NEW CHROMOSOME COUNTS AND KARYOTYPE STUDY OF FOUR ONOBRYCHIS SPECIES FROM IRAN

F. Ansari, P. Ahmadian, A. Nasirzadeh & A. Hatami

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Karyotypic observations showed that 4 species including *O. scorbiculata, O. melanotricha, O. aucheri* subsp. *teheranica* and *O. oxyptera* have 2n=16 chromosomes and according to the basic number of x=8, they are diploids. *O. oxyptera* has got the least total length (31.07) and average chromosome length (3.88) but the most TF% of 36.43% which refers to its most asymmetrical karyotype. The karyotypes can be divided as 1A (*O. scorbiculata* and *O. melanotricha*), 1B (*O. aucheri* subsp. *teheranica*) and 2B (*O. oxyptera*) due to Stebbins classification.

Feridoon Ansari, Agronomy Department, Agriculture Faculty, Azad University of Karaj, Karaj, Iran. -Parichehreh Ahmadian, Agronomy Department, Agriculture Faculty, Tehran University, Karaj, Iran. -Abdolreza Nasirzadeh and Ahmad Hatami, Fars Research Center for Natural Resources, Shiraz, Iran.

Key words. chromosome, cytogenetic, flora of Iran, karyotype, *Onobrychis*.

شمارش کروموزومی و بررسی کاریوتایپ ٤ گونه اسپرس برای اولین بار از ایران

فریدون انصاری، پریچهره احمدیان، عبدالرضا نصیرزاده و احمد حاتمی

مطالعه کاریوتایپی گونه‌های موجود یکی از گام‌های اساسی در شناسایی دقیق‌تر این گیاهان از نظر تاکسونومی و به‌نژادی می‌باشد. بررسی کاریوتایپ گونه‌ها نشان داد که ٤ گونه *O. aucheri* subsp. *teheranica*، *O. scorbiculata*، *O. melanotricha* و *O. oxyptera* دارای ١٦ کروموزوم هستند و براساس عدد پایه کروموزومی (x=8) گونه‌هایی دیپلوئید محسوب می‌گردند. در میان گونه‌های مورد مطالعه، گونه *O. oxyptera* دارای کمترین طول کل ژنوم اما بیشترین TF% بود. همچنین براساس گروه‌بندی Stebbins، گونه *O. scorbiculata* و *O. melanotricha* در گروه 1A، زیرگونه *O. aucheri* subsp. *teheranica* در گروه 1B و گونه *O. oxyptera* در گروه 2B قرار گرفتند و بر این اساس گونه *O. oxyptera* در میان گونه‌های موجود با داشتن کمترین میزان TF% نامتقارن‌ترین کاریوتایپ را دارا بود.

Introduction

Onobrychis Miller is one of the valuable plant genera in Iran because of Its vast variation. It has 56 species in different parts of the country (Rechinger 1984). Sainfoin is nonbloat-inducing, resistant to alfalfa weevil, comparable to alfalfa in both forage quality and average daily gain of cattle. Sainfoin provides earlier spring grazing or hay production than alfalfa, but generally has lower regrowth and total seasonal yield than alfalfa (Kidambi & al. 1990).

A vigourous breeding program and detailed biosystematic analysis of the genus *Onobrychis* can not be carried out due to limitations of time and facilities. However, basic biosystematic information describing parental materials may be useful, particularly in relatively unstudied species, in developing a well directed breeding program (Chapman & Yuan 1968). The chromosome number of 11 species of *Onobrychis* which exist in Iran flora, have been reported (Table 1). The first chromosome report was about *Onobrychis crista-galli* (Darlington & Wylie 1955). The earliest study was a report on *O. chorassanica* (Rahimi & al. 1999).

In this paper, we present chromosome number and karyotype of 4 *Onobrychis* species in flora of Iran, which belongs to Fars province. These 4 species have not been studied before.

Materials and Methods

Table 2 gives an overview of the species used in our study and their locations. All plants and seed samples were collected from different habitats of Fars province. Voucher specimens are deposited in the Herbaria of TARI and Fars Natural Resource Center (Shiraz). Seed samples were germinated on damp filter paper in petri dishes. The best results were obtained when fresh root tips were pretreated with 8-hydroxychinolin 0.003 M for 3 h. Other steps in cytological preparation followed the method reported earlier (Sheidai, Ahmadian & Poorseyedi 1996) using 2% aceto orcein. 50 mitotic cells at the stage of metaphase were chosen to determine the number of chromosomes of a species and 10 well metaphase cells were photographed by an Olympus BX 40 photomicroscope to determine the lengths of long and short arms of chromosomes of each species.

