Comparative karyological features of several populations of *Populus euphratica* Oliv., grown under different environmental conditions in Iran

Fereshteh Asadi-Corom [ID], Hossein Mirzaie-Nodoushan [ID] and Mohsen Calagari [ID]

Research Institute of Forests and Rangelands of Iran, Agricultural Research, Education and Extension Organization (AREEO), Tehran, I.R. Iran

Abstract

Understanding genetic structure and chromosomal characteristics is essential for developing effective breeding programmes and improving plant species. This research compared karyotypic features of 10 plant populations of *Populus euphratica* from various regions of Iran. Fresh roots grown from cuttings of the populations were used to get metaphase cells. Then several chromosomal parameters were recorded and analysed using a nested statistical model. All the studied populations were diploid, with $2n = 38$ chromosomes, consisting of medium and sub-medium chromosome types. Significant differences ($P \leqslant 0.01$) were observed between the plant populations in chromosomal dimensions and arm ratios, suggesting chromosomal rearrangements. Chromosome lengths in the studied populations ranged from 0.69 to 3.38 µm. Intra-chromosomal index ($A1$) showed clear asymmetrical differences between the plant populations. Furthermore, using Stebbins's standards, the studied populations classified as 1A and 1B classes, demonstrated more asymmetry than those categorized as 1B and 2B, respectively. Cytological differences between the plant populations, collected from different parts of the country, showed that chromosome structural rearrangements are responsible for the speciation and adaption of the species against the mentioned variable ecological conditions and play a key role in response to diverse climatic and geographical conditions.

Introduction

Poplar genus, scientifically referred to as *Populus* L., belongs to the Salicaceae family, Salicoideae subfamily and clade C. Poplar's trees and shrubs are dioecious, naturally cross-pollinated and draw from various evolutionary origins (Zhang *et al*., 2022).

According to genome sequencing, poplars diverged from willows (*Salix*) around 6 million years after genome duplication, establishing them as sister genera (Tuskan *et al*., 2006; Dai *et al*., 2014). Following genome duplication in the *Populus* genus, a significant portion of genomic components was deleted (Zhang *et al*., 2021). After genome duplication, environmental factors via genetic and epigenetic changes contributed to speciation and adaptation in poplar genus (Dai *et al*., 2014; Zhang *et al*., 2021). As a result, new traits emerged, including resistance to adverse environmental conditions such as drought, cold and salinity, leading to adaptation of species like *Populus euphratica* and *Populus alba* to cold and drought regions (Zhang *et al*., 2021).

P. euphratica Oliv. was classified under Turanga section, and research revealed that the oldest poplar trees are found within the Turanga and Leuce sections (Cervera *et al*., 2005; Wang *et al*., 2014; Zhang *et al*., 2019). *P. euphratica* grows in tropical and semi-tropical regions (Wang *et al*., 2019) and exhibits a broad distribution across Asia, including its native habitat in Iran (Maassoumi *et al*., 2011), along with introductions to Egypt and Spain (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:776672-1). In tropical regions of Iran, *P. euphratica* and *Tamarix* species have formed extensive forests along riverbanks, playing vital roles in fodder provision, wood production and environmental equilibrium (Calagari *et al*., 2000). The species is distributed naturally in expansive regions throughout the country, from sub-tropical areas like Khuzestan to colder zones like Azerbaijan and Zanjan provinces (Sabeti, 1976). The largest growth areas are concentrated in Khuzestan province, particularly along the banks of major rivers such as Karun, Karkheh and Dez. These regions hold significant economic importance as they serve as crucial sources of timber, fodder and wildlife shelter (Calagari *et al*., 2000). Furthermore, the species exhibits remarkable resistance to drought, salinity and alkalinity. Geographic and climatic variations within the habitats of *P. euphratica* influence the morphology of its leaves, such as size and shape, across different populations.

With over 50 million hectares of desert in Iran, the plantation of salinity and drought-resistant species like *P. euphratica* holds significant ecological and economic values. This diversity highlights adaptability to various environmental conditions and underscores the importance of considering local genetic variation in conservation.

