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Cytogenetic study of some populations of *Foeniculum vulgare* (*Umbelliferae)* in Iran

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Abstract — Chromosome pairing, chiasma frequency and distribution as well as chromosome segregation were analyzed in 13 populations of *Foeniculum vulgare*. All populations possessed n = 11 (2n = 2x = 22) chromosome number forming mainly bivalents with some amount of quadrivalents possibly due to the occurrence of heterozygote translocations. Cytomixis and desynapsis occurred in all populations leading to the formation of unreduced pollen grains. Cluster analysis of meiotic data showed distinctness of the populations in their meiotic behavior.

Key words: chromosome pairing, cluster analysis, *Foeniculum vulgare*.

INTRODUCTION

Bitter fennel plant (*Foeniculum vulgare* Miller subsp. *capilaceum* (GILIB.) Holmboe var. *vulgare* (*Umbelliferae*) is considered as an important medicinal and edible plant throughout the world and is grown wild in several regions of Iran. In spite of economic importance of Bitter fennel, less attention has been paid to its cultivation and no report is available on its genetics or cytogenetic in the country.

Foeniculum vulgare is native to southern Europe and the Mediterranean region (PARSONS 1973). It has been used for medicinal and culinary purposes at least since Roman times. It has become naturalized in temperate areas around the world, especially in limey soil near the sea. The oils of both sweet and bitter fennel seed, obtained by steam distillation, contain anethole, fenchone, camphene, sabenine, limonene and several other volatile constituents as well as a fixed oil. Fennel seed is used in the food and flavor industry for addition to meats, vegetable products, fish sauces, soups, salad dressings, stews, breads, pastries, teas, and alcoholic beverages. As a medicinal plant, fennel seed has been used as an antispasmodic, carminative, diuretic, expectorant, laxative, stimulant, and stomachic. Fennel has also been used to stimulate lactation, as a remedy against colic, and to improve the taste of other medicines (SIMON *et al*. 1984).

In general there have been very limited cytogenetic reports on *F. vulgare* ssp. *vulgare* throughout the world (SILVESTRE 1976; LENTINI *et al*. 1988) and no report is available from Iran. Therefore the present paper provides basic cytogenetical information of some natural populations of *F. vulgare* in Iran for the first time with regard to chromosome pairing and chiasma frequency.

MATERIALS AND METHODS

Young flower buds were collected from plants belonging to 13 populations of *F. vulgare* growing wild in different regions of Iran (Table 1), and fixed in glacial acetic acid: ethanol (1:3) for 24 hrs. They were then washed and preserved in 70% ethanol at 4°C until used (SHEIDAI *et al*. 1996). Cytological preparations used a squash technique and 2% aceto-orcein as the stain.

One hundred pollen mother cells (PMCs) were analysed for chiasma frequency and distribution at diakinesis metaphase and 1000 PMCs were analysed for chromosome segregation during anaphase and telophase. Pollen fertility was checked by staining a minimum of 1000 pollen grains from each taxon using 2% acetocarmine: 50% glycerin (1:1) for 1 hr. Well stained and perfect pollen grains were regarded here as fertile

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Table 1 — Meiotic characteristics of *F. vulgare* populations.

Population	TX	IX	TOX	RB	RD	I	IV	ST	CY	UN
1 Ashteyan	18.36	0.86	19.21	7.79	0.86	1.00	0.86	22.34	24.82	18.42
2 Imamzadeh	18.50	0.86	19.36	7.79	1.00	1.00	0.93	24.12	22.94	10.34
3 Tafresh	18.64	1.00	19.64	7.86	0.93	0.71	1.00	21.40	23.12	11.42
4 Bidhand	17.71	1.00	18.71	7.36	0.59	1.43	1.00	19.63	23.72	16.74
5 Vasf	17.50	1.14	18.64	6.86	1.43	0.71	1.14	20.30	22.83	14.33
6 Ghahan	18.00	1.14	19.14	7.50	0.71	0.71	1.14	19.82	20.14	8.74
7 Qom	17.71	1.07	18.79	7.71	0.57	1.14	1.07	19.94	16.32	8.74
8 Karmejgan	18.21	1.07	19.29	7.71	0.50	1.29	1.07	18.30	17.43	7.14
9 Khaveh	18.29	1.00	19.29	7.71	0.93	0.86	1.00	16.53	15.22	6.32
10 Hamedan	18.50	0.79	19.29	7.93	0.79	1.29	0.79	15.20	15.18	4.32
11 Vashneh	18.00	1.00	19.00	7.36	1.36	1.14	1.00	16.12	22.12	9.48
12 Fardoo	18.21	0.86	19.07	7.79	1.00	0.86	0.86	16.43	19.84	8.73
13 Isfahan	18.64	0.86	19.50	7.93	1.00	1.00	0.86	14.20	15.14	2.34

