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# B-CHROMOSOMES IN IRANIAN POMEGRANATE (*PUNICA GRANATUM* L.) CULTIVARS

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**Abstract**

Programs for screening the genetic and cytogenetic diversity among *Punica granatum* L., cultivars of Iran are in hand, therefore cytogenetical study of 22 *Punica granatum* cultivars was carried out for the occurrence and effect of B-chromosomes in genetic recombination of the cultivars possessing them. The study revealed the presence of 0-5 B-chromosomes in 17 cultivars out of 22. These accessory chromosomes were smaller than the A-chromosomes and did not pair with them or among themselves. B-chromosomes could arrange themselves on the equatorial plane and move to the anaphase poles however they lagged in some cases controlling their accumulation in the daughter nuclei. Statistical analysis showed a significant increase in the mean number of chiasmata when B-chromosomes are present in some of the cultivars leading to a change in genetic recombination.

## Introduction

Pomegranate (*Punica granatum* L.) of the family Punicaceae is native from Iran to the Himalayas in northern India, cultivated and naturalized over the whole Mediterranean region since ancient times (Facciola, 1990). Pomegranate is one of the most important endemic horticultural plants of Iran growing in most of the regions through out the country and grows well in arid and semiarid regions due to its adaptation to adverse ecological conditions. About 764 cultivars of *Punica granatum* have been collected during a germ plasm collection and grown in Saveh and Yazd cities, all of which possess their specific fruit characteristic 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, Sheidai *et al*., 2005) and in spite of great 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. In the present study the occurrence and effects of B-chromosomes in 17 *Punica granatum* var. *sativa* accessions of Iran were carried out for the first time and their possible role in breeding this important horticultural plant is discussed.

## Materials and Method

Cytogenetic studies were performed originally in 22 *P. granatum* cultivars for two successive years (2002-2003), out of which B-chromosomes were observed in 17 cultivars. For cytogenetical studies 50 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 (1:3) for 24 hours which were then washed thoroughly and transferred to ethanol until used (Sheidai *et al*., 2002).

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Chromosome pairing and chiasma frequency was determined by using minimum 100 meiocytes showing diakinesis-metaphase-I stages, while chromosome segregation was studied in minimum 500 anaphase-I and II stages. Comparison of meiotic data was performed among the cells possessing B-chromosomes and those devoid of Bs using t-test analysis (Sheidai *et al*., 2003).

In order to study meiotic similarities of the cultivars studied, both when possessing B-chromosomes and devoid of Bs, Cluster analysis and ordination based on principal components (PCA) analysis was performed (Sheidai *et al*., 2003). Statistical analyses was carried out using SPSS Ver. 9 (1998).

**Results and Discussion**

Out of 22 *Punica granatum* cultivars studied, B-chromosomes (0-5) occurred in 17 of them (Table 1). Previously, the occurrence of 0-7 B-chromosomes (Bs) has been reported only in Indian cultivars of *Punica granatum* (Gill *et al*., 1981) but their effect on chiasma frequency and genetic recombination has not been discussed. The highest number of Bs (5) occurred in Germeznarshirin cultivar collected from Shabastar city.

B-chromosomes (Bs) are accessory chromosomes reported in more than 1300 species of plants and almost 500 species of animals (Camacho *et al*., 2000). The B-chromosomes show numerical polymorphism and when present in high number affect negatively the growth and vigor of the plants, while in low number may benefit the plant possessing them.

The Bs observed in *Punica granatum* cultivars were much smaller than the A-chromosomes, round in shape and did not pair with the A-chromosomes or among themselves (Figs. 1-3). They could arrange themselves in the equatorial plane and move to anaphase poles to be included in the daughter cells. However in several instances they were lagged and possibly excluded from the daughter cells. This has been considered as a controlling mechanism avoiding B-chromosome accumulation detrimental to the plants possessing them (Sybenga, 1992).

The Bs may affect the frequency and distribution of chiasma as well as chromosome association either directly or by affecting the genes controlling meiosis present on the A-chromosomes (Camacho *et al*., 2000). Details of chiasma frequency and chromosome pairing in the cells possessing B- chromosomes and the cells devoid of Bs is presented in Table 1.

