Karyotype Analysis of Five *Iris* L. (Iridaceae) Species from Turkey

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Summary  The chromosome numbers and karyotypes of five species of the genus *Iris* distributed in Turkey were analyzed. These taxa are *I. aucheri* (Baker) Sealy, *I. reticulata* var. *reticulata* M. Bieb., *I. persica* L., *I. peshmeniana* Güner & T. Hall, *I. sari* Schott ex Baker. Two of them (*I. sari* and *I. peshmeniana*) are endemic in Turkey. The chromosome numbers of studied taxa were determined as 2n=18 (*I. persica*), 2n=20 (*I. sari*), 2n=24 (*I. peshmeniana* and *I. aucheri*), and 2n=36 (*I. reticulata* var. *reticulata*). Haploid chromosome lengths varied from 53.45 μm (*I. persica*) to 130.78 μm (*I. reticulata* var. *reticulata*) among species. Karyotype analysis indicated that *Iris* taxa generally have median (m), submedian (sm), and subterminal (st) chromosomes. In addition, only *I. sari* has two terminal (T) chromosome pairs. The karyotype of *I. peshmeniana* was determined for the first time.

Keywords  Iridaceae, *Iris*, Chromosome number, Karyotype, Turkey.

The irises (*Iris* sp.) are from the family Iridaceae and the categories monocots. These plants are resistant to changes in temperature and have long been used as a symbol of beauty, and valued as an ornamental plant in landscaping, cut flowers, and potted and due to their medicinal properties, they have been considered valuable medicinal plants. About 300 species of wild *Iris* are found worldwide. There is great uncertainty among scientists to identify and classify them (Wendelbo 1704). Moreover, flowers of some taxa (*I. histrio*, *I. aucheri*, *I. nezahatiae*, *I. caucasica*, *I. reticulata*, and *I. persica*) are consumed as food in rural areas (Kandemir and Engin 1998, 2000).

The classification of *Iris* is indeed a difficult task to tackle; botanists and taxonomists are still far from reaching a consensus on this issue. This problem is certainly reflected through the different subgeneric and sectional classifications established based on morpho-anatomical features, and ecological and cytogenetic traits. The most recent taxonomic revision recognizes six subgenera: *Nepalensis* Dykes, *Xiphium* (Miller) Spach, *Scorpiris* Spach, *Hermodactyloides* Spach, *Iris* L. and *Limniris* Tausch (Wu and Cutler 1985, Doronkin 1987).

Chromosomal changes play a major role in plant evolution and are thus important in diversification and speciation in angiosperms. Variations of chromosome numbers and ploidy levels have been frequently analyzed for a better understanding of evolutionary patterns and species relationships in plants. Among monocots, *Iris* is an excellent system in which to study the evolutionary patterns of chromosome number evolution because this group has an exceptionally high diversity of chromosome numbers (*n*=7–13, 15–22, 24, 25, 27, 36, 54) (Choi et al. 2020).

Several multidisciplinary studies such as anatomy, palynology, molecular phylogeny, and karyology are needed to clarify the taxonomy of the genus. Therefore, the karyology of *Iris* species is currently being studied to clarify their taxonomy and make contributions to other multidisciplinary studies on the genus.

Materials and methods

Plant materials of five *Iris* species were collected from natural habitats during the fruiting season between 2019 and 2021. Their names, localities, geographical position, altitude, and voucher numbers are presented in Table 1. The voucher specimens were deposited at the Herbarium of Firat University.
The karyological studies are conducted on the meristematic cells of five bulbs of each species. The bulbs were germinated at 25°C. The actively growing root tips were pretreated with 0.05% colchicine for 4.5 h at room temperature. Afterward, the root tips were fixed with Carnoy fixative (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolyzed in 1 M HCl at 60°C for 10–15 min, then rinsed in tap water for 3–5 min. Finally, they were stained in the Feulgen reagent for 1 h and mounted in 45% acetic acid (Kiran et al. 2012). Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope and an Olympus Camedia C-4000 digital camera.

The number of somatic chromosomes, chromosome length range, haploid chromosome length, arm ratio, and relative length were measured. The karyotype formula was determined based on the centromere position using a system by Levan et al. (1964). The idiograms of these taxa are arranged in decreasing lengths according to the chromosome size in the metaphase (Martin et al. 2009).

Results and discussion

The chromosome number, chromosome length range, haploid chromosome length, arm ratio, relative length, and karyotype formula were determined from 14 metaphase cells in each species and given in Table 2. Metaphase chromosomes are shown in Fig. 1 and idiograms in Fig. 2.

The somatic chromosome number of *I. aucheri* was 2n=24 (Fig. 1a). The karyotype formula was 6m+4sm+2st. The chromosome length ranged from 5.11 to 12.95 µm and the haploid chromosome length was 97.53 µm. Arm ratios were 1.07–4.13 and relative lengths were 5.24–13.27% (Table 2). The idiogram is given in Fig. 2a. The somatic chromosome number of *I. reticulata var. reticulata* was 2n=36 (Fig. 1b). The karyotype formula was 4m+10sm+4st. The chromosome length ranged from 5.16 to 13.01 µm and the haploid chromosome length was 130.78 µm. Arm ratios were 1.09–3.73 and relative lengths were 3.94–9.95% (Table 2). The idiogram is given in Fig. 2b.

