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RESEARCH ARTICLE

Analysis of karyotype and chromosome characteristics of Iranian accessions of *Falcaria vulgaris* Bernh.

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ABSTRACT: Plant karyotyping provides ordering and pairing all the chromosomes that can be used to determine the origin and genetic background of accessions. In this study, the karyotyping of 28 Iranian *Falcaria vulgaris* Bernh. accessions were evaluated for the first time. All accessions detected with the same number of chromosomes (2n=2x=22). However, in all samples, differences were found in the position of the centromere on chromosome pair 11. Our results demonstrated that the all chromosomes mainly have metacentric and sub-metacentric morphology. Karyotype asymmetry (F19) was observed among the accessions with 2A and 3A for *F. vulgaris*. So that the maximum asymmetry index (AsK) was related to F19 accession (74.95 %). Among the studied accessions, differences were found in chromosomes lengths, symmetry of karyotypes, relative length of chromosome (RL%), total form percentage (TF%), coefficient of variation of chromosome length (CVcl), average arm ratio (AR), centromeric index (CI), and ideogram chromosomes. The results indicated high cytological diversity of Iranian *F. vulgaris* accessions, suggesting that the geographical and environmental factors might have an effective role in the genetic structure and evolution of *F. vulgaris* chromosomes.

KEYWORDS: *Falcaria vulgaris* Bernh., Karyotyping, Cytogenetic, Chromosome, Ideogram.

INTRODUCTION

The Apiaceae family is one of the most important families of flowering plants [1]. This family is more commonly detected in northern temperate areas and high altitudes in the tropics [1]. Among the members of this family, cumin (*Cuminum cyminum* L.), caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* Mill.), anise (*Pimpinella anisum* L.), dill (*Anethum graveolens* L.), parsley (*Petroselinum crispumcan* L.) and Ghaziaghi (*Falcaria vulgaris* Bernh.) can be mentioned [1]. *F. vulgaris* is locally known "Paghazou" and Ghaziaghi in Iran [3] and is also distributed across the southern and central Europe, central Asia and the Middle East, Africa and North and South America [15]. This biennial plant has a whitish coating, many branches and a spindle shaped root. Stem height is 30–60 cm. The lower leaves have long petioles are divided into two parts, each with 4-5 serrated lobes. The upper leaves are ternate, less divided and sheathing around the stem. Many umbels have 5–10 rays and more or less flat topped [3]. *F. vulgaris* have been traditionally consumed as dry powder in west and south-west of Iran to cureduodenal and gastric ulcers [21], microbial and skin disease including peptic ulcer, liver diseases, stones

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of kidney and bladder and use as a vegetable in some areas for last decades and is also used as a vegetable in most areas [21]. The aerial parts essential oil of F. vulgaris contain carvacrol, spathulenol, α-bisabolol and phytol as its major constituents [16].

Karyotype analysis provides chromosomal information that can be used to determine the origin and relationship of accessions [20]. Piya reported that the basic chromosome number of *F. vulgaris* is x=11 (2n=22) [14], Several studies on *F. vulgaris* have focused on its antioxidant and antibacterial activities [7, 20, 15] but there are no reports about the karyotype of *F. vulgaris* accessions and despite the medicinal importance of *F. vulgaris*, there is very little information about this plant. Therefore, the aim of this study was to determine the karyotype of samples collected from different parts of Iran, which will lay a foundation for genetics of this species.

MATERIALS AND METHODS

Plant material preparation

Seeds of 28 accessions of *F. vulgaris* (Voucher species number: E1029-FUMH have been deposited in the Herbarium of Ferdowsi University of Mashhad, Iran) were collected during harvest season (August and September) from different locations of Iran, including Fars, Kerman, Isfahan, Markazi, Kermanshah, Razavi Khorasan, Kohkiluyeh and Boyer-Ahmad, North Khorasan and Ardabil provinces (Table 1). The seeds were washed, imbibed in distilled water for 48 h and germinated on wet filter papers in Petri dishes in dark at 25°C. After one week, the germinated seeds were prepared for meiotic analysis.

