Type I- Altitude up to 500 m above Mean Sea Level, Type II- More than 500 m above Mean Sea Level. [b]Rainfall: Pattern I- Both the southwest and northeast monsoons are active and moderately distributed. Southwest monsoon with June maximum, Pattern II- Poorly distributed rainfall; southwest monsoon with July maximum and concentrated in 3-4 months. Northeast monsoon relatively weak.

between accessions and level of significance was determined. To determine how much of phenotypic variations in quantitative traits are due to genetic factors and how much is due to environmental factors, the genetic parameters like variance components (σ^2_g, σ^2_e, σ^2_p), and phenotypic and genotypic coefficient of variations (PCV and GCV) were determined for the quantitative characters under study as per Wricke and Weber (1986)and Singh and Chaudhary (1985). Heritability in broad sense (H^2B) for each character was calculated as the ratio of genetic variance to the total phenotypic variance as suggested by (Allard, 1960) and expressed as percentage.

Patterns of morphological similarity and differences were analyzed by Multivariate Statistical Package version 3.1 (Kovach, 2007) using Principal component analyses (PCA), Principal Co-ordinate Analysis (PCoA) and Cluster analysis based on Unweighted Pair Group Method with Arithmetic Average (UPGMA). Both the Principal component analysis and the Principal coordinate analysis followed by construction of scatter plots were based on orientation of accessions and characters. PCA will be indicative that, the first principal component accounts for maximum possible variably in the data and each succeeding principal components explain the remaining possible maximum variance with respect to the preceding components. Cluster analysis was performed on the basis of genetic similarity matrix and the resulting similarity coefficients were used for the construction of dendrogram to explain the

Table 2. List of morphological characters with abbreviations recorded for 20 accessions of *A. racemosus*

No	Character	Abbreviation
1	Number of cladode clusters	CC
2	Number of cladodes per whorl	CPW
3	Cladode length (mm)	CL
4	Cladode width (mm)	CW
5	Cladode thickness (mm)	CT
6	Cladode length/width ratio	CL/W
7	Cladode fresh weight (g)	CFW
8	Cladode dry weight (g)	CDW
9	Length of spines (mm)	LS
10	Length of internodes (mm)	LI
11	Stem diameter (mm)	STEM_DIA
12	Length of cladode clusters (mm)	LCC
13	Branch diameter (mm)	BD
14	Branch length (cm)	BL
15	Length of fresh spear (cm)	LFS
16	Breadth of fresh spear (cm)	BFS
17	Circumference of fresh spear (cm)	CFS
18	Plant height (cm)	PLANT_HGT
19	Cladode shape	CLAD_SHP
20	Cladode arrangement	CLAD_ARR
21	Cladode color	CLAD_CLR
22	Spine shape	SPIN_SHP
23	Stem color	STEM_CLR
24	Stem shape	STEM_SHP
25	Branching type	BRN_TYP

Table 3. List of qualitative variables with abbreviations and descriptions used for the evaluation of 20 accessions of *A. racemous*

No.	Character	Abbreviation	Description
1	Cladode shape	CLAD_SHP	1-Needle like, 2-Oblong, 3-Subulate, 4-Curved, 5-Flattened,6-Setaceous, 99-Others
2	Cladode arrangement	CLAD_ARR	1-Errect, 2-Sparsely, 99-Others
3	Cladode color	CLAD_CLR	1-Yellowish green, 2-Pale green, 3-Green, 4-Dark green
4	Spine shape	SPIN_SHP	1-Straight, 2-Recurved, 99-Others
5	Stem color	STEM_CLR	1-Grayish white, 2-Green, 3-Pink purple, 99-Others
6	Stem shape	STEM_SHP	1-Angular, 2-Cylindrical, 99-Others
7	Branching type	BRN_TYP	1-Sparsely, 2- Profusely, 99- Others

morphological diversity. The evaluation of morphological traits can lead to indication of similarity and differences between accessions and further to confirm the diversity in the genetic level through molecular markers.

Results and Discussion

All the quantitative characters subjected to one way ANOVA revealed significant ($P < 0.05$) variations among the 20 accessions for all the traits studied (Table 4) indicating quantitative variations among the accessions. The average number of cladodes per whorl was found to be more in Ar2 (10 Nos). Ar15 had very shortest spines (0.52mm) compared to all other accessions, while Ar11 had longest spines (1.89mm). Plant height records revealed Ar16 as the shortest accession. Average cladode length ranged from 7.45 mm for Ar4 to 14.7 mm for Ar5 and the average cladode width varied from 0.93mm for Ar16 to 1.67mm (Ar20). It was observed that plant height is the most variable character which ranged from 75 cm in Ar16 to 483.8 cm in Ar1.

All the accessions studied for qualitative characters showed similarity only in the traits cladode shape, cladode arrangement and stem shape (Table 5). Cladode color present in the accessions are yellowish green, pale green, green and dark green. Stem color vary from grayish white, green, pink purple and yellowish green, other than one in the descriptors. Out of the 20 accessions studied, 19 have recurved spines and Ar3 only had straight spine. These variations existed in the germplasm separated the accessions in to different subsets.

