KARYOMORPHOLOGICAL STUDIES IN FOUR SEED SPICES OF UMBELLIFERAE

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Abstract: Karyomorphological studies were performed in four seed spices of Umbelliferae (the species also possesses immense therapeutic uses) namely, *Apium graveolens* L. (celery), *Cuminum cyminum* L. (cumin), *Foeniculum vulgare* Mill. (fennel) and *Trachyspermum amni* L. (ajowan) and it revealed six (celery: $2n = 22 = 4D^{sc}_{sm} + 2D_{sm} + 2C^{sc}_{m} + 10C_{m} + 2J_{m} + 2K_{sm}$; cumin: $2n = 14 = 2D_{sm} + 2E^{sc}_{st} + 4E_{st} + 2G_{sm} + 2H_{st} + 2I_{t}$ and ajowan: $2n = 18 = 2A^{sc}_{sm} + 2B_{st} + 2C^{sc}_{m} + 4C_{m} + 2D_{sm} + 6E_{st}$) and four (fennel: $2n = 22 = 8C_{m} + 4D^{sc}_{sm} + 2D_{sm} + 8F_{m}$) morphologically distinct chromosome types. Metacentric chromosomes were prevalent in celery and fennel; while, a telocentric pair was located in cumin. Characteristically two long and two short pairs of chromosomes were marked in ajowan and celery respectively. Total haploid chromatin length was noted to be 30.41 µm ± 2.30 in celery, 19.04 µm ± 1.61 in cumin, 29.12 µm ± 2.73 in fennel and 32.45 µm ± 3.52 in ajowan. Celery and fennel were found to possess symmetric karyotypes. Satellites in all the cases were associated to short arms.

Keywords: Karyomorphology, Seed spices, Umbelliferae

INTRODUCTION

Apium graveolens L. (celery), *Cuminum cyminum* L. (cumin), *Foeniculum vulgare* Mill. (fennel) and *Trachyspermum amni* L. (ajowan) are important seed spices of the family Umbelliferae not withstanding their significance as potential medicinal herbs (Pruthi 1998; Masoud *et al.* 2007). The spices are inadequately explored from the cytological point of view (Paul 2005; Ghaffari and Tajik 2007; Masoud *et al.* 2007; Zhao *et al.* 2011); although, cytological information is a prerequisite for crop improvement through efficient breeding methodologies. Paul and Datta (2003) provided a preliminary report on the cytological status of the above mentioned seed spices. Present investigation describes karyomorphology of celery, cumin, fennel and ajowan with the view to gather cytological information for effective exploration of the spices for further research on cytogenetical aspects, and subsequently crop improvement.

MATERIAL AND METHOD

Seeds of celery (*Apium graveolens* L.), cumin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* Mill.) and ajowan (*Trachyspermum amni* L.) belonging to the family Umbelliferae (Apiaceae) were obtained from Zonal Adaptive Govt. Research Station, Krishnanagar, West Bengal, India, and for karyomorphological analysis seed samples were presoaked (over night in distilled water) and allowed to germinate (18ºC ± 1ºC) in petriplates lined with moist filter papers. Healthy root samples (2-3 mm in sizes) were pretreated (supersaturated para-dichlorobenzene and aesculine mixture for 3 to 4 hours at 16ºC ± 1ºC; initially samples were kept at 0ºC - 4ºC for 10 minutes), fixed (propiono-alcohol 1:3, v/v), stained (2% orcein-1N HCl mixture 9:1 following warming for 10–12 mins.) and subsequently the root tips were squashed in 45% acetic acid in a glass slide before observing under the microscope. Well scattered and properly condensed metaphase plates (on an average 3 to 5) were scored for each plant species. Data for karyotype analysis was computed from camera lucida drawings (magnification- × 1500).

Metaphase chromosomes of four seed spices were classified as metacentric (F%: 40.0 to 50.0), sub-metacentric (F%: 30.0 to 39.99), sub-telocentric (F%: 20.0 to 29.99) and telocentric (F%: 1.0 to 19.99) as per Hirahara and Tatuno (1967). Karyomorphological attributes analyzed were mean length of individual chromosome measured in µm, relative length of each chromosome represented as per cent length of longest chromosome, total form per cent (TF%) represented the total length of short arms as per cent of total chromosome complement (Huziwara 1962), percentage of total chromatin length (TCL) determined from total length of a chromosome divided by total length of chromosome complement × 100, S per cent representing relative length of the smallest chromosome × 100 and total haploid chromatin length measured in µm. Karyotype formulas were also assessed.

On the basis of chromosome length (long: >3.75 µm, medium: 2.5 µm to 3.75 µm, small: >1.5 µm to <2.5 µm and very small: ≤1.5 µm), F% and presence or absence of secondary constriction the chromosomes in the species were morphologically classified into following types: type A – long sub-metacentric chromosomes with satellites; type B – long sub-telocentric chromosomes; type C – medium metacentric chromosomes with or without satellites; type D – medium sub-metacentric chromosomes with or without satellites; type E – medium sub-telocentric chromosomes with or without satellites; type F – small metacentric chromosomes; type G – small sub-

metacentric chromosomes; type H – small sub-telocentric chromosomes; type I – small telocentric chromosomes; type J – very small metacentric chromosomes; type K – very small sub-metacentric chromosomes.