Populus species are notable for their small, consistent chromosome numbers, and similar chromosome sizes. However, karyotypic studies on *Populus* species are limited, as highlighted by recent literature (Kim *et al.*, 2020; Xin *et al.*, 2020). The presence of a few documented reports regarding the chromosome count of *P. euphratica* originating from Iran (Mofidabadi *et al.*, 2001) and worldwide, without any comprehensive karyotypic details, emphasizes the need for further research into the genetic diversity, evolutionary history and conservation of the mentioned important species through chromosome count and karyotypic analysis. Numerous studies have been conducted to understand the genetic characteristics of poplar species (Dai *et al.*, 2014; Liu *et al.*, 2017). Additionally, research has shown that in poplars, triploid plants exhibit higher growth and performance than diploid trees. Therefore, the development of forestry with triploid plants has gained attention. These plants result from the fertilization of unreduced ($2n$) gametes. These gametes are produced either spontaneously in nature (at a low rate) or using artificial methods (such as colchicine and high temperature) (Wang *et al.*, 2013). On the other hand, karyological differences between the plant populations reveal that chromosome structural rearrangements are responsible for the speciation and adaption of plants. Therefore, chromosome number variation and karyomorphological information provide valuable knowledge for adaptation and improving reforestation programmes. Hence, the present study particularly focused on cytotype patterns and diversity within the species across different regions of Iran for the first time.

Materials and methods

During late winter and before bud opening, cuttings from 10 plant populations of the species, across various provinces in Iran (Fig. 1) were prepared and placed in water containers at room temperature to stimulate root growth. Fresh root tips were then pre-treated with 0.5% α-bromonaphthalene for 1 h at 4°C during early morning hours. Subsequently, they were fixed overnight in a mix of ethanol 96% (three parts) and acetic acid glacial (one part) and finally stored in 70% ethanol. Chromosome preparation and staining were conducted using 1 N HCl (for 8 min, at 60°C) and haematoxylin agents. Metaphase cells were carefully selected and chromosomal parameters: L: long arm; S: short arm; CL: chromosome length; arm ratio (AR: L/S); r-value (S/L); form percentage of chromosome (F%: $S/\sum CL \times 100$); centromeric index (CI: S/CL) and relative length of chromosome (RL%: CL/$\sum CL \times 100$) were measured, in three replicates, using Ideokar 1.2 software. Various symmetrical indices such as intrachromosomal ($A1$) and inter-chromosomal ($A2$) asymmetry indices (Zarco, 1986), Stebbins category based on a proportion of chromosomes with arm ratio <2:1 and ratio largest/smallest chromosomes (Stebbins, 1971) and total form percentage (TF%: $\sum S/\sum CL \times 100$) (Huziwara, 1962) were calculated. Karyotypic formulas were determined based on centromere position and Levan's category (Levan *et al.*, 1964).

Based on a completely randomized design, nested model analysis was used to compare populations and chromosomes nested within populations. Plant populations were classified using Duncan multiple range test performed with SAS 9.4 software. Cluster analysis was conducted using the Ward method with JMP 13.2.0 software based on chromosome measures and karyotypic values.

Results

As presented in Fig. 2, the mitotic chromosome number revealed that all plant populations were diploid with $2n = 38$ chromosomes. Data analysis results indicated that, except for RL% and F%, other chromosomal parameters including chromosome dimensions, arm ratios and centromeric index significantly differed among the studied plant populations ($P \leqslant 0.01$) (Table 1). Chromosome lengths varied from 0.69 to 3.38 μm in the studied populations (Table 3). The lowest and highest grand means of chromosome lengths were 1 μm in Kerman and 1.59 μm in Semnan plant populations, respectively (Table 2). The minimum values for the total haploid complement length were observed in Kerman and Golestan plant populations (approximately 8.2 μm); on the other hand, the maximum value of the complement length was found in plant populations collected from Semnan and Gilan provinces (approximately 12 μm) (Table 3). Additionally, satellites were observed on short arms of one pair of chromosomes of three studied plant populations (Fig. 2).

While most chromosomes of the studied species were observed to be metacentric, variations in karyotype formula and characteristics were evident across different regions. Semnan province exhibited an asymmetrical karyotype with 24 metacentric and 14 sub-metacentric chromosomes, whereas the most symmetrical karyotypes were observed on the plant populations collected from Isfahan (38 m), Tehran (38 m), Khuzestan-Gotvand (38 m + 2 sat) and Gilan (38 m + 2 sat) provinces (Table 3).

