Original article

Karyotypic study of wild pear species of Fars Province, Iran

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Abstract — Introduction. Iran, with more than 10 species of *Pyrus*, is one of the important genetic resources for this genus in the world, and Fars province is one of the centers of origin. In our research, cytogenetical investigation of native wild pear species of Fars province, including *P. glabra* Boiss., *P. syriaca* Boiss. and, probably, a natural hybrid of them, (*P. glabra* × *P syriaca*), was carried out using a video analysis system. **Materials and methods**. Seed germination of different species was done on damp filter paper in petri dishes, and root tips were collected for karyotypical investigation. After pretreatment, fixation, hydrolysis and staining, samples were prepared for observation under the microscope and chromosome morphology was studied. Analysis of the species genome (length of each chromosome, length of long and short arm, ratio of long arm to short arm and ratio of short arm to long arm) was done and the chromosomal type of each species was determined using Levan *et al.*'s method. Then the karyotypic symmetry of species was studied by Stebbins' method. **Results and discussion**. The results revealed that all of the genotypes, with $2n = 2x = 34$, were diploid. Based on Stebbins' table, genotypes 1 and 2 of *P. glabra* were classified into group 1A, *P. glabra* × *P. syriaca* into group 2A and *P. syriaca* into group 2B. Genotype plots, according to the A1 and A2 parameters and Stebbins' cross classes, revealed the same results. Similarity and differences of species in chromosomal aspects were investigated and the results shown in a dendrogram.

Iran Islamic Republic / indigenous organisms / species / *Pyrus glabra* / *Pyrus syriaca* / chromosomes / cytogenetics

Étude caryotypique d'espèces de poiriers sauvages de la province de Fars, en Iran.

Résumé — Introduction. L'Iran avec plus de 10 espèces de *Pyrus* est un pays important pour les ressources génétiques de ce genre dont la province de Fars est l'un des centres d'origine. Pour nos recherches, des études cytogénétique sur des espèces de poiriers indigènes de la province de Fars, dont *P. glabra* Boiss., *P. syriaca* Boiss. et, probablement, l'un de leurs hybrides naturels (*P. glabra* × *P syriaca*), ont été effectuées a en utilisant un système d'analyse visuel. **Matériel et méthodes**. La germination de graines des différentes espèces a été faite sur papier filtre humide en boîtes de Pétri et des pointes de racine ont été prélevées pour des études caryotypiques. Après prétraitement, fixation, hydrolyse et coloration, des échantillons ont été préparés pour observation au microscope et la morphologie des chromosomes a été étudiée. L'analyse du génome des espèces (longueur de chaque chromosome, longueur du bras long et du bras court, rapport du bras long au bras court et rapport du bras court au bras long) a été effectuée et le type chromosomique de chaque espèce a été déterminé en utilisant la méthode de Levan *et al*. Puis la symétrie caryotypique des espèces a été étudiée par la méthode de Stebbins. **Résultats et discussion**. Les résultats ont indiqué que tous les génotypes, avec $2n = 2x = 34$, étaient diploïdes. En se basant sur la table de Stebbins, les génotypes 1 et 2 de *P. glabra* ont été classifiés dans le groupe 1A, *P. glabra* × *P syriaca* dans le groupe 2A et *P. syriaca* dans le groupe 2B. Les groupes de génotypes, selon les paramètres A1 et A2 et les classes croisées de Stebbins, ont révélé les mêmes résultats. La similitude et les différences des espèces à partir des caractéristiques chromosomiques ont été étudiées et les résultats ont été représentés dans un dendrogramme.