Preparation of meiotic chromosomes

Chromosome pretreatment followed by previously reported works [10, 12] with some modifications. The seeds with roots (5 to 8 mm in length) were placed in ice at 4 °C for 48 h (at least 20 healthy root tips of each accession) followed by fixed for 24 h in Carnoy's solution (3:1 v/v absolute ethanol: acetic acid) in darkness and then washed in distilled water twice for 3 min. The fixed samples were hydrolyzed in HCl (1N) at 60 °C for 5 min and washed in distilled water twice for 45 s. Subsequently stained by aceto-orcein (45%) for 90 min at 25 °C [18].

Table 1. Origin, location and voucher number of *F. vulgaris* accessions.

Acc. No.	Origin	Location	Altitude (m)	Voucher No.
FV/1	Sedeh	51° 57′24″E; 30° 43′09″N	2078	E1029-1 FUMH
FV/2	Mashhad	59° 33′14″E; 36° 48′43″N	1747	E1029-2 FUMH
FV/3	Hasanabad	52° 25′33″E; 30° 31′02″N	2139	E1029-3 FUMH
FV/4	Mashhad	59° 32′48″E; 36° 48′48″N	1811	E1029-4 FUMH
FV/5	Tafresh	50° 03′11″E; 34° 41′22″N	2026	E1029-5 FUMH
FV/6	Eslamabad-e Gharb	46° 30′32″E; 34° 07′23″N	1299	E1029-6 FUMH
FV/7	Mashhad	59° 39′53″E; 36° 39′08″N	1297	E1029-7 FUMH
FV/8	Eqlid	52° 34′50″E; 30° 24′54″N	2166	E1029-8 FUMH
FV/9	Eqlid	52° 24′20″E; 30° 24′04″N	2190	E1029-9 FUMH
FV/10	Eqlid	52° 00′04″E; 30° 53′52″N	2303	E1029-10 FUMH
FV/11	Yasuj	51° 33′29″E; 30° 40′05″N	1768	E1029-11 FUMH
FV/12	Safashahr	53° 12′03″E; 30° 36′28″N	2300	E1029-12 FUMH
FV/13	Eqlid	52° 41′34″E; 30° 54′26″N	2204	E1029-13 FUMH
FV/14	Isfahan	51° 48′10″E; 32° 43′12″N	1546	E1029-14 FUMH
FV/15	Bojnord	57° 27′58″E; 37° 30′23″N	973	E1029-15 FUMH
FV/16	Bardsir	56° 48′50″E; 29° 31′29″N	2817	E1029-16 FUMH
FV/17	Bojnord	57° 11′16″E; 37° 29′53″N	1304	E1029-17 FUMH
FV/18	Bojnord	56° 39′53″E; 37° 25′44″N	1152	E1029-18 FUMH
FV/19	Bojnord	56° 43′14″E; 37° 27′05″N	952	E1029-19 FUMH
FV/20	Zarqan	52° 42′35″E; 29° 46′07″N	1604	E1029-20 FUMH
FV/21	Sedeh	52° 10′34″E; 30° 43′17″N	2174	E1029-21 FUMH
FV/22	Parsabad	47° 52′48″E; 39° 38′37″N	33	E1029-22 FUMH
FV/23	Shiraz	52° 35′12″E; 29° 44′11″N	1782	E1029-23 FUMH
FV/24	Faridan	50° 24′15″E; 32° 58′40″N	2274	E1029-24 FUMH
FV/25	Eqlid	52° 45′11″E; 30° 32′29″N	2314	E1029-25 FUMH
FV/26	Ardabil	48° 24′12″E; 38° 19′49″N	1310	E1029-26 FUMH
FV/27	Ardabil	48° 17′29″E; 38° 12′38″N	1387	E1029-27 FUMH
FV/28	Ardabil	47° 59′10″E; 38° 01′38″N	1622	E1029-28 FUMH

Slide Preparation

After staining, actively growing root tips (1 mm in length) were excised from the germinated seeds and were placed on the slide. Then, one drop of acetic acid (45%) was added to the sample and lamella was placed on the slide and subsequently were squashed. The slides were heated on the Bunsen burner for 1s before evaluation [13]. The photographs were taken using Nikon Eclipse Ni-u (Tokyo, Japan) photomicroscope equipped with Nikon Ds-Fi3 digital camera.