Table 3 presents chromosome numbers and some karyotypic features of the species. Total length (TL) of a genome, Average Chromosome Length (ACL), Total Form Percentage (TF%) and mean of r-index for each karyotype were measured. The degree of asymmetry/symmetry of karyotypes has been estimated (Stebbins 1958). Drawing Idiograms of the species were made in Microsoft Excel 97.

Results and Discussion

The somatic chromosome number, 2n=16, were observed in *O. scorbiculata, O. melanotricha, O. aucheri* subsp.. *teheranica* and *O. oxyptera* (Fig. 1). total length and average chromosome length varied from 31.07 and 3.88 μm in *O. oxyptera* to 39.88 and 4.98 μm in *O. gauba*, respectively. The longest and the shortest chromosomes also can be seen in *O. scorbiculata* and *O. oxyptera* respectively. TF% ranged from 36.43% in *O. oxyptera* to 41.36% in *O. aucheri* subsp. *teheranica* (Table 3). The karyotypes can be divided as 1A (*O. scorbiculata* and *O. melanotricha)*, 1B (*O. aucheri* subsp. *teheranica*) and 2B (*O. oxyptera*) due to stebbins method. The most asymmetrical karyotype is depicted by *O. oxyptera* (Fig. 1; Table 3). Karyotype formula shows that many of chromosomes are m type and some of them are sm type.

Table 1. some chromosome number reports of *Onobrychis* species for flora of Iran.

Species	Chromosome number	Source
O. crista-galli (L.) Lam.	32	Goldblatt & Johnson 1998
O. crista-galli (L.) Lam.	16	Goldblatt & Johnson 1998
O. chorassanica Bge.	14	Rahimi & al. 1999
O. cornuta (L.) Desv.	14	Goldblatt & Johnson 1991
O. galegifolia Boiss.	16	Goldblatt 1981
O. haussknechtii Boiss.	16	Goldblatt 1981
O. micrantha Schrenk	16	Goldblatt & Johnson 1995
O. ptolemaica (Delile) DC.	16	Zohary 1972
O. pulchella Schrenk	16	Goldblatt & Johnson 1995
O. sintinesii Bornm.	14	Goldblatt 1981
O. tavernieraefolia Stocks ex Boiss.	16	Goldblatt 1981

Table 2. *Onobrychis* taxa and their locality in Fars province.

Species	Locality
O. aucheri Boiss. subsp. *teheranica* (Bornm.) Rech. f.	Fars: Abadeh, Shorjestan.
O. scorbiculata Boiss.	Fars; Abadeh, Dehbid, 2300 m, Nassirzadeh & Hatami 77814.
O. melanotricha Boiss.	Fars; Shiraz, Bamoo National park.
O. oxyptera Boiss.	Fars: Arsanjan, Arsanjan, 1750 m, Hatami & Nassirzadeh 78413.

Talbe 3. Karyotype details of *Onobrychis* species (T. L=Total Length of a genome, L=longest chromosome, S=shortest chromosome, ACL=Average Chromosome Length, KF=Karyotype Formulae, TF%=Tatal Form Percentage).

Species	2n	T.L.	L	S	ACL	KF	TF%	Class
O. scorbiculata	16	39.88	6.39	3.27	4.98	7m+1sm	39.24	1A
O. oxyptera	16	31.07	5.14	2.52	3.88	5m+3sm	36.43	2B
O. aucheri	16	31.58	5.33	2.67	3.95	7m+1sm	41.36	1B
O. melanotricha	16	33.04	5.19	2.80	4.13	3m+5sm	36.48	1A

By reviewing the literature, many of the species had 2n=16 and 2n=14 but there were some species with 2n=32 (Table 1). This fact can be seen in this study. It means that all species showed 2n=16 chromosomes. Acoording to the literature, the basic chromosome number of the species studied, X, appears to be 7 and 8 (Darlington & Wylie 1955). So the species with 2n=16 chromosome including *O. scorbiculata, O. melanotricha, O. aucheri* subsp. *thehranica* and *O. oxyptera* are diploids.

The gradual alteration of TF% values from 36.43% to 41.36% might be due to structural alterations in the genome. The structural alterations of the different species might be due to duplication or translocation between chromosomes at a very early stage of evolution (Das, Basak & Das 1998).

