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Karyotype analysis of *Aeluropus* species (Poaceae)

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Summary. *Aeluropus*, a member of Poaceae subfam. *Chloridoideae*, includes six species, three of which occur in Iran. They are perennial halophytes of deserts and coastal marshlands of Iran. The genus is considered as a rich genetic source for gene manipulation and using it for crop improvement. Previous studies showed that members of *Chloridoideae* have small chromosomes and the base chromosome number $n = 10$. There are few chromosome records for *Aeluropus* species. Somatic metaphases of seven populations of three *Aeluropus* species were studied. The first chromosome counts ($2n = 20$) based on Iranian material for three species, *A. macrostachyus*, *A. littoralis* and *A. lagopoides,* are concordant with previous records outside Iran; mitotic number for *A. macrostachyus* is recorded here for the first time.

Анализ кариотипа видов *Aeluropus* (Poaceae)

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Ключевые слова: Иран, митоз, популяция, число хромосом, *Chloridoideae*.

Аннотация. Род *Aeluropus* подсемейства *Chloridoideae* семейства Poaceae представлен тремя видами в Иране и шестью – в мире. Это многолетники, обитатели соленых болот и пустынь. Считается, что виды рода обладают ценными свойствами и являются перспективными объектами для генетических исследований и использования их для улучшения свойств сельскохозяйственных культур. Предыдущие работы показали, что представители трибы *Cynodonteae* обладают небольшими хромосомами и базовым числом $n = 10$. Подсчётов хромосомных чисел для видов рода *Aeluropus* в литературе приводится немного. Нами изучены метафазные пластинки соматических хромосом у трех видов рода из семи популяций. Для *A. macrostachyus, A. littoralis* и *A. lagopoides* числа хромосом $2n = 20$ определены первый раз на материале из Ирана, при этом для спорофита *A. macrostachyus* оно указывается впервые.

Introduction

Aeluropus Trin. (*Chloridoideae* Kunth ex Beilschm., *Cynodonteae* Dumort., Poaceae Barnhart) includes three species in Iran and six in the world (Bor, 1970; Watson et al., 1992; POWO, 2021). They are halophytic elements of central and tropical Asia, Africa, and Europe (Bor, 1970). *Aeluropus littoralis* (Gouan) Parl. as a perennial halophyte is native to the central desert and coastal marshlands

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of Iran. This species is considered as a rich genetic source for gene manipulation and crop improvement (Modarresi et al., 2012). *Aeluropus lagopoides* (L.) Trin. ex Thwaites is known as a salt-secreting, rhizomatous perennial halophyte distributed on dry and salty soils of Iran. This species survives in harsh environments due to the vigorous seed production, epicuticular wax layer, salt glands, and small leaves (Mohsenzadeh et al., 2006). *Aeluropus macrostachyus* Hack. is of potential fodder value in Southwest Asia (Öztürk et al., 2019). Due to the presence of hybrids, subspecies, and ecotypes in the studied taxa, *Aeluropus* identifications are somehow difficult (Abivardi et al., 2010).

Previous records indicated that members of *Cynodonteae* possess small chromosomes and the base chromosome number $n = 10$. Tarnavschi and Lungeanu (1982) and Kožuharov and Petrova (1991) recorded $2n = 20$ for sporophyte of *A. littoralis*. Moreover, $2n = 30$ and $2n = 60$ were reported for this taxon (Tarnavschi, 1948; Matevosian, 1975). In *A. lagopoides*, $2n = 20$ (Murin, Chaudhri, 1970; Nagabhushana, 1980) and $2n = 50$ (Baquar, Saeed, 1969) for sporophyte and $n = 20$ (Moinuddin et al., 1994) for gametophyte have been recorded. Khatoon and Ali (1993) recorded $x = 10$ for *A. littoralis* and *A. macrostachyus*. There are no records for the mitotic number of *A. macrostachyus*.

Variations in the species chromosomes provide useful data for biosystematic and breeding studies. In this project, the chromosome counts and karyotype parameters for the Iranian species of *Aeluropus* are studied for the first time.

Material and methods

In this project, 7 accessions of three *Aeluropus* species in Iran were gathered from nature (Table 1). Vouchers are deposited in the herbarium of Alzahra University (ALUH) and the herbarium of the Research Institute of Forests and Rangelands (TARI).