According to the Stebbins asymmetry index, populations were classified into 1A and 2B classes. In such cases, the intrachromosomal asymmetry index ($A1$) and the other indices are utilized to uncover a few differences within the studied samples. The Gotvand population in Khuzestan province exhibited the most symmetrical karyotype based on the smallest $A1$ index value ($A1 = 0.177$) and the highest value of total form percentage (TF% = 44.537). Conversely, the plant population collected from Semnan province displayed an asymmetrical karyotype ($A1 = 0.299$ and TF% = 40.192) (Table 3). Furthermore, based on the $A1$ and TF% indices, the 1B karyotype (Golestan) demonstrated more asymmetry compared to the 2B karyotypes (Hamidiyeh, Sarakhs and Kerman) (Table 3). Based on cluster analysis, the populations were classified into three groups. Regarding the results of cluster analysis, plant populations located in the same cluster are expected to be more similar than plant populations located in other clusters (Fig. 3).

Discussion

This research and previous studies confirmed that *P. euphratica* species with $2n = 38$ chromosomes, maintain a consistent chromosome number (Chen *et al.*, 2005; Shou-Gong *et al.*, 2005; Xin *et al.*, 2020). Generally, in the *Populus* genus, diploidy is the most common ploidy level (Anamthawat-Jónsson and Sigurdsson, 1998; Shou-Gong *et al.*, 2005), and observing other ploidy levels in nature is rare. However, in several poplar species such as *Populus nigra*, *P. tremula*, *P. tomentosa*, *P. canadensis*, etc. in China, Canada and Sweden, alongside diploid populations,
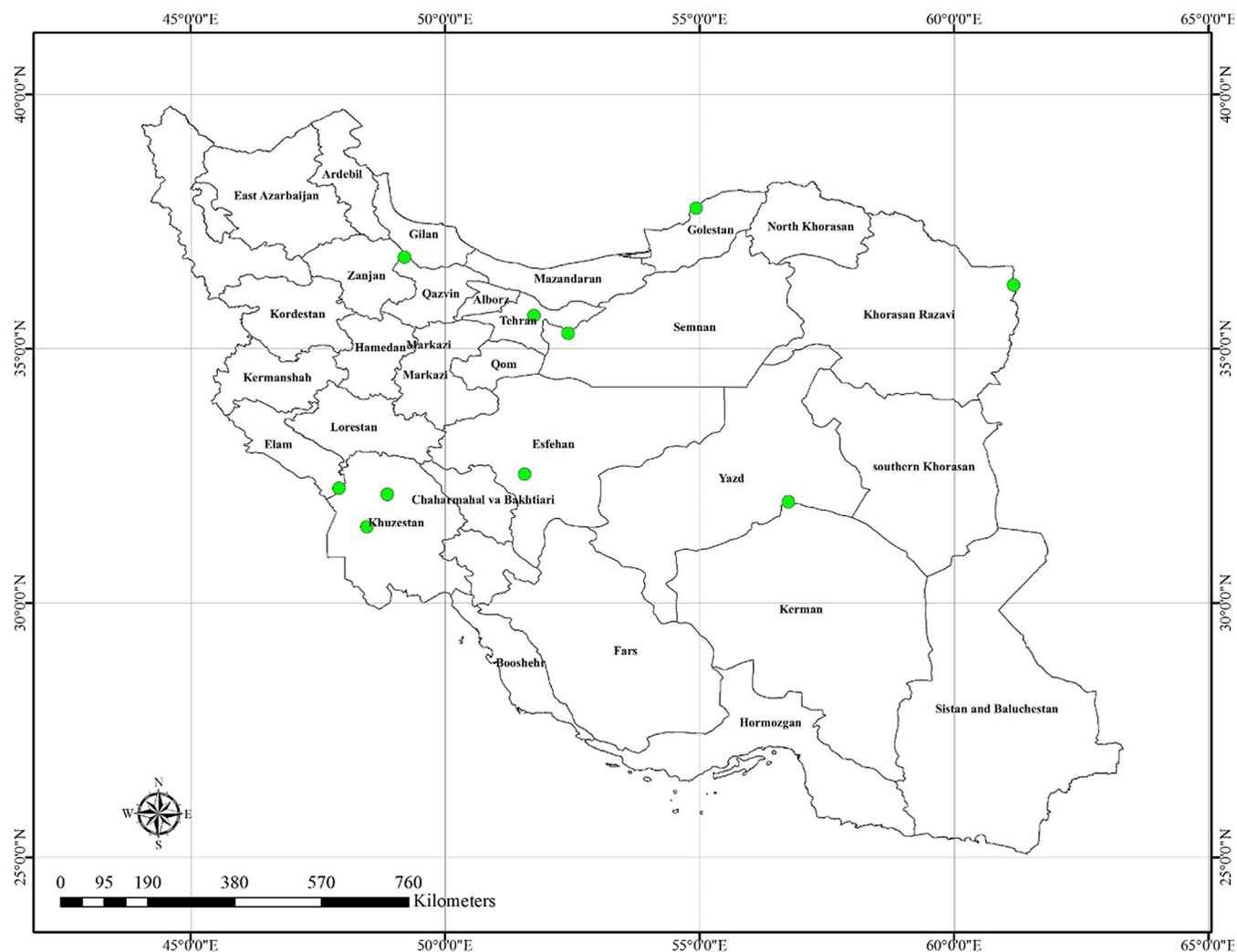
Figure 1. Distribution of sampling sites within Iranian provinces.