Iran République islamique / organisme indigène / espèce / *Pyrus glabra* / *Pyrus syriaca* / chromosome / cytogénétique

1. Introduction

Pyrus, with about 22 species, is an important genus of Rosaceae [1]. With more than 10 species, Iran is one of the important genetic resources of *Pyrus* in the world [2]. Schönbeck-Temesy reported that 12 species of *Pyrus* are growing in the Iranian plateau, including *Pyrus glabra* Boiss. and *Pyrus syriaca* Boiss [2]. Khatamsaz also reported that 12 species of *Pyrus* are growing in Iran [3]. According to Khatamsaz's report, *Pyrus glabra* Boiss. and *Pyrus syriaca* Boiss. are growing in the jungle of Dehkohne, Sepidan, Fars province, Iran. Although Fars province is one of the *Pyrus* origin centers, there is not enough information about the cytogenetics of this species. The growing area of wild pear in this province of Iran is about 35 000 ha, of which 30 000 ha is located in Sepidan county, North of Shiraz.

Karyotypic studies of *Pyrus* revealed that the genus is diploid, with $x = 17$ [4]. Cytogenetic studies of *Pyrus* in 22 provinces of China showed that the chromosome number in the studied species was $2n = 34$, they were diploid and only a small percentage of them were triploid [5]. Pu *et al.* reported that the chromosomal number of *P. pyrifolia* is $2n = 2x = 34$, with a karyotypic formula which includes 10 pairs of metacentric chromosomes, 6 pairs of submetacentric chromosomes and 1 pair of telocentric chromosomes [6].

Our research was carried out to study the karyotypic characteristics of the species *P. glabra* Boiss., *P. syriaca* Boiss., and the hybrid *P. glabra* × *P. syriaca*. This work is the first karyotypic study on the wild pear species in Iran.

2. Materials and methods

We studied Fars native species of wild pear (*table I*), including Anchochak (*P. glabra*), Hermou (*P. syriaca*) and a local type which is probably a natural hybrid of the two above-mentioned species (*P. glabra* × *P. syriaca*). Several trees of the different wild pear species were identified and selected from the same location in natural jungle located in the Dehkohne region (lat. 51° 45', long. 30° 25', alt. 2080 m to 2890 m above sea level, average annual precipitation 1069 mm), 15 km west of Sepidan, Fars province, Iran. Herbarium vouchers were also prepared and are available in the herbarium of the Research Center for Agriculture and Natural Resources of Fars Province (Iran).

Fruits of each tree were harvested separately at the end of July and transferred to the laboratory. Three hundred seeds of each tree (one tree for each species or genotype) were extracted from fruits and washed with tap water. To break the dormancy, seeds were stratified in moist sand for 60 d at 5 °C. Seeds were surface-sterilized with 10% commercial bleach for 20 min before being washed three times with tap water. Germination of seeds was done on damp filter paper in petri dishes at (15 ± 2) °C, 70% relative humidity and in dark conditions; root tips were then collected for karyotypic study. After pretreatment with saturated solution of 1% α-bromonaphthalene, fixation (Levitsky solution) [7], hydrolysis (NaOH 1N) and staining (hemotoxylin), samples for microscopic observation were prepared by the squash method, and the morphology of chromosomes in metaphase was photographed [7]. The chromosomal studies were done using a video analysis system with resolution ×1880, preparing the karyotype for each genotype with MicroMeasure software; at least five cells (each cell as a replicate) were selected from each studied slide; cytogenetic parameters, including chromosome length, relative length percentage of each chromosome, length of long arm, relative length percentage of long arm, length of short arm, relative length percentage of short arm, arm ratio [length of long arm / length of short arm], and the centromeric index which indicates the ratio between length of short arm and total length of chromosome, were calculated. Stebbins' table was used for determining evolutionary state and karyotypic symmetry of the studied genotypes [8]. Some parameters such as:

– difference in the relative length percentage of the biggest and the smallest chromosomes,

