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Chromosome Number Variation Along with Modest Morphological and Biochemical Differentiation among Wild *Cyclamen* Accessions (Myrsinaceae) in Iran

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ABSTRACT

Cyclamen, a popular decorative plant, is commonly found in gardens and pots. Since wild *Cyclamen* are endangered, a study was conducted to explore the genetic potential of wild *Cyclamen*, focusing on their morphology and chromosomal diversity among eight accessions. A total of 23 phenotypic-biochemical traits were evaluated along with chromosome counting. The morphological measures showed a humble range of variation, particularly in the number of flowers, peduncle diameter, number of leaves, hypocotyl length, and hypocotyl weight, with the Kordkuy accession being the largest and the Deylaman accession the most seeded. *Cyclamen* chromosome counting indicated 15 chromosomal levels, implying a significant chromosomal diversity among the accessions, with the Pasand accession exhibiting the highest level of chromosomal diversity (14 chromosomal levels). Furthermore, only the Pasand accession had the greatest chromosomal level (2n=48) among the accessions. By describing the results based on the median, the smallest chromosomal diversity was recorded in the Kordkuy accession. *Cyclamen* accessions were divided into two main clusters and a single accession. The Kordkuy accession was separated from the other accessions. HCA analysis confirmed the separation of the Kordkuy accession from the other accessions, as expected from its superiority in most morphological traits. The current findings can be used for improving *Cyclamen* breeding programs as well as evolutionary studies. We suggest more focus on the utilization of wild genetic resources to conserve *Cyclamen* varieties and strengthen their genetic background.

Introduction

Cyclamen once belonged to the Primulaceae family but was recently relocated to the Myrsinaceae family (Yesson et al., 2009; Curuk et al., 2015). There are 21 (Compton et al., 2004), 22 (Takamura et al., 2005), and 23 (Mahomoodally et al., 2021) species of this genus listed in various studies. *Cyclamen* species have their origins in the Mediterranean basin, and they can be found from Europe to the east of Iran, with one species even appearing in Somalia (Thulin and Warfa,

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1989; Curuk et al., 2015). The classification of the genus *Cyclamen* has received much attention over the past 85 years, and nine different classifications are in use (Curuk et al., 2015).
The native growth habitats of many plant species, including *Cyclamen*, have been devastated due to anthropogenic activity in their environment over the last 50 years (e.g., construction of roads and dams, tourism activities, and growing in agricultural and residential areas). Collecting tubers for medical uses, intense grazing, and plant exports are all human-made stressors on *Cyclamen* populations. As a result, conservation is critical for *Cyclamen*'s survival and evolution (Taşkin et al., 2012).
Estimating the level and distribution of variation in endemic and rare populations and developing a strategy to protect genetic variety within the species are the main objectives of conservation genetics (Taşkin et al., 2012). Enriching a genetic pool of commercially produced C. *persicum* requires different *Cyclamen* species that can be utilized and incorporated into breeding programs. For example, C. *coum* has mountainous origins and good frost resistance. It is a significant genetic resource for breeders of *Cyclamen* species. (Prange et al., 2010). C. *coum* has a wide geographic distribution. It thrives in Syria, Palestine, the Mediterranean region, Southwest Asia (Turkey), and Southern and Southeastern Europe (Bokov et al., 2020). It is also collected from the north of Iran (Naderi et al., 2009).
Knowing natural variations across characteristics may encourage new variations for desired features, in which case further genetic alterations can boost crops (Li et al., 2023). Variety assessments can be crucial for maximizing breeding program effectiveness. Therefore, studying morphological and molecular properties can benefit genetic diversity assessments. Plant morphology reflects the genetic and environmental adaptations that the species has undergone. Assessing the genetic diversity will aid in determining the conservation status and tactics applied to these priceless resources. It helps us to identify more allelic variation sources to broaden the geographic range and crop productivity (Salazar et al., 2019).
Besides morphological studies, cytological investigations employing chromosome numbers are widely acknowledged as conclusive species characteristics, providing taxonomists with a handy tool for defining species relationships (Vimala et al., 2021). In their natural habitat, wild C. *persicum* plants consistently exhibit a diploid state, characterized by a chromosome count of 2n=2x=48. However, this species has numerous cultivated varieties with diploid (2n=2x=48) and autotetraploid (2n=4x=96) characteristics. These cultivated varieties result from selecting natural mutants from wild plants and subsequent crossings with other plant varieties (Legro, 1959). C. *greacum* (2n=84), C. *persicum* (2n=x=24, 2x=48, and 4x=96), and their interspecific hybrids (2n=45, 3x=66, 4x=90) were studied cytologically. Findings showed that C. *greacum* is an autotetraploid. The microspore-derived *Cyclamen* (2n=45) constitutes a polyhaploid, the hybrid (2n=3x=66) was a sesquidiploid, and the hybrid (2n=4x=90) was an amphidiploid (Ishizaka, 2003). In another investigation, Ishizaka et al. (2009) discovered that C. *rohlfsianum* is an autotetraploid (2n=4x=96) and that the C. *rohlfsianum* × C. *purpurascens* hybrid is an allotriploid (2n=3x=65) generated by crossing an autotetraploid with a diploid. Cytotaxonomic data have offered substantial insights into evolutionary connections among natural groupings of species, bolstering results based on morphological criteria (Vimala et al., 2021).
Since wild *Cyclamen* are endangered species, researching their diversity is essential to conservation efforts. It is a valuable genetic source for ornamental plants (Curuk et al., 2015). Morphological, cytogenetic, and biochemical indicators can benefit genetic diversity assessments. Cytogenetic approaches supplement traditional methods such as morphological and physiological characteristic analysis. Thus, this study investigated wild *Cyclamen* accessions growing under natural conditions. The objectives were to study morphological and biochemical traits, explore chromosomal diversity, and describe relationships among accessions based on morphological features, biochemical specifics, and chromosome numbers.

Materials and Methods

Plant material sampling

Cyclamen plants with intact hypocotyls were collected from naturally growing areas in Iran (Fig. 1). The samples (15 samples from each site) were collected in late February to early April. The altitude and name of each collection area are given in Table 1. All morphological traits were assessed at the site. Parts of the collected plant material were taken to the laboratory for biochemical examination and chromosome counting.

