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Research Article

KARYOTYPE ANALYSIS OF FIVE SPECIES OF ALLIUM

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ABSTRACT
Karyotype of some species and varieties of *Allium* was studied. Except *Allium stracheyi*, all other species showed 8 as basic chromosome number. Cultivars of same species did not vary considerably in their karyotypes, although they were morphologically different. UPGMA based dendrogram and Principal co-orninate analysis were also employed to assess the phylogenetic relationships among the species and varieties. The dendrogram clearly separated the bulbous and non-bulbous forms of *Allium*. Principal co-ordninate analysis, however, slightly differed from the dendrogram and clearly separated *A. stracheyi* from other species.

Key words: *Cytology, Imagej, Phylogeny, PCOORDA, UPGMA*

INTRODUCTION
Karyotype analysis has been extensively carried out in plant phylogenetics and diversity studies for more than hundred years. In this era of modern molecular techniques, cytology is still a valuable tool for taxonomy, phylogeny and diversity studies. The information like chromosome number, size and morphology has been of considerable value in understanding interrelationships and delimitation of taxa (Naruhashi and Iwatsubo, 1991). Comparative karyotype analysis of related species has been used in many cases to describe patterns and directions of chromosomal evolution within a group and to infer the evolutionary role of karyotype changes (Sharma and Sharma, 1959; Stebbins, 1971; Watanabe *et al*., 1995; Das *et al*., 1999; Vanzela *et al*., 2000; Shan *et al*., 2003). Karyotype analysis has been proved to be useful in many cases including *Borago* (Selvi *et al.*, 2006), *Sideritis* (Esra *et al*., 2008); *Secale* (Masoud and Ali-Jarrahei, 2008); *Artemisia* (Naseri *et al*., 2009); *Lathyrus* (Badr *et al.*, 2009) and so on. Karyotype analysis has been successfully employed at the intraspecific level in several cases including the study of cultivars of *Agave tequilana* (Guadalupe *et al*., 2008); *Gossypium hirsutum* (Sheidai *et al.*, 2008) and populations of *Bidens pilosa* (Maria *et al.*, 2008).
The chromosomes of *Allium* have been studied for decades (Sharma and Aiyangar, 1961; Koul and Gohil, 1970; Konvička and Levan, 1972; Gohil and Kaul, 1980; Puizina and Papeš, 1996; Fritsch *et al*., 2001; Cui *et al*., 2008) for their diversity in size, structure and number. The variations in karyotypes are common both between and within species (Cui *et al.*, 2008). Vijayavalli and Mathew (1990) reported the existence of intraspecific polyploidy within several species of *Allium*. They have also reported that chromosomal difference is also associated with morphological difference in some cases.
In the present study, karyotyping along with dendrogram and scatter plots was conducted in some economically important species and varieties of *Allium* in order to investigate the applicability of karyotype data for the phylogenetic relationship and affinities of the different species.

MATERIALS AND METHODS
Materials
Five species of *Allium* were investigated in this study. These included three varieties including eight cultivars of *Allium cepa* L., five cultivars of *Allium sativum* L., four cultivars of *Allium porrum* L. and two other species of *Allium* (*Allium tuberosum* Rottl. and *Allium stracheyi* Baker). Name of the *Allium* species, varieties and cultivars along with their brief morphological features are given in Table 1.

Table 1: List of the *Allium* species investigated the present study along with their morphological features.