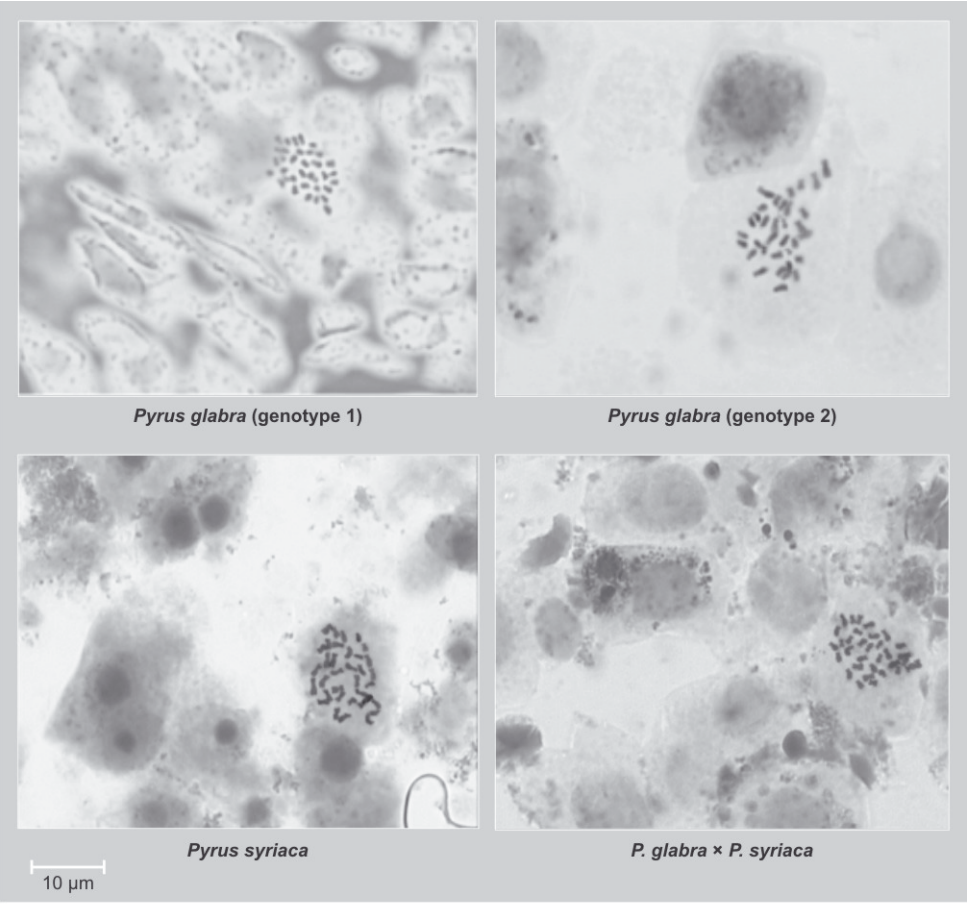

Figure 1.
Mitotic metaphase of somatic cells of two *Pyrus* species and a hybrid (2*n* = 34, Fars province, Iran).

– inter-chromosomal asymmetry index,

– intra-chromosomal asymmetry index [9],

– total form percentage [10],

were calculated.

Levan's method was used for determining the chromosome type [11]. A completely randomized design with five replicates was used for statistical analysis of data obtained from karyotypic parameters. To categorize the studied species, cluster analysis was performed by the UPGMA (Unweighted Pair Group Method With Arithmetic Averages) method using JMP [12] and SAS [13] software.

3. Results and discussion

No differences were found among the different species for the number of chromosomic stocks (x = 17). All species were diploid with $2n = 2x = 34$ (*figures 1, 2*). This is in agreement with the reports of Shenghua and Chenequan [5], Hummel [14], Darlington and Wylie [4], Pu *et al.* [6], and Quentin *et al.* [15].

Based on the results obtained in this investigation, all genotypes had metacentric and submetacentric chromosomes that indicate karyotypic symmetry. Species and genotypes were placed in the related symmetry classes using Stebbins' table. The results showed that *P. glabra* and [*P. glabra* × *P. syriaca*] had the highest values of the intra-asymmetry chromosomal index. The hybrid [*P. glabra* × *P. syriaca*] and the two genotypes of *P. glabra* were placed in class 2A and class 1A, respectively, and *P. syriaca* was placed in class 2B (*table II*). The maximum (4.86) and minimum (3.56) value of difference in range relative length of the biggest and the

Table I.
Morphological characteristics of two wild *Pyrus* species of Fars Province, Iran. Both species are 5-10-m trees, with spiny branches, roundish or cuneate leaf base and 3-6-mm sepals, and they were studied in the Zagros region.