Karyotype analysis

In order to accurate measurement of lengths, chromosomes from five metaphase plates were measured for each accession. Karyotype formula was determined by chromosome morphology based on centromere position [10]. The parameters including long arm (LA), short arm (SA), total length (TL), average arm ratio (AR), chromosome length range, average chromosome length, longest chromosome length/ shortest chromosome length (Lt/St), coefficient of variation of chromosome length (CV_{cl}), relative length of chromosome (RL%) and average centromeric index (CI) were estimated to characterize the karyotypes numerically. Various karyomorphological parameters, symmetry and asymmetry indices, were calculated including the total form percent (TF%) [8], karyotype asymmetry index (AsK%) [2], intrachromosomal asymmetry index (A1), interchromosomal asymmetry index (A2), symmetry class (SC), type and karyotype asymmetry degree according to Stebbins's classification [19]. Data analysis was done using Ideokar software (Fig 3).

RESULTS AND DISCUSSION

All *F. vulgaris* accessions in the present study showed the same basic chromosome number x=11 and same polyploidy level, which was consistent with the previous report [14]. Our findings showed a significant difference among the accessions in terms of long arm length (1.04-3.08 µm), short arm length (0.47-1.25 µm) and total chromosome length (1.49-4.16 µm) (Table 2). This suggested that there was a difference in the chromatin content among the studied accessions. The longest and shortest chromosomes were observed in F3 (3.48 µm) and F25 (1.41 µm) and length ratio of the longest to shortest chromosome (Lt/St) varies from 3.93 (F27) to 7.97 (F6) (Table 2). These differences are associated to different growing conditions [6]. In the present study, *F. vulgaris* accessions showed differences in relative chromosome length (RL%) and length ratio of the longest to shortest chromosome (Lt/St). In a study on *Ferula gumosa* of populations indicated differences in arm length and centromeric index (CI), that this type of intraspecific variation can be caused by differences in stage of cell division [6]. The coefficient of variation of chromosome index (CV_{CI}) is a parameter that provides information about centromere heterogeneity [22]. In the present study, CV_{CI} value ranged from 8.34 (FV27) to 15.95 µm (FV11). It means that karyotypes are mostly asymmetric (variation in position of centromeres). Based on comparative analysis, the smallest and largest chromosomes were observed in F18 (ranging from 1.11 to 5.52 µm) and F11 (ranging from 3.50 to 8.09µm) accessions.

Assessment of basic cytogenetic characteristics and variability of *F. vulgaris* accessions is substantial for an efficient long-term breeding plan. In the present study chromosomal characteristics and quantitative traits of *F. vulgaris* accessions were assessed under experimental field condition. Analysis of cytological data is of great importance supporting species designation [11]. Relatively high variation in chromosome size and metacentric and sub-metacentric chromosomes are characteristics of an asymmetric karyotype [17]. Asymmetric karyotypes have been shown with shifts in centromere position and additions/deletions [17]. Conceptually, inter-chromosomal asymmetry tendencies result from heterogeneity in chromosome sizes between taxa while intra-chromosomal asymmetry is dependent on relative position of the centromeres [9]. In the present study, the karyotype of F19 showed the lowest CI (0.25) and TF (24.84) and the highest A1 (0.64) and A2 (0.12), while F4 accession exhibit the highest values for CI (0.38) and TF (38.1) and the lowest values for A1 (0.36), A2 (0.14). So, it could be concluded that F19 accession had more asymmetric karyotype than F4 accession, then, F19 accession was one of the most asymmetric karyotypes.

Probably, the production of asymmetric karyotypes can be related to the loss of chromosomal fragments after polyploidy, which leads to the formation of shorter chromosomes. In addition, unequal translocations or differential amplification of heterochromatic regions can also cause this event [12]. All of these events, increase intra-chromosomal asymmetry (TF%, AsK%, A1 and A2) by increasing morphological discontinuities between chromosomes in a karyotype [12]. The average arm ratio

Table 2. Karyotype analysis of the chromosomes in *F. vulgaris* Bernh.