T-test analysis (Table 1) revealed that the presence of B-chromosomes significantly increased the mean values of intercalary and total chiasmatas in the cells possessing B-chromosomes compared to those devoid of Bs in some of the cultivars. For example in 3 cultivars of Golmagasi, Malas-Shahsavar 2 and Torshnar a significant increase in the mean value of total chiasmata occurred in the presence of B-chromosomes (Table 1) while the presence of Bs significantly increased the mean value of intercalary chiasmata in cultivars Neitalkhi, Golmagasi and Malas-Shasavar.

A non-significant increase in the number of ring bivalents was observed in the cultivars Neitalkhi, Alaktorsh, Berit, Golmagasi, Malas-Shahsavar 2 and Torshnar (Table 1). Moreover, a significant decrease in the number of univalents was noticed in the cells possessing B- chromosomes.

**Table 1. Meiotic characteristics in *Punica granatum* cultivars.**

| | Cultivar | Locality | | TX | IX | TOX | RB | ROB | QU | I | BN |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Neitalkhi | Bandarabbas | -B | 14.80 | 0.35 | 15.15 | 6.84 | .098 | 0.04 | 0.15 | 0-2 |
| | | | +B | 15.00 | .066 | 15.66 | 7.00 | 1.00 | 0.00 | 0.00 | |
| 2. | Ghermeznarshirin | Shabastar | -B | 13.85 | 0.42 | 14.27 | 6.44 | 1.28 | 0.00 | 0.71 | 0-5 |
| | | | +B | 12.75 | .050 | 13.25 | 5.12 | 2.78 | 0.00 | 0.25 | |
| 3. | Malas-Shasavar1 | Bafgh | -B | 13.54 | 0.24 | 13.58 | 6.66 | 1.70 | 0.00 | 0.52 | 0-2 |
| | | | +B | 14.44 | 0.11 | 14.55 | 6.66 | 1.10 | 0.00 | 0.44 | |
| 4. | Dadashpoostkoloft | Shabastar | -B | 13.63 | 0.12 | 13.75 | 6.00 | 1.55 | 0.03 | 0.73 | 0-2 |
| | | | +B | 13.60 | 0.00 | 13.60 | 5.60 | 2.40 | 0.00 | 0.44 | |
| 5. | Golnar | Saveh | -B | 13.45 | 0.22 | 13.67 | 6.00 | 1.45 | 0.03 | 0.97 | 0-3 |
| | | | +B | 13.60 | 0.00 | 13.60 | 6.00 | 1.60 | 0.00 | 0.80 | |
| 6. | Shahitorsh | Kerman | -B | 14.40 | 0.10 | 14.50 | 6.60 | 1.10 | 0.00 | 0.40 | 0-1 |
| | | | +B | 13.60 | 0.00 | 13.60 | 5.80 | 2.00 | 0.00 | 0.40 | |
| 7. | Torshpoostkoloft | Ardel | -B | 13.74 | 0.54 | 14.28 | 6.54 | 1.15 | 0.00 | 0.61 | 0-2 |
| | | | +B | 13.40 | 0.57 | 13.97 | 6.30 | 1.14 | 0.00 | 1.14 | |
| 8. | Malastoghi | Gorgan | -B | 13.37 | 0.43 | 13.80 | 6.00 | 1.52 | 0.03 | 0.80 | 0-3 |
| | | | +B | 13.22 | 0.66 | 13.88 | 5.78 | 1.90 | 0.00 | 0.66 | |
| 9. | Malastorsh | Saveh | -B | 13.75 | 1.35 | 15.10 | 6.52 | 1.00 | 0.00 | 0.72 | 0-4 |
| | | | +B | 13.10 | 1.31 | 14.41 | 6.00 | 1.53 | 0.00 | 0.92 | |
| 10. | Malasshirin | Saveh | -B | 9.80 | 1.44 | 11.24 | 3.94 | 3.00 | 0.00 | 1.64 | 0-2 |
| | | | +B | 10.67 | 1.00 | 11.67 | 3.83 | 3.66 | 0.00 | 1.00 | |
| 11. | Alaktorsh | Saveh | -B | 12.66 | 1.63 | 14.29 | 5.44 | 1.89 | 0.00 | 0.78 | 0-3 |
| | | | +B | 13.00 | 1.10 | 14.10 | 5.50 | 2.20 | 0.00 | 0.60 | |
| 12. | Berit | Kazeron | -B | 11.90 | 1.00 | 12.90 | 4.63 | 2.84 | 0.10 | 0.63 | 0-4 |
| | | | +B | 10.83 | 1.42 | 12.25 | 4.75 | 2.50 | 0.25 | 0.93 | |
| 13. | Golmagasi | Taft | -B | 10.85 | 2.60 | 13.45 | 5.30 | 1.23 | 0.00 | 2.60 | 0-2 |
| | | | +B | 10.50 | 5.50 | 16.00 | 5.50 | 2.50 | 0.00 | 1.00 | |
| 14. | Seyah | Saveh | -B | 14.14 | 1.00 | 15.14 | 6.85 | 0.60 | 0.00 | 1.10 | 0-3 |
| | | | +B | 12.80 | 1.00 | 13.80 | 5.80 | 0.80 | 0.20 | 1.20 | |
| 15. | Malas-Shahsavar2 | Bafgh | -B | 13.40 | 1.93 | 15.33 | 6.27 | 1.40 | 0.00 | 0.55 | 0-2 |
| | | | +B | 13.44 | 2.67 | 16.44 | 6.66 | 1.33 | 0.00 | 0.00 | |
| 16. | Redki | Bafgh | -B | 14.40 | 1.60 | 16.00 | 6.75 | 1.00 | 0.00 | 0.50 | 0-1 |
| | | | +B | 13.50 | 2.00 | 15.50 | 6.50 | 1.50 | 0.00 | 0.00 | |
| 17. | Torshnar | Shabastar | -B | 14.80 | 1.87 | 16.67 | 6.67 | 0.82 | 0.00 | 0.96 | 0-1 |
| | | | | 15.25 | 2.00 | 17.25 | 7.25 | 0.75 | 0.00 | 0.00 | |

