Chromosome studies on pistachio (*Pistacia vera* L.) from Iran

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SUMMARY – The pistachio tree (*Pistacia vera* L.) is a member of the Anacardiaceae family and originates in Central Asia. Two centres of diversity have been described for *P. vera*, an area from Iran into eastern Turkey and an area in northern Afghanistan and southern Turkmenistan. Pistachio is the only species in this genus, successfully grown in orchards, which produces edible nuts large enough to be commercially acceptable. Iranian species of *P. vera* is the subject of a chromosomal study. Frequently, meiosis in this species was regular and showed 15 bivalents at the first metaphase. Most bivalents had two terminal chiasmata. The mean number of chiasmata was estimated at 1.35 for each bivalent at the first metaphase. One to five pairs of chromosomes were associated with the nucleolus in diakinesis. The observation of chromosomal behaviour in this species suggests that the chromosomal coiling is very fast in a manner that zygotene chromosomes are short and thick and gathered around nucleolus like a synizetic knob. Because of this coiling of chromosomes we could not observe any marked difference, except for the presence of nucleolus between diakinesis and the first metaphase. Occasionally in some cells, laggard chromosomes, univalent and multivalent chromosomes were observed. The conclusions obtained from mitotic studies approved the meiotic studies and the chromosome complement (2n = 30). In the interphase, two chromocentres were observed clearly, which were also observable in the prophase and metaphase. It seems that they are candidates for sex chromosomes in this taxon. Meiotic behaviour for this taxon is reported here for the first time.

Key words: Chromosome, cytogenetics, pistachio, *Pistacia vera.*

RESUME – *"Etudes des chromosomes du pistachier (*Pistacia vera *L.) d'Iran". Le pistachier (*Pistacia vera *L.) est un membre de la famille Anacardiaceae et est originaire de l'Asie centrale. Deux centres de diversité sont définis pour* P. vera*, une région à partir d'Iran et à l'est de la Turquie et une région au nord d'Afghanistan et au sud du Turkménistan. Le pistachier est la seule espèce de ce genre, qui croît favorablement dans les vergers et produit des noix comestibles, assez grandes pour être commercialement acceptables. L'espèce iranienne de* P. vera *fait l'objet d'une étude chromosomique. Fréquemment, la méiose de cette espèce était régulière et présentait 15 bivalents au cours de la première métaphase. La plupart des bivalents avaient deux chiasmas terminaux. Le nombre moyen de chiasmas a été estimé à 1,35 pour chaque bivalent à la première métaphase. Une à cinq paires de chromosomes ont été associées avec le nucléole à la diacinèse. L'observation de la conduite chromosomique dans cette espèce suggère que l'enroulement chromosomique est tellement rapide que les chromosomes du zygotène deviennent courts et épais, assemblés autour du nucléole comme un bouton synizétique. Comme conséquence de la présence de l'enroulement chromosomique, nous n'avons pas pu observer une différence marquée excepté la présence du nucléole entre diacinèse et la première métaphase. Occasionnellement, dans quelques cellules, des chromosomes traînants, des chromosomes univalents et multivalents ont été observés. Les conclusions obtenues par les études mitotiques sont en accord avec les études méiotiques et le complément chromosomique (2n = 30). A l'interphase, deux chromocentres ont été observés clairement et ils étaient aussi observables à la prophase et métaphase. Ils sembleraient être les candidats pour les chromosomes sexuels dans ce taxon. La conduite méiotique pour ce taxon a été rapportée ici pour la première fois.*

Mots-clés : *Chromosome, cytogénétiques, pistache,* Pistacia vera.

Introduction

The pistachio tree (*Pistacia vera* L.), a member of the family Anacardiaceae is one of the eleven species of the genus *Pistacia*. Pistachio originated in Central Asia. Two centers of diversity have been described for *P. vera*, an area from Iran into eastern Turkey, and an area in northern

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Afghanistan and southern Turkmenistan (Whitehouse, 1957; Joley, 1969; Rechinger, 1969). The scientific name of *Pistacia* genus derives from its Persian name "Pisteh" or "Pesteh" (Abrishami, 1995). Existing documents show that pistachio (*P. vera*) has been cultivated since 3000-4000 years ago in Iran and was introduced into Mediterranean Europe at approximately the beginning of the Christian Era (Crane, 1978).

