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Karyological Studies on Some Wild Species of Family Cruciferae in Egypt

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Abstract: The present study on some wild taxa belonging to family Cruciferae has been carried out from the cyctological view-point. The somatic chromosome counts for *Matthiola arabica* 2n = 14, *M. livida* 2n = 10 and 12, *Erucaria hispanica* 2n = 14 and *Eremobium aegyptiacum* 2n = 18 and 20 were the new reports. Karyotype analyses showed that the examined taxa had no identical chromosome sets. *Cakile maritima* (2n = 18) collected from Rashid had 6 M, 6 nm and 6 nsm(-) chromosomes, from Baltim 2 M, 8 nm, 6 nsm(-) and 2 nsm(+) chromosomes meanwhile specimens from Alexandria had 2 M, 10 nm and 6 nsm(-) chromosomes. *Erucaria hispanica* (2n = 14) had 2 M, 4 nm, 6 nsm(-) and 2 nsm(+) chromosomes and (2n = 16) had 2 M, 6 nm, 6 nsm(-) and 2 nsm(+) chromosomes. *Matthiola arabica* (2n = 14) had 2 M, 4 nm, 4 nsm(-), 2 nsm(+) and 2nst(+) chromosomes. *M. livida* (2n = 10) had 2 M, 4 nm and 4 nsm(-) chromosomes and 2n = 12 had 2 M, 4 nm and 6 nsm(-) chromosomes. Finally *M. longipetala* (2n = 12) had 2 M, 6 nm, 2 nsm(-) and 2 nsm(+) chromosomes. That the total complement length was the highest in *Matthiola longipetala* (15.66 µm) and the lowest in *Erucaria hispanica*, 2n = 14 (8.54 µm). The arm ratio ranged from 4.25 to 1.00. All of them had symmetrical karyotype except in *Matthiola arabica* where nst(-) chromosomes appeared and consequently showed affinity to asymmetry. With regard to the growth habit of the genus *Matthiola*, it is also evident that the perennial species had low TF% and asymmetric karyotype. Chromosomal aberrations were observed in mitotic division. Only *Matthiola* species in this study showed the mitotic chromatin bridge, irregular distribution of chromosomes, laggards and stickiness. The present work may throw light on possibility of using the studied taxa as natural genetic resources which are broadly used today in the field of conservation biology.

Key words: Cruciferae, chromosome number, karyotype, chromosomal aberrations, genetic resources

Introduction

Cytological characters, including chromosome number and karyotype analysis have been considered as reliable guides in studies of taxonomic and evolutionary relationships by many authors (Davis and Heywood, 1963; Moore, 1968 and Stace, 1980). A range of examples has been reviewed by Moore (1968), Stace (1980) and Elkington (1984) showing that chromosome studies, especially when combined with hybridization and genetic analysis, have provided essential clues in tracing the origin and the evolutionary history of plant species. The number, size and shape of chromosomes were used to characterize the karyotypes of plants and define the taxonomic differences between them.

In *Cruciferae*, in particular the cytology of a number of genera have been studied in detail, leading to a much clearer understanding of their variation patterns, for example *Cochlearia* (Gill, 1965; 1976; Gill *et al*., 1978), *Brassica* (Stebbins, 1971 and Harberd, 1972, 1976) and *Matthiola*.

Chromosomal morphology is usually studied on the basis of the position of the primary constriction (centromere or kinetochore) (Battaglia, 1955; Huziwara, 1958; Levan *et al*., 1964 and Adhikary, 1974). A modification of all the previous systems was proposed by Abraham and Prasad (1983). In this system four fixed points and six intermediate regions are recognized in each chromosome segment. Thus according to this last system, the chromosomes can be labeled effectively and it can be successfully used in determining the karyotype more precisely than other systems.

The present study is carried out on some Cruciferous taxa. The objectives of this work are to standardize the cytological analysis of mitotic chromosomes, construct karyotypes of the species and to reveal the types of mitotic irregularities if present and their frequencies in Cruciferae taxa.

Materials and Methods

The present study was carried out on some Cruciferous taxa. These are *Cakile maritima* Scop. ssp. *aegyptiaca* (Willd.) Nyman and *Erucaria hispanica* (L.) Druce within tribe Brassiceae, *Eremobium aegyptiacum* (Spreng.) Schweinf. Et Asch. ex Bioss in tribe Hesperideae, *Matthiola arabica* Boiss, *M. livida* (Del.) DC. and *M. longipetala* (Vent.) DC. in tribe Matthioleae.