Species/variety	Cultivar	Morphological features
Allium cepa L. var *cepa* Helm.	Punjab selection	Bulb 5-6 cm wide, globular, red skin
	Pusa white round	Bulb 4-6 cm wide, flatish round, white skin
	Agrifound light red	Bulb 4-6 cm, gobular, light red skin
	Pusa red	Bulb 4-6 cm, flat to globular, bronze red skin
	Sukh Sagar	bulb Long, 4-7 cm wide, tapering towards the neck, dark red skin
	Patna red	Bulb 4-6 cm, globular, pinkish red skin
	Puna red	Bulb 3-5 cm wide, round, dark red skin
	Spring onion	Non bulbous, clustered, leaf sheathes form a long, white, stem like structure at the base
Allium cepa L. var. *aggregatum* G. Don	---------	Small cluster of 3-4 bulbs, bulbs 1-1.5 cm wide, skin red
A. cepa L. var. *viviparum* (Metzger) Alefeld.	---------	Bulb tunicate, small, 2-3 cm wide, pale red
Allium sativum L.	Yamuna Safed	Bulbs compact, creamy white, bulb diameter 3.5 – 4.0 cm; cloves sickle shaped, 25 – 30 cloves per bulb, moderately pungent.
	Agrifound Parvati	Larger bulb, 5.0 – 6.0 cm in diameter; cloves 1.5 – 1.8 cm, brownish white, 10 – 16 cloves per bulb, moderately pungent.
	Bote lasun small	Bulbs small, 3.0 – 3.5 cm in diameter; 10 – 13 cloves per bulb, sometimes with aerial bulblets, highly pungent.
	Bote lasun large	Bulb large, 5.0 – 6.0 cm in diameter, with aerial bulblets on a long, hard stalk developed from the centre of the bulb, highly pungent.
	Single clove	Single small bulb; 3 – 3.5 cm in diameter, highly pungent.
Allium porrum L.	Armor	Pseudostem diameter 2.3-2.7 cm; Pseudostem length 13-15 cm; Leaf diameter 2.8-3.1 cm
	Alto	Pseudostem diameter 2.8-3.1 cm; Pseudostem length 20-21 cm; Leaf diameter 2.7-3.1 cm
	Selecta	Pseudostem diameter 1.8-2.2 cm; Pseudostem length 17-19 cm; Leaf diameter 2.1-2.2 cm
	Maridor	Pseudostem diameter 2.6-2.8 cm; Pseudostem length 10-12 cm; Leaf diameter 2.2-2.4 cm
Allium stracheyi Baker	-----	Perennial slender herb; leaves 3-12; narrowly linear; flowers light yellow
Allium tuberosum Rottl.	-----	Perennial rhizomatous herb; with tubers, leaves flat, 5 cm wide, typically bend outwards at the tip, flowers white

Mitotic squash preparations

Mitotic squash preparations were carried out on root tip meristems of the investigated taxa. Roots were generated by sowing the bulbs of the investigated taxa of *Allium* in sand. Roots of *Allium tuberosum* were collected from germinated seedlings. Root tips of the related taxa of *Allium* were collected from the potted plants. All the root tips were collected at the periods showing peak mitotic frequency i.e. between

10 a.m. to 11 a.m. The root tips were pretreated with 0.05 % colchicine for 2 hours at 12°C. The pretreated root tips were then 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 hydrolysed in 1N HCl at 60°C for 5-7 minutes as suitable 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, 1980.

Karyotype analysis

Prepared slides were examined under a compound microscope under oil immersion lens (x 100). Photomicrographs were taken from the well spread preparations. All measurements were taken using the software ImajeJ (Abramoff *et al*., 2004) downloaded from http://rsbweb.nih.gov/ij/download.html.

The following numerical values were measured for each investigated taxa

1) Total chromosome length (TCL)
2) Average chromosome length (ACL)
3) Arm ratio (AR) was calculated according to Kutarekar and Wanjari (1983) 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).

1) Disparity index (DI) was calculated according to Mohanty *et al.* (1991) by the formula

$$DI = \text{x } 100 \quad \frac{\text{Longest chromosome – shortest chromosome}}{\text{Longest chromosome + shortest chromosome}}$$

2) The total forma percentage or the mean centromeric index value (TF%) was calculated in each taxa following Huziwara (1962), by the formula

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

Based on the data relating to the length, the idiograms were presented. The chromosomes were arranged according to their length, arm ratio and position of the secondary constrictions, if present.