P. vera (2n = 30) is the only species in this genus, successfully grown in orchards, which produces edible nuts large enough to be commercially acceptable. Other species and subspecies, producing smaller nuts, which are mainly used as rootstocks or for oil, agroforestry, timber production and carpentry (Barghchi and Alderson, 1989). Commercial scion cultivars are obtained from selected mature lines. Selection of desirable forms is still being actively pursued (Chernova and Olekhnovich, 1977; Bolotov, 1979; Popov, 1979). Pistachio cultivation plays an important role in the agricultural economy of arid and semi-arid countries such as Iran, a country that leads the world in the production of these nuts. The most genetic diversity of pistachio is found in Iran and the most valuable quality of pistachio in the world is originated from Iran.

Chromosomal data has long been a valuable tool for cytogeneticists and breeders. Chromosome studies are often useful in suggesting taxonomic and phylogenetic relationships (Raven, 1975; Stuessy, 1990). Fasihi Harandi (1996), Fasihi Harandi *et al.* (1996) and Fasihi Harandi and Shahsavan Behboodi (1997) evaluated species and subspecies of Iranian pistachio to find their ecological distribution and their karyotype. They gave the chromosome complement of *P. vera* as 2n = 30, *P. khinjuk* as 2n = 24 and the other subspecies of *P. atlantica* as 2n = 28.

This work could be difficult with pistachio since its chromosomes were extremely small, frequently having only a few cell divisions visible in a single root tip. Meiotic analysis was more successful but required plants at the flowering stage. In this paper we studied the meiotic chromosome behaviour of *P. vera*. However, information on chromosome numbers and karyomorphology in *Pistacia* is incomplete and inconsistent.

Materials and methods

Chromosome studies carried out on material collected from Rafsanjan (Kerman province, Iran). Flower buds were sampled at the stage, which meiotic division occur in the microsporocytes and immediately fixed in the field in the Piennar's fixing fluid (6 ethanol 96% : 3 chloroform : 2 propionic acid v/v) for 24 hours at room temperature. They were then washed and preserved in 70% ethanol and stored at 4°C until slide preparations were made. Meiotic analysis was accomplished using the acetocarmine squash technique. Chromosome counts were carried out from the meiotic microsporocytes. At first metaphase, univalents and bivalents were analysed in 110 cells.

For mitotic studies, seed of pistachio were germinated following stratification root tips 1-2 cm in length were cut and pre-treated in colchicine 0.5% for 3 hours at room temperature and fixed in Piennar's solution for 24 hours. The fixed roots were refrigerated (4°C) until they were made into slides. The root tips were hydrolysed in 1N HCL at 60°C for 12 min. Staining was carried out with the Feulgen reaction enhanced by squashing in 2% acetocarmine. All slides were made permanent by the Venetian turpentine (Wilson, 1945). Chromosomes were taken on an Olympus photomicroscope at initial magnification of 330X.

Results and discussions

Chromosome studies in this paper showed that *P. vera* is a diploid species with 2n = 30 and n = 15, which agree with previous reports given by Zohary (1952), Bochantseva (1972), Fasihi Harandi (1996) and Fasihi Harandi *et al.* (1996). Meiosis studies in pollen mother cells showed that in leptotene, chromosomes gathered around the nucleolus like a synizetic knob (Fig. 1). This stage is necessary for the pairing of homologous chromosomes, and it will produce 15 bivalents in zygotene. Chromosome coiling in this species is considerably fast, thus the morphology of bivalents in diakinesis and first metaphase is so similar that only by presence of nucleolus, the difference of these two stages could be distinguished (Figs 3 and 4). In diplotene, one to five pairs of chromosomes, associated with the nucleolus (Fig. 2). Meiosis in this species was regular and showed (15-15) segregation of diads and monads in first and second anaphase (Figs 5 and

10). In the end of second division of meiosis, Telophase II and tetrad of microsporocytes were observed (Figs 11 and 12). Occasionally in some cells laggard chromosomes were observed (Fig. 6). Telophase I, Prophase II and Metaphase II in all cells were normal (Figs 7, 8 and 9).