triploid clones and hybrids have also been observed and reported (Peto, 1938; Johnsson, 1940; Shou-Gong *et al.*, 2005). Also, besides diploidy, tetraploidy has been found in *Populus balsamifera* (Chen *et al.*, 2005). Additionally, aneuploidy has been reported in some varieties of *P. alba* (http://www.tropicos.org/Project/IPCN). On the other hand, the type of chromosomes of *P. euphratica* species revealed that most chromosomes were metacentric (m) and sub-metacentric (sm), the same as other *Populus* species. However, sub-telocentric (st) type has been reported in some species, such as *Populus caspica*, *P. alba* and *P. tremula* (Johnsson, 1940). Meanwhile, one pair of satellites on short arms of sub-metacentric or sub-telocentric chromosomes was observed for *P. euphratica* species similar to other species in the *Populus* genus (Kim *et al.*, 2020).

Although the number of chromosomes often acts as the primary constraint on karyotype evolution, in closely related species with the same chromosome count, chromosome rearrangements, such as inter-chromosomal translocations and inversions, are typically the main forces behind karyotype evolution. Therefore, it appears that the differences between the species in *Populus* genus are related to changes in chromosome structure rather than the number of chromosomes.

Fluorescence *in situ* hybridization mapping has shown that inter-chromosomal rearrangements are not readily detectable between five *Populus* species in five different sections. This suggests that the karyotypes of the species remain structurally conserved and also recommended that structural rearrangements in the studied species are likely to be included in chromosomal sections of sub-megabase size (Xin *et al.*, 2020).

DNA content and natural mutations like single-nucleotide substitutions contribute to species distinctions. Variations in nuclear DNA content, indicate potential genomic size differences and gene dosage variations that can lead to diverse expression profiles and traits in different *Populus* species (Greilhuber *et al.*, 2005; Wang *et al.*, 2014; Chase *et al.*, 2016; Zhang *et al.*, 2021).