Genotype	Hair of buds	Leaf shape	Leaf size (cm)	Leaf apex	Petiole length (cm)	Sepal shape	Petal shape	Petal size (mm)	Style number	Fruit size (cm)	Fruit shape
Pyrus glabra Boiss.	Glaber	Oblong-lanceolate	2.5–11 × 0.5–2.5	Obtuse or cuspidute	1–4	Lanceolate	Obovate or oblong	10 × 8	3–4	1.5–2.5 (diameter)	Subspheroidal
Pyrus syriaca Boiss.	Floccose-tomentose	Obovate or ovate or lanceolate	2.5–10 × 1.5–3.5	Obtuse or apiculate or acute	1–5	Triangular - ovate Triangular - acuminate	Obovate or sub orbicular	15 × 8	5	2.5–3.0 (length) × 2.0–2.5 (width)	Pyriform to subspheroidal

Table II.
Karyotypic details of different wild *Pyrus* species and genotypes of Fars Province, Iran.
Mean chromatin length = 17 µm, 2*n* = 34.

Genotype	Intra-asymmetry chromosomal index	Inter-asymmetry chromosomal index	Symmetry classes	Difference in range relative length	Total form percentage	Value of relative chromatin	Karyotype formulae[1]
P. glabra (genotype 1)	0.35	0.15	1A	3.56	39.50	3.32	32 m + 2 sm
P. glabra (genotype 2)	0.37	0.17	1A	4.01	38.36	2.10	32 m + 2 sm
P. syriaca	0.44	0.19	2B	4.86	35.70	3.05	10 m + 24 sm
P. glabra × *P. syriaca*	0.48	0.17	2A	3.66	33.87	3.08	4 m + 30 sm

[1] m: Metacentric chromosomes; sm: submetacentric chromosomes.

smallest chromosomes belonged to the *P. syriaca* species and the genotype 1 of *P. glabra*. Differences in range relative length and inter-chromosomal asymmetry index were compared in the genotypes and they showed a positive correlation and similar trend (*figure 3*). The total form percentage and intra-asymmetry chromosomal index as intra-chromosomal asymmetry parameters also had negative correlation (*figure 4*).

A dispersion diagram of different wild pear species and genotypes according to the intra-asymmetry chromosomal index (A1) and inter-asymmetry chromosomal index (A2) revealed that, with karyotypic evolution, the studied species and genotypes were placed in three completely separate groups. Genotypes 1 and 2 of *P. glabra* were placed in one group, and *P. syriaca* and *P. glabra* × *P. syriaca* were placed in another two groups (*figure* 5).

Based on Stebbins table results, the studied genotypes were placed in three classes: 1A, 2A and 2B. The genotype of class 2B was more evolutional in comparison with the genotypes of classes 1A and 2A. A significant difference was observed between genotypes for some chromosomal characteristics (*table III*). The length of long arm of chromosomes in [*P. glabra* × *P. syriaca*] was significantly greater than that of *P. glabra* genotypes 1 and 2. Arm ratio in [*P. glabra* × *P. syriaca*] and *P. syriaca* was significantly greater than that of *P. glabra* genotypes 1 and 2. The centromeric index of *P. glabra* genotypes 1 and 2 was significantly greater than those of [*P. glabra* × *P. syriaca*] and *P. syriaca*. No significant difference was observed among the different species for short arm length of chromosome.

To categorize the studied species and genotypes, cluster analysis was performed by the UPGMA method (*figure 6*). According to the dendrogram obtained, genotypes 1 and 2 of *P. glabra* belonged to one species, and, with a chromosome number of $2n = 2x = 34$, they were placed in one cluster (class 2). *Pyrus syriaca* and [*P. glabra* × *P. syriaca*] were placed in another cluster (class 1).

In our research, among the studied genotypes and species, the least genetic distance was between the species *P. syriaca* and the hybrid [*P. glabra* × *P. syriaca*]; the two genotypes of *P. glabra* had the most divergent genetic distance.

