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Karyotype analysis of some species of Asparagales

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Abstract

The order Asparagales is characterized by the presence of raphides and a pigment called phytomelan in the seed coat. There are several taxonomic controversies within this order. In this study, karyotypes of some species from different families of the order Asparagales were studied to assess the phylogenetic relationships and to solve taxonomic problems among these species. These species varied greatly in their chromosome number and morphology. Two parameters namely total chromosome length and average chromosome length were positively correlated. Unweighted pair group method with arithmetic average (UPGMA) based dendrogram and Principal co-orninate analysis (PCOORDA) was also employed to assess the phylogenetic relationships among the species. The dendrogram clearly separated *Ornithogalum virens* from the rest of the species. Principal co-ordninate analysis, however, slightly differed from the dendrogram. This study suggests that *Agapanthus* should not be included in the family Alliaceae. Members of Amaryllidaceae and Hyacinthaceae also showed great variability in their karyotypes. This study shows that using proper statistical data, phylogenetic relationships can be drawn with chromosomal data.

Keywords: *cytology, ImageJ, phylogeny, dendrogram, PCOORDA, PSPP*

INTRODUCTION

The order Asparagales was first circumscribed by Huber [1] on the basis of seed coat characters. Dahlgren *et al.* [2] placed several families including Alliaceae, Hemerocallidaceae, Hyacinthaceae, Amaryllidaceae Asparagaceae etc. in Asparagales. Some of the important characters of Asparagales are the presence of raphides and a pigment called phytomelan which is encrusted in the outer epidermis of testa of capsular fruits (but also in some berry-fruited members) [2]. Additonally, the terminal part of the seed coat is usually collapsed to form a reddish brown or colourless membrane. Relationships among different members within the order have been investigated previously [2-4]. According to Dahlgren *et al.* (1985) [2], *Agapanthus* is included in the family Alliaceae which along with Amaryllidaceae and Hyacinthaceae form one clade within Asparagales. However, APG [5] placed members of Alliaceae, Amaryllidaceae and Agapanthaceae under a single family i.e. Amaryllidaceae. They placed members of Hyacinthaceae under Asparagaceae. Another genus *Hemerocallis* was placed in a separate family, Hemerocallidaceae [2, 6]. Later, it was placed under Xanthorrhoeaceae [5]. Again, the family Hemerocallidaceae has been resurrected [4]. Thus, there are several controversies concerning the relationships among the plants of the order Asparagales.

Karyotype analysis has been proved to be very effective for assessing taxonomic relationships in many cases [7-12]. Comparative karyotype analysis of closely related species has been performed in many cases to explain patterns and directions of chromosomal evolution and to deduce the evolutionary role of karyotype changes [13-18]. Considering the importance of karyotype analysis, the present study was carried out to investigate the effectiveness of karyotype data for the assessment of phylogenetic relationship and to solve the taxonomic controversies among some members of the order Asparagales. This work may form a basis for future studies for assessing phylogenetic relationships in Asparagales with DNA based molecular markers.

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MATERIALS AND METHODS

Materials

Ten species from the order Asparagales were investigated in this study. The name of these species and the families they belong according to Dahlgren *et al*. (1985) [2] are given in table 1.

Methods

Mitotic squash preparations

Root tip meristems of the investigated taxa were used for carrying out mitotic squash preparations. Root tips were collected from the bulbs of the investigated taxa from the potted plants. The root tips were pretreated with 0.05 % colchicine for 2 hours at 12°C. Then, the pretreated root tips were thoroughly washed with distilled water and fixed in 1:3 acetic acid – ethyl alcohol mixture overnight, followed by 3-7 minutes treatment in 45% acetic acid. Root tips were then hydrolysed in 1N HCl at 60°C for 5-7 minutes depending on the optimum results obtained for different species. Squash preparations of the apical 0.5-1.0 mm root tips were made in 2% aceto-orcein following Sharma and Sharma [19].