Abbreviations: +B = cells possessing B-chromosome, -B = Cells devoid of B-chromosome, TX = Terminal chiasmata, IX = Intercalary chiasmata, TOX = Total chiasmata, RB = Ring bivalent, ROB = Rod bivalent, QU = Quadrivalent, I = Univalent. BN = Number of B-chromosomes. (* = Significant at 0.05).

An increase in chiasma number may bring about more genetic variation for the plant possessing B-chromosomes, this holds true particularly for those cultivars having increase in their intercalary chiasmata, as the genes located in the intercalary positions of the chromosome arms also become involved in genetic recombination. An increase in the number of ring bivalents and decrease in the number of univalents also may help in better separation of chromosomes in anaphase leading to a higher pollen fertility in the cultivars with B-chromosomes.

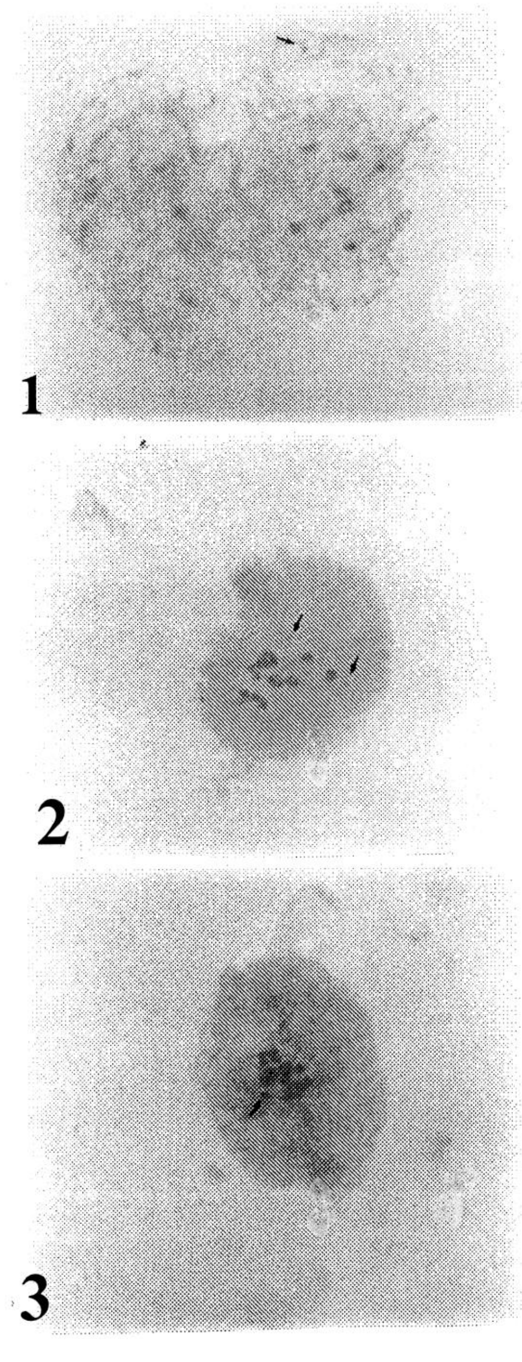

Fig. 1-3. Representative meiotic cells showing the presence of B-chromosomes in *Punica granatum* cultivars. Metaphase-I cells showing B-chromosomes (Arrow) in the cultivars 1- Alaktorsh, 2- Malastoghi and 3- Anarseyah respectively. Scale bar = 10 μm.

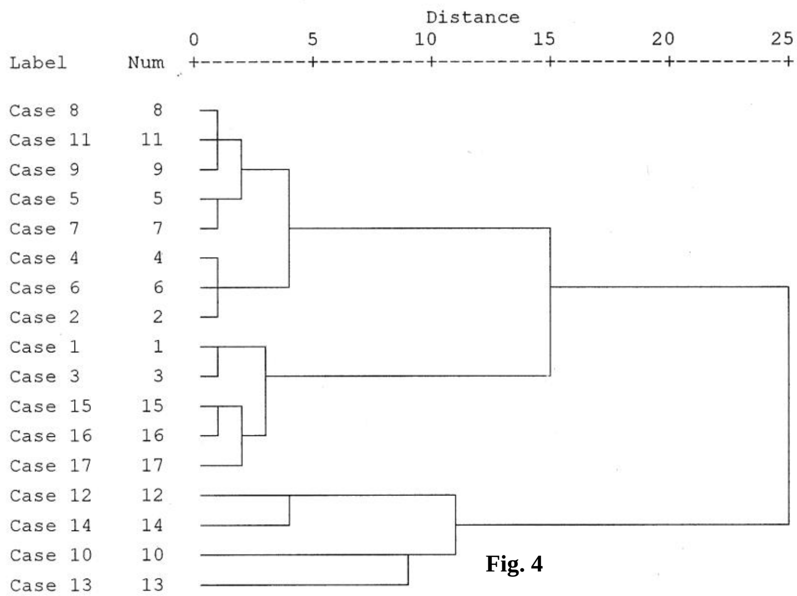


**Fig. 4**

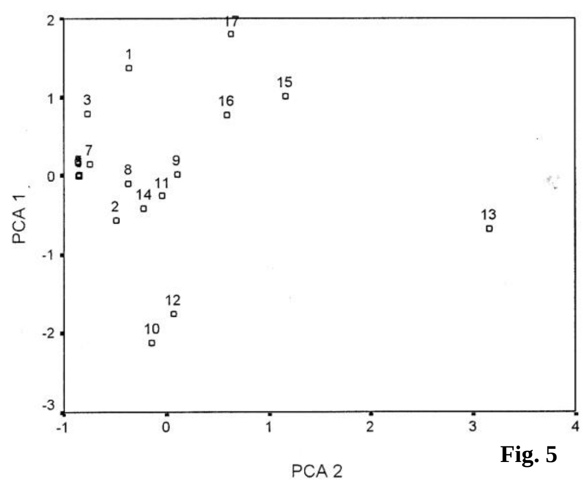


**Fig. 5**

Figs. 4 & 5.UPGMA cluster analysis and PCA ordination of *Punica granatum* cultivars in the presence of B- chromosomes. (Cultivars number as in Table 1).