Coefficient of variation can be used to compare the variation of a trait in two or more populations or the variation of different traits in a population (Sokal and Rohlf, 2012).The range of coefficient of variation (CV) revealed broad variability for all the quantitative descriptors studied. In the present study, coefficient of variation ranged from

Table 4. Morphological variation in different quantitative characters of 20 accessions of *A. racemosus*

ACC No.	CC (nos.)	CPW (nos.)	CL (mm)	CW (mm)	CT (mm)	CL /W	CFW (g)	CDW (g)	LS (mm)	LI (mm)	Stem_ DIA (mm)	LCC (mm)	BD (mm)	BL (cm)	LFS (cm)	BFS (cm)	CFS (cm)	Plant_ HGT(cm)
Ar1	7.00^{f}	5.16^{de}	11.8^{bcd}	1.17^{def}	0.18^{fg}	10.3^{cde}	0.45^{de}	0.37^{h}	1.13^{efg}	6.89^{de}	1.11^{s}	19.1^{ghij}	1.31^{j}	24.7^{cdef}	185.3^{b}	0.311^{f}	19.5^{f}	483.8^{a}
Ar2	7.90^{b}	9.57^{a}	12.0^{bc}	1.43^{bc}	0.34^{ab}	8.44^{fg}	0.42^{e}	0.39^{gh}	1.19^{defg}	6.17^{efg}	6.21^{a}	25.3^{bcde}	1.55^{efghij}	25.5^{bcde}	68.4^{l}	0.308^{g}	19.3^{g}	185.4^{f}
Ar3	10 .2^{d}	5.28^{d}	11.3^{cdef}	1.32^{bcde}	0.27^{c}	8.58^{fg}	0.46^{de}	0.43^{cdefg}	1.56^{b}	5.11^{i}	3.06^{o}	9.57^{l}	1.46^{ghij}	21.0^{ghi}	202.7^{a}	0.303^{i}	19.0	381.6^{b}
Ar4	8.00^{b}	3.33^{h}	7.45^{h}	1.18^{def}	0.18^{fg}	6.32^{jh}	0.47^{cd}	0.45^{cdef}	1.54^{b}	5.50^{ghi}	2.43^{r}	12.4^{kl}	1.67^{defg}	22.9^{efgh}	113.4^{d}	0.246^{o}	15.4^{o}	316.2^{c}
Ar5	7.80^{d}	3.47^{h}	14.7^{a}	1.37^{bcde}	0.33^{b}	11.0^{bcd}	0.48^{cd}	0.40^{fgh}	1.64^{b}	6.64^{def}	2.43^{qr}	14.4^{jk}	1.61^{efghi}	22.2^{fgh}	87.8^{h}	0.264^{m}	16.5^{m}	183.9^{f}
Ar6	6.90^{f}	4.72^{def}	11.1^{def}	1.33^{cde}	0.13^{h}	8.23^{fg}	0.53^{ab}	0.40^{fgh}	1.22^{cdef}	6.59^{def}	4.15^{f}	22.5^{defg}	2.36^{b}	33.5^{a}	96.2^{g}	0.425^{a}	26.6^{a}	144.9^{i}
Ar7	7.90^{d}	4.33^{fg}	11.0^{def}	1.17^{def}	0.17^{fg}	10.1^{de}	0.57^{a}	0.42^{efg}	0.85^{hi}	6.65^{def}	3.24^{m}	28.2^{bc}	1.33^{ij}	25.4^{bcde}	147.8^{c}	0.331^{d}	20.7^{d}	240.8^{d}
Ar8	8.90^{c}	7.55^{b}	12.7^{b}	0.99^{fg}	0.21^{def}	12.7^{a}	0.45^{de}	0.42^{efg}	1.02^{fgh}	6.03^{efg}	4.14^{g}	16.9^{hijk}	1.40^{ghij}	27.5^{b}	97.2^{g}	0.262^{n}	16.4^{n}	147.2^{j}
Ar9	6.90^{f}	6.30^{c}	11.4^{cde}	1.14^{efg}	0.35^{ab}	10.4^{cd}	0.54^{ab}	0.52^{a}	0.72^{ij}	8.23^{a}	5.31^{b}	21.5^{efgh}	1.77^{de}	25.8^{bcd}	59.4^{m}	0.307^{h}	19.2^{h}	152.4^{i}
