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Chromosome variation in *Pistacia* genus

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SUMMARY – *Pistacia* genus belongs to the Anacardiaceae family, and comprises only dioecious species. These plants are distributed between 29° and 42° north. All species of this genus are diploid with chromosome numbers of 2n = 24, 28 and 30. According to literature, the basic chromosome number in this genus is x = 12, 14 and 15. *Pistacia khinjuk* with a chromosome complement of 2n = 24 has a symmetric karyotype with a median and submedian centromere position. Because ancestral species have a symmetric karyotype, it seems that x = 12 is the initial basic chromosome number in this genus and the x = 14 and x = 15 derived from x = 12. No polyploid races have been reported in *Pistacia* so far, so it seems that diploidy phenomena played an important role in evolution and speciation.

Key word: Anacardiaceae, chromosome number, pistachio, *Pistacia atlantica, Pistacia khinjuk, Pistacia vera.*

RESUME – *"Variation chromosomique chez le genre* Pistacia*". Le genre* Pistacia *appartient à la famille des Anacardiacées, et toutes ses espèces sont dioïques. Cette plante est distribuée entre 29° et 42° degrés Nord. Toutes les espèces de ce genre sont diploïdes avec un nombre chromosomique 2n = 24, 28 et 30. Les références montrent que le nombre chromosomique de base pour ce genre est de x = 12, 14, et 15.* Pistacia khinjuk *avec un complément chromosomique à 2n = 24 possède un karyotype symétrique avec une position centromérique médiane ou submédiane. Etant donné que les espèces ancestrales ont un karyotype symétrique, il semble que x = 12 soit la base initiale de ce genre et x = 14 et x = 15 dérivent de x = 12. Jusqu'à présent la polyploïdie n'est pas rapportée chez* Pistacia, *il semble donc que la diploïdie joue un rôle important dans l'évolution de ce genre.*

Mots-clés : *Anacardiaceae*, *nombre de chromosomes, pistache,* Pistacia atlantica, Pistacia khinjuk, Pistacia vera.

Introduction

The genus *Pistacia* L. with approximately 15 species in the world is an important genus of the Anacardiaceae family, which is distributed in some parts of Asia, Europe and north of Africa. These plants are found in a range of 29 to 42 degree of north latitude in the world. Pistachio is the only species in this genus, successfully grown in orchards, which produces edible nuts large enough to be commercially acceptable. According to the literature, from 15 species, which are mentioned in some floras, only seven species are real *Pistacia* species, since some of them are synonyms.

Previous works indicated that all species of *Pistacia* are diploid with chromosome numbers of 2n = 24, 28 and 30 (Zohary, 1952; Mehra and Sareen, 1969; Nilsson and Lassen, 1971; Bochantseva, 1972; Mehra, 1976; Natarajan, 1977, 1978; Gill *et al.,* 1984; Huang *et al*., 1986; Sandhu and Mann, 1988; Huang *et al*., 1989; Ghaffari and Fasihi Harandi, 1999; Ghaffari and Fasihi Harandi, 1999, 2002; Fasihi Harandi and Ghaffari, 2001). Two different chromosome counts (2n = 24 – 30) are reported only for *P. lentiscus* (Zohary, 1995; Nilsson and Lassen, 1971; Natarajan, 1977, 1978).

During the past five years my research has focused on *Pistacia* in Iran, a region where about 3 species and 3 subspecies are known to occur. This study is a further contribution towards providing basic chromosome number and mechanisms of chromosome variation in the genus *Pistacia.*

Materials and methods

Between 1998 and 2002, buds, seeds and herbarium vouchers of 2 species (*P. khinjuk* and *P. vera*) and 3 subspecies (*P. atlantica* subsp*. cabulica, P. atlantica* subsp*. kurdica, and P. atlantica* subsp*. mutica*) were collected from their native habitats in various areas of Iran. Seeds and cytological specimens of cultivated *P. vera* were obtained from some orchards of Rafsanjan city. Floral buds for chromosome studies were fixed in absolute ethanol:chloroform:propionic acid (6:3:2) for 24 hours and subsequently washed and stored in ethanol (70%) until analysed. Anthers were macerated in ferric acetocarmine (1%) and counts were made from microsporocytes in various stages of meiosis.