Table 1

Details of *Aeluropus* population and taxa studied

Locality, collector, voucher number	Species
Azerbaijan, Tabriz to Ahar, Beside Salt Lake, Zare, Abivardi, 8511 (ALUH)	
Kerman, Jazmorian, Zehkelot, Khodashenas, 859 (ALUH)	*Aeluropus littoralis*
Markazi, Saveh, Keshavarzi, Abivardi, 8512 (ALUH)	
Semnan, Garmsar, Abivardi, 8513 (ALUH)	
Qom, Qom, Foroughi, 1097 (TARI)	*A. lagopoides*
Semnan, Touran Protected Region, Abbas Abad, Freitag, 14152 (TARI)	
Kerman, Jazmorian, Basiri, Nourouzi, 1516 (TARI)	*A. macrostachyus*

The plants were collected during the years 2007–2017 in their natural habitats (Table 1). For the somatic chromosome study, the seeds were germinated on moist filter paper in the laboratory (ca. 21–24 ºC). In order to vernalize seeds, they were put at 4 ºC in a refrigerator (48–72 h), then transferred to room temperature. The growing root tips of ca. 0.7–1.0 cm long were cut and pre-treated in a saturated 0.002 M water solution of 8-hydroxyquinolin at 4 ºC in a refrigerator (2–4 h) and fixed in a cold mixture of ethanol and acetic acid (3 : 1) for 24 h. Root tips were macerated in two ways: 1) 1N HCl was used for 3 hours (Cold Hydrolysis) at room temperature; 2) Hot Hydrolysis was used by means of 1N HCl for 6 min. in 60 ºC bath. Temporary slides were made by squashing the segments and staining in 2% aceto-orcein for 30–45 min. Good metaphase plates were photographed with Olympus microscope equipped with DP12 digital camera and measured Ideokar software ver. 1.2.

Chromosomes were identified based on Levan et al. (1964). For karyotype symmetry, the coefficient of variation of chromosome length (CV_{CL}) based on Paszko (2006) and mean centromeric asymmetry (M_{CA}) based on Peruzzi and Eroğlu (2013) were determined. A2 index of Zarco (1986) and coefficient of variation of the chromosome size (CV) were also calculated.

Results

The chromosome counting in seven accessions of different species of *Aeluropus* in Iran showed only one ploidy level ($2n = 20$). The best time to catch the highest somatic metaphases at root tips was 10 a. m. to 01 p. m. in the species studied. The summary of karyotype features is shown in Table 2.

The size of the shortest chromosome varied from 0.54 to 0.91 µm while the size of the longest varied from 1.19 to 1.67 µm. The range of total length of

the haploid chromosome varied from 17.99 to 24.06 µm. The highest value of chromosome size variation (CV) occurred in *A. littoralis* and the lowest one in *A. lagopoides*. The coefficient of variation of chromosome length (CV_{CL}) was the highest in *A. littoralis* and the lowest in *A. macrostachyus*. *Aeluropus littoralis* and *A. lagopoides* showed 3B Stebbins' symmetry class while *A. macrostachyus* showed 3A. *Aeluropus lagopoides* and *A. macrostachyus* had metacentric chromosomes while *A. littoralis* had nine metacentric and one sub-metacentric chromosome (Figs. 1–3).

Table 2

Karyotype features of *Aeluropus* taxa studied

KF	ST	CV	M_{CA}	CV_{CL}	A2	L/S	TL	S	L	2n	Species
10 m	3B	21	9.9	21.34	0.21	2.16	17.99	0.55	1.19	20	*A. lagopoides*
9 m + 1sm	3B	26	13.7	26.25	0.26	2.72	19.41	0.54	1.47	20	*A. littoralis*
10 m	3A	20	8.99	19.72	0.20	1.83	24.06	0.91	1.67	20	*A. macrostachyus*

Abbreviations: TL – total chromatin length (µm), L/S – ratio of the longest to shortest chromosome (µm), A2 – inter-chromosomal asymmetry index (Zarco), CV_{CL} – coefficient of variation of chromosome length, M_{CA} – mean centromeric asymmetry, CV – coefficient of variation, ST – Stebbins' symmetry class, KF – karyotypic formulae.

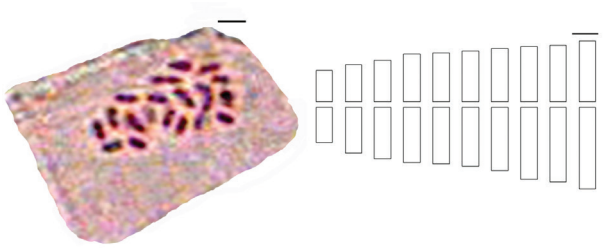

Fig. 1. Somatic metaphases in *Aeluropus lagopoides* in Iran (2n=20) (Scale bar: 3 µm).

