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Cytogenetical study of some Iranian pomegranate (*Punica granatum* L.) cultivars

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Abstract — Meiotic studies were performed on 22 *Punica granatum* cultivars of Iran considering ploidy level, chiasma frequency and chromosomes association. All the cultivars studied possessed n = 8 (diploid) chromosome number but varied significantly in their chiasma frequency, chromosome pairing and segregation indicating their genomic difference. A low number of quadrivalents were formed in some of the cultivars possibly due to translocations. Cluster and ordination based on Principal Components Analysis grouped those cultivars showing meiotic similarities.

Key words: Chiasma frequency, chromosome pairing, cluster analysis, *Punica granatum*

INTRODUCTION

The pomegranate (*Punica granatum* L.) of the family *Punicaceae* is native from Iran to the Himalayas in northern India and was cultivated and naturalized over the whole Mediterranean region since ancient times. It is widely cultivated throughout India and the drier parts of Southeast Asia, Malaya, the East Indies and tropical Africa (FACCIOLA 1990).

Pomegranate is one of the most important horticultural plants of Iran growing in most of the regions throughout the country and grows well in arid and semiarid regions due to its adaptation to adverse ecological conditions. About 550000 hectares of lands has been devoted to the cultivation of pomegranate in Iran, producing about 570000 tones of fruit. About 764 cultivars of *Punica granatum* have been collected during a germplasm collection and grown in Saveh and Yazd cities. All of these cultivars possess their specific fruit characteristics, such as size, color, time of ripening, disease resistance, taste, etc.

In general there have been very limited cytogenetic studies of pomegranate accessions in the world (RAMAN *et al*. 1971; GILL *et al*. 1981; XUE *et al*. 1992) and in spite of grate economic importance of this horticultural plant in Iran having large number of cultivated accessions in the country, there has been no genetic/ cytogenetic report on them. Therefore the present study considers cytogenetical characteristics of 22 *Punica granatum* var. *sativa* accessions of Iran for the first time, dealing with ploidy level, chiasma frequency and chromosome pairing.

MATERIALS AND METHOD

Cytogenetic studies were performed in 22 *P. granaum* cultivars for two successive years (2002-2003). Fifty to hundred young flower buds were collected randomly during 9-12 A M. from 10 randomly selected plants of each cultivar and fixed in glacial acetic acid: ethanol 70% (1:3) for 24 hrs. These were then washed thoroughly and transferred to 85% ethanol until used (SHEIDAI *et al*. 2003).

Chromosome pairing and chiasma frequency were determined by using minimum 100 meiocytes showing diakinesis/ metaphase-I stages, while chromosome segregation was studied in minimum 500 anaphase-I and II stages.

Pollen stainablity as a measure of fertility was determined by staining minimum 1000 pollen grains with 2 % acetocarmine: 50 % glycerin (1:1) for about ½ hr. Round/ complete pollen grains which were stained were taken as fertile, while incomplete/ shrunken pollens with no stain were considered as infertile (SHEIDAI *et al*. 2003).

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Analysis of variance (ANOVA) followed by the least significant difference test (LSD) was performed on cytogenetic characteristics including chromosome pairing, chiasma frequency as well as distribution to indicate any significant difference among the cultivars studied (SHEIDAI *et al*. 2003).

Different methods of cluster analysis including UPGMA (Unweighted Paired Group Mean Average) and WARD (minimum variance spherical clusters) as well as ordination based on Principal Components Analysis (PCA) was performed to identify the cultivars showing similarities in their meiotic characteristics (DIGBY and KEMPTON 1994). For cluster and principal components analysis, standard values (mean = 0, variance = 1) were used. Squared Euclidean distance was use as a measure of similarity in cluster analysis (SHEIDAI *et al*. 2003).

In order to detect the effects of B-chromosomes on chiasma frequency and distribution, t-test analysis was performed among the cells possessing B-chromosomes and those devoid of Bs. The statistical analyses were performed with SPSS ver.9 software.

RESULTS AND DISCUSSION

The names and locality of the *P. granatum* cultivars studied as well as their cytogenetic characteristics are presented in Tables 1 & 2 and Fig. 1. All *P. granatum* cultivars studied possessed n = 8 (diploid) (Fig. 1, a, d, g) supporting the earlier reports from other parts of the world (XUE *et al.* 1992; GILL *et al.* 1981).