Table 1. Locations and coordinates of *Cyclamen* accessions gathered from Iran.

Number	County	Collection site	Longitude	Latitude
1	Nur	Ab-Pari	36.2839° N	51.5451° E
2	Gorgan	Qarnabad	36.4923° N	54.3525° E
3	Nur	Lavij	36.2228° N	52.0218° E
4	Kordkuy	Kordkuy	36.4531° N	54.0722° E
5	Chalus	ShahCheshmeh	36.4142° N	51.1800° E
6	Chalus	Sinava	36.3745° N	51.2346° E
7	Siahkal	Deylaman	36.5317° N	49.5447° E
8	Behshahr	Pasand	36.4101° N	43.3650° E

Fig. 1. Eight native Iranian *Cyclamen* accessions gathered and investigated in this study.
In order from top left to right: Ab-Pari, Qarnabad, Lavij, Kordkuy, ShahCheshmeh, Sinava, Deylaman, and Pasand.

Morphological traits

Morphological traits including number of flowers per plant, petal width (cm), petal length (cm), petal length/petal width, number of seeds per capsule, peduncle length (mm), peduncle diameter (mm), peduncle length/peduncle diameter, number of leaves per plant, petiole length (cm), leaf width (cm), leaf length (cm), leaf length/leaf width, hypocotyl length (cm), and hypocotyl weight (g) were measured.

Biochemical analyses

Preparation of extract

Leaf samples were cut off at the point where the petiole connects the hypocotyl, washed with distilled water, and then dried on paper in a cool, dark atmosphere. Flower samples (petioles with flowers) were also taken from the plant and dried similarly to leaves. Powdered leaves and flowers (30 g) were extracted by maceration containing 210 mL of hexane, acetone, and methanol (1:1:1) and stirred at 130 rpm for 48 h without exposure to light. Whatman filter was used for refining the end product of extraction. At a bath temperature of 40 °C, rotary evaporation was employed to eliminate the solvent from the fraction. The finished product was kept at 4 °C until further use.

Carbohydrate contents
An amount of 300 mg of ground leaf and flower samples were combined with 7 mL of 70% ethanol and stirred for 5 min on ice before being centrifuged at 6,500 g for 10 min at 4 °C. The anthrone solution was composed of 0.5 g of anthrone, 12.5 mL of distilled water, and 250 mL of 95% H2SO4. After adding 200 mL of the supernatant to this mixture, the absorbance at 625 nm was measured spectrophotometrically (Frølund et al., 1996).

Total phenols
Aqueous Na_2CO_3 (4 mL, 1 M) and Folin Ciocalteu solution (5 mL, 1:10 diluted with distilled water) were combined with a diluted extract (0.5 mL of 1:10, v/v) or phenolic standard. Total phenols were measured colorimetrically at 765 nm after 15 min of solution heating in a water bath at 45 °C. Gallic acid equivalents (mg g^{-1} dry mass), which is a popular reference chemical, are used to express total phenol levels (McDonald et al., 2001).

Antioxidant activity
According to Marsoul et al. (2020), we assessed the antiradical activity of methanolic extracts of *Cyclamen* using diphenyl picrylhydrazyl (DPPH) as a stable free radical. Antioxidants change the purple hue of diphenyl picryl-hydrazyl into yellow, and this coloration correlates negatively with environmental antioxidant capacity. We combined 100 mL of extract solutions with 1300 mL of diphenyl picryl-hydrazyl (DPPH) (0.004% produced in methanol) as a negative control. In 30 min of incubation at room temperature (RT) in the dark, the absorbance at 517 nm was measured against a blank for each concentration. For the positive control, the ascorbic acid absorbance (standard) appeared under the same circumstances as the analyzed samples and for each concentration (Marsoul et al., 2020).

Anthocyanin contents
To measure the anthocyanins, 0.1 g of plant material was ground and mixed with 10 mL of methanol that had been acidified with HCl at a ratio of 99:1. After centrifuging samples at 10000 rpm for 20 min, each sample supernatant was kept for 24 h at RT and in the dark. The anthocyanin content was determined by employing an extinction coefficient of 33000 M^{-1} cm^{-1} at 550 nm absorbance (Krizek et al., 1998).

Chromosome counting
To perform chromosome counting, the tips of the fresh roots of each *Cyclamen* were harvested. Ten mm long pieces of freshly cut root tips were pretreated in 2 mM 8-hydroxyquinoline at 20 °C 4 h before being fixed in a 3:1 solution of ethanol: acetic acid for 24 h. After proper cleaning in distilled water, the fixed roots were macerated in 1N HCl for 20 min. The material was stained with 2% aceto-orcein for around 30 min and then squashed (Legro, 1959; Ishizaka et al., 2009). We used a light microscope (Motic BA300) to examine the stained chromosomes. Chromosomes in well-dispersed metaphase were chosen and captured through photography while employing an Olympus CX31 microscope. To record chromosomal numbers, at least 164 mitotic metaphase plates with relatively healthy chromosomes spread from 6 slides. The chromosomes were counted in each of the areas.

Statistical analysis
Morphological and biochemical data were analyzed in three replicates using Randomized Complete Block Design (RCBD), and the means were compared by applying the Least Significant Difference (LSD) test in SAS software version 9.1 (SAS Institute, Cary, NC, USA). The 5% significance threshold was used for all statistical tests. Data are shown as mean values with standard error. Chromosome number analysis and reports on specific chromosomal levels were comparable based on the median and standard deviation. Principal components analysis and cluster analysis were done using Factoextra (Kassambara and Mundt, 2017) and cluster R packages (Maechler, 2019). Data were subjected to Agglomerative Hierarchical Clustering Analysis (AHC), with Ward's hierarchical clustering method to form clusters.