Table 1: List of investigated taxa with their families (as per Dahlgren *et al.* 1985)

No.	Name of the plant	Family
1	*Nothoscordum fragrans* Kunth.	Alliaceae
2	*Agapanthus africanus* L.	Alliaceae
3	*Hemerocallis fulva* L.	Hemerocallidaceae
4	*Asparagus racemosus* Willd.	Asparagaceae
5	*Amaryllis belladonna* L.	Amaryllidaceae
6	*Haemanthus multiflorus* Martyn	Amaryllidaceae
7	*Zephyranthes rosea* Lindl	Amaryllidaceae
8	*Scilla indica* Baker	Hyacinthaceae
9	*Urginea indica* Kunth.	Hyacinthaceae
10	*Ornithogalum virens* L.	Hyacinthaceae

Karyotype analysis

Prepared slides were examined under a compound microscope under oil immersion lens (x 100). Photomicrographs were taken from the well spread mitotic preparations. Chromosomes were measured with the help of the image processing software ImageJ [20]. Several numerical values were measured for each investigated species. These are Total chromosome length (TCL), Average chromosome length (ACL), Arm Ratio (AR), Disparity index (DI) and total forma percentage or the mean centromeric index value (TF%).

AR was calculated according to Kutarekar and Wanjari [21] by the formula

AR = (Short arm length of the chromosome/ Long arm length of the chromosome)

The chromosomes having the arm ratio less than 0.51 were termed as subtelocentric (st), 0.51 to 0.75 as submetacentric (sm) and 0.76 to 1.0 as metacentric (m).

DI was calculated according to Mohanty *et al.* [22] by the formula

$$D\,I = \frac{\text{Longest chromosome} - \text{shortest chromosome}}{\text{Longest chromosome} + \text{shortest chromosome}} \times 100$$

TF% was calculated in each taxa following Huziwara [23], by the formula

$$TF\% = \frac{\text{Sum of short arm length}}{\text{Sum of total chromosome length}} \times 100$$

Based on the chromosomal data involving the length, the idiograms were built. The chromosomes were arranged according to their length and arm ratio.

Phylogenetic study

Based on the karyotype data, a cluster analysis was carried out to examine karyotype similarity among the species. A data matrix of 10 OTUs (operational taxonomic units) X 8 variables was used. The variables were chromosome number, TCL, ACL, TF %, DI, number of m (metacentric), sm (submetacentric) and st (subtelocentric) chromosomes. To analyze data obtained from the binary matrices, the NTSYS-pc version 2.1 statistical package [24] was used. The similarity matrices were then used to construct dendrograms using Unweighted pair group method with arithmetic average (UPGMA) method. Cophenetic matrix was derived from the dendrogram using the COPH (cophenetic values) program, and the goodness-of-fit of the clustering was calculated by comparing the original similarity matrix with the cophenetic value matrix using the Mantel matrix correspondence test [25] in the MXCOMP program. Principal co-ordinate analysis (PCOORDA) was performed based on the similarity coefficient using the DCENTER module to transform the symmetric similarity matrix to scalar product form. Then, the EIGEN module was used to extract eigenvectors resulting into a three dimensional plot. Linear regression analyses among the 8 variables were also performed using GNU PSPP 0.7.9 statistical package [26].

Results

In the present investigation, 10 species of the order Asparagales were investigated. The metaphase chromosomes of different species are shown in figure 1. Detailed chromosomal measurements of the investigated species are given in table 2. The idiograms are shown in figure 2.