In this study, significant differences in arm ratios and centromeric index within natural populations are evidence of inversions, possibly driven by a high ratio of crossing-over. Inversions can result in chromosomes with deleted or duplicated segments, potentially leading to structural rearrangements that may influence the reproductive fitness of individuals within the studied populations by abortion of pollen and ovules (Singh, 2017). Also, research has demonstrated that variations in size and structure of chromosomes can impact the intensity of crossover
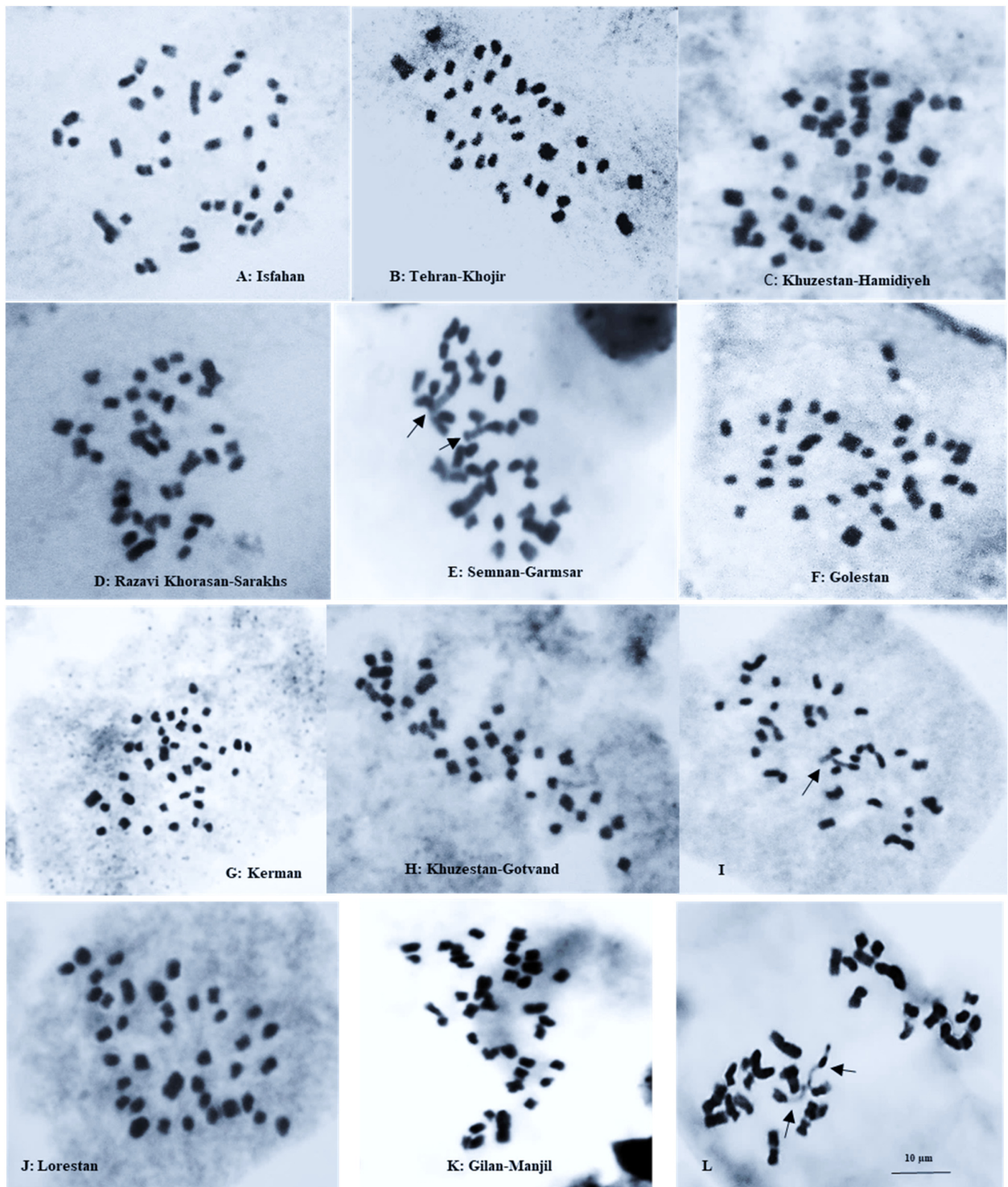
Figure 2. Mitotic chromosomes of different plant populations of *P. euphratica* (arrows show satellites and scale bar = 10 μm).

interference. Notably, in *P. euphratica* species, a high degree of crossover interference has been detected, posited as instrumental for the species' adaptation and reproductive success in challenging saline habitats (Wang *et al.*, 2019). It is also reported that in *P. euphratica*, inversions in small segments of the sex-linked regions, by inducing hyper-methylation, lead to the formation of male plants and inversions contribute to the sexual differentiation observed in the species (Zhang *et al.*, 2022).

Table 1. Mean squares resulted from nested model analysis of variance of chromosome traits of *P. euphratica* plant populations

Source of variations	df	L (μm)	S (μm)	CL (μm)	AR	*r*-Value	RL%	F%	CI
Plant populations	9	0.845**	0.345**	2.219**	0.58**	0.068**	0.005[ns]	0.062[ns]	0.009**
Chromosome(plant populations)	180	0.145**	0.063**	0.38**	0.18[ns]	0.03[ns]	1.65**	0.286**	0.003[ns]
Error	380	0.01	0.007	0.013	0.154	0.024	0.023	0.027	0.002
CV%		14.91	17.45	9.98	28.45	20.25	5.68	14.43	12.74

L, long arm; S, short arm; CL, chromosome length; AR, L/S; *r*-value, *S/L*; RL%, relative length of chromosome; F%, form percentage of chromosome; CI, centromeric index.
**Significant difference at the 1% level; *significant difference at the 5% level; ns, no significant difference.