Among the 8 bivalents usually formed during metaphase I of meiosis, one chromosome pair is much bigger that the others (Fig. 1, d), while the other chromosome pairs showed little size variation. In spite of their small size, chromosomes of *P. granatum* provide suitable material for cytogenetic studies.

The highest value of terminal chiasmata occurred in the cultivars Jazipoostsefid and Radki (14.34) while the lowest value occurred in Malasshirin (9.92). Similarly the highest value of intercalary chiasmata occurred in the cultivar Golmagasi (3.00) and the lowest value occurred in Anarseyah (1.00).

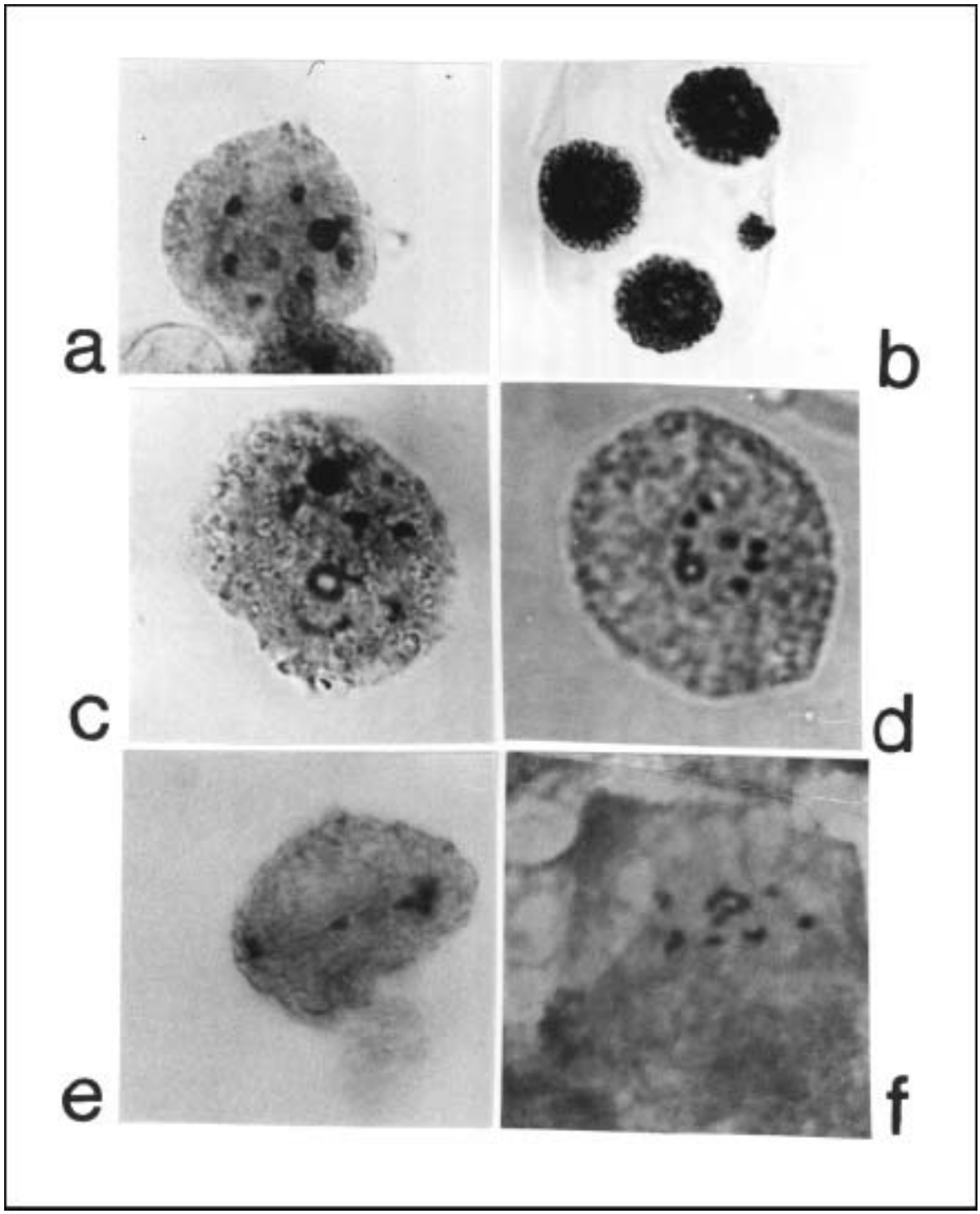
The highest amount of ring bivalents occurred in the cultivars Redki and Anartorsh (6.72 & 6.71 respectively) while the lowest value occurred in Malasshirin of Saveh (3.92). The highest value of rod bivalents occurred in Malasshirin (3.10) and the lowest value occurred in Anarseyah cultivars (0.66).

