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AN IMPROVED TECHNIQUE FOR THE KARYOTYPE STUDY OF AN ECONOMICALLY IMPORTANT FAMILY UMBELLIFERAE

INDIA has got a large number of Umbelliferous species many of which are economically important as spices, vegetables and medicinal plants. Chromosome number of Umbellifers has been studied by many authors (*Wanscher, 1933; *Delay, 1947; *Garde and Garde, 1949, 1954; *Hakansson, 1953; *Sharma and Ghosh 1954; *Sharma and Bhattacharyya, 1959; *Bell and Constance, 1960, 1966; *Gadella and Kliphuis, 1967; *Cauwet, 1971). With one or two exceptions detailed studies of chromosome morphology of the members of this family which can elucidate structure and behaviour of karyotype and their interrelationships are yet to be made. Moore[3] pointed out that Umbelliferous cytotaxonomy is still at the 'alpha' level. This work involved the evolution of special pretreatment and fixation schedules for a proper study of chromosome morphology of members of Umbelliferae and their cytological implications.

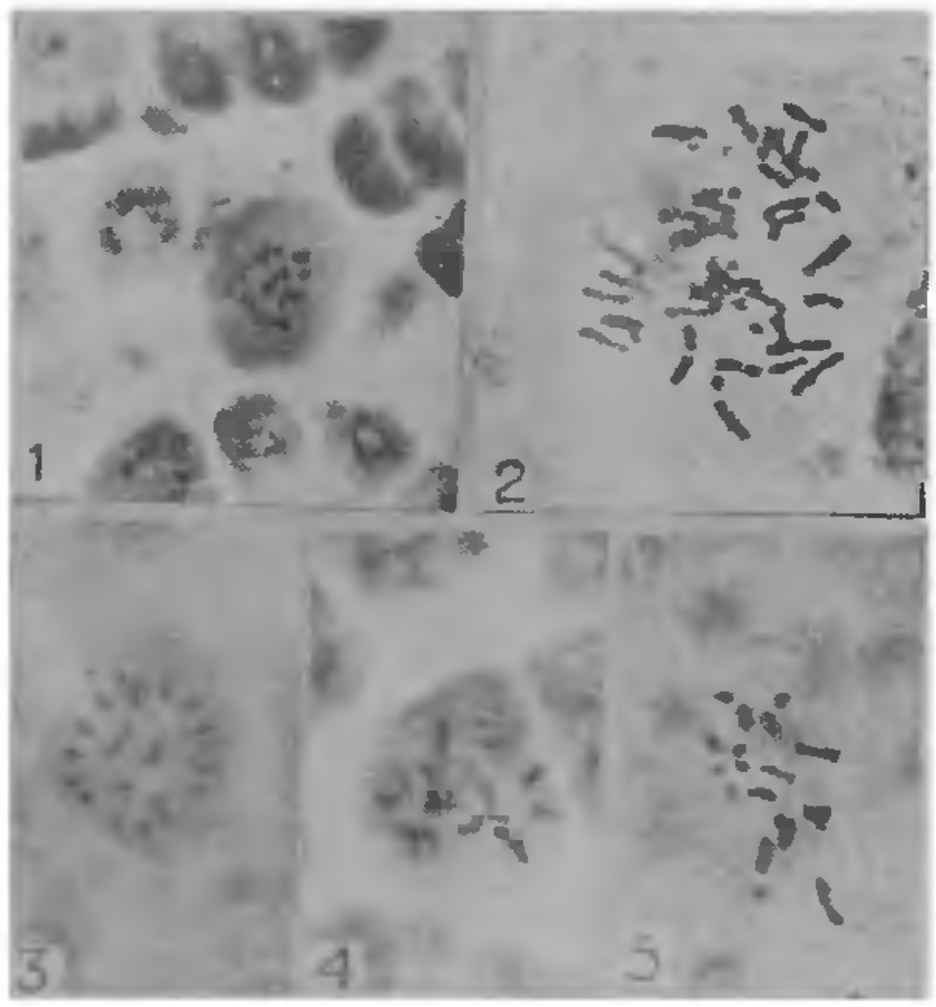
Different species and members of 18 genera were included in the study. Effect of chemical pretreatment, duration of pretreatment temperature as well as nature of fixative and period of fixation on cytological preparation were studied.