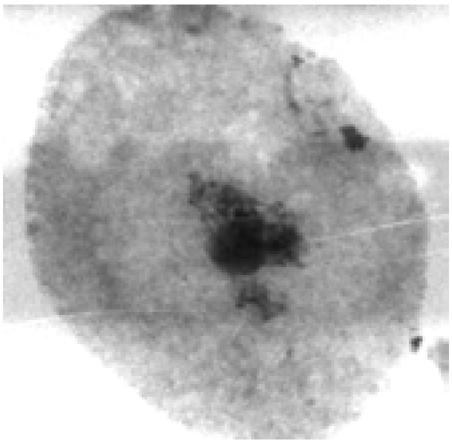

Fig. 1. Leptotene: chromosomes are clumped around nucleolus like a synizetic knob

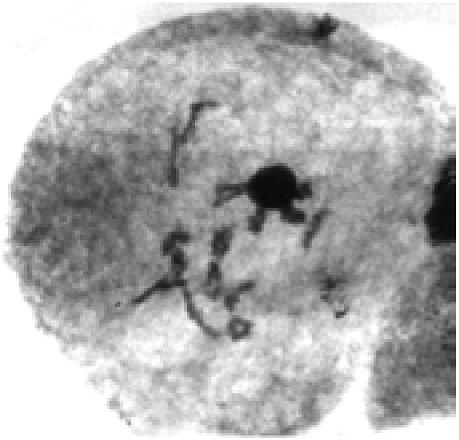

Fig. 2. Diplotene: showing 15 bivalents, which four of them are associated with nucleolus

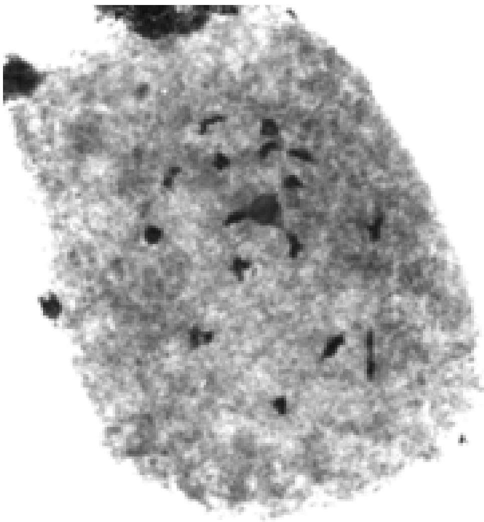

Fig. 3. Diakinesis: showing 15 bivalents, which two of them are associated with nucleolus

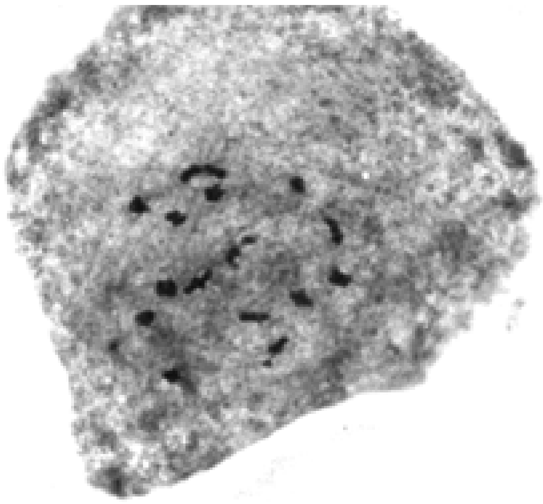

Fig. 4. Metaphase I.

The progress of terminalization of chiasmata at the end of zygotene and first metaphase showed 15 pairs of bivalents, which most of them appeared in a ring conformation and with two terminal chiasmata. Interstitial chiasmata were observed with a low frequency. The mean number of chiasmata in 110 cells was estimated 1.35 for each bivalent at first metaphase. In some cells the repulsion force followed by chiasmata terminalization causes the segregation of bivalents and therefore the appearance of univalents.

The conclusions obtained from mitotic studies in meristematic cells of root tips were in accordance with our observations of meiotic studies. These two studies showed that *P. vera* is a diploid species with 2n = 30 chromosome compliment.

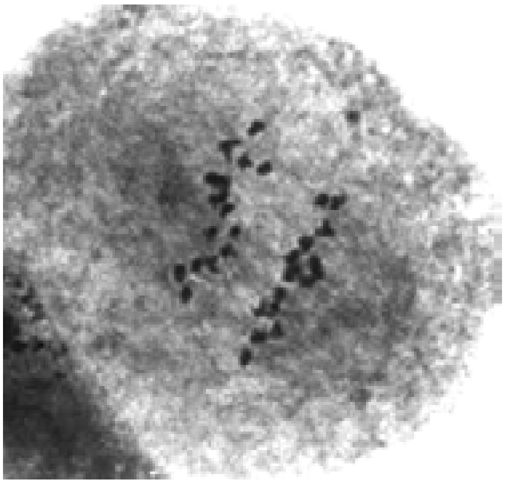

Fig. 5. Anaphase I. Showing (15-15) segregation.