It is interesting to mention that although *P. granatum* is diploid and it is expected to form only bivalents in metaphase I of meiosis, a low value of quadrivalents occurred in the Brit, Malastoghi

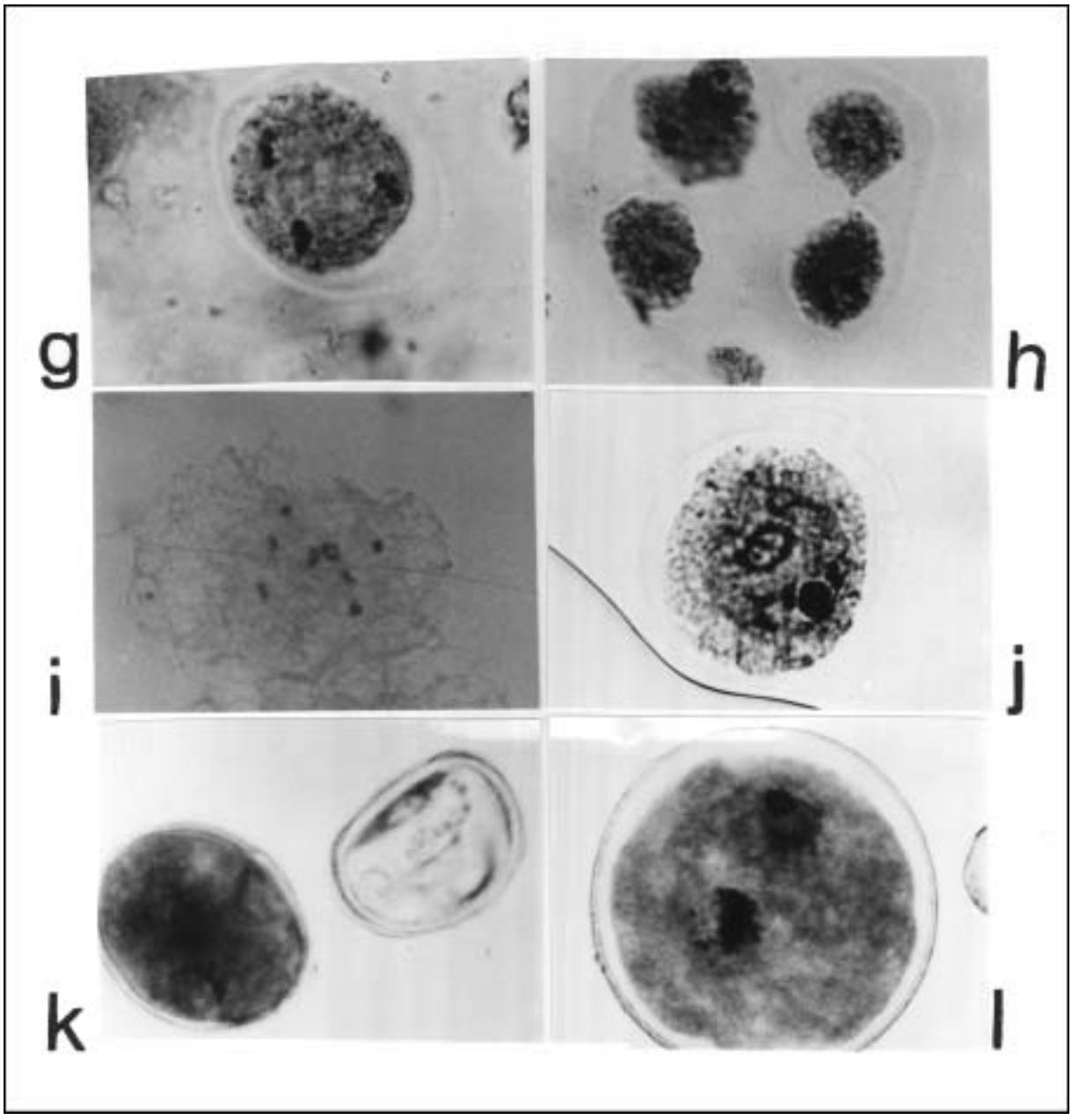
Table 1 — Meiotic characteristics of *Punica granatum* cultivars studied.