The chromosome number, chromosome length range, haploid chromosome length, arm ratio, and relative length were measured. The karyotype formula was determined based on the centromere position using a system by Levan et al. (1964). The idiograms of these taxa are arranged in decreasing lengths according to the chromosome size in the metaphase (Martin et al. 2009).

Table 1. Localities and voucher numbers of the studied *Iris* taxa.

| Taxa         | Locality                  | Voucher Number |
|--------------|----------------------------|----------------|
| *I. aucheri* | B7/Elazig: Karakoçan, 1250m | Dogan, 2543    |
| *I. reticulata var. reticulata* | B7/Elazig: Çiiti Village, 1240m | Dogan, 2558    |
| *I. persica* | B7/Elazig: Cip Village, 1050m | Dogan, 2562    |
| *I. peshmeniana* | B7/Malatyas: Pelitli, 1280m | Dogan, 2548    |
| *I. sari*    | B7/Elazig: Harput, 1250 m  | Dogan, 2561    |

Table 2. Karyological features of the studied *Iris* taxa.

| Taxa                     | 2n | Chromosome length (µm) | Haploid chromosome length (µm) | Arm ratio | Relative length (%) | Karyotype formula |
|--------------------------|----|------------------------|-------------------------------|-----------|---------------------|-------------------|
|                          |    | Min       | Max       | Min       | Max       | Min       | Max       | Min       | Max       | Min       | Max       |                        |
| *I. aucheri*             | 24 | 5.11      | 12.95     | 97.53     | 1.07      | 4.13      | 5.24      | 13.27     | 6m+4sm+2st            |
| *I. reticulata var. ret.*| 36 | 5.16      | 13.01     | 130.78    | 1.09      | 3.73      | 3.94      | 9.95      | 4m+10sm+4st           |
| *I. persica*             | 18 | 4.44      | 7.49      | 53.45     | 1.09      | 3.34      | 8.30      | 14.01     | 4m+4sm+1st            |
| *I. peshmeniana*         | 24 | 3.73      | 8.19      | 66.65     | 1.16      | 3.89      | 5.59      | 12.29     | 7m+4sm+1st            |
| *I. sari*                | 20 | 4.01      | 9.40      | 64.87     | 2.41      | 7.57      | 6.18      | 14.49     | 1sm+7st+2T             |

Chromosome lengths show wide variation among species. The mean chromosome length varies between 3.73 µm (*I. peshmeniana*) and 13.01 µm (*I. reticulata var. reticulata*). Haploid chromosome length ranges from 53.45 µm (*I. persica*) to 130.78 µm (*I. reticulata var. reticulata*). The arm ratio was determined minimum of 1.07 in *I. aucheri*, and a maximum of 7.57 in *I. sari*, whereas the lowest relative length was observed in *I. reticulata var. reticulata* (3.94%), and the highest relative length was found in *I. sari* (14.49%).

The somatic chromosome number of *I. aucheri* has been reported as 2n=24 (Bareka and Kamari 1999) and 2n=22 (Hall et al. 2001, Kocyigit et al. 2013). The chromosome number determined for this species is in agreement with previous studies. Numerous studies have been
conducted on *I. reticulata* and the somatic chromosome number of this species is generally reported as $2n=16, 18, 20, 26$ (Gustafsson and Wendelbo 1975, Johnson and Brandham 1977, Karihaloo 1978, Senel and Ozyurt 1990, Ozhatay 2002, Jozghasemi *et al.* 2014, 2016).

According to the literature, the chromosome number of *I. persica* species has been reported as $2n=20, 24, 38$ (Gustafsson and Wendelbo 1975, Hall *et al.* 2001, Ozhatay 2002, Jozghasemi *et al.* 2014, 2016). The chromosome number of *I. persica* populations in Syria and Turkey were reported as $2n=20$ and $2n=22$ by Kocyigit *et al.* (2013) and Hall and Seisums (2014) and respectively. Also, chromosome numbers in the Lebanon populations were found $2n=24$ by Abdel Samad *et al.* (2016).
To date, only one study has been conducted on the chromosome number of the *I. sari*. In this study conducted by Avishai and Zohary (1977), the chromosome number of the species was reported as $2n=20$. Our investigations also determined the same $2n=20$. Also, this study determined for the first time the karyotype of *I. peshmeniana*.

In this study, chromosome numbers and karyotypes of five *Iris* taxa were established and will contribute to further studies on the karyotype properties and implications on the systematic of *Iris* taxa. A combination of detailed karyotype analysis with molecular phylogenetic studies is needed to overcome the taxonomic problems of *Iris* genus.

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