Ar10	5.60^{h}	3.12^{h}	10.9^{ef}	1.36^{bcde}	0.38^{a}	8.20^{fg}	0.48^{cd}	0.45^{cdef}	1.49^{b}	5.90^{fgh}	3.21^{n}	15.4^{jk}	1.93^{cd}	19.0i	81.5^{j}	0.177^{q}	11.1^{q}	215.5^{e}
Ar11	7.60^{de}	5.19^{de}	10.8^{efg}	1.52^{ab}	0.33^{b}	7.43^{gh}	0.54^{ab}	0.47^{bcd}	1.89a	7.37^{cd}	3.54^{k}	24.0^{cdef}	2.15^{bc}	27.3^{bc}	48.2^{n}	0.228^{p}	14.3^{p}	118.9^{j}
Ar12	8.00^{d}	3.56^{h}	12.0^{bc}	1.20^{cdef}	0.26^{c}	10.0^{de}	0.52^{ab}	0.48^{abc}	1.49^{b}	7.80^{abc}	3.61^{j}	27.2^{bcd}	1.73^{def}	21.0^{ghi}	83.4^{i}	0.305^{j}	19.^{i}	162.6^{h}
Ar13	10.8^{a}	3.79^{gh}	10.0^{g}	1.32^{bcde}	0.25^{cd}	8.09^{fg}	0.46^{de}	0.43^{defg}	1.53b	7.26^{cd}	2.73^{p}	25.9^{bcde}	1.64^{efgh}	20.1^{hi}	75.1^{k}	0.278^{j}	17.4^{k}	110.5^{k}
Ar14	7.00^{f}	3.33^{h}	10.5^{fg}	1.33^{bcde}	0.25^{cde}	7.91^{fg}	0.55^{ab}	0.52^{a}	0.94ghi	6.13^{efg}	3.69^{j}	24.7^{bcde}	1.48^{efghij}	23.6^{defg}	105.8^{f}	0.366^{c}	22.9^{c}	163.0^{h}
Ar15	8.80^{c}	5.04^{def}	12.7^{b}	1.40^{bcd}	0.25^{cd}	9.04^{ef}	0.51^{bc}	0.46^{e}	0.52j	5.84^{fgh}	3.49^{l}	14.9^{jk}	1.60^{efghi}	21.3^{ghi}	46.7^{n}	0.268^{l}	16.8^{l}	171.0^{g}
Ar16	6.20^{g}	3.63^{gh}	10.8^{efg}	0.93^{g}	0.20^{efg}	11.9^{ab}	0.54^{ab}	0.50^{ab}	1.10fgh	6.13^{efg}	3.74^{h}	38.3^{a}	1.40^{ghij}	19.2^{i}	34.5^{p}	0.152^{r}	9.54^{r}	76.0^{l}
Ar17	8.00^{d}	5.37^{d}	9.99^{g}	1.37^{bcde}	0.26^{cd}	7.55^{gh}	0.45^{de}	0.30^{i}	1.42bcd	6.23^{efg}	3.49^{l}	23.3^{cdefg}	1.58^{efghij}	25.5^{bcde}	108.2^{e}	0.308^{g}	19.3^{g}	183.1^{f}
Ar18	10.3^{ab}	3.71^{gh}	12.2^{bc}	1.18^{def}	0.24^{cde}	10.5^{cd}	0.44^{de}	0.30^{i}	1.46bc	4.92^{j}	4.28^{e}	19.8^{fghi}	1.36^{hij}	21.2^{ghi}	43.3^{o}	0.315^{e}	19.7^{e}	126.2^{j}
Ar19	6.10^{gh}	3.41^{h}	12.5^{b}	1.07^{fg}	0.17^{gh}	11.6^{abc}	0.45^{de}	0.42^{efg}	0.70ij	8.11^{ab}	4.93^{c}	29.3^{b}	1.34^{ij}	24.4^{def}	105.5^{f}	0.380^{b}	23.8^{b}	322.0^{c}
Ar20	7.20^{ef}	4.50^{ef}	12.1^{bc}	1.67^{a}	0.27^{c}	7.27^{fg}	0.54^{ab}	0.48^{abc}	1.37bcde	6.09^{efg}	4.35^{d}	23.5^{cdefg}	2.82^{a}	34.6^{a}	83.0^{ij}	0.331^{d}	20.7^{d}	240.7^{d}
F value	1.643	45.82	26.09	6.08	24.83	15.71	10.71	15.69	18.46	11.38	18.8	23.99	19.41	23.03	46.7	54.8	90.76	131.6
Df (n-1) =19	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