Table 2. Mean values of chromosomal and karyological traits in the studied populations of *P. euphratica*

Plant populations	L (μm)	S (μm)	CL^{M} (μm)	AR	*r*-Value	RL%	F%	CI
Isfahan	0.573[e]	0.445[c]	1.018[d]	1.307[bcd]	0.794[ab]	2.67[a]	1.169[a]	0.439[ab]
Tehran-khojir	0.674[cd]	0.466[b]	1.187[b]	1.33[bcd]	0.778[ab]	2.67[a]	1.152[a]	0.434[ab]
Khuzestan-Hamidiyeh	0.619[cd]	0.466[bc]	1.086[bc]	1.38[bcd]	0.77[ab]	2.657[a]	1.141[a]	0.429[ab]
Khuzestan-Gotvand	0.65[cd]	0.522[b]	1.173[bc]	1.245[d]	0.823[a]	2.672[a]	1.19[a]	0.449[a]
Razavi Khorasan-Sarakhs	0.698[cd]	0.512[b]	1.211[b]	1.4[bcd]	0.762[abc]	2.692[a]	1.138[a]	0.426[ab]
Kerman	0.58[de]	0.429[c]	1.009[d]	1.477[ab]	0.75[bc]	2.667[a]	1.131[a]	0.42[bc]
Semnan-Garmsar	0.951[a]	0.639[a]	1.59[a]	1.582[a]	0.7[c]	2.677[a]	1.074[a]	0.403[c]
Golestan	0.599[de]	0.431[c]	1.03[bc]	1.446[abc]	0.735[bc]	2.67[a]	1.117[a]	0.418[bc]
Lorestan	0.606[cd]	0.468[bc]	1.07[bc]	1.314[bcd]	0.788[ab]	2.665[a]	1.161[a]	0.437[ab]
Gilan-Manjil	0.827[b]	0.641[a]	1.469[a]	1.303[cd]	0.793[ab]	2.682[a]	1.173[a]	0.439[ab]

L, long arm; S, short arm; CL^{M}, grand mean of chromosome length; AR, L/S; *r*-Value, S/L; RL%, relative length of chromosome; F%, form percentage of chromosome; CI, centromeric index.
Common letters in each column indicate no significant difference between the populations at the 5% level.

Table 3. Karyotypic formula and symmetry indices in studied plant populations of *P. euphratica*

Plant populations	Ploidy levels	Stebbins	Range of chromosome length (μm)	KF	HCL	*A*1	*A*2	TF%
Isfahan	2n = 2x = 38	1B	0.69–2.09	38 m	8.459	0.205	0.129	43.720
Tehran-khojir	2n = 2x = 38	1B	0.91–1.97	38 m	9.742	0.221	0.085	43.183
Khuzestan-Hamidiyeh	2n = 2x = 38	2B	0.79–1.77	36 m + 2 sm	8.871	0.230	0.044	42.964
Khuzestan-Gotvand	2n = 2x = 38	1B	0.83–2.5	38 m (2 sat)	9.927	0.177	0.051	44.537
Razavi Khorasan-Sarakhs	2n = 2x = 38	2B	0.84–2.42	34 m + 4 sm	9.738	0.238	0.039	42.325
Kerman	2n = 2x = 38	2B	0.77–1.62	30 m + 8 sm	8.156	0.250	0.074	42.517
Semnan-Garmsar	2n = 2x = 38	2B	0.95–3.38	24 m + 14 sm (2 sat)	12.148	0.299	0.098	40.192
Golestan	2n = 2x = 38	1B	0.73–1.62	34 m + 4 sm	8.201	0.264	0.041	41.872
Lorestan	2n = 2x = 38	1A	0.83–1.65	36 m + 2 sm	8.896	0.211	0.055	43.555
Gilan-Manjil	2n = 2x = 38	1B	0.94–2.99	38 m (2 sat)	12.191	0.207	0.105	43.672

KF, karyotypic formula; HCL, total chromosome length of the haploid complement; *A*1, intra-chromosomal asymmetry index; *A*2, inter-chromosomal asymmetry index; TF%, total form percentage; sat, satellite; m, metacentric; sm, submetacentric.