For cytological study seeds were raised in earthenware containing sand and soil mixture. When the roots attained 1 length, 1·5–2 mm apical lengths of roots were taken, washed in water and transferred to pretreatment chemicals of varying concentrations for different durations. Pretreated root tips were fixed in mixture of acetic alcohol (1 : 2) for 1–2 hours. After fixation, they were heated in a mixture of 2% aceto-orcein (N) HCl (9 : 1) solution for 5–6 seconds and kept in the mixture for 6–24 hours. Subsequently, the root tips were squashed in 45% acetic acid sealed and observed. The best schedules for different genera are given in Table I.

It is seen that L-bromonaphthalene proved most suitable for the majority of the species and varieties investigated. The response of chromosomes to chemical pretreatment varied from genus to genus as well as within the species and even within the varieties of the same species.

Pretreatment with a mixture of half saturated solution of aesculine and 0·002 M oxyquinoline (1 : 1) in cold (8° –14° C) proved effective in Hydrocotyloideae and Saniculoideae.

In the genera *Eryngium* and *Centella* however after fixation treatment with saturated solution of pectinase for 15 minutes at 60° C was necessary to get well-defined chromosome morphology.



FIGS. 1-5. Fig. 1. Somatic metaphase with $2n=22$ chromosomes in *Ammi majus*. Fig. 2. Somatic metaphase with $2n=38$ chromosomes in *Heracleum wallichii* D.C. Fig. 3. Somatic metaphse with $2n = 24$ chromosomes in *Coriaudrum sativum* var. $W.B_4$. Fig. 4. Somatic metaphase with $2n = 22$ chromosomes in *Coriandrum sativum*var. Madras. Fig. 5. Somatic metaphase with $2n = 16$ chromosomes in *Eryngium foetidum*.

Pretreatment with a saturated solution of L-bromonaphthhalene at 8–23° C for 75 minutes to 150 minutes proved satisfactory in members of the subfamily Apioideae.

For leaf tip chromosome preparations, an additional step after pretreatment, *viz.*, keeping in a mixture of Newcomers fluid and Carnoy's fluid (1 : 1) for 2–3 hours was very helpful in clearing the heavy cell content.

For feulgen staining of Umbelliferous materials standardization of time of hydrolysis (with N. HCl at 60° C) was found to be very important.

* References cited from recent compilation of Federov *et al.* [2].

TABLE I

Optimum pretreatment and fixation schedule of the different genera studied

Name of the genera	Pretreatment			Fixation	
	Pretreating chemicals	Duration of pretreatment	Temperature	Fixative	Period of fixation
Centella L.	Saturated solution of aesculine : oxyquinoline (0·002 M) mixture (1 : 1)	2 hrs.	14° C–16° C	1·2 Acetic alcohol	1 hr. 30 min.
Hydrocotyle L. (2 species)	do.	3–3½ hrs.	do.	do.	do.
Eryngium L.	do.	2 hrs. 15 min.	do.	do.	do.
Sanicula L.	do.	do.	12° C–16° C	do.	do.
Apium L. (7 varieties)	do.	1–2 hrs.	12° C–19° C	do.	do.
Carum L.	Saturated solution of paradichlorobenzene : oxyquinoline (0·002 M) (1 : 1); also saturated L-bromonaphthalene	2 hrs. 15 min. to 2 hrs. 45 min.	14° C–16° C	1·1 Acetic alcohol	do.
Coriandrum L. (20 varieties)	Saturated solution of L-bromonaphthalene	do.	do.	1·2 Acetic alcohol	do.
Heracleum wallichii D.C.	do.	do.	do.	do.	do.
Anthriscus Pers.	do.	do.	do.	do.	do.
Foeniculum L. (5 varieties)	do.	do.	do.	do.	do.
Petroselinum Hoffm.	do.	do	do.	do.	do.
Pastinaca L.	do.	do.	do.	do.	do.
Peucedanum L.	do.	do.	do.	do.	do.
Ferula L.	do.	do.	do.	do.	do.
Daucus L. (8 varieties)	Half saturated, aesculine : oxyquinoline (0·002 M) (1 : 1)	1 hr. and 30 min	8° C–14° C	do.	do.
Ammi L.	do. Also saturated L-bromonaphthalene	1½ hrs. to 2 hrs.	9° C–28° C	do.	do.
Oenanthe L.	do.	2 hrs. and 30 min.	10° C–14° C	do.	do.
Torilis Adans.	do.	2 hrs.	do.	do.	do.

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