***Significant at P < 0.001 level;**Significant at P < 0.01level;*Significant at P < 0.05 level

12.46% for cladode fresh weight to 53.51% for length of cladode clusters. While studies on Iranian wild asparagus (Sarabi et al., 2010) reported coefficient variation ranged between 2.2% for number of cladodes to 79% for spear number. The highest coefficient of variation (Table 6) estimated for length of cladode clusters (53.51 %) followed by plant height (47.98%), length of fresh spear (46.57%) and number of cladodes per whorl (36.71%) signify the existence of high degree of variability with regard to these traits. It is clear that these characters can be used for distinguish these accessions. Whereas coefficient of variation was lowest for cladode fresh weight (12.46%), cladode length (14.38%) and cladode dry weight (16.99%) indicating that quantitative characters of individual cladodes are least important in distinguishing the accessions. Similar to the present study by Mousavizadeh et al. (2015), reported 48.74% variation in plant height in edible *Asparagus* species. However, qualitative cladode characters have some role in characterizing the accessions particularly cladode color and cladode arrangement under stem as clear by PCA.

The genotypic (σ^2_g), environmental (σ^2_e) and phenotypic variance (σ^2_p), genotypic and phenotypic coefficient of variations (GCV and PCV) and broad sense heritability (H^2B) of *A. racemosus* accessions were estimated (Table 7). A wide range of variations were observed with regard to different traits. The contrast in range was high among collections in characters such as plant height (411 cm), length of fresh spear (172 cm), length of cladode clusters (33.26 mm) and circumference of fresh spear (17.15 cm).

Table 5. Morphological variations in different qualitative characters among 20 accessions of *A. racemosus*

ACC.No.	CLAD_SHP	CLAD_ARR	CLAD_CLR	SPIN_SHP	STEM_CLR	STEM_SHP	BRN_TYP
Ar1	4	99	4	2	99	2	2
Ar2	4	99	4	2	99	2	2
Ar3	4	99	3	1	3	2	1
Ar4	4	99	3	2	2	2	1
Ar5	4	99	2	2	2	2	2
Ar6	4	99	2	2	3	2	1
Ar7	4	99	3	2	1	2	2
Ar8	4	99	3	2	3	2	1
Ar9	4	99	1	2	99	2	2
Ar10	4	99	1	2	99	2	1
Ar11	4	99	3	2	99	2	2
Ar12	4	99	2	2	2	2	2
Ar13	4	99	4	2	2	2	2
Ar14	4	99	3	2	3	2	1
Ar15	4	99	3	2	99	2	1
Ar16	4	99	1	2	99	2	1
Ar17	4	99	1	2	99	2	2
Ar18	4	99	2	2	99	2	2
Ar19	4	99	3	2	1	2	2
Ar20	4	99	4	2	3	2	2

Table 6. Range, Mean, Standard deviation, and Coefficient of Variation and F value for 18 characters in the *A. racemosus* accessions

Characters	Range	Mean	Std. Deviation	CV (%)	F values for accessions
Number of cladode clusters	5.00 -11.00	7.85	1.47	18.72	56.45
Number of cladode per whorl	2.00 -10.00	4.72	1.73	36.71	45.82
Cladode length (mm)	7.45-14.70	11.44	1.64	14.38	26.09
Cladode width (mm)	0.93-1.67	1.27	0.27	21.49	6.08
Cladode thickness (mm)	0.13-0.38	0.25	0.07	30.78	24.83
Cladode length/width ratio	6.30-12.79	9.33	2.16	23.20	15.71
Cladode fresh weight (g)	0.42-0.57	0.49	0.06	12.46	10.71
Cladode dry weight (g)	0.30-0.52	0.43	0.07	16.99	15.19
Length of spines (mm)	0.52-1.89	1.24	0.43	34.91	18.46
Length of internodes (mm)	4.90-8.23	6.48	1.21	18.67	11.38
Stem diameter (mm)	1.11-6.21	3.65	1.08	29.67	18.89
Length of cladode clusters (mm)	5.06-38.32	19.41	10.38	53.51	23.55
Branch diameter (mm)	1.31-2.82	1.67	0.45	27.48	19.41
Branch Length (cm)	19.08-34.69	24.33	4.87	20.04	23.03
Length of fresh spear (cm)	31.00 -203.00	93.25	43.43	46.57	46.70
Breadth of fresh spear (cm)	1.52-4.25	2.93	0.62	21.27	11.02
Circumference of fresh spear (cm)	9.54-26.69	18.41	3.91	21.26	90.76
Plant height (cm)	75.00 -486.00	205.55	98.64	47.98	131.60

Table 7. Genetic parameters of 18 quantitative characters in *A. racemosus* accessions

Character	Mean	σ^2_g	σ^2_e	σ^2_p	GCV (%)	PCV (%)	H^2B
Number of cladode clusters	7.85	1.90	0.03	1.94	17.58	17.73	98.21
Number of cladodes per whorl	4.72	2.55	0.05	2.60	33.84	34.22	97.80
Cladode length (mm)	11.44	2.00	0.07	2.08	12.37	12.61	96.15
Cladode width(mm)	1.27	0.02	0.00	0.03	12.74	13.93	83.70
Cladode thickness (mm)	0.25	0.00	0.00	0.00	26.30	26.88	95.74
Cladode length/width ratio	9.33	2.86	0.19	3.06	18.15	18.75	93.63
Cladode fresh weight (g)	0.49	0.00	0.00	0.00	8.77	9.22	90.47
Cladode dry weight (g)	0.43	0.00	0.00	0.00	13.20	13.60	94.28
Length of spines (mm)	1.24	0.12	0.00	0.13	28.23	29.04	94.55
Length of internodes (mm)	6.48	0.76	0.07	0.83	13.48	14.11	91.21
Stem diameter (mm)	3.65	1.23	0.00	1.23	30.38	30.38	100.00
Length of cladode clusters (mm)	19.41	77.19	3.42	80.61	45.26	46.25	95.75
Branch diameter (mm)	1.67	0.14	0.00	0.14	22.38	22.98	94.82
Branch Length (cm)	24.33	16.90	0.76	17.66	16.89	17.27	95.66
Length of fresh spear (cm)	93.25	1976.4	0.00	1976.40	47.67	47.67	100.00
Breadth of fresh spear (cm)	2.93	0.40	0.00	0.40	21.77	21.77	100.00
Circumference of fresh spear (cm)	18.41	16.05	0.00	16.05	21.76	21.76	100.00
Plant height(cm)	205.55	10192.78	0.00	10192.78	49.12	49.12	100.00