Results
Biochemical variation
Both flowers (CCF) and leaves (CCL) had significantly variable carbohydrate contents in accessions from various areas, and neither organ showed a consistent pattern. For CCF content, the highest value was observed in Kordkuy (219.92 mg g^{-1} DW) and reached the minimum average of 57.14 mg g^{-1} DW in Qarnabad. Meanwhile, a

different pattern was observed in the leaf carbohydrate content (CCL), as the highest and lowest values were observed in Pasand and Qarnabad, respectively. After that, Lavij, Kordkuy, and Shah Cheshmeh accessions had the lowest amount of leaf carbohydrates without significant differences among them (Table 2).
The flower extracts of Deylaman (18.69 mg GAE g^{-1} DW), followed by Lavij, Ab-Pari, and Qarnabad had higher total phenolic contents (TPC). The highest TPC was recorded in Ab-Pari (25.84 mg GAE g^{-1} DW) and ShahCheshmeh (23.08 mg GAE g^{-1} DW). Also, Pasand and Deylaman accessions had the lowest amount of phenolic content in the flowers (14.50 mg GAE g^{-1} DW and 10.12 mg GAE g^{-1} DW), respectively (Table 2).
Concerning total anthocyanin content in flowers (TACF), Deylaman (2.47 mg g^{-1} DW) accession was ranked the first with a significant difference, while Sinava (0.20 mg g^{-1} DW) and ShahCheshmeh (0.39 mg g^{-1} DW) stand as the accessions with the least anthocyanins. A different pattern was observed for total leaf anthocyanin content (TACL), so the highest and lowest values were recorded for Pasand and Lavij, respectively (Table 2).
By measuring the antioxidant activity of leaves (AAL), the highest activity was obtained in Sinava (0.445 mg mL^{-1}), followed by Deylaman (0.254 mg mL^{-1}). Other groups had no significant differences. Regarding the antioxidant activity of the flower (AAF), the pattern was very different, as all the accessions except Qarnabad and Lavij were placed in completely distinct groups. The difference was significant in that the antioxidant activity of the Kordkuy (4.583 mg mL^{-1}) flower compared to Lavij (0.410 mg mL^{-1}) was more than 11 times larger (Table 2).

Table 2. Values of different biochemical indices in different *Cyclamen* accessions.

Accession	Total phenol content (mg GAE g^{-1} DW)		Total anthocyanin content (mg g^{-1} DW)		Carbohydrate content (mg g^{-1} DW)		Antioxidant activity (IC_{50}) (mg mL^{-1})	
	Flower	Leaf	Flower	Leaf	Flower	Leaf	Flower	Leaf
Ab-Pari	15.93^{c}	25.84^{a}	0.63^{d}	7.88^{c}	89.13^{bc}	38.83^{d}	0.910^{e}	0.012^{d}
Qarnabad	15.93^{c}	16.41^{c}	1.29^{b}	6.76^{c}	57.14^{d}	28.97^{f}	0.570^{f}	0.076^{cd}
Lavij	16.98^{b}	20.22^{b}	0.96^{c}	2.98^{e}	75.05^{cd}	35.01^{de}	0.410^{f}	0.018^{cd}
Kordkuy	15.55^{d}	14.41^{c}	1.00^{c}	6.45^{cd}	219.92^{a}	35.01^{de}	4.583^{a}	0.066^{cd}
Shah Cheshmeh	14.98^{e}	23.08^{ab}	0.39^{e}	4.68^{de}	69.01^{cd}	34.81^{e}	1.380^{d}	0.076^{cd}
Sinava	14.41^{f}	16.22^{c}	0.20^{f}	11.36^{b}	69.01^{cd}	44.87^{c}	2.117^{c}	0.445^{a}
Deylaman	18.69^{a}	10.12^{d}	2.47^{a}	7.70^{c}	77.06^{cd}	59.15^{b}	2.720^{b}	0.254^{b}
Pasand	5.17^{g}	14.50^{c}	1.18^{b}	20.98^{a}	107.24^{b}	64.99^{a}	1.247^{d}	0.060^{cd}

Morphological variation

For the majority of the tested traits, significant levels of variation were detected among accessions. Only the petal width (PetW) revealed no significant differences among accessions. The traits related to vegetative organs (leaf and hypocotyl) exhibited more morphological variation in *Cyclamen* accessions (Table 3). In all traits related to leaves and hypocotyl, except for the ratio of leaf length to width (Lle/Lwi), Kordkuy *Cyclamen* obtained the highest values. The situation was a little different in the case of indicators related to flowers. Although the Kordkuy accession was still the top in most of the indices, the highest value of petal length (PetL) and the ratio of petal length to width (PetL/PetW) were recorded in Lavij. Also, the highest number of seeds was counted in the *Cyclamen* of the Deylaman region, although it was not significantly different from the C*yclamen* of the Sinava region.

Correlation among variables

Flower count correlated positively with peduncle diameter, peduncle length, number of leaves, petiole length, leaf length, leaf width, hypocotyl length, and hypocotyl weight. This finding implied that vigorous vegetative plants can produce more flowers. On the other hand, it was found that as the number of flowers increased, the diameter of the petals decreased. Also, the characteristics of the hypocotyl and leaves correlated positively. This favorable relationship encourages a simultaneous improvement of these traits (Fig. 2). Total anthocyanin content and carbohydrate content correlated positively in the leaves.

Table 3. Different morphological parameters in *Cyclamen* accessions.

Accession	Number of flowers	Petal length (cm)	Petal width (cm)	Petal length/Petal width	Number of seeds per capsule
Ab-Pari	18.00^{bc}	1.64^{bc}	1.01^{a}	1.72^{b}	26.67^{bc}
Qarnabad	15.33^{bc}	1.68^{bc}	1.01^{a}	1.68^{b}	20.33^{c}
Lavij	16.67^{bc}	2.09^{a}	0.89^{a}	2.39^{a}	27.00^{bc}
Kordkuy	39.00^{a}	1.57^{c}	0.88^{a}	1.84^{b}	31.33^{b}
Shah Cheshmeh	4.67^{d}	1.63^{bc}	1.11^{a}	1.61^{b}	21.00^{c}
Sinava	10.33^{cd}	1.59^{bc}	1.08^{a}	1.57^{b}	40.67^{a}
Deylaman	22.00^{b}	1.69^{b}	1.14^{a}	1.54^{b}	44.67^{a}
Pasand	12.00^{b-d}	1.67^{b}	1.11^{a}	1.57^{b}	26.67^{bc}