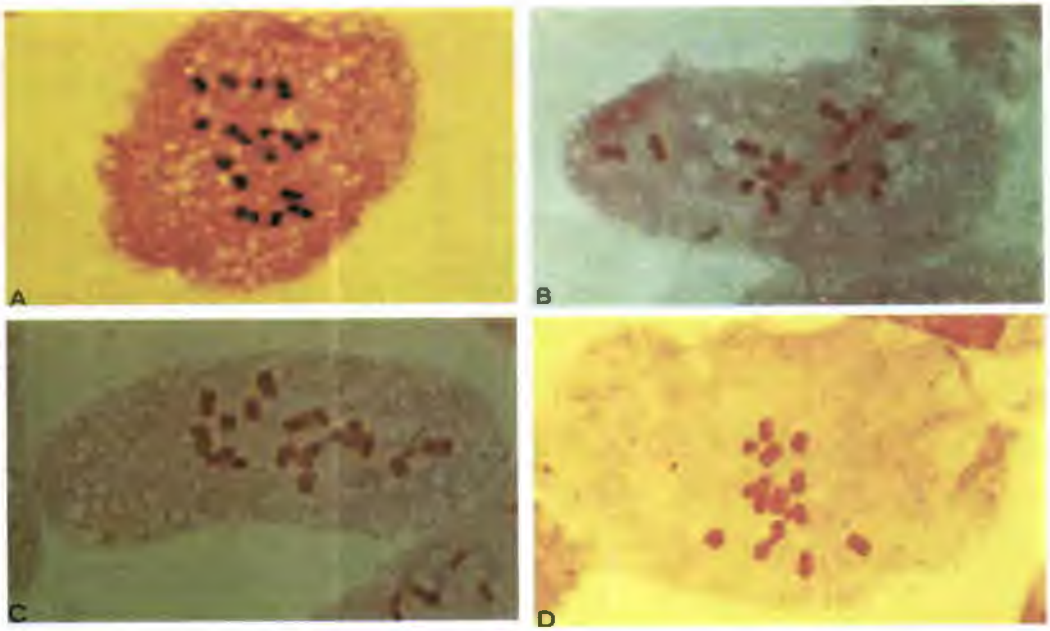

Fig. 1. Karyotypes of 4 *Onobrychis* taxa. -A. *O. aucheri* subsp. *teheranica,* B. *O. scorbiculata*; C. *O. melanotricha;* D. *O. oxyptera.*

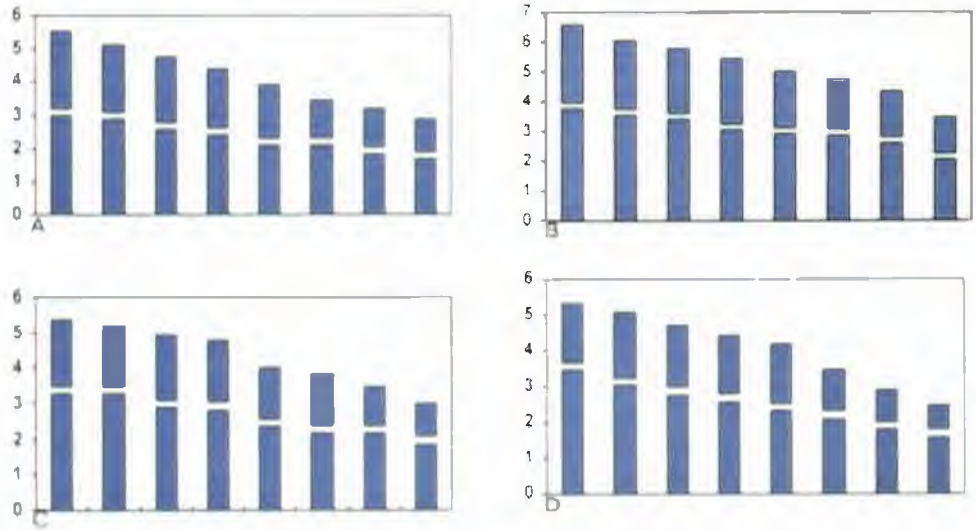

Fig. 2. Idiograms of 4 *Onobrychis* taxa in µm. A. *O. aucheri* subsp. *teheranica;* B. *O. scorbiculata;* C. *O. melanotricha;* D. *O. Oxyptera.*

The present results clearly showed that the *Onobrychis* species differ from each other in definite karyotypic features (Fig. 1 and 2 and Table 3). Remarkably, the maximum karyotypic asymmetry is associated with the least TF%. If an increase in karyotypic asymmetry indicates phylogenetic advantage, then the direction of evolution can be inferred towards decreasing TF%.

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