The estimates of genetic variances calculated for all the quantitative traits were smaller than their respective phenotypic variances and greater than their respective environmental variances. Maximum genotypic and phenotypic variations were obtained for plant height and length of fresh spear, while moderate variation was obtained for length of cladode clusters, branch length and circumference of fresh spear. Lower values of genotypic and phenotypic values were noticed in cladode fresh weight, cladode dry weight and cladode thickness. In general, there was a very close difference between phenotypic and genotypic variance for all the characters except stem diameter, length of fresh spear, breadth of fresh spear, circumference of fresh spear and plant height. For these characters genotypic and phenotypic variances were observed to be equal and these characters can be considered stable based on Jonah et al. (2013). This is an indication that genotypic component of variation was the major contributor to the total variation. Also, the environmental variances of these traits were observed to be very small indicating that the environment had little effect on the observed phenotypic variations of the traits.

Genetic parameters such as genotypic coefficient of variation and phenotypic coefficient of variation are useful in detecting the amount of variability present in the germplasm (Reddy et al., 2013). The genotypic coefficient of variation provides a measure for comparing genetic variability in various quantitative characters, while phenotypic coefficient of variation measures total relative variations. In the present study GCV in almost all the characters showed great resemblance to PCV. The characters *viz.*, plant height, circumference of fresh spear, breadth of fresh spear, length of fresh spear, stem diameter, length of cladode clusters, dry weight, length, number of cladode clusters, cladode thickness and cladode length/width ratio had very close GCV and PCV values. These results were similar to the findings by Reddy et al. (2013) which suggests that, the variations observed in all the characters was the result of genetic effect and the environment had little effect on those character's expression and was in conformity with the observations of Sattar et al. (2007) and Ashfaq et al. (2014). In contrast to this Fantaw et al. (2014) reported the influence of environment in the phenotypic variance of *Xanthosoma sagittifolium*.

The highest GCV was recorded for plant height (49.12%), while moderate for stem diameter (30.28%), spine length (28.23%), cladode thickness (26.30%), branch diameter (22.38%), breadth of fresh spear

(21.77%), circumference of fresh spear (21.76%), cladode length/width ratio (18.15%), number of cladode clusters (17.58), branch length (16.89) and the lowest for cladode fresh weight (8.77). The highest value of both GCV and PCV for plant height clearly indicated a high degree of genetic variability in the plant height among accessions. PCV was highest for plant height (49.12%) followed by length of fresh spear (47.67%) and length of cladode clusters (46.25%). Whereas, stem diameter (30.38) followed by spine length (29.04%), cladode thickness (26.88%), branch diameter (22.98%), breadth of fresh spear (21.77%), circumference of fresh spear (21.76%), cladode length width ratio (18.75%), number of cladode clusters (17.73%) and branch length (17.21%) recorded moderate PCV and the lowest for cladode fresh weight (9.22%).

The estimation of heritable variation with the help of GCV alone may be misleading. The GCV together with heritability estimates will give a better picture of the extent of heritable variation (Burton,1953). Heritability estimates were interpreted as low (less than 3%), medium (30-60%) and high (more than 60%) as per the classification of Johnson et al. (1955). In the present investigation the heritability estimates obtained were high (> 60%) for all the characters representing minimum environmental influence in the expression of these characters (Table 7). The value ranged from 83.7% for cladode width to 100% for stem diameter, length of fresh spear, breadth of fresh spear, circumference of fresh spear and plant height. Highest values were also obtained by cladode length (96.15%), number of cladodes per whorl (97.8%) and numbers of cladode clusters (98.21%). Among the high heritability estimates, moderate heritability was recorded by cladode dry weight (94.28%) and length of spines (94.55%). The lowest estimate of broad sense heritability was recorded by cladode width (83.7%). Rest of the characters recorded heritability estimates between 90-95%. Similar results were obtained by Naegele et al. (2016) in *Capsicum annum* with respect to heritability. Ogunniyan and Olakojo (2015) had also reported high heritability for some relevant agronomic characters in maize.