Accessions	Chr. no.	SA (µm)	LA (µm)	S+L (µm)	CLR (µm)	CL (µm)	Lt/St	CV_{cl}	CI
FV1	22	0.7	1.42	2.12	1.20-3.41	2.13	6.52	13.02	0.33
FV2	22	0.51	1.04	1.55	1.07-2.09	1.54	4.08	13.03	0.32
FV3	22	1.25	2.34	3.59	1.32-4.79	3.48	4.22	14.05	0.34
FV4	22	0.72	1.17	1.89	1.11-2.91	1.89	4.82	13.88	0.38
FV5	22	0.71	1.26	1.97	1.23-2.82	1.97	4.56	12.4	0.35
FV6	22	0.73	1.39	2.12	1.40-4.48	2.13	7.97	14.61	0.33
FV7	22	0.57	1.29	1.86	1.11-3.09	1.87	7.67	14.04	0.31
FV8	22	0.53	1.15	1.68	1.11-2.72	1.68	6.77	13.69	0.31
FV9	22	0.59	1.04	1.63	1.12-2.49	1.64	5.45	14.58	0.36
FV10	22	0.68	1.21	1.89	1.38-3.24	1.98	5.25	14.92	0.36
FV11	22	0.61	1.09	1.70	1.28-3.65	1.72	4.05	15.95	0.35
FV12	22	1.08	3.08	4.16	3.11-4.98	2.20	5.16	12.62	0.26
FV13	22	0.51	1.17	1.68	1.15-2.57	1.69	7.37	11.78	0.30
FV14	22	0.67	1.29	1.96	1.24-3.09	1.97	5.35	13.5	0.34
FV15	22	0.61	1.21	1.82	1.37-2.56	1.82	5.54	12.56	0.33
FV16	22	0.64	1.05	1.69	1.15-2.58	1.70	4.22	12.98	0.37
FV17	22	0.62	1.22	1.84	1.12-2.77	1.86	4.57	12.24	0.33
FV18	22	0.47	1.02	1.49	1.02-2.36	1.50	5.44	9.54	0.30
FV19	22	0.51	1.5	2.01	1.24-2.64	2.03	6.96	12.68	0.25
FV20	22	0.76	1.33	2.09	1.35-3.15	1.75	5.52	15.25	0.36
FV21	22	0.64	1.11	1.75	1.42-3.08	1.76	5.12	14.29	0.36
FV22	22	0.69	1.45	2.14	1.52-3.08	1.79	5.38	12.13	0.31
FV23	22	0.8	1.82	2.62	1.77-3.36	2.63	6.02	14.55	0.30
FV24	22	0.86	1.77	2.63	1.70-4.20	2.63	6.51	12.22	0.32
FV25	22	0.5	1.18	1.68	1.21-2.71	1.41	6.03	12.63	0.29
FV26	22	0.64	1.42	2.06	1.33-3.85	1.72	7.40	18.17	0.31
FV27	22	0.66	1.34	2	1.69-2.36	2.01	3.93	8.34	0.32
FV28	22	0.68	1.35	2.03	1.33-3.30	2.03	4.97	10.94	0.32

SA: Short arm length; LA: Long arm length; L+S: Total chromosome length; CLR: Chromosome length range; CL: Average chromosome length; Lt/St: Longest chromosome length/Shortest chromosome length; CVcl: Coefficient of variation of chromosome length; CI: Centromeric index.