For *Matthiola longipetala* and *Erucaria hispanica* the materials were collected from barley fields of Burg-El-Arab in Alexandria, while *Matthiola livida* and *Eremobium aegyptiacum* from Cairo-Suez desert road, El-Salhia Ismalia and Belbies desert. The specimens of *Matthiola arabica* were collected from rocky mountains in Sant-Kathrin (Sinai). Finally, *Cakile maritima* specimens were collected from three different localities namely: coastal salt marshes of Burg Rashid, Baltim and Alexandria.

Seeds of the taxa were collected at the fruiting time. Germination at 17-20°C was found to give the highest germination percentage. Root tips 1-1.5 cm long were collected, pre-treated with 0.002 µ of 8-*hydroxyquinoline* (Tjio and Levan, 1950) for 2-4 hours for karyotype analysis. However for the analysis of mitotic chromosomal aberrations root tips were not pre-treated.

Different staining techniques were tried like, 2% aceto-orcein (La Cour, 1941) and 2% aceto-orcein after acid treatment (Chattopadhyay and Sharma, 1988). It was found that aceto-orcein stain after acid treatment gave the best results.

Well spread metaphase plates were selected and photographed. The negatives were developed, magnified using a photo enlarger and the image was projected onto a flat table surface. By careful focussing a sharp image obtained and Karyograms were drawn, lengths of long arm (L) and short arm (S) were measured for karyotype analysis.

Types of chromosomes were identified and classified according to Abraham and Prasad (1983). The total form percent (TF%) i.e. the average degree of symmetry over the whole karyotype was calculated according to Huziwara (1962).

Results

All the taxa under study were diploid. In the analyzed species, 10, 12, 14, 16, 18 and 20 chromosomes were observed in somatic cells (Plate 1).

Karyotype analyses include chromosome number, arm ratio, total complement length as well as karyotype formula carried out for all the taxa under study, data is summarized (Table 1). In case of *Eremobium aegyptiacum* the very small apparent size of the chromosomes did not permit a detailed study of their morphology. Karyograms of the species were illustrated (Plates 2 & 3). The types and proportions of abnormalities observed at mitotic division are summarized and shown (Table 2, Plate 1), respectively.

Discussion

Genera and species examined in this study were diploid. No polyploidy were encountered in different investigated taxa. Some taxa under study were characterized by more than one