A non-significant reduction in the mean values of chiasma frequency and ring bivalents were observed in some the cultivars e.g., Dadashpoostkoloft, Torshpoostkoloft, Malastoursh, Berit and Redki. A significant decrease in total chiasmata in the cultivar Seyah was also noticed (Table 1). Therefore the effects of B-chromosomes vary in different *Punica granatum* cultivars studied, which may be due to the difference in their genetic background. According to our knowledge this is the first report on the effects of B-chromosomes on chiasma frequency and chromosome pairing in *Punica granatum*.

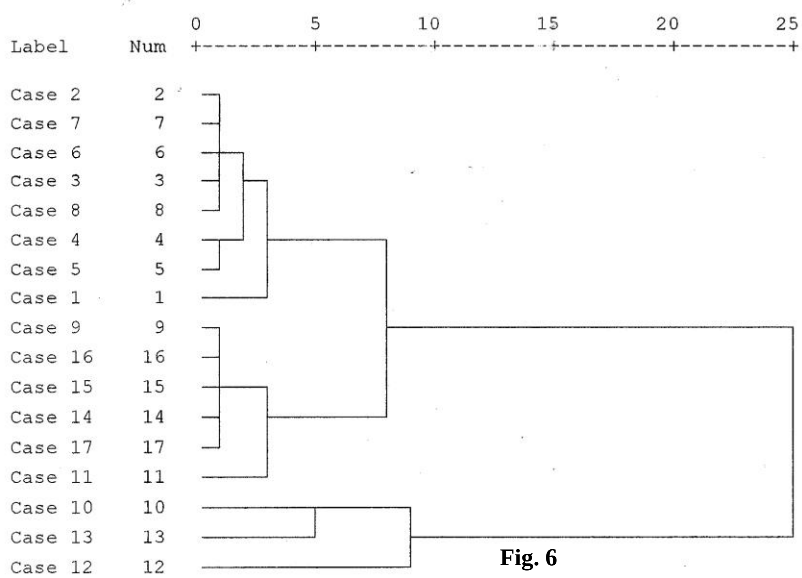


**Fig. 6**

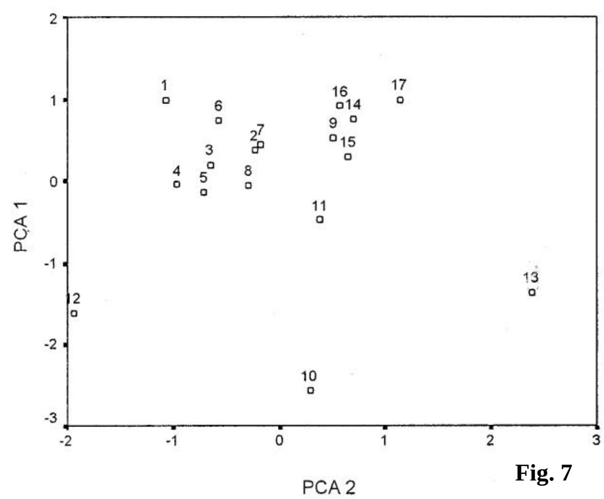


**Fig. 7**

Figs. 6 & 7. UPGMA cluster analysis and PCA ordination of *Punica granatum* cultivars in the absence of B-chromosomes. (Cultivars number as in Table 1).

In order to study meiotic similarities of the cultivars both when B-chromosomes are present and when they are absent, cluster analysis and ordination based on the first two PCA axes was performed (Figs. 4-7). Both analyses produced almost similar results in which 3 major clusters or groups were formed. The first and second major clusters or groups are comprised of the cultivars 1-9, 11 and 15-17 (Table 1) showing more similarity in their meiotic characteristics (although very little difference exists in the members of two clusters in the presence of B-chromosomes compared to when Bs are absent). The third major cluster or group is comprised of cultivars 10, 12 and 13 in both analyses. Therefore the presence of B-chromosomes does not change the cytogenetic relationships (showing partly genetic relationships) of the *Punica granatum* cultivars studied. The present findings may be used in planning breeding and hybridization programs of pomegranate cultivars.

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