Based on the karyotype data, a cluster analysis was carried out to examine karyotype similarity among species and varieties. For *Allium cepa* var. *cepa*, *Allium sativum* and *Allium porrum*, average values of the variables for all cultivars were used. A data matrix of 7 OTUs (operational taxonomic units) X 9 variables was used. The variables were TCL, ACL, TF %, DI, number of m, sm, st chromosomes and number of satellite pairs. To analyze data obtained from the binary matrices, the NTSYS-pc version 2.1 statistical package (Rohlf, 2000) was used. The similarity matrices were then used to construct dendrograms using Unweighted pair group method with arithmetic average (UPGMA) method. Cophenetic matrices were derived from the dendrograms using the COPH (cophenetic values) program and the goodness-of-fit of the clustering was calculated by comparing the original similarity matrices with the cophenetic value matrices using the Mantel matrix correspondence test (Mantel, 1967) in the MXCOMP program. Principal co-ordinate analysis (PCOORDA) was performed based on the similarity coefficient using DCENTER module to transform the symmetric similarity matrix to scalar product form and then EIGEN module was used to extract eigenvectors resulting into a two dimensional plot.

RESULTS

In the present investigation, 5 species of *Allium* with their varieties and cultivars were investigated. Within *Allium*, only *A. stracheyi* showed 2n = 14 metaphase chromosomes (figure 1). *Allium cepa* and *A. sativum* showed 2n = 16. *A. porrum* and *A. tuberosum* were tetraploid (2n = 4x = 32). *A. cepa* var. *viviparum* showed 24 metaphase chromosomes. In *A. cepa* and *A. sativum*, one pair of chromosome showed secondary constrictions. *A. cepa* var. *viviparum* showed 24 chromosomes of which 16 chromosomes formed eight pairs but rest of the eight chromosomes remained unpaired (figure 2).

Table 2: Different chromosomal indices of all the investigated species and cultivars of *Allium*. TCL: Total chromosome length; ACL: Average chromosome length; TF: mean centromeric index value; DI: Disparity index; m: number of metacentric chromosomes, sm: number of submetacentric chromosomes; st: number of subtelocentric chromosomes; SAT: number of pairs containing satellites.

Species	Chromosome number	cultivar	TCL (µm)	ACL (µm)	TF %	DI	m	sm	st	SAT
Allium cepa var. *cepa*	16	Punjab selection	148.68	9.29	43.91	34.20	14	0	2	1
		Pusa white round	137.85	8.62	48.24	37.08	14	0	2	1
		Agrifound light red	161.67	10.10	43.96	29.20	14	0	2	1
		Sukh Sagar	150.69	9.42	43.90	38.49	14	0	2	1
		Pusa red	174.20	10.89	43.79	29.75	14	0	2	1
		Puna red	156.41	9.78	44.03	34.33	14	0	2	1
		Patna red	176.23	11.01	43.73	26.72	14	0	2	1
		Spring onion	187.90	11.74	42.97	27.70	14	0	2	1
		Average value	**161.70**	**10.11**	**44.32**	**32.18**	**14**	**0**	**2**	**1**
Allium cepa var. *aggregatum*	16	---------	175.50	10.97	44.02	27.84	14	0	2	1
Allium cepa var. *viviparum*	24	---------	253.57	10.56	41.36	32.85	11	10	3	0
Allium sativum	16	Single clove	205.60	12.85	45.63	36.25	14	2	0	1
		Yamuna Safed	199.35	12.46	45.93	39.25	14	2	0	1
		Bote lasun small	181.49	11.34	45.74	29.76	14	2	0	1
		Bote lasun large	204.34	12.77	45.92	34.46	14	2	0	1
		Agrifound Parvati	185.49	11.59	45.66	30.04	14	2	0	1
		Average value	**195.25**	**12.20**	**45.78**	**33.95**	**14**	**2**	**0**	**1**
Allium porrum	32	Armor	298.72	9.33	43.51	49.13	28	4	0	2
		Alto	268.55	8.39	42.04	45.40	28	4	0	2
		Selecta	259.33	8.10	43.32	60.86	28	4	0	2
		Maridor	258.82	8.09	43.38	60.26	28	4	0	2
		Average value	**271.36**	**8.48**	**43.06**	**53.91**	**28**	**4**	**0**	**2**
Allium stracheyi	18	---------	244.78	14.48	44.11	18.75	8	6	0	0
Allium tuberosum	32	---------	323.16	10.10	42.55	45.02	22	6	4	0

Detailed chromosomal measurements of the *Allium* species are given in table 2. Relationships among different *Allium* species are shown in figure 3.