For the study of mitotic chromosomes root types were placed in 0.5% colchicine for 3 hours at room temperature and then fixed in 6:3:2 absolute ethanol:chloroform:propionic acid at 4ºC for 48 hours. After rinsing in distilled water, root types were hydrolyzed in the hydrochloric acid 1 N at 60ºC for 10-12 minutes. Staining was carried out with the Feulgen reaction enhanced by squashing in 2% acetocarmine.

Chromosomes were studied under oil immersion on a phase contract microscope at a magnification of 1000x. All slides were made permanent by the venetian turpentine (Wilson, 1945). Nomenclature adopted by Levan *et al*. (1964) was followed for recognizing chromosome types. For the previous chromosome counts, we used the following references: Fedorov (1969), Moore (1973;1977), Goldblatt (1981;1988), Goldblatt and Johnson (1990; 1991).

Results

Pistacia atlantica Desf.

The origin centre of this taxon is the Iranian plateau but, it has been spreading continuously to Europe, Africa and Canary islands. In Flora Iranica 3 subspecies have been mentioned for this taxon.

P. atlantica subsp. *cabulica* (Stocks) Rech.f.

Our previous chromosome count for this subspecies was 2n = 28 (Ghaffari and Fasihi Harandi, 2002). The somatic chromosome number in the new sample from Genu mountain was 2n = 28. Two large metacentric chromosomes, which have the heterochromatic nature, were observed (Figs 1, 2 and 3).

Fig. 1. Metaphase of mitosis, showing 2n = 28.

P. atlantica subsp. *kurdica* (Zohary) Rech.f.

This plant is distributed in Turkey, Iran, Iraq and Syria. Complementary information, from mountain

Genu plants, gave a chromosome number of 2n = 28, which is in agreement with previous reports (Ghaffari and Fasihi Harandi, 2002). In the present study two large heterochromatic chromosomes were observed (Figs 4 and 5).

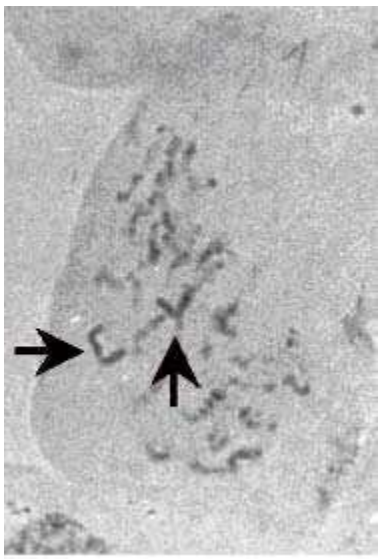

Fig. 2. Prophase of mitosis, showing two heterochromatin chromosomes (arrows).

Fig. 3. Idiogram of *Pistacia atlantica.* Subsp. *cabulica.*

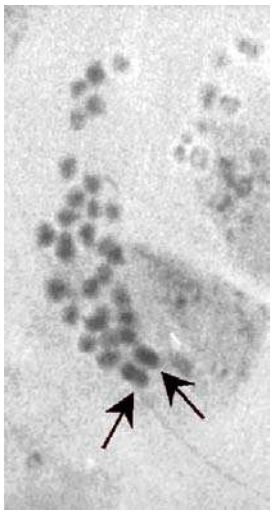

Fig. 4. Metaphase of mitosis, showing two heterochromatin chromosomes (arrows).

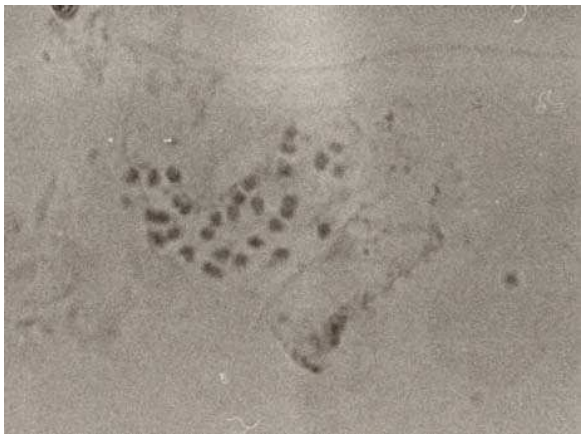

Fig. 5. Metaphase of mitosis (2n = 28).