Metaphase plates were photomicrographed from temporary squash preparations.

FIGURE LEGENDS

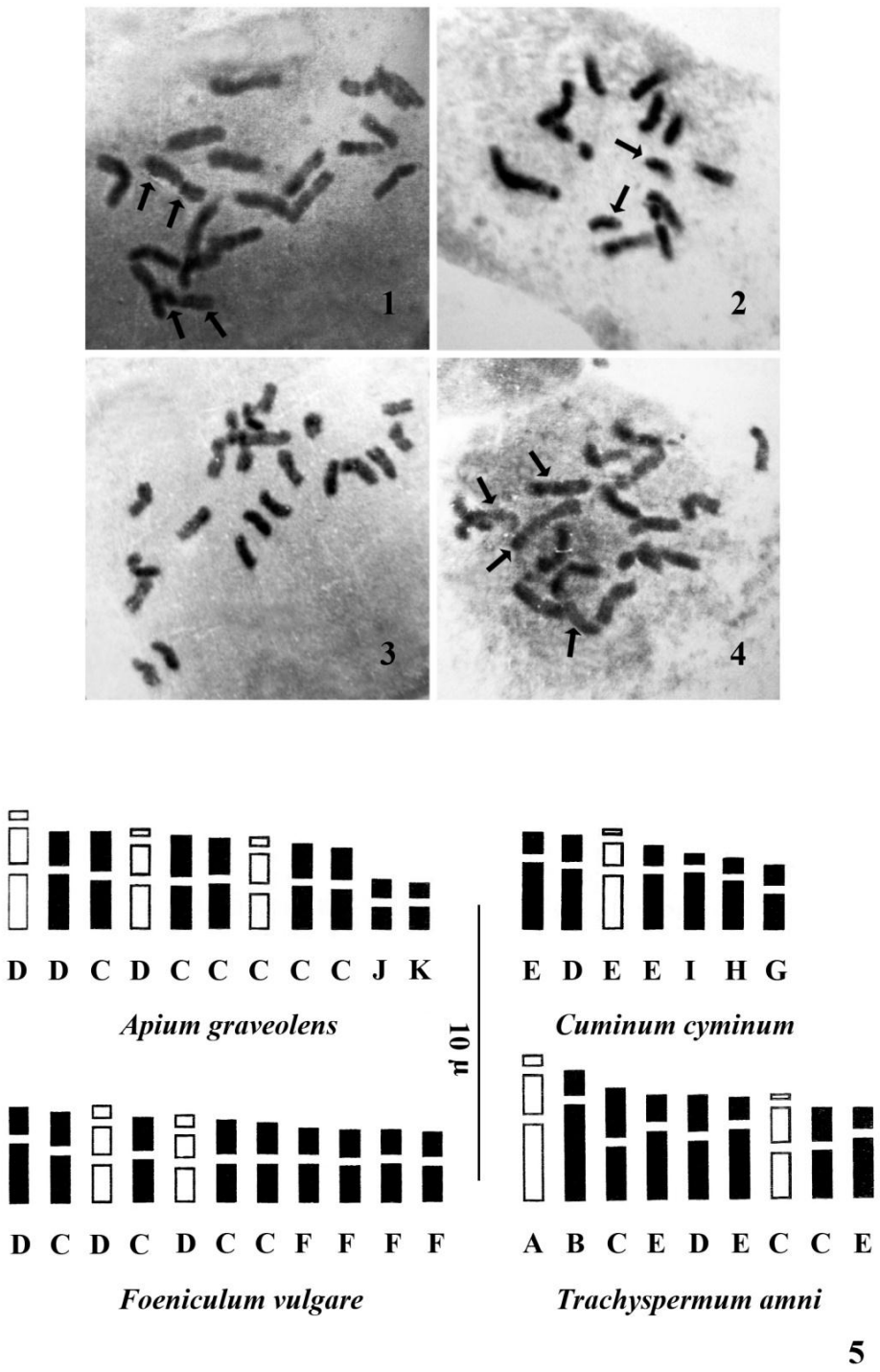

Figure plate I (1-5) showing metaphase chromosome complements **(1-4)** and idiograms **(5)** in seed spices of Umbelliferae. **(1)** *Apium graveolens* – 2n=22; **(2)** *Cuminum cyminum* – 2n=14, a telocentric pair marked; **(3)** *Foeniculum vulgare* – 2n=22; **(4)** *Trachyspermum amni* – 2n=18; **(5)** idiograms.