The UPGMA based dendrogram (figure 3a) clearly divides the *Allium* species in two groups. The first group contains *A. tuberosum*, *A. styracheyi*, *A. porrum* and *A. cepa* var. *viviparum*. The secoond group consists of *A. sativum*, *A. cepa* var. *cepa* and *A. cepa* var.

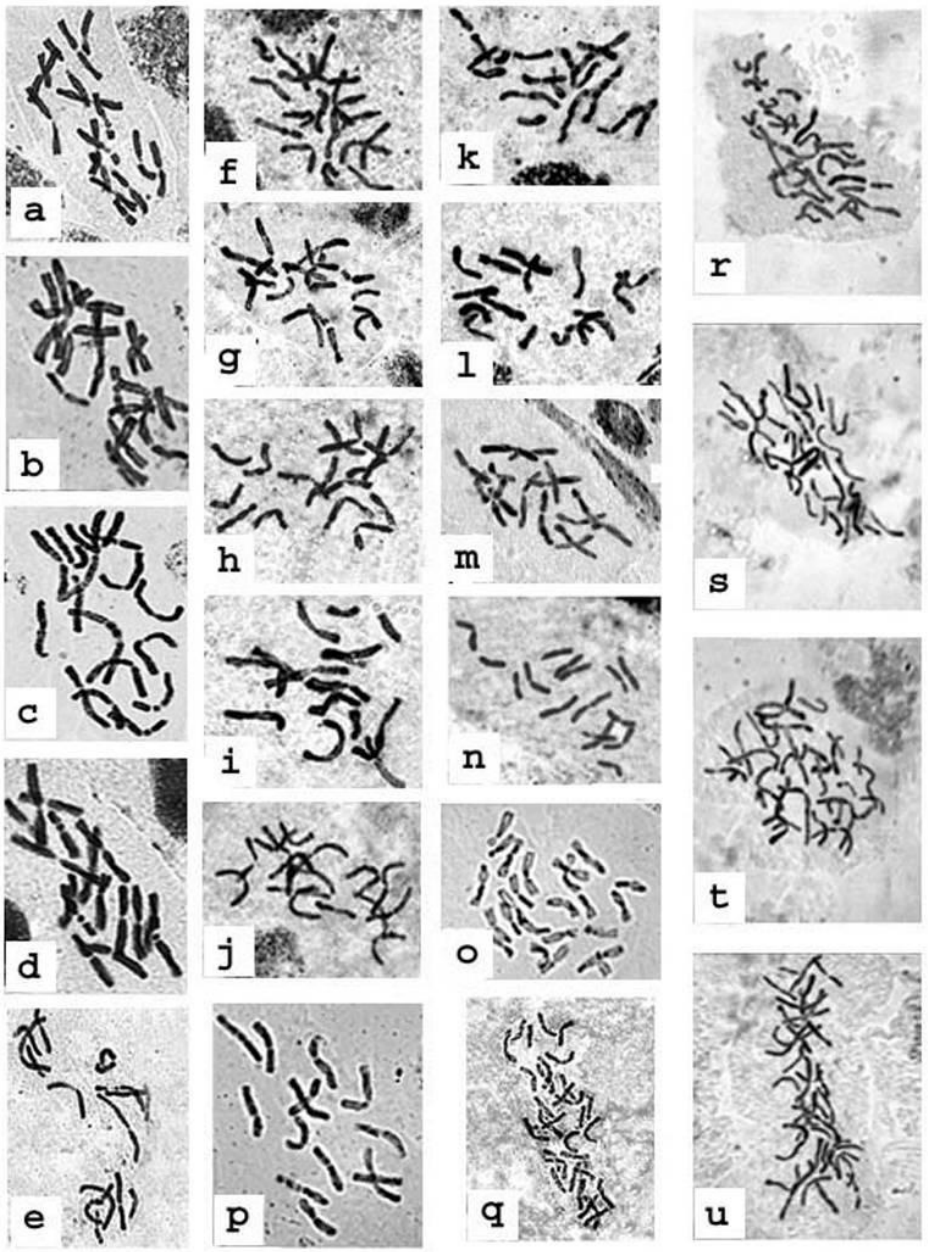

Figure 1: Mitotic metaphase plates of the *Allium* species. a-e: *a:* *sativum* cultivars. a: Single clove; **b:** Yamuna Safed; **c:** Bote lasun small; **d:** Bote lasun large; **e:** Agrifound parvati. **f-m:** *A. cepa* var. *cepa* cultivars. **f:** Punjab selection; **g:** Pusa white round; **h:** Agrifound light red; **i:** Sukh Sagar; **j:** Pusa red; **k:** Puna red; **l:** Patna red; **m:** Spring onion; **n:** *A. cepa* var. *aggregatum*; **o:** *A. cepa* var. *viviparum*; **p:** *A. stracheyi*, **q:** *A. tuberosum*; **r-u:** *A. porrum* cultivars. **r:** Armor; **s:** Alto; **t:** Selecta and **u:** Maridor.

aggregatum. It is interesting that *A. cepa* var. *viviparum* did not group with other varieties of *A. cepa*. Its chromosome number, TCL, ACL, TF and DI are also different from other varieties of *A. cepa* (Table 2). However, the PCOORDA plot showed that *A. cepa* var. *viviparum* is different from all other taxa (figure 3b). This also clearly separated *A. stracheyi* from other species.