Principal Component Analysis (PCA)

PCA leads to realization of actual morpho-traits that are responsible for the highest percentage of total variance of the experimental data. The PCA grouped 25 morphological characters into 10 components, which accounted for the entire variability among the accessions studied. The percentage of variation was high for the first four components (Table 8). The first PC accounted for maximum variability (97.23%) having an Eigen value 27.9, mainly due to variations in the cladode length (CL), length of cladode clusters (LCC), branch length (BL), length of fresh spear (LFS), circumference of fresh spear (CFS), Plant height (PLANT_HGT), cladode arrangement (CLAD_ARR), and stem color (SREM_CLR). The second PC accounted for 2.11% of the variability with high contributing factor loading for stem color (STEM_CLR). The third and fourth PCs exhibited 0.29 % and 0.11% of variations respectively, which dominated by traits of number of cladodes per whorl (CPW), stem diameter (STEM_DIA), length of cladode clusters (LCC), length of fresh spear (LFS), plant height (PLANT_HGT) and cladode color (CLAD_CLR). Studies conducted by Chaudhary (2012) on morphological variability among 13 *A. racemosus* accessions collected from different geographical locations in India showed 80.93% variance by considering the first four components, whereas in the present study, 99.76% of total variance

Table 8. Principal Component Analysis in 20 accessions of *A. racemosus*. Eigen values, percentage variation and cumulative percentage by the four principal components (highly loaded variables given in bold font)

Character	PC1	PC2	PC3	PC4
CPW	0.145	0.029	-0.124	-0.411
CL	0.212	-0.019	0.049	-0.156
STEM_DIA	0.127	0.00	0.179	-0.433
LCC	0.244	-0.065	0.767	0.366
BL	0.271	-0.047	0.069	-0.178
LFS	0.375	-0.186	-0.388	0.341
CFS	0.248	-0.084	0.019	-0.177
PLANT_HGT	0.441	-0.117	-0.338	0.359
CLAD_ARR	0.338	-0.041	0.103	-0.085
CLAD_CLR	0.104	-0.086	-0.192	-0.302
STEM_CLR	0.231	0.960	-0.057	0.073
Eigen values	27.937	0.608	0.084	0.032
Percentage variation	97.238	2.117	0.293	0.112
Cumulative percentage	97.238	99.355	99.648	99.76

on first four components was scored (Table 8). Studies on Iranian *Asparagus* populations, *A. officinalis* (wild and cultivated) and *A. persicus* (wild) (Sarabi et al., 2010) showed that the first four PCs explained only 66.19% of total variance which is contributed by the plant height, number of secondary branches, intermodal length of primary and secondary branches, diameter of main stem, percent of dry matter on the spear and length and width of the spear scales among the 22 morphological characters evaluated and it was 53.75% in pomegranate as reported by Martinez-Nicolas et al. (2016). In the present study STEM_CLR, LCC, PLANT_HGT, STEM_DIA, CPW, LFS, CLAD_ARR, CLAD_CLR, BL, CFS and CL were inferred as significant characters from PCA.

Principal Co-ordinate Analysis (PCoA)

Principal Co-ordinate Analysis (PCoA) explores and visualizes similarities and dissimilarities of data. It starts with dissimilarity matrix (distance matrix) and assigns for each accession a location in scatter plots (Fig.2). In PCoA the similarity is denoted by very small degree of dissimilarity index. The minimum divergence exhibited by accessions of Chingoli (Alappuzha, Ar6) and Vandoor (Malappuram, Ar14) indicating their close relationship with dissimilarity index (0.30). They are similar in majority of morphological traits except for cladode color, which is pale green for Ar6 and green for Ar14. *Asparagus racemosus* from Mundur (Ar12), Mannarkkad (Ar13), Vandoor (Ar14), and Kareelakkulangara (Ar5) were found to be very identical in morphology as revealed very less dissimilarity index with Ar12 (Mundur-Palakkad).They showed lesser amount of variability in highly loaded characters like CPW, LCC, STEM_DIA, PLANT_HGT and STEM_CLR. Ar14 (Wandoor), Ar20 (Ranipuram), Ar12 (Mundur), Ar13 (Mannarkkad) and Ar5 (Kareelakkulangara) are also less variable to Ar6 and is included in the same cluster. Variations in plant height, cladode color and branching type contributed to more similarity between Ar6 and Ar14 than Ar20 and Ar6. Ar6 have sparsely branching, pale green cladodes and is much shorter than Ar20 with dark green cladodes and profuse branches. Silent Valley accession (Ar11) and Thaliparamba accession (Ar18) are more similar with respect to most of their characters. The quantitative characters which differs between them is CC, CPW, CL, CL/W TL and LI. Ar11 had LI, TL and CPW more than Ar18, with slightly lengthier cladodes than Ar11. Though the accessions Ar9 and Ar17 were included in the same cluster, they are less similar to Ar11 than Ar18. From distance matrix it is clear that Ar11 (Silent Valley) and Ar18 (Thaliparamba) are more similar than Ar 9 and Ar17. Accessions from Punalur (Ar 3) and Kanhangad (Ar19) were found to be highly diverse to Janakikkadu (Ar16) with a dissimilarity index of 1.98. They differ highly with respect to length of cladode clusters, length of fresh spear, plant height, spine shape and also in color of cladode and stem. Ar3 had green cladodes with pink purple stem and Ar16 had yellowish green cladodes, with yellowish green stem color, other than one in the descriptors. They differ in the qualitative characters considered except cladode color, stem color and branching type. Ar19 had lengthy spear than Ar16. Also Ar1 was found to be shorter in plant height than Ar19. Being significant characters for variability as evident from PCA, here PLANT_HGT, CLAD_CLR and STEM_CLR were critical factors for the divergence of Ar19 from Ar16. Ar7 (Kozhencherry) and Ar4