	Cultivar	Locality	TX	IX	TOX	RB	ROB	QU	I
1	Neytalkhi	Bandarabbas	14.83	0.41	15.22	6.87	0.98	0.00	0.15
2	Ghermeznarshirin	Shabastar	13.61	0.44	14.06	6.14	1.56	0.00	0.61
3	Malasshasavar1	Bafgh	13.69	0.22	13.91	6.15	1.60	0.00	0.51
4	Dadashpostkoloft	Shabastar	13.63	0.11	13.74	5.97	1.66	0.00	0.63
5	Golnar	Saveh	13.47	0.19	13.67	6.00	1.47	0.00	0.94
6	Shahitorsh	Kerman	14.24	0.08	14.28	6.44	1.28	0.00	0.40
7	Torshpostkoloft	Ardel	13.70	0.54	14.24	6.50	1.15	0.00	0.70
8	Shirinhasteriz	Shiraz	14.16	0.12	14.42	6.47	1.30	0.00	0.47
9	Malastoghi	Gorgan	13.35	0.46	13.82	5.97	1.57	0.01	0.80
10	Malaspeyvandi	Askzar	14.06	0.21	14.27	6.48	1.00	0.01	0.79
11	Malastorsh	Saveh	13.62	1.34	14.95	6.42	1.14	0.00	0.76
12	Malasshirin	Saveh	9.92	1.38	11.30	3.92	3.10	0.00	1.55
13	Aghamohamadali	Saveh	13.12	2.94	16.05	5.88	1.52	0.00	0.94
14	Alaktorsh	Saveh	12.73	1.52	14.25	5.45	1.95	0.00	0.75
15	Alakshirin	Saveh	13.75	1.44	15.12	6.40	1.12	0.00	0.81
16	Berit	Kazeron	11.48	1.19	12.70	4.67	2.70	0.16	0.70
17	Golmagasi	Taft	10.80	3.00	13.80	5.33	1.40	0.00	2.40
18	Anarseyah	Saveh	13.58	1.00	14.58	6.41	0.66	0.00	1.16
19	Malashahsavar2	Bafgh	13.41	2.06	15.53	6.34	1.38	0.00	0.44
20	Redki	Bafgh	14.32	1.64	15.95	6.72	1.04	0.00	0.45
21	Torshnar	Shabastar	14.16	1.88	16.01	6.71	0.82	0.00	0.89
22	Jazipoostsefid	Kerman	12.92	1.30	14.21	5.39	2.26	0.00	0.52

Abbreviations: TX = Terminal chiasmata, IX = Intercalary chiasmata, TOX = Total chiasmata, RB = Ring bivalent, ROB = Rod bivalent, QU = Quadrivalent, I = Univalent.



Explanation to Fig. 1 —
a, d & i = Meiocyte showing 8 bivalents in the cultivars Golnar, Malasshirin and Anarseyah respectively.
b, c & j = Metaphase I cell showing quadrivalent/s in the cultivars Berit, Malastoghi and Malaspeyvandi respectively.
e = Meiocyte showing anaphase I laggard chromosome in the cultivar Neytalkhi.
f & h = Abnormal tetrads in the cultivars Malasshasavar 1 and Malaspeyvandi respectively.
g = A tripolar cell in the cultivar Malaspeyvandi.
k = Fertile reduced pollen grain (stained) and infertile pollen grain (unstained) in the cultivar Malaspeyvandi.
l = A potential unreduced (2n) pollen grain in the cultivar Malaspeyvandi.
Scale bar = 10 μm



and Malaspeyvandi cultivars (Table 1., Fig. 1, b, c & j.). A possible reason for this may be the occurrence of translocation between two pairs of chromosomes and as the size of quadrivalents formed was always large, it seems that the largest chromosome pair of the genome is involved.