Accession	Peduncle length (mm)	Peduncle diameter (mm)	Peduncle length/Peduncle diameter	Number of leaves/plant	Petiole length (cm)
Ab-Pari	15.79^{bc}	1.37^{d-e}	12.84^{b}	21.67^{b-d}	13.04^{bc}
Qarnabad	14.93^{bc}	2.01^{ab}	8.14^{c}	16.33^{c-e}	9.16^{d}
Lavij	12.81^{c}	1.40^{c-e}	9.94^{bc}	24.33^{bc}	12.29^{bc}
Kordkuy	24.72^{a}	2.29^{a}	11.52^{bc}	44.33^{a}	19.17^{a}
Shah Cheshmeh	13.21^{c}	1.03^{e}	12.86^{b}	8.67^{e}	10.88^{cd}
Sinava	18.58^{b}	1.12^{e}	17.53^{a}	12.33^{de}	14.07^{b}
Deylaman	14.60^{bc}	1.61^{b-d}	9.64^{bc}	23.33^{bc}	11.94^{bc}
Pasand	18.83^{b}	1.83^{bc}	9.61^{bc}	30.00^{b}	13.00^{bc}

Accession	leaf length (cm)	Leaf width (cm)	leaf length/Leaf width	Hypocotyl length (cm)	Hypocotyl weight (g)
Ab-Pari	4.16^{b}	4.29^{bc}	0.98^{b}	3.37^{a-c}	48.33^{ab}
Qarnabad	3.41^{c}	3.64^{cd}	0.94^{b}	2.50^{de}	20.00^{c}
Lavij	4.37^{b}	4.30^{bc}	1.02^{ab}	3.77^{ab}	43.33^{a-c}
Kordkuy	5.06^{a}	5.70^{a}	0.89^{b}	4.00^{a}	63.33^{a}
Shah Cheshmeh	3.39^{c}	3.02^{d}	1.13^{a}	2.20^{e}	20.00^{c}
Sinava	4.36^{b}	4.34^{b}	1.01^{ab}	2.80^{c-e}	26.67^{bc}
Deylaman	4.19^{b}	4.39^{b}	0.96^{b}	3.47^{a-c}	46.33^{ab}
Pasand	4.72^{ab}	5.13^{a}	0.93^{b}	3.13^{b-d}	48.33^{ab}

Chromosome number analysis

Cyclamen chromosomal counting revealed 15 chromosomal numbers among these accessions (Table 4, Table S1). Figure 3 depicted the chromosomal status of mitosis in the meristem region of the root tip of each of the examined accessions. Given that 14 chromosomal numbers between 2n=20 and 2n=48 were identified, the findings demonstrated the highest level of chromosomal diversity in the Pasand accession (Fig. 4, Table 4). Describing the results based on the median, the highest and lowest number of chromosomes were recorded in Qarnabad and Kordkuy, respectively (Fig. 3). In the Qarnabad area, no chromosome count was fewer than 28. Additionally, the Pasand area had the greatest chromosomal level (2n=48) among the accessions (Fig. 3).

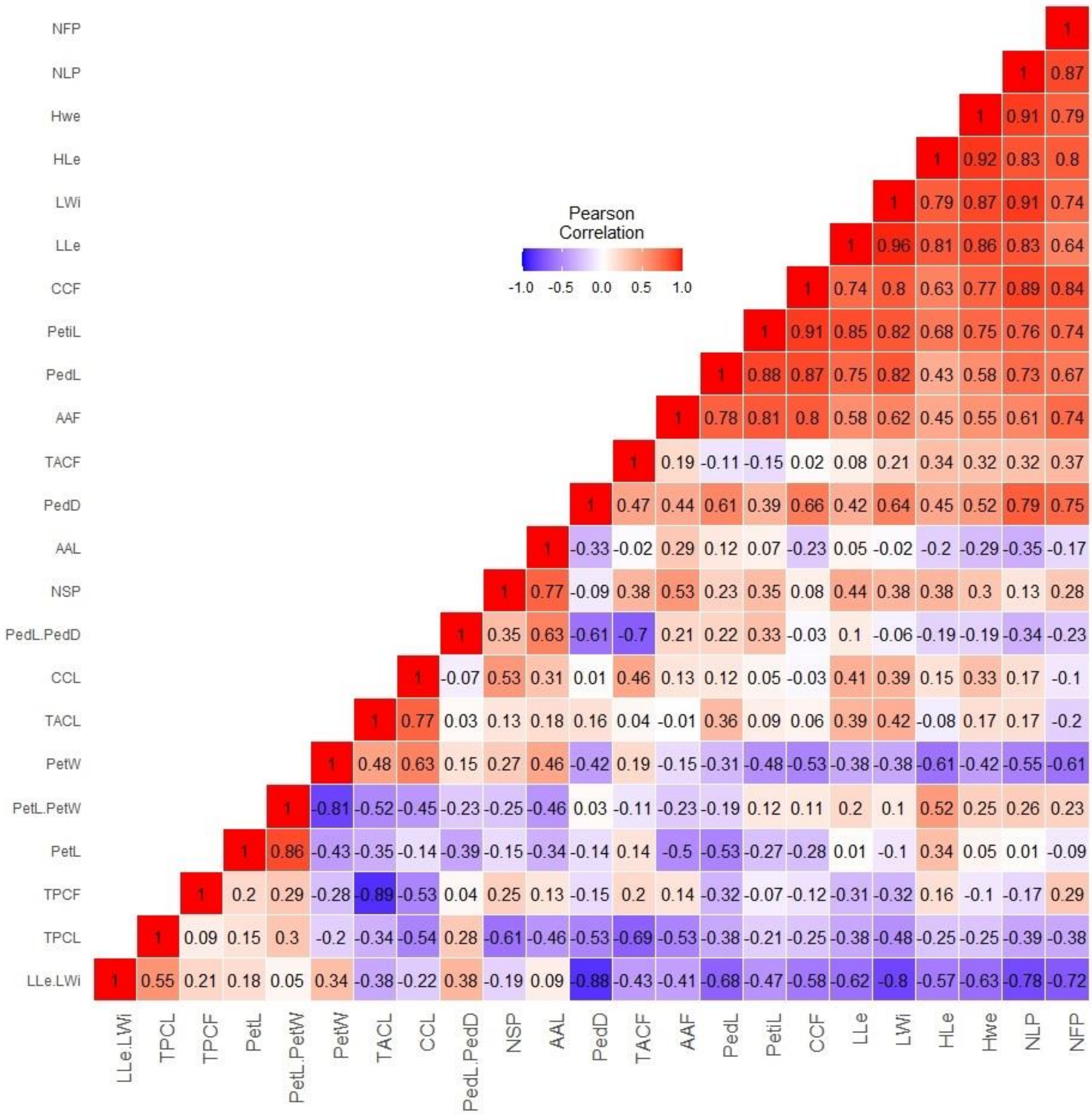

Fig. 2. Correlation heatmap among morphological and biochemical variables examined in this study. Red represents a tight correlation whereas blue suggests a more distant correlation.