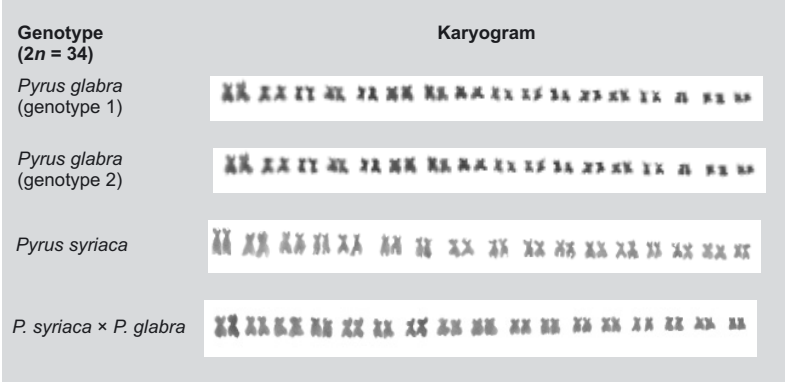

Figure 2.
Karyograms of wild *Pyrus* species according to chromosome length (resolution x1880).

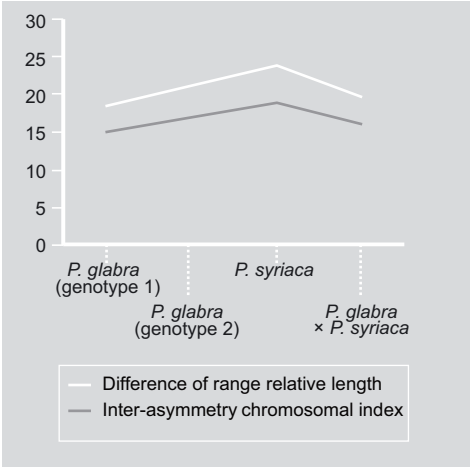

Figure 3.
Difference in range relative length and inter-asymmetry chromosomal index trend in different wild *Pyrus* species (Iran).

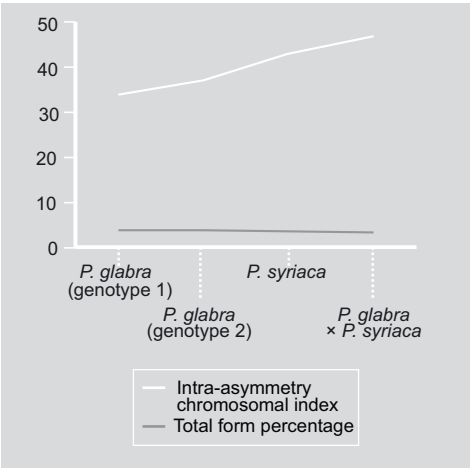

Figure 4.
Intra-asymmetry chromosomal index and total form percentage trend in different wild *Pyrus* species (Iran).

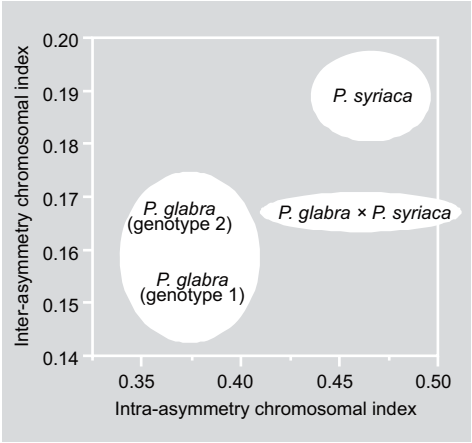

Figure 5.
Dispersion diagram of different wild *Pyrus* species according to the intra-asymmetry chromosomal index and inter-asymmetry chromosomal index.

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Table III.
Karyotypic characteristics of different wild *Pyrus* genotypes of Fars province, Iran.