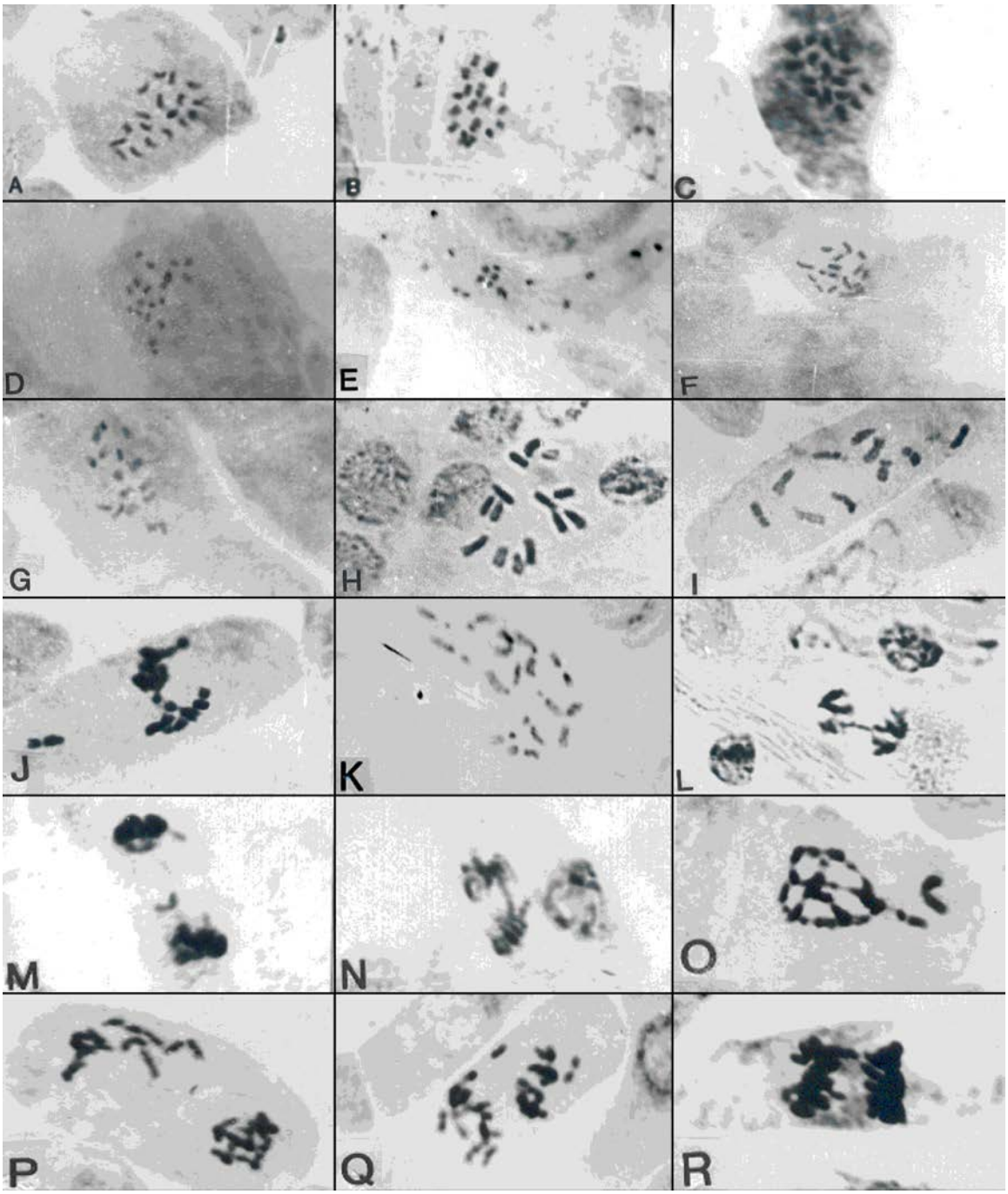

Plate 1: Somatic chromosomes of : (A) *Cakile maritima* (Alexandria), (B) Rashid, (C) Baltim, (D) *Eremobium aegyptiacum* (2n= 18), (E) 2n= 20, (F) *Erucaria hispanica* 2n= 14, (G) 2n= 16, (H) *Mathiola arabica,* (I) *M. livida* (2n= 10), (J) 2n= 12 and (K) *M. longipetala;* (L,N,R) Bridge in anaphase stage, (M,Q) laggard chromosome (O) stickiness and (P) Irregular distribution of chromosomes. (X = 1700)

Table 1: Chromosome data for all the species examined (somatic chromosome number, average size, TF%, total complement length and karyotype formulae)

Taxon	Source	Somatic chromosome number	Average length of chromosome		TF%	Total complement length (µm)	Karyotype formula
			Longest	Shortest			
Cakile maritima	Rashid	2n= 18	0.72	0.46	38.02	10.61	6M, 6nm, 6nsm (-)
	Baltim	2n= 18	0.90	0.52	35.37	12.70	2M, 8nm, 6nsm(-),2nsm(+)
	Alexandria	2n= 18	0.70	0.51	37.20	11.14	2M, 10 nm, 6nsm(-)
Erucaria hispanica	Burg-El Arab	2n= 14	0.71	0.46	32.95	8.54	2M, 4nm, 6nsm(-), 2nsm(+)
		2n= 16	0.80	0.54	34.23	10.18	2M, 6nm, 6nsm(-), 2nsm(+)
Matthiola arabica	Sant-Kathrin	2n= 14	1.15	0.99	31.70	15.00	2M, 4nm, 4nsm(-), 2nsm(+), 2nst(-)
M. livida	Cairo-Suez	2n= 10	1.29	1.05	35.48	11.77	2M, 4nm, 4nsm(-)
	Desert road	2n= 12	1.40	0.51	32.57	11.37	2M, 4nm, 6nsm(-)
M. longipetala	Burg-El Arab	2n= 12	1.40	1.12	34.09	15.66	2M, 6nm, 2nsm(-), 2nsm(+)

Table 2: Mitotic abnormalities observed in species of *Matthiola* studied

Taxon	Total no. of cells examined	Normal cells	% of Abnormalities			
			Bridge	Stickiness	Laggards	Irregular distribution of chromosomes
Matthiola arabica	144	138	4.16	-	-	-
M. Livida	132	129	1.52	-	0.75	-
M. longipetala	433	419	0.70	2.08	0.20	0.23

Plate 2: Karyograms of: (A) *Cakile maritima* (Rashid), (B) Baltim, (C) Alexandria, (D) *Erucaria hispanica* 2n = 14 and (E) *E. hisponica* 2n = 16