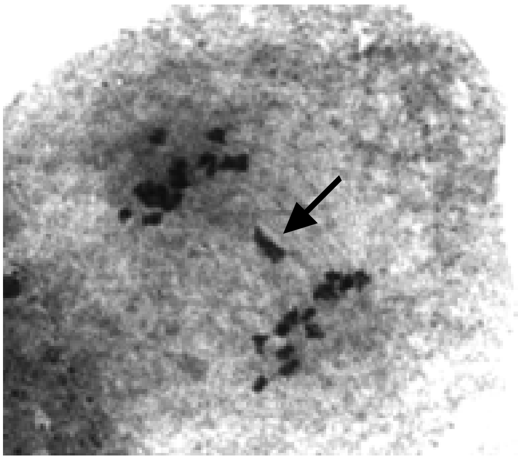

Fig. 6. Anaphase I. Showing laggard chromosomes (arrow).

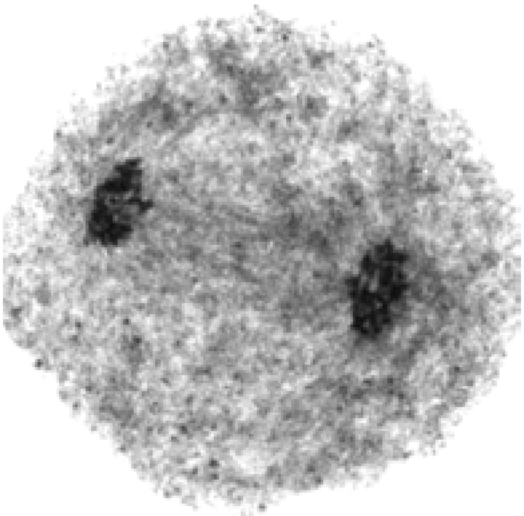

Fig. 7. Telophase I.

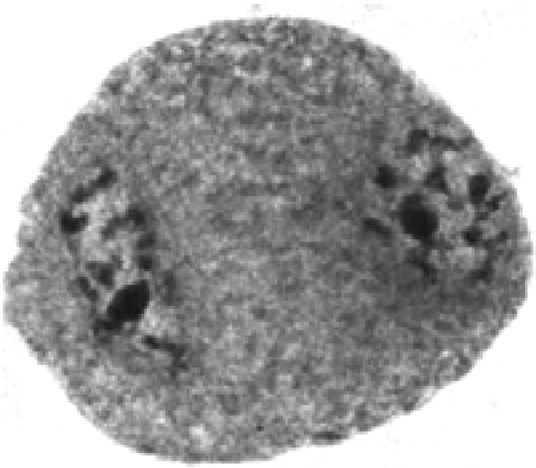

Fig. 8. Prophase II.

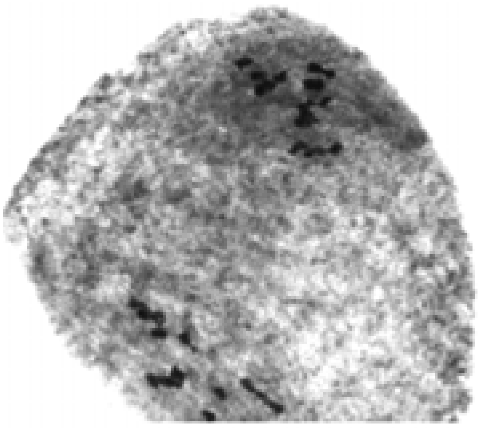

Fig. 9. Metaphase II.

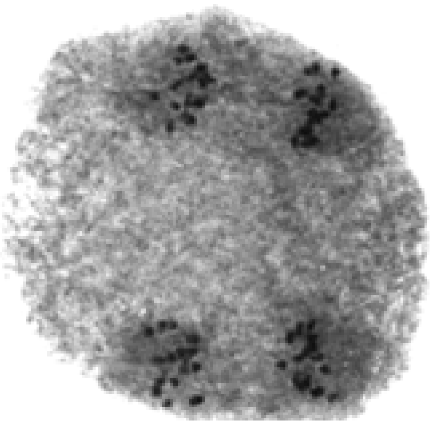

Fig. 10. Anaphase II. Showing (15-15) segregation of monads.