Univalents were formed in a low frequency in all the cultivars studied (Table 1). The highest value was observed in Golmagasi t (2.40) and the lowest value occurred in cultivar Shahitorsh (0.40). The regular occurrence of univalents in the cultivars studied may be due to small size of chromosome and early terminalization of chiasmata in them. Such univalents may be one of the reason for the formation of laggard chromosomes observed in the cultivars studied (Table 2).

ANOVA test revealed the presence of a significant difference ($p < 0.01$) for chiasma frequency and distribution as well as bivalent formation among the cultivars studied. Therefore at least two cultivars differ significantly in their meiotic behavior. The LSD test showed that such significant difference is present in most of the cultivars studied, particularly those cultivars which are placed in different clusters/ groups in cluster analysis and ordination based on PCA (explained in the following paragraphs).

Table 2 — Meiotic characteristics of *Punica granatum* cultivars studied.

	Cultivar	MIS	MIIS	AIS	AIIS	AIL	AIIL	MP	PF
1	Neitalkhi	6.15	0.00	3.11	0.00	6.49	0.00	0.00	72.00
2	Ghermeznarshirin	7.10	1.10	2.15	0.00	5.13	0.00	0.00	72.00
3	Malasshasavar1	0.00	0.00	2.10	0.00	4.10	0.00	3.33	73.50
4	Dadashpoostkoloft	6.10	0.00	0.00	0.00	0.00	0.00	0.00	75.30
5	Golnar	7.85	5.40	6.15	5.20	0.00	0.00	0.00	60.30
6	Shahitorsh	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.00
7	Torshpoostkoloft	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.00
8	Shirinhastehriz	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.00
9	Malastoghi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.00
10	Malaspeyvandi	0.00	0.00	0.00	0.00	5.80	12.55	0.00	74.00
11	Malastorsh	2.00	0.00	0.00	0.00	0.00	0.00	0.00	99.00
12	Malasshirin	8.50	0.00	0.00	0.00	6.60	1.30	0.00	74.00
13	Aghamohamadali	2.50	0.00	0.00	0.00	3.30	1.00	0.00	98.40
14	Alaktorsh	2.50	0.00	0.00	0.00	14.60	0.00	0.00	96.60
15	Alakshirin	1.00	0.00	0.00	0.00	5.50	1.00	0.00	90.00
16	Berit	1.37	0.00	0.00	0.00	28.00	10.00	0.00	60.00
17	Golmagasi	3.00	0.00	0.00	0.00	2.30	4.00	1.00	72.00
18	Anarseyah	9.00	0.00	0.00	0.00	7.00	7.60	5.00	60.00
19	Malashahsavar2	8.00	0.00	0.00	0.00	3.30	0.00	0.00	90.00
20	Redki	0.00	0.00	0.00	0.00	7.60	0.00	0.00	97.00
21	Torshnar	8.50	0.00	0.00	0.00	3.50	0.50	0.00	95.00
22	Jazipoostsefid	3.30	0.00	0.00	0.00	3.60	3.40	0.00	90.00

Abbreviations: MIS = Metaphase I stickiness, MIIS = Metaphase II stickiness, AIS = Anaphase I stickiness, AIIS = Anaphase II stickiness, AIL = Anaphase I laggard, AIIL = Anaphase II laggard, MP = Multipolar cell, PF = Pollen fertility.

The frequency and distribution of chiasma is under genetic control (Rees and Jones 1977) and the heritable adjustment in the frequency and distribution of chiasma and recombination, as well as their effects on the variability of progenies and populations, is established in both experimental and natural populations (Rees and Jones 1977). Therefore, presence of a significant difference in chiasma frequency and distribution as well as ring and rod bivalents among the cultivars grown under uniform conditions may indicate genomic differences (Sheidai *et al*. 2002).

Data concerning chromosome segregation is provided in Table 2. Although in most of the cases normal chromosome segregation occurred during anaphase and telophase stages, some amount of irregularities occurred which were mainly of chromosome stickiness and the formation of laggard chromosomes (Fig. 1, e) and micronuclei.