Abbreviations: TX = Terminal chiasmata, IX = Intercalary chiasmata, TOX = Total chiasmata, RB = Ring bivalent, RD = Rod bivalent, I = Univalent, IV = Quadrivalent, ST = Chromosome stickiness (%), CY = Cytomixis (%), UN = Unreduced gametes (%).

while unstained or empty pollens were considered as infertile.

In order to detect a significant difference among different populations, analysis of variance (ANOVA) followed by the least significant test (LSD) test was performed on meiotic characters (SHEIDAI *et al*. 1996). In order to group the species and populations according to similarity in meiotic behavior, UPGMA (unweighted paired group with arithmetic average) and Ward's minimum variance clustering methods as well as ordination based on principals components analysis (PCA) were used (ROMESBURG 1984). For cluster analyses variables (characters) were standardized (mean = 0, variance = 1: SHEIDAI *et al*. 1996). In order to determine the most variable meiotic characters factor analyses based on PCA were performed on standardized data. Multivariate statistical analyses used SPSS Ver. 9 (1998).

RESULTS AND DISCUSSION

The meiotic analysis of *Foeniculum vulgare* populations studied showed a deviant course of meiosis-I prophase sub-stages i.e. the occurrence of synezetic knot stage instead of leptotene and zygotene. In the early synezetic knot stage, thin chromatin strands surround the nucleolus till covering it totally. Latter on paired chromosomes (now thick strands) unraveled from the knot, entering the pachytene stage. End to end attachment of chromosomes in pachytene is a feature reported in those taxa showing synezetic knot stage. However despiralization of chromosomes occurred after pachytene, commencing diffuse stage (Fig. 1).

The occurrence of diffuse stage has been reported in several plant species (SYBENGA 1992). Diffuse may be of complete type in which the whole chromosomes decondense or it may be partial in which some parts of the genome show decondensation. The present study showed the occurrence of partial diffuse in *F. vulgare* populations.

Various reasons have been suggested for the occurrence of diffuse stage. These are: high synthetic activity analogous to the lampbrush stage in amphibian oocyte; shedding of the lateral elements in the synaptonemal complex; the post pachytene elimination or modification of histone proteins and meiotic arrest to withstand the adverse environmental conditions (SHEIDAI and INAMDAR 1991). As *F. vulgare* populations grow wild in different environmental conditions, it may be suggested that adaptation to such adverse environmental conditions may be the reason for the occurrence of diffuse stage in *F. vulgare* populations.

Data with regard to ploidy level, chiasma frequency and distribution, as well as chromosome pairing is presented in Table 1 (Fig. 1). All the populations studied possessed n = 11 (2n = 2x = 22) chromosome number supporting the earlier reports (SILVESTRE 1976; LENTINI *et al*. 1988). Tafresh population possessed the highest value of total and terminal chiasmata (19.64 and 18.64 respectively), while Bidhand population possessed the lowest value of total chiasmata (18.71) and Vasf population showed the lowest value of terminal chiasmata (17.50). The highest value of intercalary chiasmata occurred in Vasf and Ghahan populations (1.14).

The populations studied formed both ring as well as rod bivalents with highest values observed in Hamedan (7.93) and Vasf (1.43) populations