Genotype	Chromosome length[1] (µm)	Length of long arm (µm)	Length of short arm (µm)	Arm ratio	Centromere index (Ci)
P. glabra (genotype 1)	2.32 a	1.40 bc	0.92 a	1.56 b	0.39 a
P. glabra (genotype2)	2.10 a	1.30 c	0.81 a	1.61 b	0.38 a
P. syriaca	3.05 a	1.96 ba	1.09 a	1.84 a	0.35 b
P. syriaca × *P. glabra*	3.08 a	2.03 a	1.04 a	1.95 a	0.34 b

[1] Chromosome length = total length.

Values in each column followed by the same letter are not significantly different ($P < 0.05$).

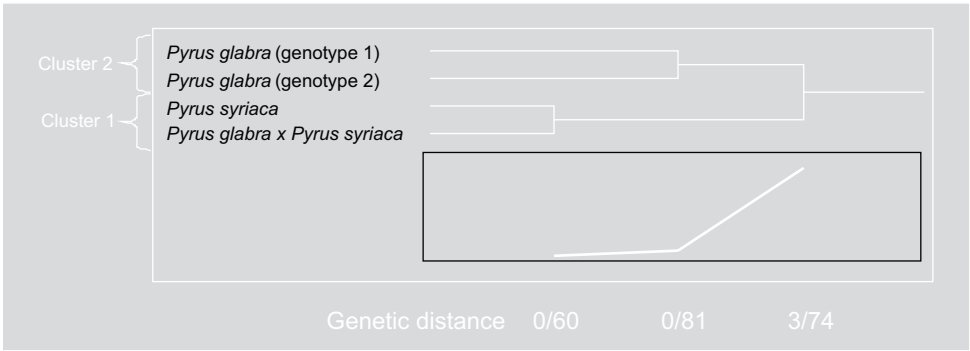

Figure 6.
Dendrogram cluster analysis of different wild *Pyrus* species by UPGMA method according to the intra-asymmetry chromosomal index and inter-asymmetry chromosomal index.

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Estudio cariotípico de especies de perales silvestres de la provincia de Fars, en Irán.

Resumen — Introducción. Irán, con más de 10 especies de *Pyrus* es un país importante para los recursos genéticos de este tipo, cuya provincia de Fars es uno de los centros de origen. Para nuestras investigaciones se llevaron a cabo, gracias al empleo de un sistema de análisis visual, unos estudios citogenéticos sobre especies de perales indígenas de la provincia de Fars, de los cuales *P. glabra* Boiss., *P. syriaca* Boiss. y, probablemente, uno de sus híbridos naturales (*P. glabra* × *P. syriaca*). **Material y métodos**. La germinación de semillas de las diferentes especies se realizó sobre papel filtro húmedo en cajas de Petri y se tomaron muestras de puntas de raíz para estudios cariotípicos. Tras pretratamiento, fijación, hidrólisis y coloración, se prepararon muestras para observación en microscopio y se estudió la morfología de los cromosomas. El análisis del genoma de las especies (longitud de cada cromosoma, longitud del brazo largo y del brazo corto, relación del brazo largo con el brazo corto y relación del brazo corto con el brazo largo) se realizó y el tipo cromosómico de cada especie se determinó gracias al empleo del método de Levan *et al*. Después se estudió la simetría cariotípica de las especies mediante el método de Stebbins. **Resultado y discusión**. Los resultados indicaron que todos los genotipos, con $2n = 2x = 34$, eran diploides. Partiendo de la base de la tabla de Stebbins, los genotipos 1 y 2 de *P. glabra* se clasificaron en el grupo 1A, *P. glabra* × *P. syriaca* en el grupo 2A y *P. syriaca* en el grupo 2B. Los grupos de genotipos, de acuerdo con los parámetros A1 y A2 y las clases cruzadas de Stebbins, mostraron los mismos resultados. La similitud y las diferencias de las especies a partir de las características cromosómicas se estudiaron y se representaron los resultados en un dedrograma.

Iran República Islámica / organismos indígenos / especies / *Pyrus glabra* / *Pyrus syriaca* / cromosomas / citogenetica