Chromosome stickiness and late separation was observed in some of the cultivars (Table 2). Sticky chromosomes were observed from early stages of prophase and continued to the final stages of meiosis. Genetic and environmental factors as well as genomic-environmental interaction have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao 1996). However Baptista-Giacomelli *et al*. (2000), reported a difference in the percentage of cells showing stickiness in Brazilian *Avena sativa* cultivars and suggested a genomic-environmental interaction as the main reason for the occurrence of chromosome stickiness, this may hold true for the *P. granatum* cultivars studied.

The highest value of anaphase I laggards occurred in Alaktorsh (14.63%) and the lowest value occurred in Malastorsh with no laggard chromosomes. The highest value of anaphase II laggards occurred in Berit cultivar while cultivars of Malastorsh, Alaktorsh, Malasshahsavar and Redki showed no laggards in anaphase II. (Table 2).

Paired sample χ^2 test performed for chromosome segregation among the cultivars revealed a significant difference among most of the cultivars studied, further supporting their genomic differences.

An interesting observation was the occurrence of multipolar telophase-I and II cells in most of the cultivars studied (Fig. 1, g). The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during metaphase. Any distortion or breakage in the spindle may result in random sub-grouping. Different reasons have been suggested for the occurrence of spindle abnormalities including: duality of nucleus in foreign cytoplasm, environmental

influence and disharmonious gene interaction (NIRMALA and RAO 1996).

In several instances spindle abnormalities have led to the production of aneuploid and polyploid/ unreduced (2n) gametes for example in polyploidy hybrids and derivatives of *Aegilops* × *Triticum* hybrids, amphiploid Triticineae, amphiploids of *Solanum* hybrids, etc (VILLEUX 1985). The presence of giant grains has been seen as an indication of the production of 2n pollen (VORSA and BINGHAM 1979). In fact pollen grains (potential 2n pollens) of significantly larger size compared to the normal pollen grains were observed in low frequency (1-2 %) in the pomegranate cultivars of Aghamohamadali and Malaspeyvandi (Fig.1, k, l.), which may have been formed due to multipolar cells occurring in these cultivars. Several abnormal tetrads were also formed in the cultivars studied (Fig.1, f & h), which may also be the result of such multipolar cells.

Pearson coefficient of correlation determined between pollen fertility and cytogenetic characters studied revealed a positive significant correlation between total chiasmata and pollen fertility indicating that with an increase in chiasma number increase in pollen fertility occurs. However a negative significant correlation was observed between pollen fertility and meiotic abnormalities like chromosome stickiness in metaphase I and anaphase I, laggard chromosomes in anaphase I and multipolar cell formation (Table 3). Therefore theses meiotic abnormalities are partly the reason for reduction in pollen fertility of the pomegranate cultivars studied.

Cluster and ordination analyses of cultivars produced similar results (Figs. 2 & 3). In general three major cluster/ groups were formed, separating the cultivars studied. The first major cluster consists of two sub-clusters. The cultivars Shahitorsh, Shirinhasteriz, Torshpostkoloft, Malaspeyvandi, Neytalkhi, Golnar, Malastoghi, Ghermeznarshirin, Malasshahsavar and Dadashpostkoloft show more similarity in their meiotic characteristics and form the first sub-cluster while two cultivars of Alaktorsh and Jazipoostsefid form the second sub-cluster.

The cultivars Redki, Torshnar, Alakshirin, Malastorsh and Malasshahsavar and Aghamohamadali are comprised in the second major cluster, in which the cultivar Aghamohamadali shows some distance to the other cultivars due to its cytogenetic difference.

The third major cluster is formed by the three cultivars Berit, Golmagasi and Malasshirin. The Berit cultivar is joined to the other two cultivars with some distance due to its meiotic difference.

It is interesting to mention that the cultivars of the second major cluster belong to different regions of Saveh and Bafgh cities while the cultivars comprising the second major cluster except Mala-

Table 3 — Correlation between pollen fertility and meiotic abnormalities in *Punica granatum* cultivars. (Meiotic characters as in Table 2).