Current research identified clear asymmetrical differences within *P. euphratica* species using the *A*1 index. Consequently, regarding the *A*1 index, populations categorized as Stebbins's classification types of 1A and 1B exhibited more asymmetry than those classified as 1B and 2B, respectively, aligning with previous findings by Zarco (1986).

Significant karyotypic differences between the studied populations showed that there is a high potential for adaptation of the studied species to different climatic and geographical conditions. Also, it seems that chromosomal structural rearrangements are responsible for the species' adaptive mechanisms and evolutionary history.
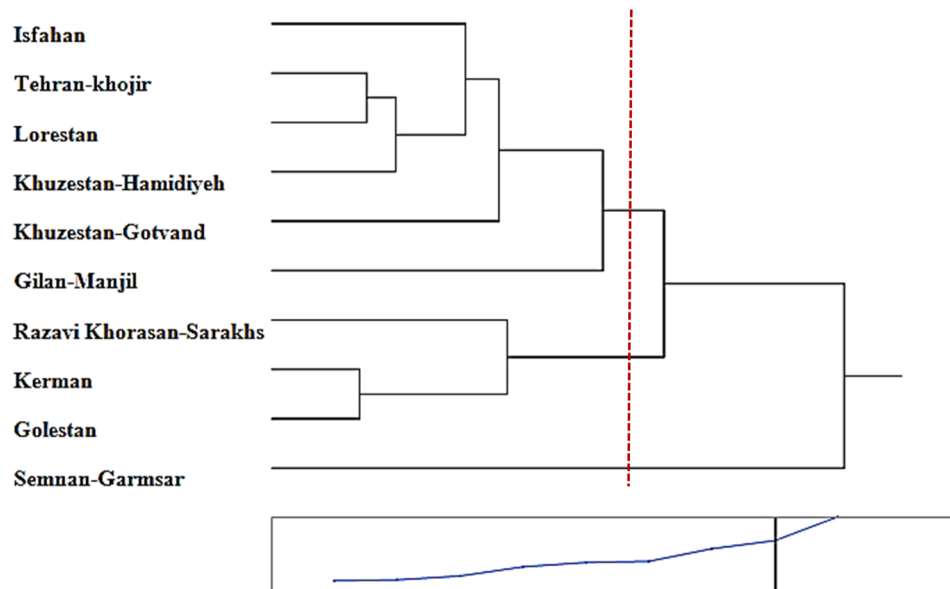
Figure 3. Classification of 10 populations of *P. euphratica* based on chromosomal and karyological parameters. (The horizontal line in the lower panel shows variation changes between the groups, with several breaking points, formed by the software. One of the breaking points suggested by the software is to be used to divide the clusters (vertical black line in the lower panel). Based on other factors, such as origins and karyotype characteristics of the plant populations, the researcher may choose other breaking points to divide the clusters. We chose the point specified by the red dotted line to divide the plant population into three clusters.)

References

Anamthawat-Jónsson K and Sigurdsson V (1998) Chromosome number of Icelandic *Populus tremula*. *Nordic Journal of Botany* **18**, 471–473.

Calagari M, Djavanshir K, Zobeiry M and Modir-Rahmati AR (2000) Study of *Populus euphratica* Oliv. community in the margin of the Karoon River. *Iranian Journal of Forest and Poplar Research* **4**, 25–52.

Cervera MT, Storme V, Soto A, Ivens B, Van Montagu M, Rajora OP and Boerjan W (2005) Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. *Theoretical and Applied Genetics* **111**, 1440–1456.

Chase MW, Christenhusz MJ, Fay MF, Byng JW, Judd WS, Soltis DE, Mabberley DJ, Sennikov AN, Soltis PS and Stevens PF (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* **181**, 1–20.

Chen C, Zhang S, Li X, Han S, Song W and Qi L (2005) A comparative study on the karyotypes among sections of *Populus*. *Guihaia* **4**, 338–340.

Dai X, Hu Q, Cai Q, Feng K, Ye N, Tuskan GA, Milne R, Chen Y, Wan Z, Wang Z and Luo W (2014) The willow genome and divergent evolution from poplar after the common genome duplication. *Cell Research* **24**, 1274–1277.