P. atlantica subsp. *mutica* (Fisch.&May.) Rech.f.

All samples, which were collected from the west of Rafsanjan, indicate a chromosome complement of 2n = 28. Similarly to the two other subspecies, we found two large heterochromatic chromosomes in this taxon at prophase stage (Figs 6 and 7).

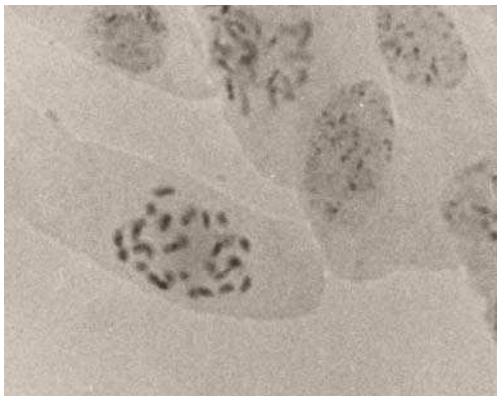

Fig. 6. Late prophase (2n = 28).

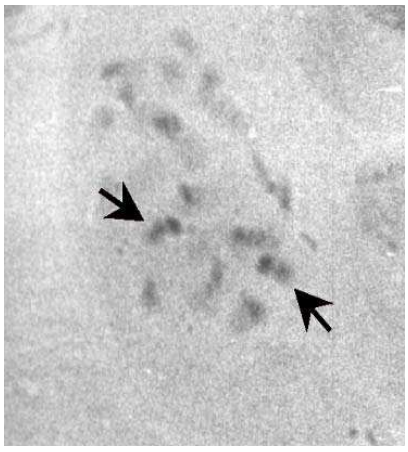

Fig. 7. Prophase of mitosis, showing two heterochromatin chromosomes (arrows).

P. khinjuk Stocks

This species exhibited the greatest distribution in Iran and another parts of Turkey, Afghanistan, Pakistan, Iraq, Syria, Palestine and North of Africa. Our previous report indicates that this taxon has a symmetric karyotype with 2n = 24 chromosomes (Ghaffari and Fasihi Harandi, 2002), which agrees with the present count. Also, two heterochromatic chromosomes were observed (Figs 8 and 9).

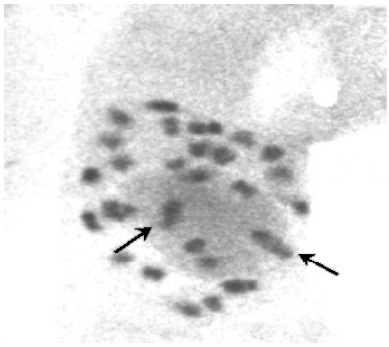

Fig. 8. Late prophase of mitosis, showing to two heterochromatin chromosomes (arrows).

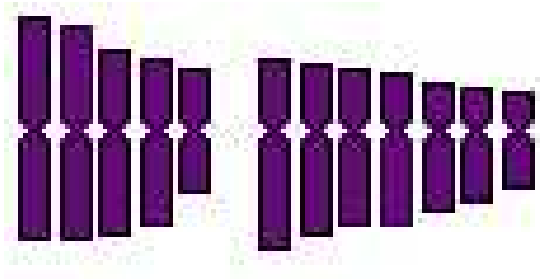

Fig. 9. Idiogram of *Pistacia khinjuk.*

P. vera L.

This plant is the only species in the genus *Pistacia,* successfully grown in orchards, which produces edible nuts large enough to be commercially acceptable. *P. vera* is distributed in wild form in North-East of Khorassan province and some parts of Central Asia. Our present and previous works on wild and cultivated plants indicates the chromosome complement of 2n = 30 in root type cells and gametic numbers (n = 15) in pollen mother cells. In both wild and cultivated plants two heterochromatic chromosomes were observed (Figs 10, 11 and 12).

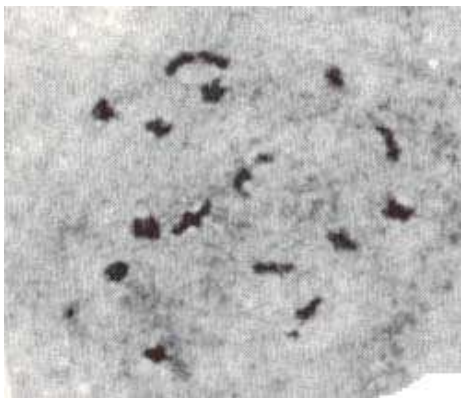

Fig. 10. Metaphase I, showing 15 bivalents.