Table 4. Chromosome number distribution within and among the collected *Cyclamen* accessions.

Accession	Chromosome number														
	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
Ab-Pari	*			*		*		*			*	*		*	
Qarnabad					*		*	*		*	*		*		
Lavij	*		*	*		*	*		*						
Kordkuy	*	*	*	*	*	*	*	*							
Shah Cheshmeh	*	*	*	*	*	*	*	*	*			*	*		
Sinava	*	*	*	*	*	*	*			*		*			
Deylaman			*	*		*	*	*	*						
Pasand	*	*	*	*	*	*	*	*	*	*	*		*	*	*

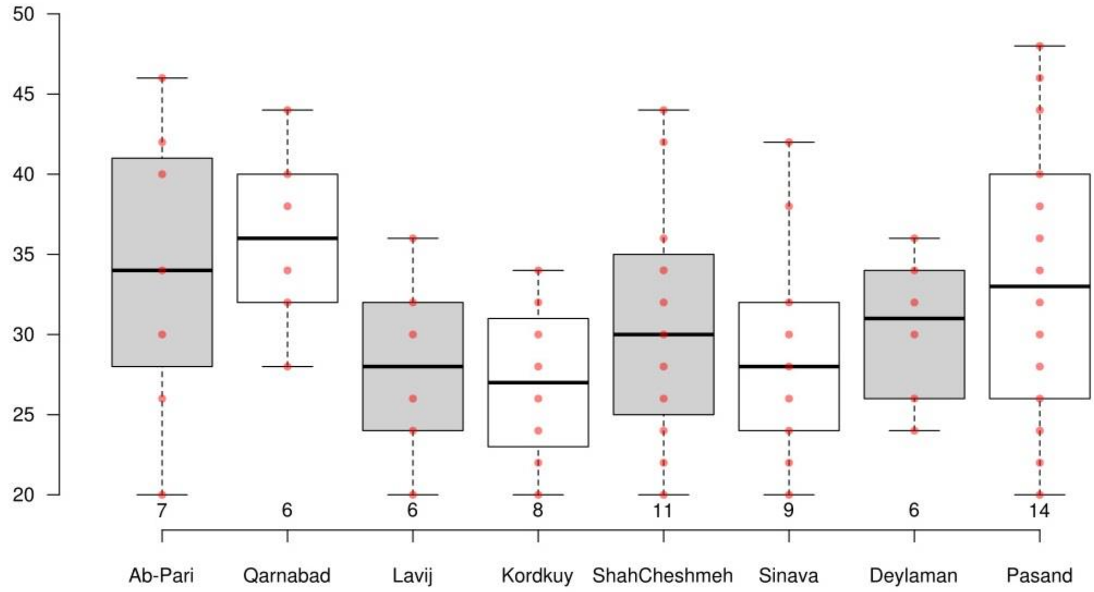

Fig. 3. Distribution and chromosomal diversity within and among *Cyclamen* accessions based on the median.

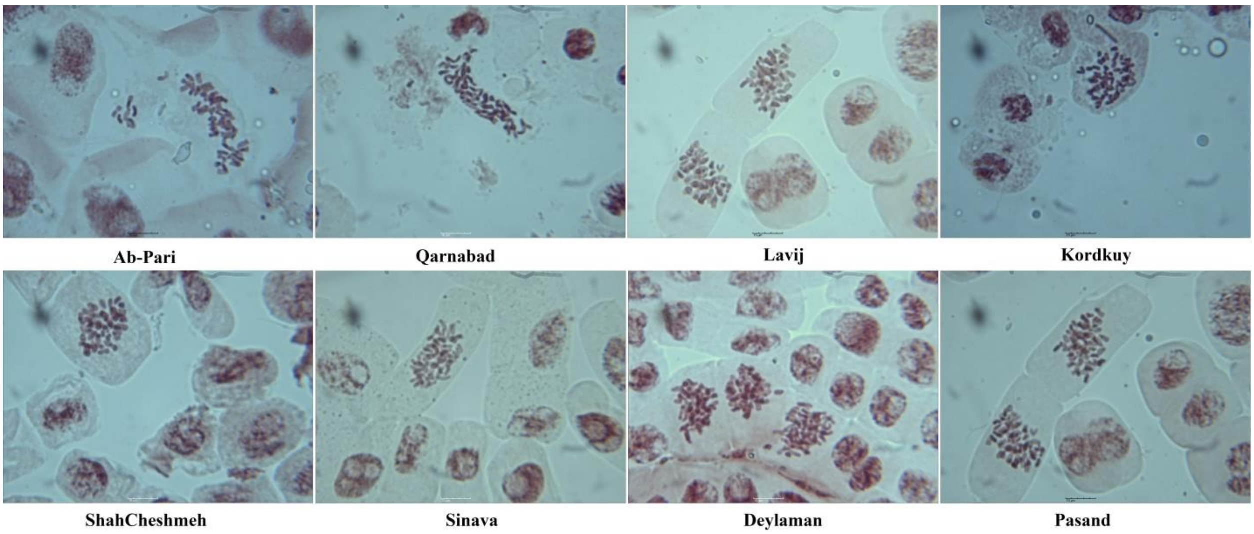

Fig. 4. Karyomorphological of mitotic metaphase chromosomes in *Cyclamen* accessions.
Scale bars in all figures are 10 µm.

Principal component analysis (PCA) and cluster analysis

Principal component analysis determined the pattern of grouping between genotypes based on 24 traits (Fig. 5). According to the PCA plots, the first component (PC1) accounted for 41.04% of the total genetic variance, and the second component (PC2) accounted for 19.38%. Thus, 60.42% of the variance could be explained by the PCA plot. The examined *Cyclamen* accessions showed relatively large variability. The Kordkuy accession had no companions and was separate from other accessions. According to the analysis, Kordkuy accession was positioned in the upper right quadrant of PC1, where plants with higher values of indicators including carbohydrate content, number of flowers, peduncle diameter, number of leaves, hypocotyl length, and hypocotyl weight were located. Ab-Pari, Qarnabad, and Lavij accessions were clustered close to the biplot's upper left quadrant, which contained the total flower phenol content, leaf phenol content, and petal length. Overall, the number of leaves per plant, leaf width, and petal length/petal width ratio contributed the most to dimensions 1 and 2.