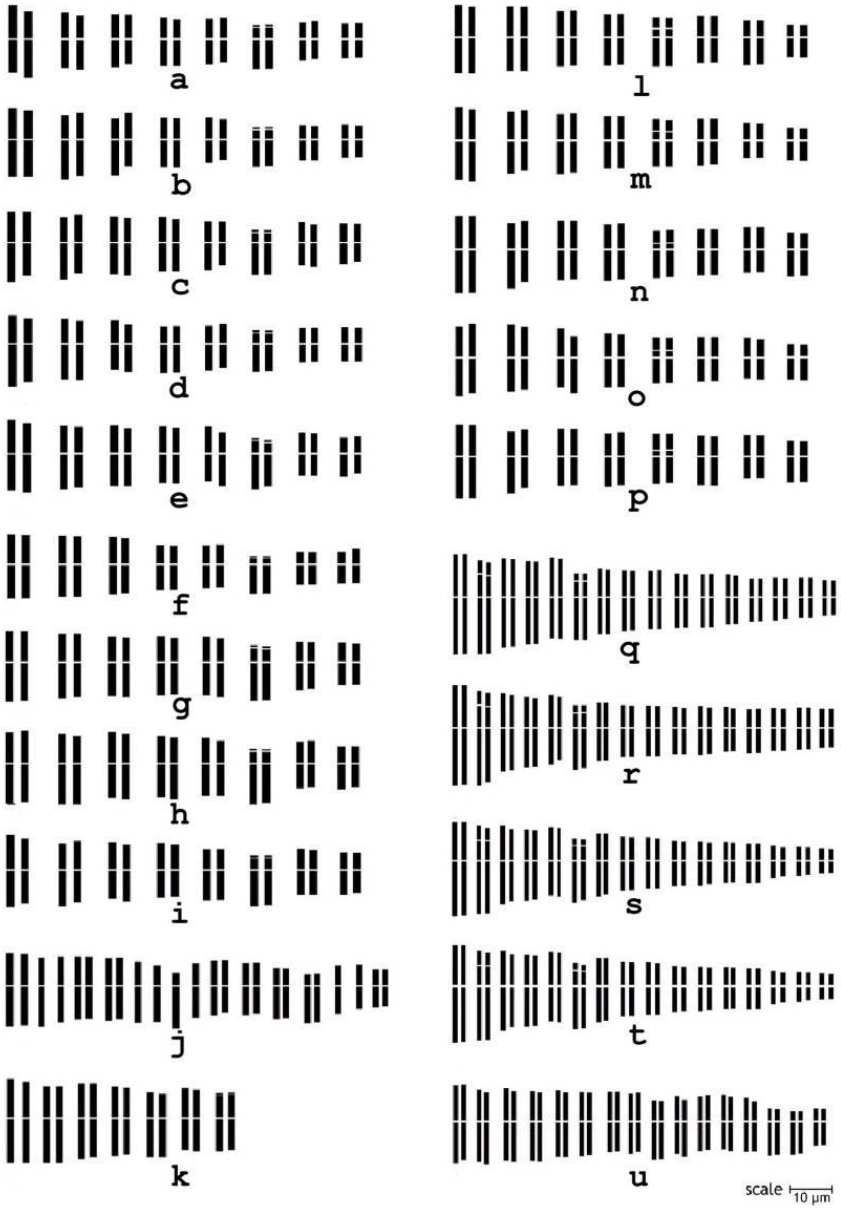

Figure 2: Idiograms of *Allium* species. a-h: a: Punjab selection; **b:** Pusa white round; **c:** Agrifound light red; **d:** Sukh Sagar; **e:** Pusa red. **f:** Puna red; **g:** Patna red; **h:** Spring onion; **i:** *A. cepa* var. *aggregatum*; **j:** *A. cepa* var. *viviparum*; **k:** *A. stracheyi*; **l-p:** *A. sativum*. **l:** Single clove; **m:** Yamuna Safed; **n:** Bote lasun small; **o:** Bote lasun large; **p:** Agrifound parvati. **q-t:** *A. porrum*. **q:** Armor; **r:** Alto; **s:** Selecta; **t:** Maridor. **u:** *A. tuberosum.*

DISCUSSION

In this study, five cultivars of *Allium sativum*, three varieties and eight cultivars of *Allium cepa*, four cultivars of *Allium porrum* and two other species of *Allium* were cytologically investigated.. Of these, *A. cepa* (except *A. cepa* var. *viviparum*) and *A. sativum* showed 2n = 16 and *A. stracheyi* showed 2n = 14. *A. cepa* var. *viviparum* showed 2n = 3x = 24. *A. tuberosum* and *A. porrum* showed 2n = 4x = 32. Thus, except *A. stracheyi*, all other species showed a basic chromosome number of x = 8.