		MIS	MIIS	A1S	AIL	AIIL	MP	PF
MIS	Pearson Correlation	1.000	.328	.325	-.031	-.079	.431*	-.469*
	Sig. (2-tailed)	.	.136	.140	.889	.727	.045	.028
	N	22	22	22	22	22	22	22
MIIS	Pearson Correlation	.328	1.000	.853**	-.169	-.140	.030	-.395
	Sig. (2-tailed)	.136	.	.000	.453	.535	.896	.069
	N	22	22	22	22	22	22	22
A1S	Pearson Correlation	.325	.853**	1.000	-.129	-.223	.036	-.497*
	Sig. (2-tailed)	.140	.000	.	.568	.319	.874	.019
	N	22	22	22	22	22	22	22
AIL	Pearson Correlation	-.031	-.169	-.129	1.000	.520	.055	-.374
	Sig. (2-tailed)	.889	.453	.568	.	.013	.809	.087
	N	22	22	22	22	22	22	22
AIIL	Pearson Correlation	-.079	-.140	-.223	.520*	1.000	.259	-509*
	Sig. (2-tailed)	.727	.535	.319	.013	.	.244	.015
	N	22	22	22	22	22	22	22
MP	Pearson Correlation	.431*	.030	.036	.055	.259	1.000	-.445*
	Sig. (2-tailed)	.045	.896	.874	.809	.244	.	.038
	N	22	22	22	22	22	22	22
PF	Pearson Correlation	-.469*	-.395	-.497*	-.374	-.509*	-.445*	1.000
	Sig. (2-tailed)	.028	.069	.019	.087	.015	.038	.
	N	22	22	22	22	22	22	22

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

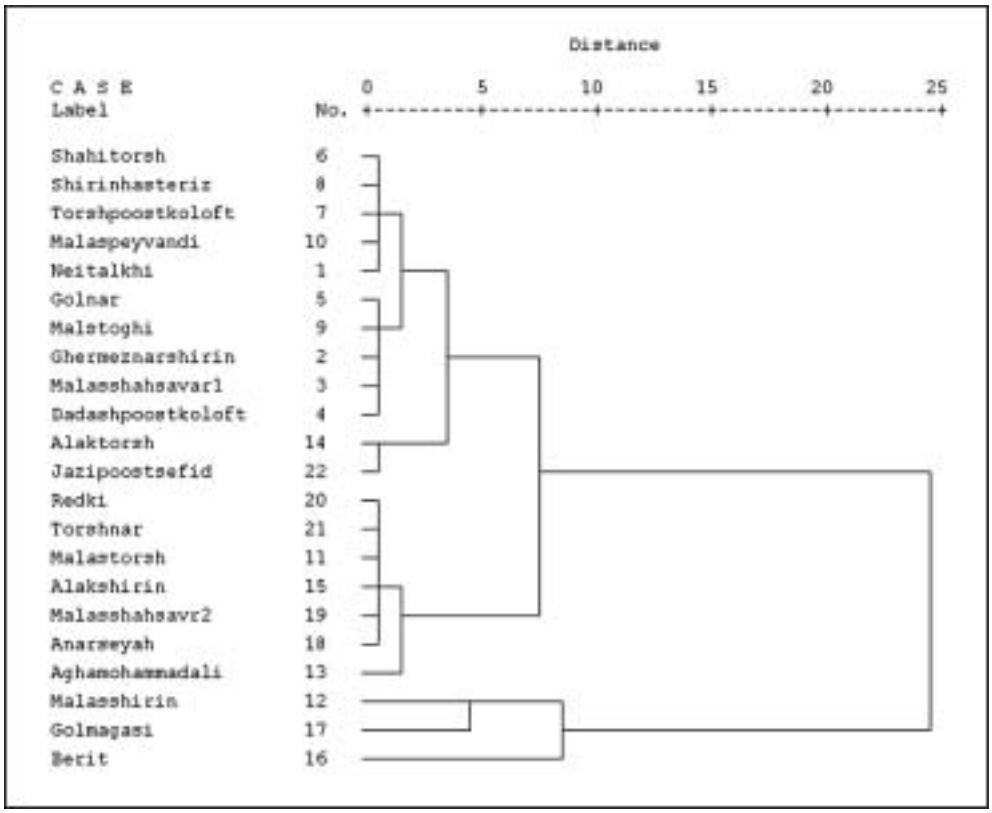


Fig. 2 — WARD cluster analysis of meiotic data in *Punica granatum* cultivars studied.