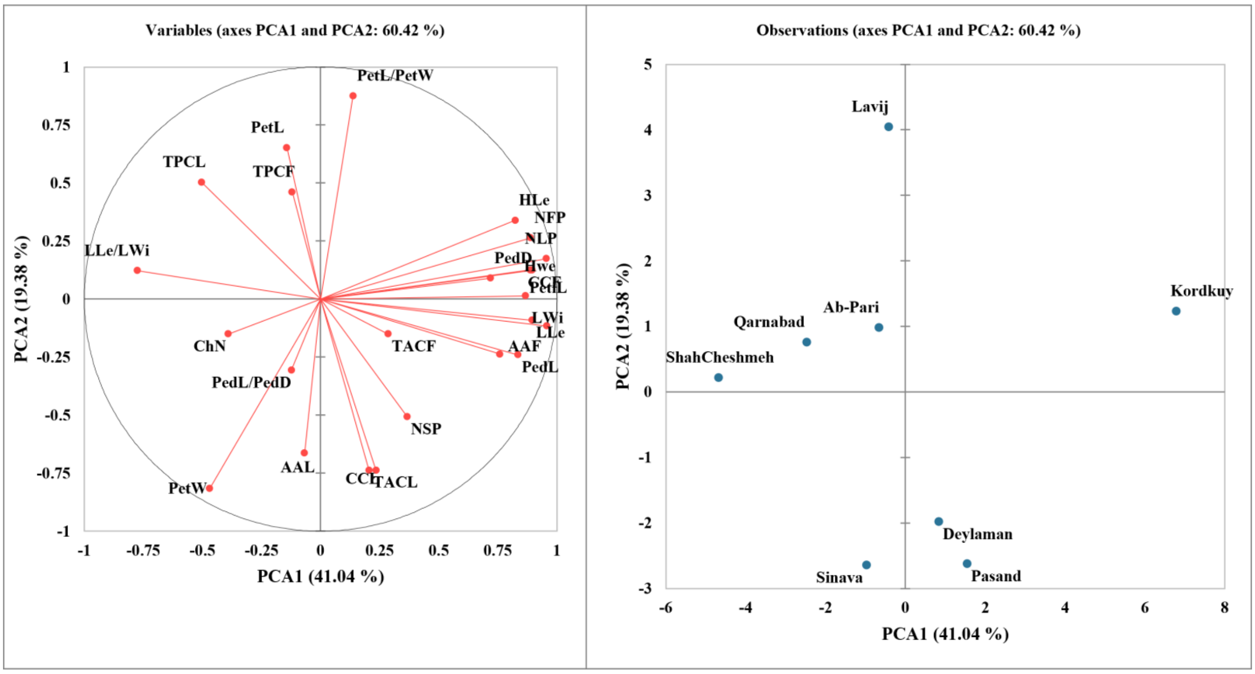

Fig. 5. Principal components analysis (PCA) based on morphological, biochemical, and chromosome number parameters. TPCF: total phenol content of flower, TACF: total anthocyanin content of flower, CCF: carbohydrate content of flower, AAF: antioxidant activity of flower, TPCL: total phenol content of leaf, TACL: total anthocyanin content of leaf, CCL: carbohydrate content of leaf, AAL: antioxidant activity of leaf, NFP: number of flowers per plant, PetL: petal length, PetW: petal width, PetL/PetW: petal length/petal width, PedL: peduncle length, PedD: peduncle diameter, PedL/PedD: peduncle length/peduncle diameter, NSP: number of seeds per capsule, NLP: number of leaves per plant, PetiL: petiole length, LLe: leaf length, LWi: leaf width, LLe/LWi: leaf length/leaf width, Hle: hypocotyl length, Hwe: hypocotyl weight, ChN: chromosome number.

Furthermore, a hierarchical cluster analysis (HCA) was employed to improve accession discrimination. Thus, using Euclidean distance, the grouping pattern of *Cyclamen* accessions for diversity revealed three major clusters (Fig. 6). Kordkuy accession was placed alone and far from other accessions. Cluster II highlights three *Cyclamen* accessions with Sinava, Deylaman, and Pasand. Cluster III had four accessions, namely, Ab-Pari, Qarnabad, Lavij, and ShahCheshmeh (Fig. 6). The superiority of plants belonging to Kordkuy in most traits such as peduncle length, peduncle diameter, number of leaves per plant, petiole length, leaf length, leaf width, hypocotyl length, and hypocotyl weight separated it from other accessions, as expected from their initial appearance. They also had the highest number of flowers per plant.

Discussion

Since combining morphological, phytochemical, and cytogenetic examination might produce superior results in classifying populations, the variety of *Cyclamen* accessions in Iran was examined using morphological, phytochemical markers, and chromosomal numbers. By examining the wild *Cyclamen* in this research, it was observed that there is an important diversity in the *Cyclamen* genus in the northern habitats of Iran. Other studies have demonstrated this variability in other *Cyclamen* species using morphological, molecular, and biochemical features (Anderberg et al., 2000; Aalaei et al., 2007; Taşkin et al., 2012).

According to the present findings, the investigated biochemical traits were less effective than the morphological traits in discovering the diversity of *Cyclamen* accessions. It also seems that among the morphological parameters, specific characteristics such as the number of leaves per plant, leaf width, petal width, and also number of flowers, hypocotyl length, and hypocotyl weight are more useful in identifying diversity in different *Cyclamen* accessions. Furthermore, petal width and petal length indicators are not suitable indicators for this purpose. Variation in morphological features is common in *Cyclamen*, as it has been documented in other species (Curuk et al., 2015).