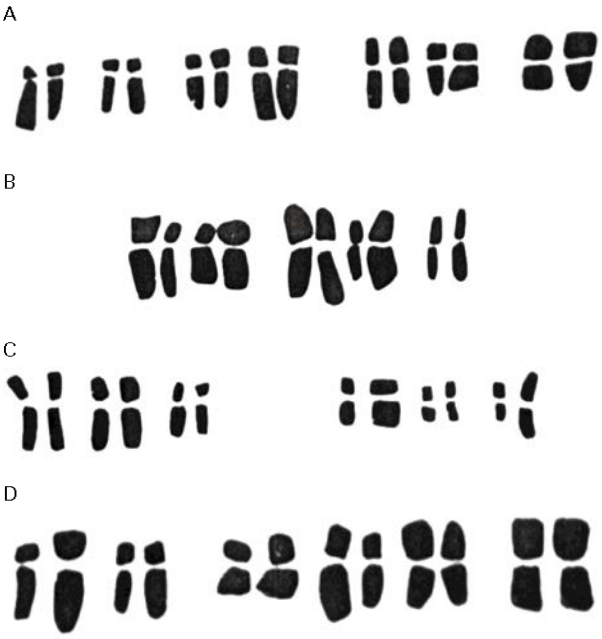

Plate 3: Karyograms of: (A) *Matthiola arabica*, (B) *M. livida* (2n = 10), (C) *M. livida* 2n = 12 and (D) *M. longipetala*

chromosomal number. This phenomenon is not rare in the family Cruciferae since in the genus *Erysimum* which is closely related to the genus *Matthiola* n = 6, 7, 8, 9, 10, 11 and 13 were reported by Favargel and Goodhue (1977). Also Bocher (1966) and Mulligan (1971 and 1974) reported dibasic number of n = 6 and 7 in the genus *Draba*.

The cytological features of the three studied species of *Matthiola* showed three basic numbers of n = 5, 6 and 7. Earlier studies of chromosome number of the genus *Matthiola* showed that the majority of the species have n = 6 or 7 (Renzoni, 1969; Larsen and Lagaard, 1971; Sharma and Sikka, 1976; Kuzmanov and Jurukova, 1977 and Soliman and Parker, 1986). *Matthiola arabica* with 2n = 14 and *M. livida* with 2n = 10 and 12 are reported for the first time in the present study. M. longipetala in the present study has 2n = 12. Most previous records reported 2n = 14 (Kuzmanov and Jurukova, 1977; Al-Shehbaz and Al-Omar, 1982) whereas it is Maassoumi (1980) reported that 2n = 12 which is confirmed by this investigation.

Cakile maritima has 2n= 18. This chromosome number had already been investigated (Delay and Petit, 1971; Strid, 1971; Gadella and Kliphis, 1973; Vanloon, 1974 and Engelskjon, 1979). For *Erucaria hispanica* two chromosome numbers were recorded, namely 2n = 14 and 16. The former (2n = 14) is a new record for this species while 2n = 16 was previously reported by Harberd (1972), Sharma and Sikka (1976).

Eremobium aegyptiacum characterized by tiny chromosomes had n = 9 and 10 (reported here for the first time). Rodman (1978) found that n = 13 which don't agree with our results. The observed variation in chromosome number as compared with other records may be due to two factors: firstly, different phytochoria and secondly, chromosomal structural alterations that might have taken place during speciation.

Different dibasic chromosome numbers reported in this study in the same root tip has been observed previously in *Sorghum purpeurosericeum* (Darlington and Thomas, 1941); *Haplopappus gracilis* (Ostergren and Frost, 1962), *Oryza* (Sampath, 1950) and in *Hymencollis calathenum* (Snoad, 1955).