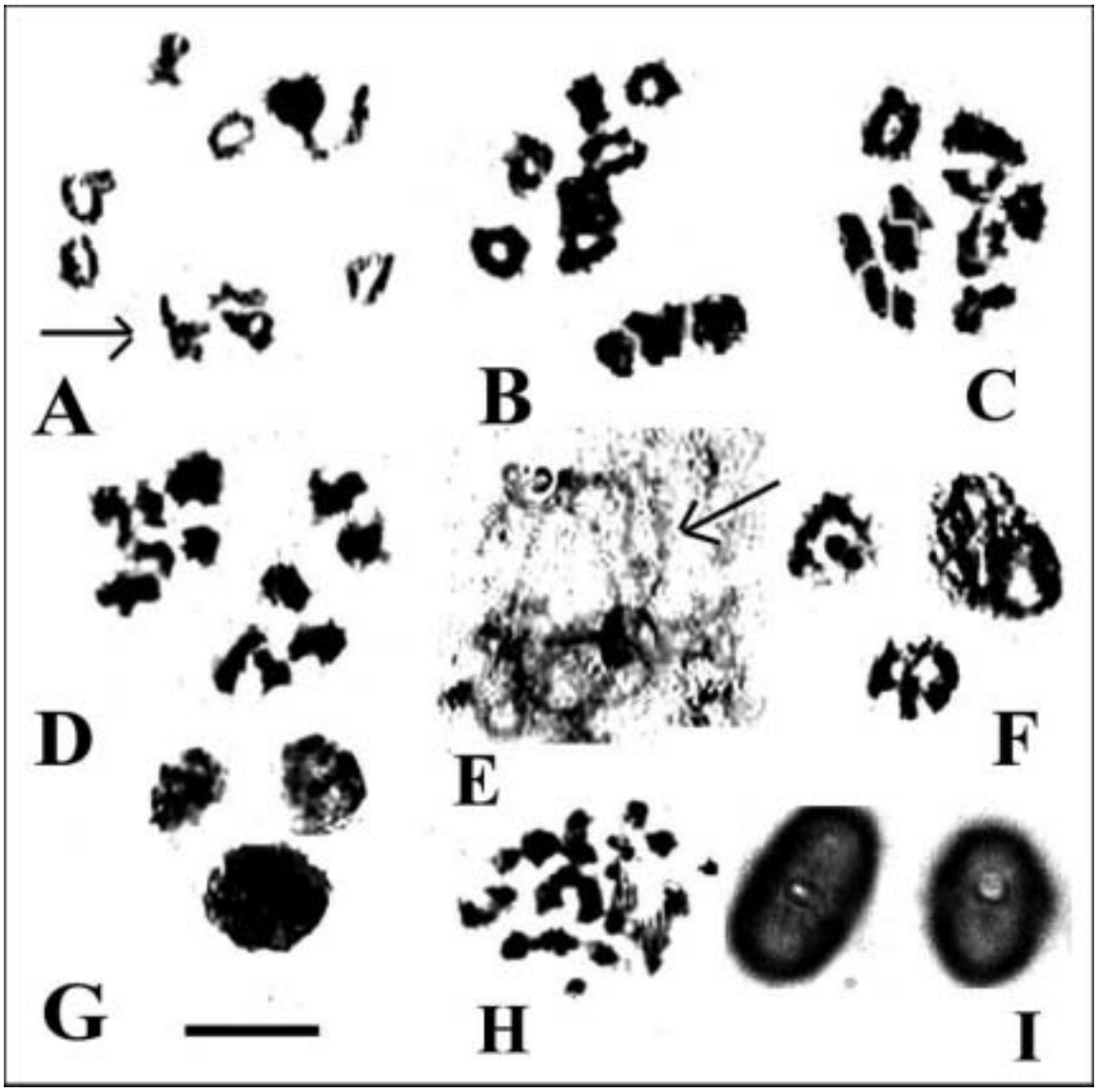
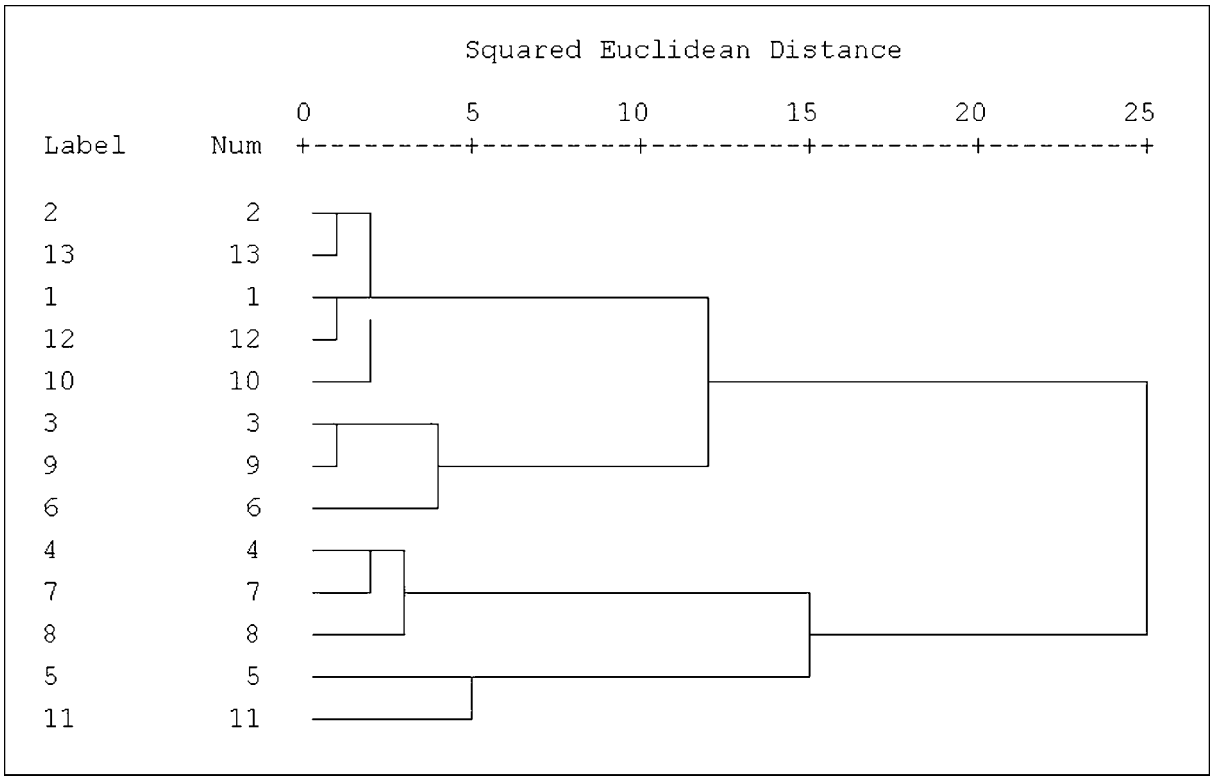