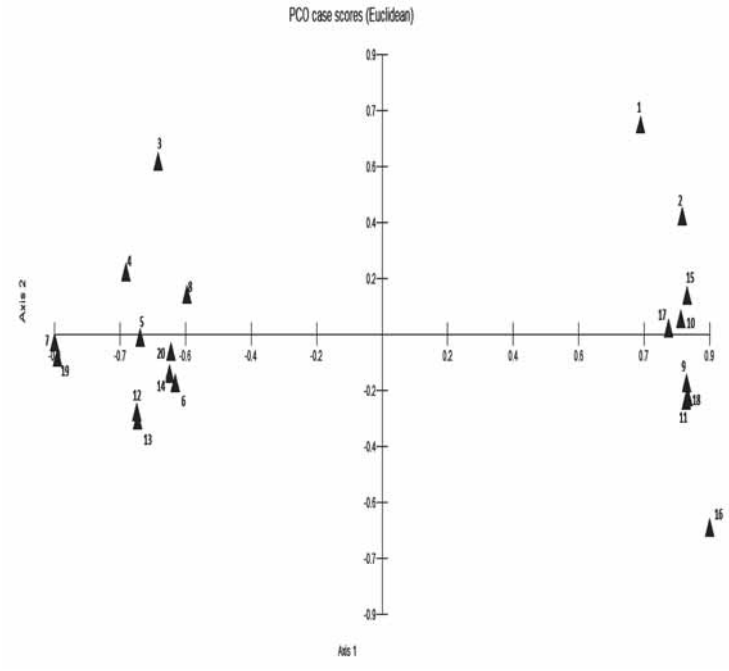

Fig.2. Principal co-ordinate analysis scatter plot based on qualitative and quantitative characters in 20 accessions of *A. racemosus*

(Arattupuzha) showed variation from Ar16 to a slightly higher degree. These variations are clearly depicted in the cluster analysis by locating Ar16 in the first principal cluster and the other diverse accessions Ar3, Ar19, Ar7, Ar4, Ar5, Ar8, Ar20, Ar13, Ar12, Ar6 and Ar14 in the second principal cluster with decreasing index of dissimilarity as reported by Anil et al.,(2011) in *Amorphophallus paeoniifolius*.

Cluster analysis

The UPGMA dendrogram with 25 variables divided the whole accessions into two principal clusters between the Euclidean distance of 0.3-1.6 (Fig.3). Similar kind of clustering was also reported by Sarabi et al. (2010) in Iranian wild *Asparagus* populations. The first principal cluster consists of 9 accessions (Ar1, Ar2, Ar9, Ar10, Ar11, Ar 15, Ar16, Ar17 and Ar18) based on similarities in cladode arrangement, stem color, branching type and plant height and was subdivided into 3 subclusters with Ar1 as an outlier. Among the traits mainly contributing for the first principal cluster, STEM_CLR is the most contributing character. Ar1 stood far apart due to its highest plant height (483.8 cm), very lengthy fresh spear (185.3 cm) and comparatively thin stems (1.11 mm) at a Euclidean distance of 0.99. Ar16 stood apart due to smallest cladode width (0.93 mm) and shortest in plant height (76 cm) and fresh spear length (34.5 cm).

The second principal cluster included remaining 11 accessions (Ar19, Ar7, Ar20, Ar14, Ar6, Ar13, Ar12, Ar5, Ar8, Ar4, Ar3) with 3 subgroups. The character responsible for the second principal cluster is plant height. All the accessions included in this cluster grow more than1m. This principal cluster was subdivided into three sub clusters in which the first group consisted of Ar19 and Ar7. The clustering of these accessions was due to