RESULT AND DISCUSSION

Karyomorphological parameters are presented in Table 1. Karyotype analysis revealed six (*A. graveolens*: $2n = 22 = 4D^{sc}_{sm} + 2D_{sm} + 2C^{sc}_{m} + 10C_m + 2J_m + 2K_{sm}$; *C. cyminum*: $2n = 14 = 2D_{sm} + 2E^{sc}_{st} + 4E_{st} + 2G_{sm} + 2H_{st} + 2I_t$ and *T. amni*: $2n = 18 = 2A^{sc}_{sm} + 2B_{st} + 2C^{sc}_{m} + 4C_m + 2D_{sm} + 6E_{st}$) and four (*F. vulgare*: $2n = 22 = 8C_m + 4D^{sc}_{sm} + 2D_{sm} + 8F_m$) morphologically distinct chromosome types in the seed spices of Umbelliferae (Figs. 1-5). Satellites in all the cases were associated to short arms. Metacentric chromosomes were prevalent in *A. graveolens* and *F. vulgare*; while, a telocentric pair was noted in *C. cyminum* (Fig. 2). Characteristically two long and two short pairs of chromosomes were marked in *T. amni* (Fig. 4) and *A. graveolens* (Fig. 1) respectively. Total haploid chromatin length, TF% and S% were observed to be 30.41 µm ± 2.30, 42.03 and 37.60 in *A. graveolens*; 19.04 µm ± 1.61, 26.63 and 63.98 in *C. cyminum*; 29.12 µm ± 2.73, 40.00 and 69.59 in *F. vulgare* and 32.45 µm ± 3.52, 33.04 and 62.92 in *T. amni* respectively. The somatic complement of *A. graveolens* and *F. vulgare* were symmetric in nature.

The chromosome number is in confirmity to earlier reports (Sharma and Ghosh 1954; Darlington and Wylie 1955; Bell and Constance 1957; Baijal and Kaul 1973; Ghaffari and Tajik 2007) but Subramanian (1986) suggested 2n = 18 chromosomes for the species. Baijal and Kaul (1973) reported seven chromosome types (A to G; four pairs A–D with sub-metacentric primary constrictions, rest had sub-telocentric constrictions) and the chromosomes length was found to vary between 2.32 µm and 4.33 µm (relative length – 63.95% to 100.0%) possessing three pairs of satellited chromosomes. On the contrary, Subramanian (1986) reported four chromosome types (4F + 4G + 6H + 4K) with one pair of chromosomes with satellites (length: 1.6 µm to 2.6 µm).

Chromosome number (2n = 22) of fennel observed in the present investigation is in accordance to earlier reports (Sharma and Ghosh 1954; Raghuvanshi and Joshi 1966; Hore 1976; Subramanian 1986; Deng *et al.* 2006). Subramanian (1986) designated three chromosome types (2n = 22; 4F + 10H + 8K) with seven pairs of chromosomes with median primary constrictions and four pairs with sub terminal centromeres. The author reported that the complement possesses one pair of chromosomes with secondary constrictions (length: 1.6 µm to 2.6 µm). Deng *et al.* (2006) described symmetric karyotype in fennel with predominance of metacentric chromosomes (ten pairs) along with a sub-metacentric pair. Masoud *et al.* (2007) also reported 2n = 22 chromosomes from 13 different populations collected from Iran. Zhao *et al.* (2011) performed karyotype analysis in *A. graveolens* (2n = 22) and the chromosome complement was found to possess three pairs metacentric, one pair sub-metacentric, four pairs acrocentric and three pairs telocentric chromosomes.

Table 1. Karyomorphological details in four spice yielding plants

Plant species	Chromosome number (2*n*)	Chromosome types	Range in the complement				Haploid chromosome length (µm)	TF%
			Chromosome length (µm)	Relative length (%)	TCL (%)	F%		
Apium graveolens	22	3D+6C+1J+1K	1.41-3.75	37.60-100.0	4.64-12.33	36.00-49.02	30.41±2.30	42.03
Cuminum cyminum	14	1D+3E+1G+1H+1I	2.06-3.22	63.95-100.0	10.82-16.91	16.60-36.41	19.04±1.61	26.63
Foeniculum vulgare	22	4C+3D+4F	2.22-3.19	69.59-100.0	7.62-10.95	31.35-46.99	29.12±2.73	40.00
Trachyspermum amni	18	1A+1B+3C+1D+3E	2.97-4.72	62.92-100.0	9.15-14.55	21.17-48.27	32.45±3.52	33.04

Karyotype formula:
Apium graveolens: $4D^{sc}_{sm}+2D_{sm}+2C^{sc}_{m}+10C_m+2J_m+2K_{sm}$
Cuminum cyminum: $4D_{sm}+2E^{sc}_{st}+4E_{st}+2G_{sm}+2H_{st}+2I_t$
Foeniculum vulgare: $8C_m+4D^{sc}_{sm}+2D_{sm}+8F_m$
Trachyspermum amni: $2A^{sc}_{sm}+2B_{st}+2C^{sc}_{m}+4C_m+2D_{sm}+6E_{st}$

CONCLUSION

Karyomorphological data obtained in four seed spices may be effectively explored in designing future research on cytogenetical aspects of the species.

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