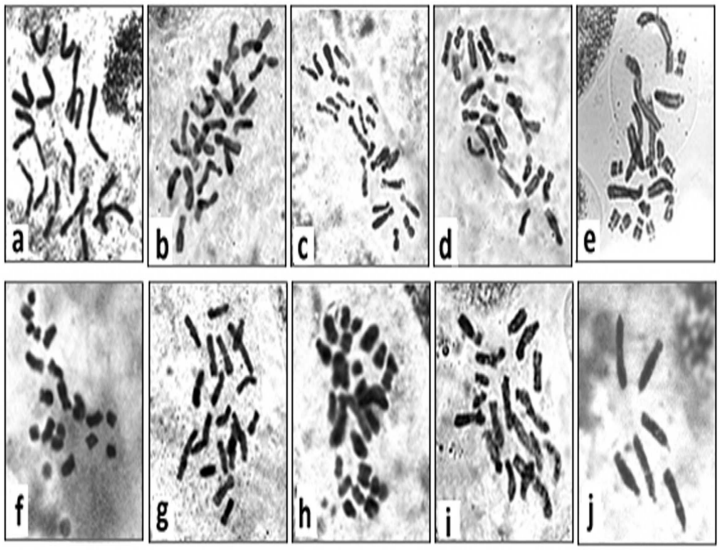

Fig. 1: Mitotic metaphases (ca. x 1000) of (a) *Nothoscordum fragrans*; (b) *Agapanthus africanus*; (c) *Amaryllis belladonna*; (d) *Zephyranthes rosea*; (e) *Haemanthus multiflorus*; (f) *Asparagus recemosus*; (g) *Hemerocallis fulva*; (h) *Scilla indica*; (i) *Urginea indica* and (j) *Ornithogalum virens*.

Table 2: Different chromosomal indices of the investigated taxa. TCL: Total chromosome length; ACL: Average chromosome length; TF: mean centromeric index value; DI: Disparity index; m: number of metacentric chromosomes

Sl. No.	Name of the plant	Chromosome number	Range of chromosome length (µm)	TCL (µm)	ACL (µm)	TF %	DI	m	Sm	st
1	*Nothoscordum fragrans* Kunth.	16	11.7 - 18.0	233.7	14.60	48.39	21.21	16	0	0
2	*Agapanthus africanus* L.	30	3.04 - 8.0	168.16	5.60	38.98	45.65	14	12	4
3	*Hemerocallis fulva* L.	22	7.5 - 18.0	253.5	11.52	38.46	41.17	6	6	10
4	*Asparagus recemosus* Willd.	20	1.6 - 4.6	57.90	2.89	26.82	48.38	4	16	0
5	*Amaryllis belladonna* L.	22	5.7 - 14.06	220.02	10.00	30.20	42.30	4	2	16
6	*Haemanthus multiflorus* Martyn	18	4.69 - 23.49	201.75	11.20	25.23	66.83	0	6	12
7	*Zephyranthes rosea* Lindl	24	3.09 - 8.5	114.69	4.77	37.30	46.67	4	14	6
8	*Scilla indica* Baker	30	1.76 - 7.76	102.4	3.41	40.70	63.02	14	14	2
9	*Urginea indica* Kunth.	20	4.6 - 10.0	163.8	8.19	27.10	36.98	0	2	18
10	*Ornithogalum virens* L.	6	5.3 - 7.0	37.1	6.18	11.85	13.82	0	0	6

Nothoscordum fragrans showed 2n = 16 with long chromosomes. *Agapanthus africanus* showed 2n = 30 with graded karyotype. The three members of the family Amaryllidaceae (*Zephyranthes rosea*, *Amaryllis belladonna* and *Haemanthus multiflorus*) showed clearly different karyotypes with each other. *Zephyranthes rosea* (2n = 24) and *Amaryllis belladonna* (2n = 22) both showed graded karyotype while *Haemanthus multiflorus* (2n = 18) showed bimodal karyotype. Range of chromosome length, TF% and DI were similar in *A. africanus* and *Z. rosea* (table 2). However, *A. africanus* showed not much similarity with other species of Amaryllidaceae. Karyotype of *Hemerocallis fulva* very much resembled with the karyotype of *Amaryllis belladonna* showing 2n = 22 with graded karyotype.