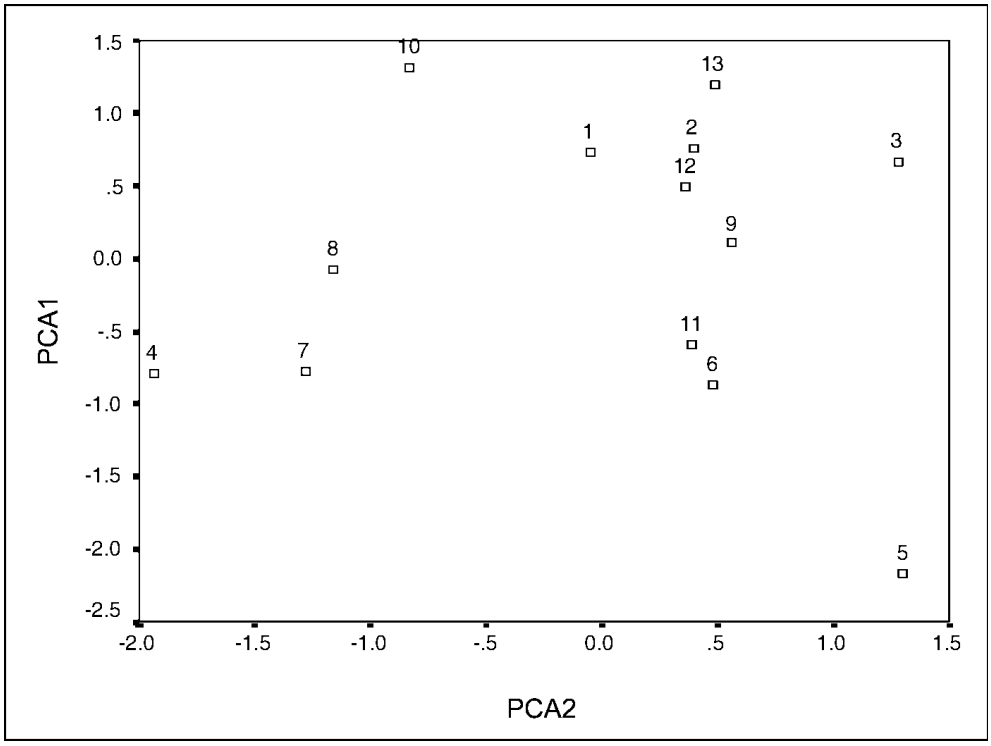
Fig. 1 — Representative meiotic cells in *F. vulgare* populations studied. (A-D) Metaphase cell showing n = 11 chromosome number in the populations of Ashteyan, Imamzadeh, Qom and Tafresh (arrow indicates quadrivalent). (E) Diffuse stage in the population of Ghahan (arrow indicates decondensed region of chromatin). (F-G) Triad formation in the populations of Karmejgan and Khaveh showing unreduced cell formation. (H) Desynaptic cell in Bidhand population. (I) Larger sized (potentially unreduced) pollen grains in Vashneh population. Scale bar = 10 μm