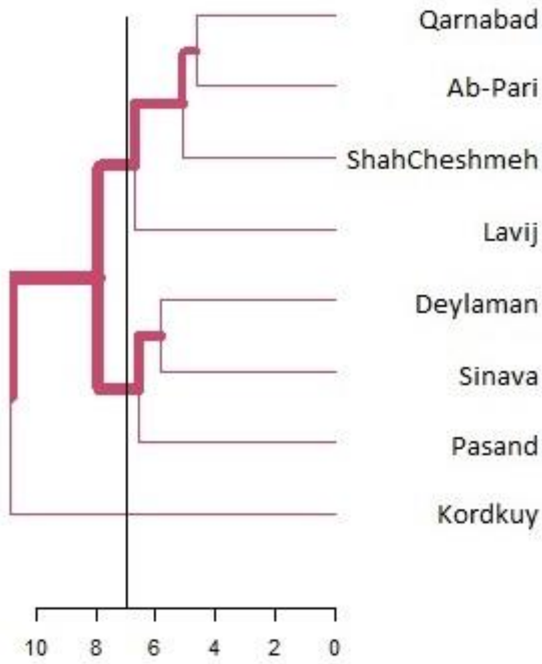

Fig. 6. Hierarchical cluster analysis (HCA) for *Cyclamen* accessions.

In a comparative analysis, (Curuk, 2017) examined three *Cyclamen* species, including *C. hederifolium*, *C. mirabile*, and *C. alpinum*, across five different regions, assessing 12 morphological traits and identifying significant differences. When contrasting the findings of the Curuk study with our own, it becomes evident that Iranian *Cyclamen* accessions display notable discrimination. For instance, while the maximum number of flowers per plant in the Curuk study was 8.8, observed in *C. mirabile* from Isparta and Aydın sites, our evaluated samples, except for the ShahCheshmeh, consistently exhibited at least 10 flowers per plant, even Kordkuy samples had an average of 39 flowers per plant. A similar pattern emerged when comparing the number of leaves per plant between the Iranian samples and those in the aforementioned study. Apart from the Shah Cheshmeh samples, all the other accessions featured a significantly higher number of leaves compared to the samples from Isparta and Aydın, Turkey. This trend held for *C. alpinum* samples from Denizli and Muğla and *C. hederifolium* samples from Aydın and İzmir sites, with the Iranian accessions displaying more abundant flowers and leaves than their counterparts in these four locations. In summary, when it comes to two of the most crucial appearance indicators for *Cyclamen*, namely the number of leaves and flowers, Iranian accessions exhibit distinct superiority over the accessions of three *Cyclamen* species, including *C. hederifolium*, *C. mirabile*, and *C. alpinum* across five different habitats in Turkey (Curuk, 2017).

Our biochemical findings exhibit consistency and comparability with the characteristics of *Cyclamen* species found in other regions. The antioxidant activity of our studied *Cyclamen* accessions is in line with *C. mirabile* Hildebri cultivated in Turkey (Sarikurkcu, 2011) and *C. africanum* growing in Jijel, Algeria (Sofiane and Wafa, 2020). The total phenol content of the Iranian samples confirms previous cases in which *C. hederifolium* was collected from Kuşadası, Aydın, Turkey (Sak et al., 2022), and *C. cilicium* from Anatolia (Zengin et al., 2020). Cornea-Cipcigan et al. (2022) estimated the total anthocyanin content of flowers in *Cyclamen* accessions of *C. persicum*, *C. mirabile*, and *C. hederifolium* to be within the range of 564-1673 μg g^{-1} DW. Consistent with their findings, the total anthocyanin content of flowers in the accessions examined in the present study also resides within 0.2-2.4 mg g^{-1} DW.

As the initial appearance assessment showed, the statistical results proved the superiority of Kordkuy *Cyclamen* in most morphological characteristics. In other words, *Cyclamen* growing in Kordkuy had the largest hypocotyls, the highest number of leaves, and the largest leaf size. The fact that these plants also had the most flowers makes this more intriguing. These findings suggest that Kordkuy accession has much room for *Cyclamen* breeding enhancement. The median-based chromosome count also revealed less variation in the number of chromosomes in the Kordkuy accession than in the others, indicating more uniformity compared to the other accessions. Following the Kordkuy accession, the biggest hypocotyls were found in the Pasand accession, which had the most chromosomes ($2n$=48). Researchers have verified that *Cyclamen* with more than 30 chromosomes, such as *C. persicum* ($2n$=48), may generate big hypocotyls (Clennett, 2002).

The genus Cyclamen has around 23 species (Mahomoodally et al., 2021) with various morphologies, chromosomal numbers, and growth behaviors (Ishizaka, 2003). In the present study, a wide range of chromosomal levels occurred within and among the collected accessions, which confirms previous studies on different *Cyclamen* species (Ishizaka, 2003). While such discrepancies in chromosome numbers have been observed in members of other plant families, including Marantaceae, in some instances, preparation artifacts and misidentification have been cited as potential contributing factors (Winterfeld et al., 2020). Understanding evolutionary processes in various species can be aided by identifying these chromosomal levels (de Carvalho et al., 2017). The unique chromosomes are most likely due to karyotype modifications throughout their evolution, affecting chromosomal features and leading to taxonomic diversification (de Carvalho et al., 2017).

The PCA results of this study identified 60% morphological, biochemical, and chromosome number variations. Aalaey et al. (2007) discovered how effective features linked to flowers, petioles, and leaves could classify *Cyclamens* into six groups that accounted for about 87% of the total variance (Aalaei et al., 2007). Different *Cyclamen* accessions in this study were grouped according to the PCA results, regardless of whether they were from the same or a different geographic origin. Genetic drift and natural selection, genetic material exchange, new accessions introduction, and human intervention can all reduce genetic and geographic variation (Zhao et al., 2007). Also, according to a cluster analysis performed using all data, accessions were not clustered into a group based on geographic origin. For example, Sinava and Shah Cheshmeh, from Chalus, were divided into two groups. This type of clustering has been reported in other studies on *Cyclamen* (Curuk et al., 2015) and other genera (Saad and Mahy, 2009). In regions with significant topographical and climatic variation, plants are almost usually confined to distinct geographic and climatic zones. But over time, fundamental evolutionary processes (such as adaptability, selection, migration, genetic drift, and self-pollination) influenced by the environment and human actions make various accessions emerge (Martins et al., 2006).