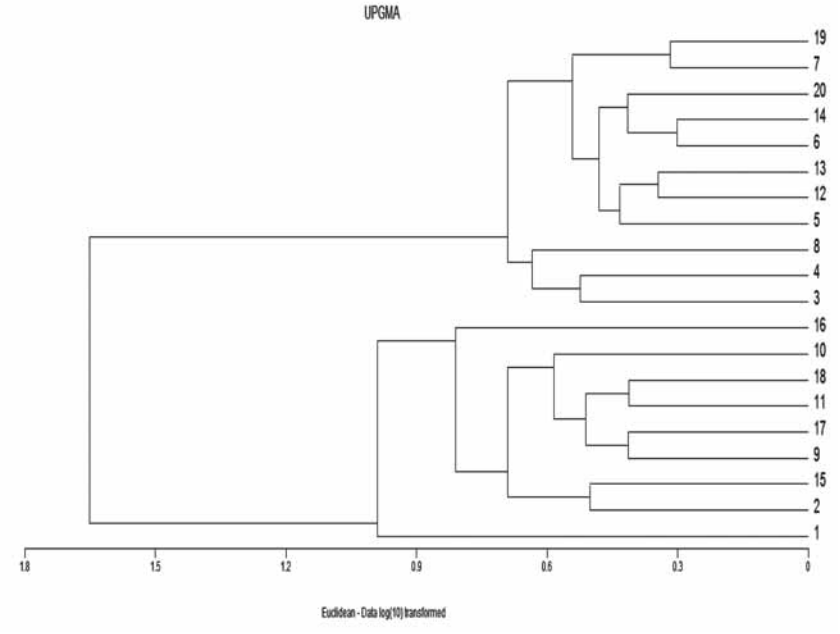

Fig.3. Dendrogram based on UPGMA analysis on qualitative and quantitative characters in 20 accessions of *A. racemosus*

similarities in most of the quantitative and all the qualitative characters with the dissimilarity index of 0.318. Both had green cladodes and grayish white stem. The second subcluster was constituted by Ar20, Ar14, Ar6, Ar13, Ar12 and Ar5. This second subcluster further divided in to two sub groups each with three accessions. One sub group consisted of Ar20, Ar14 and Ar 6. These accessions are distinct due to pink purple stem and similarities in CC, CPW, CDW, LI and LCC. Ar13, Ar12 and Ar5 constituted the second subgroup by the presence of profusely branched green stem. Among these accessions, Ar5 stands well apart at a Euclidean distance of 0.433. Ar5 is distinguished from other accessions of this sub group due to the presence of short cladode clusters and lengthy stem. The accessions Ar3, Ar4 and Ar8 constituted the third subcluster of second principal cluster. In this sub group Ar3 and Ar4 grouped together by the presence of lengthy fresh spear and highest plant height.

The cluster pattern revealed that the accessions originated from different Agro-ecological zones of Kerala got themselves grouped in the same clusters. Even though the accessions Ar4 and Ar5 are with same altitude type, rainfall pattern and soil type, they were grouped in different sub groups irrespective of geographical boundaries. Ar3 and Ar16 then Ar19 and Ar16 which were found to be most dissimilar, collected from almost same altitudes (Fig.3). Also the most similar accessions Ar6 and Ar14 are of from regions of wide difference in their geographical conditions. This differential grouping of the accessions indicates that factors other than

geographical specificity may be responsible for genetic divergence which are in conformity with the earlier works of Stebbins (1970) and Rao et al. (1980).

In addition to the morphological markers the molecular markers provide additional tools for germplasm characterization and assessment of genetic relatedness and diversity among collections. These tools have been found to be more dependable than the phenotypic observations for evaluating the variations and in the assessment of the genetic stability. So it is on demand for PCR based markers to measure and confirm the morphological as well as the genetic diversity. They have been used extensively for assessing genetic variation within the species through versatile tools like Random Amplified Polymorphic Markers, Microsatellites and DNA sequence information.

Conclusion

ANOVA revealed significant ($p < 0.05$) morphological variations among accessions in case of all the quantitative vegetative parameters recorded.The highest coefficient of variation estimated for length of cladode clusters followed by plant height, length of fresh spear and number of cladodes per whorl signify the existence of high degree of variability with regard to these traits. It was found that the estimates of genetic variances were smaller than their respective phenotypic variances and greater than their respective environmental variances and there was a close difference between phenotypic and genotypic variance indicating that genotypic component of variation was the major contributor to the total variation and the environment had little effect on the observed phenotypic variations of the traits. The highest value of GCV and PCV for plant height clearly indicated a high degree of genetic variability in the plant height in *A. racemosus* accessions. The PCA, PCoA and the cluster analysis on vegetative morphological characters revealed the existence of variability among the accessions studied. The multivariate analysis recorded considerable variations among the accessions. The results of PCA and PCoA are concurring to the cluster analysis. The analysis suggests that morphological characters such as stem color, length of cladode clusters, plant height, stem diameter, number of cladodes per whorl, length of fresh spear, cladode arrangement, cladode color, branch length, circumference of fresh spear and cladode length are highly variable characters among the accessions of *A. racemosus*. These morpho-traits of *A. racemosus* could be used in future characterization and for breeding program, which stands for the primary steps towards the elite identification of *A. racemosus* germplasm. Here the twenty accessions collected from different regions of Kerala were subjected to same environmental conditions. Therefore the observed variations could be genetic. Further studies need to be carried out based on extensive sampling, which together with molecular characterization may provide more insights to the evolutionary dynamics of this plant.

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