respectively. Univalents and quadrivalents were also formed in metaphase of meiosis I in all populations with the highest value occurring in Karmejgan (1.29) and Vasf as well as Ghahan (1.14) populations respectively (Table 1). The occurrence of quadrivalents in the diploid populations of *F. vulgare* is interesting as diploid species are expected to form only bivalents. The occurrence of heterozygote translocations may be the reason for quadrivalent formation in F. *vulgare*. Such genomic changes may play a role in adaptations of these plants to different environmental conditions they are growing.

ANOVA and LSD test revealed a significant difference in meiotic characteristics among the populations studied. Variation in chiasma frequency and localization is genetically controlled (QUICKE 1993). Such a variation in species or populations with the same chromosome number is considered as a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (REES and DALE 1974).

Different cluster analyses of *F. vulgare* populations based on meiotic data produced identical re-





Figs. 2-3 — UPGMA clustering and PCA plot of *F. vulgare* populations. (populations code: 1 = Ashteyan, 2 = Imamzadeh Ismaeil, 3 = Tafresh, 4 = Bidhand, 5 = Vasf, 6 = Ghahan, 7 = Nehalestan, 8 = Karmejgan, 9 = Khaveh, 10 = Hamedan, 11 = Vashnoh, 12 = Fardoo and 13 = Isfahan).

sults forming two major clusters (Fig. 2). The first major cluster is comprised of two sub-clusters. Populations of Tafresh, Khaveh and Ghahan form the first sub-cluster, in which the first two cultivars show more similarity and are placed close to each other. The populations of Ashteyan, Imamzadeh Ismaeil, Hamedan, Fardoo and Isfahan comprise the second sub-cluster. The populations of Bidhand, Nehalestan Qom, Karmejgan, Vasf and Vashon form the second major cluster.

Ordination of these populations based on the first two factors supported the clustering results (Fig. 3). The Factor analysis of the meiotic data revealed that the first two components comprised about 78% of the total variance. Total and terminal chiasmata and ring bivalents in the first factor and rod bivalents in the second factor possessed the highest positive correlation ($r = > 0.80$), and hence may be considered as the most variable meiotic characters among the *F. vulgare* populations studied.

The *F. vulgare* populations studied showed the occurrence of chromosome stickiness, desynapsis and cytomixis (Table 1, Fig. 1). Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originated from the pre-existing systems of plasmodesmata formed within the anther tissues. The plasmodesmata become completely obstructed by the deposition of callose but in some cases they still persist during meiosis and increase in size forming conspicuous inter-meiocytes connections or cytomictic channels that permit the transfer of chromatin (Falistocco *et al*. 1995).

Chromatin/chromosome migration occurred in different directions from the early prophase to telophase-II stages in *F. vulgare* populations studied. Several metaphase cells possessed extra/missing chromosomes showing aneuploid condition. Cytomixis leads usually to aneuploidy and reduction in fertility of the plants, therefore is considered to be of less evolutionary significance. However, it may bring about new genetic variability by producing aneuploid gametes and new phenotypic characters as reported in other plants (Sheidai *et al*. 1993). Some meiocytes showed the presence of two sets of genomes which may lead to the formation of unreduced pollen grains observed in *F. vulgare* populations studied (Fig. 1). In fact at the end of telophase II, large number of triads were formed showing the occurrence of unreduced cells (Fig. 1).

In all *F. vulgare* populations studied, morphological observations of the pollen grains showed the occurrence of bigger sized pollen grains along with smaller sized pollen grains (Fig. 1). Such bigger sized pollen grains may be potential unreduced (2n) pollen grains formed due to meiotic abnormalities such as cytomixis and desynapsis.

Different methods have been used to detect 2n gametes including morphological, flow cytometery and cytological methods. The most direct method of screening for 2n pollen involves the examination of the range of size of pollens produced by an individual, as with increase in DNA content the cell volume increases which in turn influence the pollen diameter. The presence of giant grains has been used as an indication of the production of 2n pollen (Falistocco *et al*. 1995). This is the first report on the occurrence of unreduced pollen grains in *F. vulgare*.

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