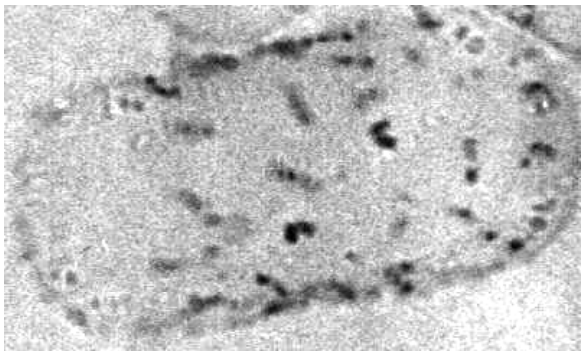

Fig. 11. Prophase of mitosis, showing two heterochromatin chromosomes (arrows).

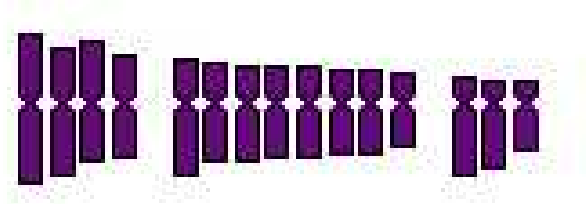

Fig. 12. Idiogram of *Pistacia vera.*

Discussion

Pistacia genus belongs to the Anacardiaceae family, from which all species are dioecious. These plants distributed in a range of 29 to 42 degree of north latitude. According to literature , all species of this genus are diploid with chromosome number of 2n = 24, 28 and 30. The conclusion obtained from the previous works (Table 1) and present counts, showed the 3 basic chromosome number of x = 12, 14 and 15 in this genus. Our previous work (Ghaffari and Fasihi Harandi, 2002) indicates that the chromosome complement of *P. khinjuk* (2n = 28) has a symmetric karyotype with a median or submedian centromere position. Because ancestral species have a symmetric karyotype, it seems that x = 12 and 15 derived from x = 14. In the genus *Pistacia* polyploidy level does not exist in any species. Almost all chromosome results available for the species of the *Pistacia* are based on one chromosome complement for each species with the exception of 2n = 24 and 30 for *P. lentiscus* (Zohary, 1952; Nilsson and Lassen, 1971; Natarajan, 1977,1978). It seems that the chromosome count of 2n = 24 is correct, as mentioned Zohary (1952) and Nilsson and Lassen (1971). The error in the chromosome count by Natarajan (1977) may be either due to a misidentification of the species or simply a wrong chromosome count. Karyotype data of 3 subspecies of *P. atlantica* (Ghaffari and Fasihi Harandi, 2002) showed a high similarity between them. The mechanism of chromosome variation in the genus *Pistacia* are not well understood. We suppose that diploidization may play a great part in the diversification of the chromosome variation and the speciation of the genus.

Table 1. Previous reports of chromosome data on *Pistacia* genus

Taxon	n	2n	References
Pistacia atlantica		28	Zohary, 1952
subsp. *cabulica*		28	Ghaffari and Fasihi Harandi, 2002
subsp. *kurdica*		28	Ghaffari and Fasihi Harandi, 2002
subsp. *mutica*		28	Ghaffari and Fasihi Harandi, 2002

Table 1 (cont.). Previous reports of chromosome data on *Pistacia* genus

Taxon	n	2n	References
P. chinensis		24	Huang *et al*., 1986
			Huang *et al*., 1989
P. integerrima	15		Mehra and Sareen, 1969
	15		Mehra 1976
	15		Gill *et al*., 1984
	15		Sandhu and Mann, 1988
P. khinjuk		24	Ghaffari and Fasihi Harandi, 2002
P. lentiscus		24	Zohary, 1952
		24	Nilsson and Lassen, 1971
		30	Natarajan, 1977
		30	Natarajan, 1978
P. terebinthus		30	Natarajan, 1978
P. vera		30	Zohary, 1952
		30	Bochantseva, 1972
	15	30	Fasihi Harandi and Ghaffari, 2001
	15	30	Ghaffari and Fasihi Harandi, 2002

Acknowledgements

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