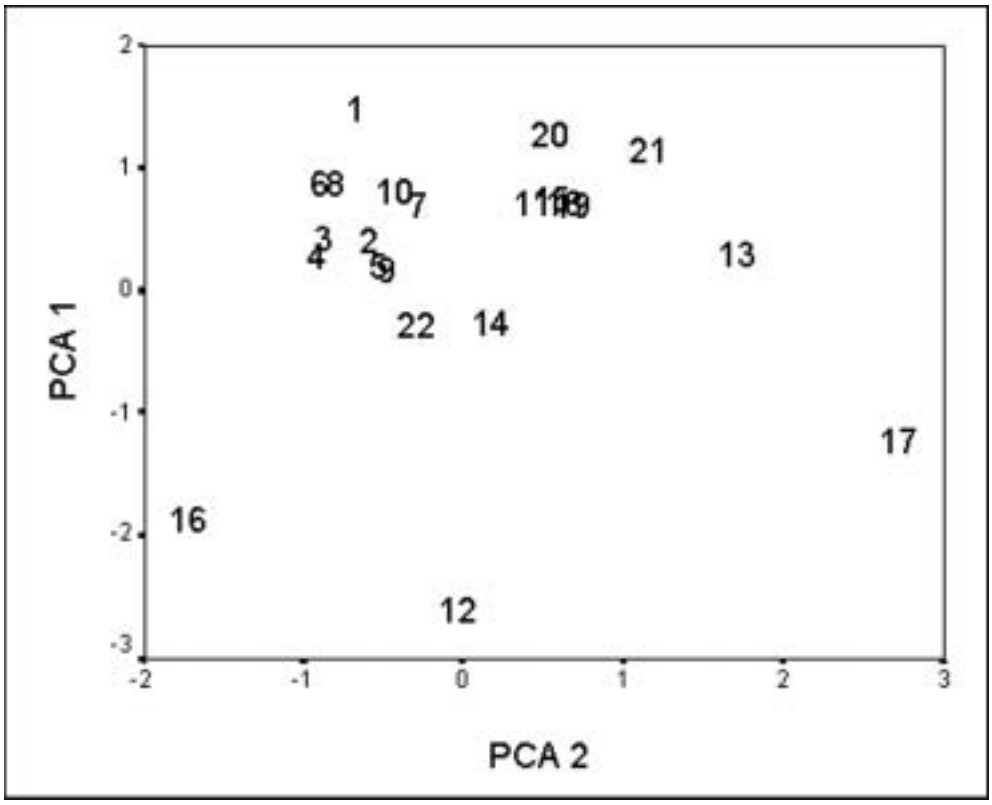


Fig. 3 — PCA ordination of *Punica granatum* cultivars studied. The cultivars number as in Table. 1.

shirin are grown in other cities (Table 1). Therefore it seems that the cytogenetic characteristics of the cultivars studied may be somewhat related to their geographical distribution.

PCA analysis of meiotic data revealed that the first 3 factors account for about 89% of total variance. In the first component, which explains about 54% of total variance, meiotic characters like ring bivalents, terminal and total chiasma possessed the highest positive correlation (>0.90), while rod bivalents possessed the highest negative correlation (>-0.80).

In the second component, which explains about 25% of total variance, intercalary chiasma possessed the highest positive and negative correlation (>0.90). Therefore these are the most variable meiotic characteristics among the cultivars studied, as also revealed by ANOVA test discussed earlier.

The first PCA factor separates mainly the cultivars Malasshirin, and Berit from the others while the second factor mainly separates the cultivars Golmagasi and Aghamohamadali from the other cultivars studied (Fig. 4).

In general, if the cytogenetic differences observed in the cultivars studied is accompanied by other agronomic differences, a better hybridization and selection program may be planned for pomegranate cultivars. This is particularly true for the cultivar Malashirin which grows in Saveh city and is placed in the third major cluster, far separate from the other cultivars of this city (in the second major cluster) due to its genomic differences. The occurrence of 2n (unreduced) gametes also should be studied in more detail as the cultivars producing such gametes may be used in hybridization program and obtain polyploid plants with some new characteristics.

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