Karyotype analysis of the taxa in this study was recorded for the first time except that of *M. longipetala* which has been studied earlier but on Russian and British accessions. Karyotypic differences were observed between *M. arabica*, *M. livida* and *M. longipetala* with regard to chromosomal morphology and chromosomal length. The chromosomes of *M. arabica* falls in 5 groups: metacentric, nearly metacentric, nearly submetacentric (-), nearly submetacentric (+) and nearly subtelocentric (-) types whereas *M. livida* had 3 groups of chromosomes: metacentric, nearly metacentric and nearly submetacentric (-) groups. In case of *M. longipetala* the types of chromosomes appeared to be metacentric, nearly metacentric, nearly submetacentric (-) and nearly submetacentric (+) (Table 1). Also the length of chromosomes showed considerable differences where the total complement length varied from 15.66 to 11.37 µm, with *M. livida* showing the lowest value. Not only the chromosomal morphology was variable but also the arm ratio exhibited a considerable variation in *Matthiola* ranging from 4.25 to 1.00. Similar results have been reported in other plant species, Pederick (1970) found differences in the chromosome lengths between karyotypes of different species of *Pinus* and considered length differences to be due were the accumulation of small duplications. In *Amaranthus*, Madhusoodanan and Nazeer (1983) and in *Hebenari*, Kashyap and Mehra (1983) have shown that variation in chromatin length as well as in the karyotypic formulae is indicative of structural chromosomal alterations in the form of repatterning and chromatic deletions or additions. Ved Brat (1965) reported that inversions have been the main cause for karyotype differentiation in the whole genus *Allium*. Numerous studies have reported that variation in mitotic chromosomes may have originated either by translocation or by pericentric inversions or both, for example: *Crotalaria* (Chennaveeraiah and Patil, 1973), *Blumea* (Matthew and Matthew, 1982), *Ornithogalum* (Vosa, 1983), *Monochoria* (Christopher, 1983), on Australian grass species (Jadhav *et al.*, 1983) and *Guizotia* (Patel *et al.*, 1983). There is therefore a strong probability that similar forces have been operative in *Matthiola*.

Karyotypic variations exist not only between different species but also within the same species with different base numbers, thus in *Erucaria hispanica* it is obvious that the most conspicuous karyological difference between 16 and 14 cells is the addition of two nearly metacentric chromosomes. Cells with 2n = 16 had 2 metacentric, 6 nearly metacentric, 6 nearly submetacentric (-) and 2 nearly submetacentric (+), while cells having 2n = 14 had 2 metacentric, 4 nearly metacentric, 6 nearly submetacentric (-) and 2 nearly submetacentric (+). The total complement length varied from 8.54 (2n = 14) to 10.18 µm (2n = 16). In *M. livida* there was an addition of a pair of chromosomes in nearly submetacentric (-) group in the cells with 2n = 12 where they have 2 metacentric, 4 nearly metacentric and 6 nearly submetacentric (-) chromosomes whereas the cells with 10 chromosomes have 2 metacentric, 4 nearly metacentric and 4 nearly submetacentric (-) chromosomes. The total complement length varied from 11.37 to 11.77 µm. Some chromosomal aberrations that are important tools in bringing about variation in chromosomal number may as well play a role in inducing such difference in chromosomal morphology.

Also, interpopulation differences were observed among *Cakile maritima* from three different areas. The total complement length varied from 10.61 to 12.70 µm. With those plants from Rashid showing the lowest value the karyotype formulae were to some extent different. Specimens from both Rashid and Alexandria were similar in the karyotype classes (three classes) with different chromosome number in each class. Also they were similar in having the longest chromosome being nearly submetacentric (-). Meanwhile, Baltim specimens have four chromosome classes as well as the longest chromosome being nearly metacentric. This karyologic differences within different cytotypes of the same species have been observed previously in some species such as *Amaryllis balladonna* (Guha, 1979) and *Astragalus* (Ashraf & Gohil, 1988).

The karyotype formulae of the studied species revealed that all of them had symmetrical karyotype except in *M. arabica* where nearly subtelocentric (-) chromosomes appeared and thus gave the asymmetric affinity. Stebbins (1971) has stated that one of the basic features which bring about karyotype asymmetry is shifting of the centromere from median to sub-median and subterminal position and it has been considered as a progressive step in karyotype evolution. Taxa with asymmetric karyotype tend to have low TF% (Huziwara, 1962). Therefore, *M. arabica* can be presumed to have more asymmetrical karyotype than other two species of *Matthiola*. The growth habit of *M. arabica* (perennial) confirms this suggestion since Sveshnikovas'work (1927) on *Vicia* species revealed that in the section Cracca of *Vicia* in which both annuals and perennials occur; the annuals have a more symmetric karyotype than the perennials.

In this study *Matthiola* species showed the mitotic chromatin bridge, irregular distribution of chromosomes, stickiness and laggards. This mitotic irregular chromosome behaviour had been reported in other plants where during the study of karyotype and meiosis of *Aloe vera* (Vig, 1968). Such abnormalities have been known to arise from abnormal metaphase spindle (Varaama, 1949). On the other hand, in study of mitosis in *Aloe aristata* (Brandham, 1970), bridges and fragment were extremely rare but the existence of them at mitosis confirms that some sort (U-type) of chromatid exchanges at meiosis can be due to breakage and reunion between sister chromatids.

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