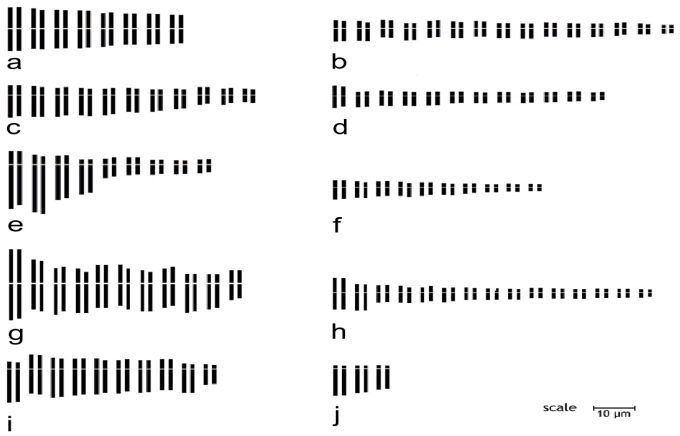

Fig. 2: Idiograms of (a) *Nothoscordum fragrans*; (b) *Agapanthus africanus*; (c) *Amaryllis belladonna*; (d) *Zephyranthes rosea*; (e) *Haemanthus multiflorus*; (f) *Asparagus recemosus*; (g) *Hemerocallis fulva*; (h) *Scilla indica*; (i) *Urginea indica* and (j) *Ornithogalum virens*.

The three members of Hyacinthaceae (*Scilla indica*, *Urginea indica* and *Ornithogalum virens*) showed completely different chromosome numbers and karyotypes. *S. indica* showed 2n = 30; *U. indica* showed 2n = 20 while *O. virens* showed 2n = 6. *Asparagus recemosus* showed 20 very small chromosomes which was unlike any other investigated species.

Linear regression of the different variables showed that only TCL and ACL were significantly correlated with a R^2 value of 0.70. No other variables were significantly related to each other.

UPGMA based dendrogram among different species is shown in figure 3. The goodness of fit between the dendrogram and the similarity matrix was high (r = 0.87950) as shown by the Mantel matrix correspondence test.

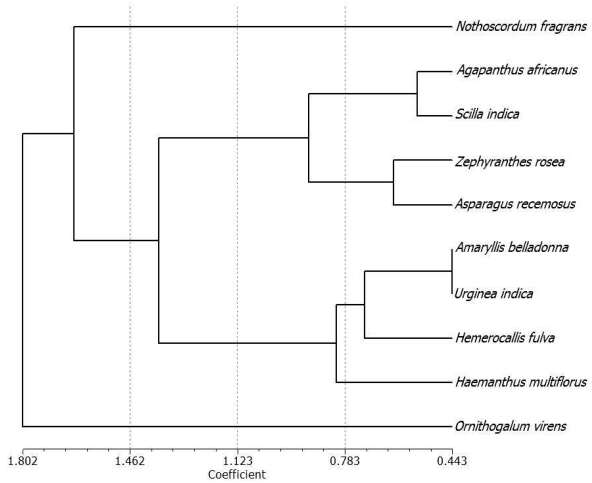

Fig. 3: Relationships among the investigated species of Asparagales using UPGMA based dendrogram based on karyotype data.

The dendrogram clearly separated *O. virens* from the other species. This species was indeed different from the other species as shown by different chromosomal parameters (table 2). Among the others, *N. fragrans* formed a separate clade. The rest of the species formed two subclusters. One subcluster was formed by *A. africanus*, *S. indica*, *Z. rosea* and *A. recemosus*. The other subcluster was formed by *A. belladonna*, *U. indica*, *H. fulva* and *H. multiflorus*. The three dimensional plot obtained with PCOORDA is shown in figure 4. The first three eigen values correspond to 40.76, 32.11 and 19.81% of the total variance, respectively with a cumulative value of 92.70%, which shows that maximum variance

has been described by the first three eigenvalues.