Greilhuber J, Doležel J, Lysák MA and Bennett MD (2005) The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. *Annals of Botany* **95**, 255–260.

Huziwara Y (1962) Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. *American Journal of Botany* **49**, 116–119.

Johnsson H (1940) Cytological studies of diploid and triploid *Populus tremula* and crosses between them. *Hereditas* **26**, 321–352.

Kim YG, Kwon SH, Kang HI, Yoem DB, Kim KW, Kim HH and Kang KS (2020) Similarity of chromosome structure among *Populus tremula* var. davidiana, *Populus alba* and their hybrids revealed by FISH karyotype analysis. *Dendrobiology* **83**, 68–74.

Levan A, Fredg K and Sandber AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* **52**, 201–222.

Liu X, Wang Z, Shao W, Ye Z and Zhang J (2017) Phylogenetic and taxonomic status analyses of the Abaso section from multiple nuclear genes and plastid fragments reveal new insights into the North America origin of *Populus* (Salicaceae). *Frontiers in Plant Science* **7**, 232494.

Maassoumi AA, Aassadi M and Hemmati A (2011) *Flora of Iran. Salicaceae, No. 74*. Tehran: Research Institute of Forests and Rangelands Publication, 87p.

Mofidabadi AJ, Jorabchi A, Shahrzad S and Mahmodi F (2001) New genotypes development of *Populus euphratica* Oliv. using gametoclonal variation. *Silvae Genetica* **50**, 275–278.

Peto FH (1938) Cytology of poplar species and natural hybrids. *Canadian Journal of Research* **16**, 445–455.

Sabeti H (1976) *Forests, Trees, and Shrubs of Iran*. Iran: Ministry of Agriculture and Natural Resources, 874p.

Shou-Gong Z, Cheng-Bi C, Su-Ying H, Xiu-Lan LI, Jian-Zhong R, Yu-Quan Z, Wen-Qin S, Rui-Yang C and Li-Wang (2005) Chromosome numbers of some *Populus* taxa from China. *Journal of Systematics and Evolution* **43**, 539.

Singh RJ (2017) *Practical Manual on Plant Cytogenetics*. Boca Raton: CRC Press, 346p.

Stebbins GL (1971) *Chromosomal Evolution in Higher Plants*. London: Edward Arnold, 216p.

Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A and Rokhsar D (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**, 1596–1604.

Wang J, Shi L, Song S, Tian J and Kang X (2013) Tetraploid production through zygotic chromosome doubling in *Populus*. *Silva Fennica* **47**, 12p.

Wang Z, Du S, Dayanandan S, Wang D, Zeng Y and Zhang J (2014) Phylogeny reconstruction and hybrid analysis of *Populus* (Salicaceae) based on nucleotide sequences of multiple single-copy nuclear genes and plastid fragments. *PLoS ONE* **9**, e103645.

Wang P, Jiang L, Ye M, Zhu X and Wu R (2019) The genomic landscape of crossover interference in the desert tree *Populus euphratica*. *Frontiers in Genetics* **10**, 440.

Xin H, Zhang T, Wu Y, Zhang W, Zhang P, Xi M and Jiang J (2020) An extraordinarily stable karyotype of the woody *Populus* species revealed by chromosome painting. *The Plant Journal* **101**, 253–264.

Zarco CR (1986) A new method for estimating karyotype asymmetry. *Taxon* **35**, 526–530.

Zhang B, Zhu W, Diao S, Wu X, Lu J, Ding C and Su X (2019) The poplar pangenome provides insights into the evolutionary history of the genus. *Communications Biology* **2**, 215.

Zhang ZS, Zeng QY and Liu YJ (2021) Frequent ploidy changes in Salicaceae indicates widespread sharing of the salicoid whole genome duplication by the relatives of *Populus* L. and *Salix* L. *BMC Plant Biology* **21**, 1–17.

Zhang S, Wu Z, Ma D, Zhai J, Han X, Jiang Z, Liu S, Xu J, Jiao P and Li Z (2022) Chromosome-scale assemblies of the male and female *Populus euphratica* genomes reveal the molecular basis of sex determination and sexual dimorphism. *Communications Biology* **5**, 1186.