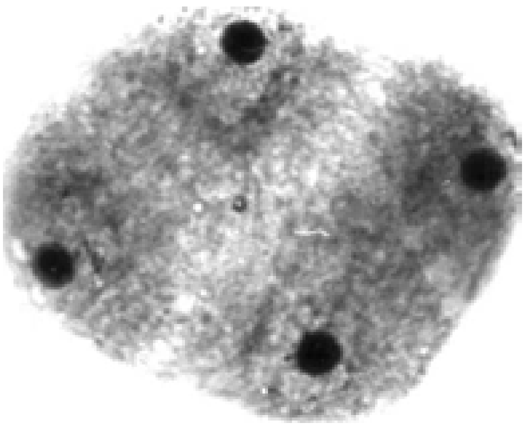

Fig. 11. End of Telophase II.

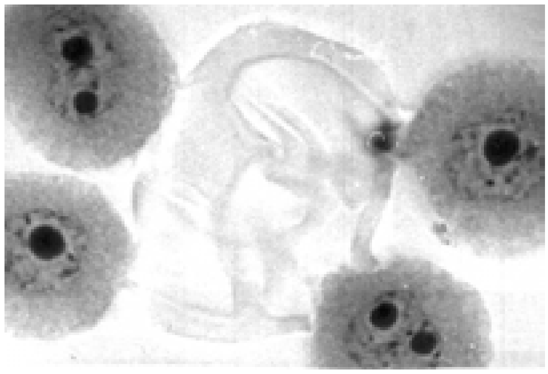

Fig. 12. End of tetrad stage with four microsporocytes.

Except two chromosomes, which were mostly consisted of heterochromatin, other chromosomes had terminal and interstitial heterochromatin bands in their length. Two above chromosomes were also observable in prophase and metaphase of mitosis and it seems that they are candidates for sex chromosomes in this taxon (Figs 13 and 14). The karyotype of this species was symmetric with predominant metacentric chromosomes (Fig. 15). Meiotic behaviour for this species is reported here for the first time.

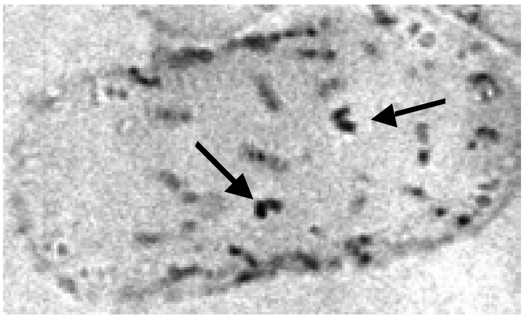

Fig. 13. Prophase of mitosis. Showing two heterochromatin chromosomes (arrows).

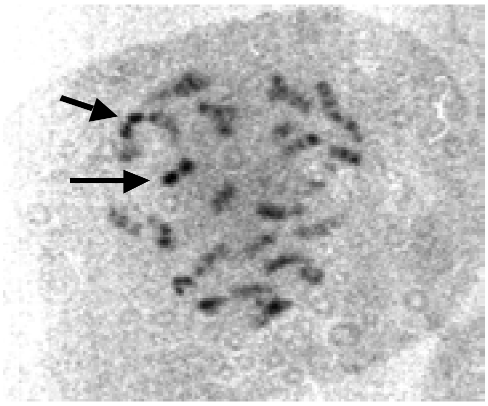

Fig. 14. Metaphase of mitosis. Showing 30 banded chromosomes, which two of them are candidates for sex chromosomes (arrows).

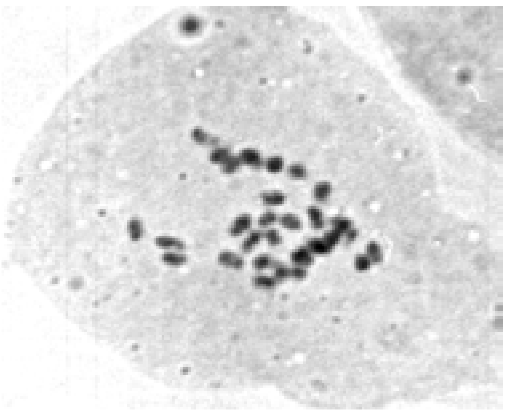

Fig. 15. Metaphase of mitosis showing 30 chromosomes, which more of them are submetacentric.

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