ranged from 1.67 (F4) to 3.03 µm (F19). So, the examined accessions were placed in 2A and 3A classes of the Stebbins karyotype system [19] and analysis of the karyotype formulae indicated that chromosomes of 28 the studied accessions of *F. vulgaris* are mostly composed of metacentric (m) along with submetacentric (sm) karyotypes type and subtelocentric (st) karyotype. The results of Stebbins classification of karyotypes in *Kelussia odoratissima* showed that chromosomes were more metacentric and submetacentric, then karyotype of populations more symmetrical was observed [5]. Thus, 15 the studied accessions of *F. vulgaris* had the most symmetrical karyotypes and more primary than the other accessions. The remaining accessions (FV1, FV2, FV8, FV13, FV22, FV23, FV24, FV25, FV26, FV27 and FV28) had asymmetric karyotypes and more advanced than other accessions. The Karyotype type all accessions are presented in Table 2 and also the karyotypic characteristics of *F. vulgaris* are presented for the first time in this study (Table 2). In a literature, it is showed that variety in climate and topography of the sample collection sites can influence distribution of species accessions and cause variation in ploidy level, chromosome numbers, chromosome structure, and asymmetry of karyotypes [4]. In other literature, significant relationships were observed between altitude and rainfall and the physico-chemical parameters of accessions soils, and effect of altitude was relatively more pronounced on the nitrogen, clay, pH and C / N ratio [4], probably, diversity in *F. vulgaris* of accessions can be attributed to this issue.

This study is the first report on the karyological traits of *F. vulgaris*. The studied accessions were diploid with 22 chromosomes. In terms of karyotype asymmetry indices, there was a significant difference among *F. vulgaris* accessions. Our results indicated high cytogenetic diversity of *F. vulgaris* accessions, suggesting that geographical location and environmental factors might

have an effect on the genetic structure and evolution of chromosomes, which is important for cytogenetic assessment and characterization of accessions, to enhance plant genetic resources. Furthermore, this variation might be associated with current growing habitats and morphological differences among of *F. vulgaris* accessions, because difference habitats are contributed to morphological distinctions and even genetic differences. Overall, there were karyotypic differences in morphology, symmetry and size of chromosomes between this species of accessions, that comparative karyotype analysis can be used to describe the chromosomal evolution patterns in a group. Our study is effective to classify Iranian accessions and further studies are essential to understand genetic patterns and chromosome structures using satellites studies and also identifying chromosome indices of *F. vulgaris* accessions might assist breeders for better programming of breeding strategies of this species. In the future, a comparison of genome size, molecular cytogenetic and molecular phylogenetic analyses will be required in order to reveal the genetic differentiation among these accessions.

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تجزیه کاریوتایپ و خصوصیات کروموزوم در ۲۸ نمونه ایرانی *Falcaria vulgaris* Bernh.

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چکیده

تجزیه کاریوتایپ اطلاعات کروموزومی را فراهم می‌کند که می‌تواند برای تعیین خواستگاه و خویشاوندی نمونه‌ها مورد استفاده قرار گیرد. در این مطالعه، کاریوتایپ ۲۸ نمونه *Falcaria vulgaris* Bernh. از ایران برای اولین بار تعیین شد. همه نمونه‌ها دارای تعداد کروموزوم مشابه (2n=2x=22) بودند. تفاوت‌هایی در موقعیت سنترومر در جفت کروموزوم ۱۱ در همه نمونه‌ها یافت شد. ما دریافتیم که کروموزوم‌ها به طور عمده دارای مورفولوژی متاسنتریک یا ساب متاسنتریک هستند. آسیمتری کاریوتایپ (F19) در بین نمونه‌ها مشاهده شد. کاریوتایپ برای *F. vulgaris*، 2A و 3A بود. حداکثر شاخص آسیمتری (AsK%) مربوط به نمونه F19 (۷۴/۹۵) بود. در میان نمونه‌های مورد مطالعه، تفاوتهایی در طول، سیمتری کاریوتایپ‌ها، طول نسبی کروموزوم (RL%)، درصد شکل کل (TF%)، ضریب تنوع طول کروموزوم (CVcl)، میانگین نسبت بازو (AR)، شاخص سنترومری (CI) و آیدئوگرام کروموزوم‌ها یافت شد. نتایج حاکی از وجود تنوع سیتولوژیکی بالا در میان نمونه‌های ایرانی *F. vulgaris* بوده و نشان داد که عوامل جغرافیایی و محیطی ممکن است نقش موثری در ساختار ژنتیکی و تکامل کروموزوم‌های *F. vulgaris* داشته باشند.

کلمات کلیدی: *Falcaria vulgaris* Bernh.، کاریوتایپینگ، سیتوژنتیک، کروموزوم، آیدئوگرام