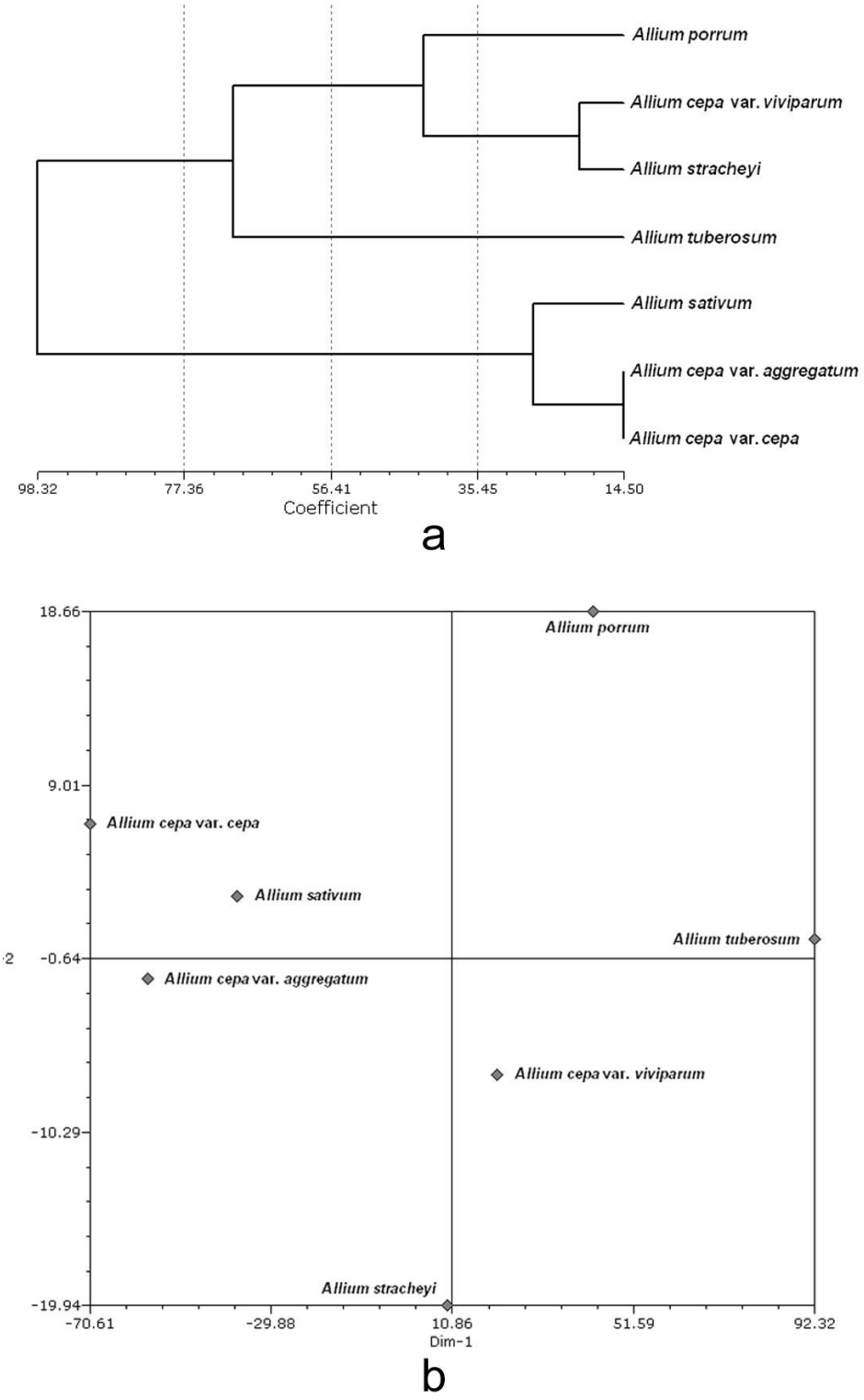

Figure 3: Relationships among different species of *Allium*. a) UPGMA based dendrogram of the *Allium* species based on karyotype data. **b)** two dimensional plot of the Principle Coordinate analysis (PCOORDA) of the *Allium* species based on karyotype data.

Cultivars of common onion (*A. cepa* var. *cepa*) varied considerably in their bulb morphology (Table 1). However, these differences have not been clearly reflected in their karyotypes. Different indices like total chromosome length, average chromosome length, disparity index and centromeric index did not vary significantly (Table 2) in these cultivars. Chromosomes of *A. cepa* var. *aggregatum* also did not vary considerably with chromosomes of *A. cepa* var. *cepa*. Except that they were slightly shorter than the later. *A. cepa* var. *viviparum* showed 24 chromosomes of which 16 chromosomes formed eight pairs but rest of the eight chromosomes remained unpaired. This observation supports the works of Puizina and Papeš (1997), who also observed 16 paired and 8 unpaired chromosomes in this plant, showing the origin of these unpaired chromosomes from distantly related species through hybridization. In *A. sativum* and *A. porrum*, chromosome morphology did not vary significantly at the intraspecific level although they were morphologically quite different.