Overall, the present study found a range of morphological features. Variations in some features may be linked to chromosomal number changes associated with polyploidy (Debussche et al., 2004). This fact is also verified by the current study and its chromosomal findings, which showed a wide range of chromosomal numbers (2n=20 to 48) in the investigated accessions with diverse morphological features.

Conclusions

A breeding effort focused on genetic improvement can benefit from morphological and genetic assessments. It can assist plant breeders in deciding which desirable features to include in their breeding strategy. This study provided a morphological-biochemical focus on several wild *Cyclamen* accessions. It also showed chromosome numbers and their large diversity in these accessions for the first time. Chromosome number and morphological feature measurements revealed a wide range of variation. Accessions had distinct differences that grouped them based solely on morphological traits and chromosome numbers, as well as AHC and PCA. The variation may be explained by the fact that these accessions are dispersed throughout many habitat types. Chromosome counts and morphological traits help discover superior accessions that may assist in future cross-breeding or inter-specific hybridization research. We believe that little attention has been paid to using wild genetic resources (particularly Iranian accessions) to improve the genetic base and preserve the diversity of *Cyclamen*. More work needs to be done in exploring and conserving wild *Cyclamen*.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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Supplementary Table

Table S1. Numerical variation of chromosomes in root tip cells of Cyclamen accessions.

Site	Slide No	Number of cells observed	Frequency of chromosome numbers observed														
			20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
Ab-Pari	1	32	5	-	-	9	-	5	-	2	-	-	3	6	-	2	-
	2	30	4	-	-	7	-	4	-	2	-	-	5	6	-	2	-
	3	37	7	-	-	7	-	8	-	4	-	-	2	5	-	4	-
	4	37	9	-	-	5	-	8	-	2	-	-	3	7	-	3	-
	5	31	5	-	-	6	-	5	-	3	-	-	4	6	-	2	-
	6	31	7	-	-	7	-	3	-	2	-	-	6	4	-	2	-
	Total	198	37	-	-	41	-	33	-	15	-	-	23	34	-	15	-
Qarnabad	1	25	-	-	-	-	2	-	2	9	-	2	3	-	7	-	-
	2	23	-	-	-	-	1	-	3	6	-	4	2	-	7	-	-
	3	31	-	-	-	-	3	-	4	6	-	5	4	-	9	-	-
	4	26	-	-	-	-	2	-	4	7	-	3	3	-	7	-	-
	5	32	-	-	-	-	4	-	3	8	-	6	3	-	8	-	-
	6	27	-	-	-	-	2	-	4	6	-	5	2	-	8	-	-
	Total	164	-	-	-	-	14	-	20	42	-	25	17	-	46	-	-
Lavij	1	30	2	-	7	6	-	9	2	-	4	-	-	-	-	-	-
	2	30	3	-	7	5	-	7	3	-	5	-	-	-	-	-	-
	3	27	2	-	6	5	-	7	2	-	5	-	-	-	-	-	-
	4	27	4	-	9	3	-	6	2	-	3	-	-	-	-	-	-
	5	28	2	-	7	7	-	8	2	-	2	-	-	-	-	-	-
	6	27	2	-	7	7	-	8	2	-	1	-	-	-	-	-	-
	Total	169	15	-	43	33	-	45	13	-	20	-	-	-	-	-	-
Kordkuy	1	51	6	3	8	3	7	9	7	8	-	-	-	-	-	-	-
	2	43	4	2	9	5	3	8	5	7	-	-	-	-	-	-	-
	3	39	4	2	9	4	4	4	7	5	-	-	-	-	-	-	-
	4	39	5	4	8	3	2	6	4	7	-	-	-	-	-	-	-
	5	44	6	2	9	6	2	3	9	7	-	-	-	-	-	-	-
	6	39	4	2	9	5	5	2	6	6	-	-	-	-	-	-	-
	Total	255	29	15	52	26	23	32	38	40	-	-	-	-	-	-	-
Shah Cheshmeh	1	44	4	2	9	4	3	6	2	5	2	-	-	3	4	-	-
	2	46	4	3	8	5	2	5	3	7	3	-	-	4	2	-	-
	3	44	3	3	9	3	1	7	3	7	1	-	-	4	3	-	-
	4	44	6	2	8	4	2	6	4	6	2	-	-	2	2	-	-
	5	40	4	1	8	3	2	5	4	5	2	-	-	3	3	-	-
	6	42	2	2	8	4	3	7	2	7	2	-	-	2	3	-	-
	Total	260	23	13	50	21	13	36	18	37	12	-	-	18	17	-	-
Sinava	1	41	2	4	6	5	3	9	8	-	-	1	-	3	-	-	-
	2	37	1	4	6	4	1	9	9	-	-	1	-	2	-	-	-
	3	33	2	5	4	3	1	7	7	-	-	2	-	2	-	-	-
	4	33	3	3	5	3	1	9	7	-	-	1	-	1	-	-	-
	5	44	4	4	8	3	2	8	9	-	-	4	-	2	-	-	-
	6	44	1	5	7	4	2	8	8	-	-	5	-	4	-	-	-
	Total	232	13	25	36	22	10	50	48	-	-	14	-	14	-	-	-
Deylaman	1	33	-	-	6	3	-	9	3	5	7	-	-	-	-	-	-
	2	31	-	-	6	2	-	9	3	5	6	-	-	-	-	-	-
	3	33	-	-	7	2	-	7	4	7	6	-	-	-	-	-	-
	4	23	-	-	5	1	-	6	2	4	5	-	-	-	-	-	-
	5	30	-	-	6	2	-	9	2	4	7	-	-	-	-	-	-
	6	27	-	-	5	2	-	9	1	3	7	-	-	-	-	-	-
	Total	177	-	-	35	12	-	49	15	28	38	-	-	-	-	-	-
Pasand	1	54	4	2	8	5	3	6	2	7	4	2	3	-	2	2	4

2	73	5	3	8	4	4	9	5	9	3	3	4	-	4	7	5
3	70	7	2	6	3	5	4	7	3	5	4	7	-	7	6	4
4	52	2	4	5	5	3	4	6	2	3	4	4	-	5	3	2
5	69	6	3	7	4	5	9	4	7	2	8	4	-	4	2	4
6	62	4	2	5	2	4	8	3	5	5	6	3	-	7	5	3
Total	380	28	16	39	23	24	40	27	33	22	27	25	-	29	25	22