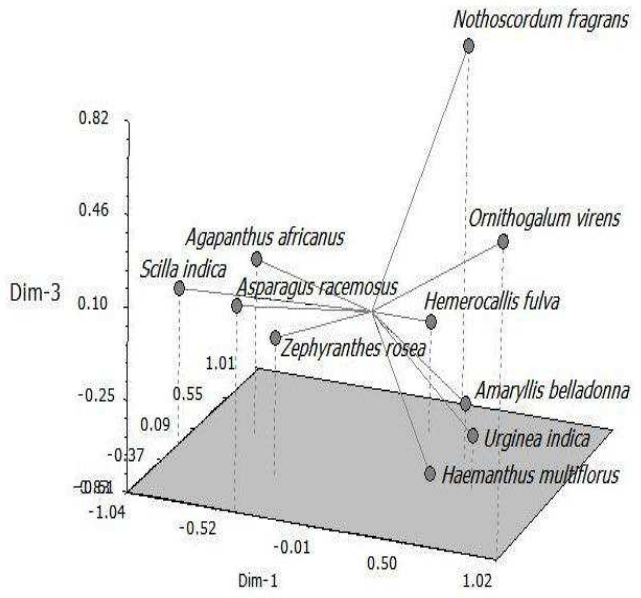

Figure 4: Three dimensional plot of the Principle Coordinate analysis (PCOORDA) of the investigated species based on karyotype data.

The spatial relationship of the 10 species (figure 4) showed similar results as obtained with UPGMA dendrogram. *N. fragrans* and *O. virens* maintained their unique spatial position relative to the other species. The plot showed that the spatial ordination of *H. fulva* was somewhat different from *A. belladonna*, *U. indica* and *H. multiflorus*. Distribution of *A. africanus*, *A. recemosus*, *A. africanus* and *Z. rosea* was similar.

Discussion

This study showed the relationships of different species under the order Asparagales on the basis of karyotype data. *Nothoscordum fragrans* and *Agapanthus africanus* were both included under Alliaceae [2]. However, they were different in several chromosomal parameters like TCL, ACL, TF% and DI, as shown by the present study. The UPGMA dendrogram as well as the three-dimensional plot showed that they are not closely related. Consensus of morphological data, habitat and analytical study supported the removal of *Agapanthus* from Alliaceae [27]. Thus, the present karyotype analysis also supports this view. On the basis of umbellate inflorescence, Hutchinson [28] placed *Agapanthus* under the tribe Agapantheae in the family Amaryllidaceae. In another study, *Agapanthus* was also transferred to Amaryllidaceae as subfamily Agapanthoideae [27]. However, these views were not supported by the present study as all the three members of Amaryllidaceae have not been grouped with *Agapanthus africanus*. Only *Z. rosea* of Amaryllidaceae along with *Asparagus recemosus* formed a sister clade of *A. africanus* in the UPGMA tree.

According to Dahlgren *et al.* [2], the affinities of *Hemerocallis* with other members of Asparagales are uncertain, although seed coat character resembles with Alliaceae. The karyotype of *H. fulva* showed some similarities with *Amaryllis belladonna* of Amaryllidaceae as investigated in the present study. In the dendrogram, *Hemerocallis fulva* grouped with two members of Amaryllidaceae and *Urginea indica*. *H. fulva*, however, maintained its unique position in the three-dimensional plot.

Scilla indica Baker, *Ornithogalum virens* L. and *Urginea indica* Kunth. was placed under the family Hyacinthaceae under the order Asparagales [2]. However, they showed remarkable differences in their karyotypes. According to Dahlgren *et al.* [2], Alliaceae is probably more closely related to Hyacinthaceae than Amaryllidaceae. However, none of the three members of Hyacinthaceae in this study resembled the karyotype of *Nothoscordum. Scilla indica*, however, grouped with *Agapanthus africanus*. APG [5] placed members of Hyacinthaceae under Asparagaceae. The members of Hyacinthaceae were not closer to *Asparagus recemosus* as revealed by the dendrogram and three-dimensional plot. Thus, this study does not support the views of APG [5] in this respect.

In conclusion, this study showed that chromosomal data along with suitable statistical methods can be applied to draw phylogenetic relationships and to solve taxonomic controversies of the plants of Asparagales. This study may be considered as a platform for future studies involving molecular markers.

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