Hanelt *et al.*, (1992) placed *Allium sativum* and *A. porrum* together under the same section Allium of the subgenus Allium of the genus *Allium*. However, the present chromosomal analysis did not reveal any significant resemblance between the karyological features of these two species. The average chromosome length (ACL) of *A. porrum* was 8.55 while ACL of *A. sativum* was 12.2. Disparity index (DI of *A. porrum* and *A. sativum* was 53.91 and 33.95, respectively) and centromeric index (TF % of *A. porrum* and *A. sativum* was 43.42 and 45.78, respectively) also varied considerably between these two species. *A. porrum* showed two pairs of satellite chromosome but they did not show any resemblance with the satellite bearing chromosome of *A. sativum*. This observation supports the previous study by Vijayavalli and Mathew (1990), who also did not found any similarity between the satellite bearing chromosomes of these two species. The dendrogram as well as scatter plot also placed these two species distantly. *Allium stracheyi* was the only member showing 2n = 14 chromosomes. Sharma and Aiyangar (1961) found B chromosomes in this species which has not been observed in the present study. Also, different indices like TCL, ACL and DI also did not match with other species of *Allium*. The dendrogram showed that the non bulbous and bulbous *Allium* species grouped separately. Thus, *A. cepa* var. *viviparum* grouped with *A. stracheyi, A. tuberosum* and *A. porrum*. This should be further studied with detail.

In conclusion, this study shows that karyotyping along with suitable statistical method was successfully applied in the phylogenetic studies of *Allium*. This syudy also showed that karyotype based dendrogram clearly differentiated bulbous and non-bulbous *Allium* species.

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