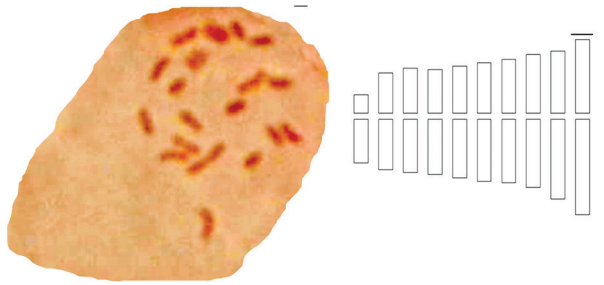

Fig. 2. Somatic metaphases of *Aeluropus littoralis* in Iran (2n=20) (Scale bar: 3 µm).

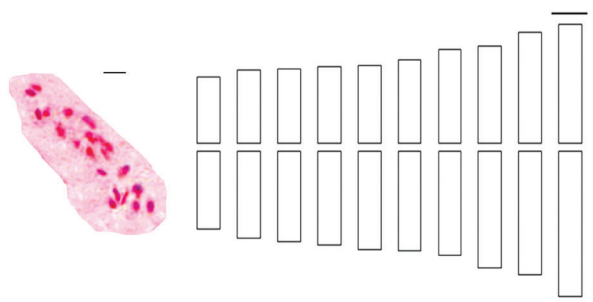

Fig. 3. Somatic metaphases of *Aeluropus macrostachyus* in Iran (2n=20) (Scale bar: 3 µm).

Discussion

Aeluropus and *Odyssea* Stapf are the members of subtribe *Aeluropodinae* P. M. Peterson of Eurasia and Africa origin (Soreng et al., 2015, 2017). *Aeluropus* is a small genus with three species in Iran. They are mainly found as members of halophytic vegetation. These species have very small chromosomes and the basic chromosome number $x = 10$.

The most common basic chromosome numbers in Poaceae are $x = 7, 9, 10, 12$ (Stebbins, 1982). It is assumed that the original basic chromosome number for Poaceae species is $x = 7$, and the larger ones are its derivatives. Another assumption is that the ancestral genome has $x = 5$ that varied by duplications and inter-chromosomal translocations to provide an intermediate ancestral genome with $x = 12$ (Shchapova, 2012).

Basic chromosome number is constant in some tribes of Poaceae. Two main and frequent basic chromosome numbers are $x = 7$ (29.5 %) and $x = 10$ (31.9 %). Avdulov (1931) considered $x = 12$ as the ancestral basic chromosome number that results in other basic chromosome numbers by reduction due to aneuploidy (Hilu, 2004), but others as Stebbins (1982) considered $x = 6$ and 7 as ancestral in primitive genera and this discussion is still being continued (Hilu, 2004; Shchapova, 2012).

Chloridoideae has $x = 9$ and 10 as the common basic numbers (Hilu, 2004); $x = 9$ is found in 86 % of the species and $x = 10$ in 13 % (de Wet, 1987). The basic chromosome number $x = 5$ has not been reported for this subfamily. Hilu and Alice (2001) believed that $x = 10$ is a plesiomorphic character in *Chloridoideae*. It is evident that aneuploid reduction

from $x = 12$ to $x = 10$ and $x = 9$ in some genera appeared in the early stages of the evolutionary history of *Chloridoideae* (Hilu, 2004). Variation in basic chromosome number, the frequency of polyploidy, and hybridization is common in grass family but there is a kind of homogeneity in the basic chromosome number in *Aeluropus*.

In this study, all the species were found to be diploid. Two species, *A. littoralis* and *A. lagopoides*, showed mitotic numbers $2n = 20$ that were in agreement with previous results (Murin, Chaudhri, 1970; Nagabhushana, 1980; Tarnavschi, Lungeanu, 1982; Kožuharov, Petrova, 1991). In *A. macrostachyus*, $2n = 20$ was also found which is the first record of the mitotic number for this species. Previously Khatoon and Ali (1993) reported the meiotic number $x = 10 + 1B$ for *A. macrostachyus*.

The inter-chromosome asymmetry index varied from 0.20 to 0.26, indicating similar chromosome length of taxa. Based on this index, *A. littoralis* had more variation in chromosome length. *Aeluropus littoralis* and *A. lagopoides* showed more similarities in karyotype characters. The close relationship of these taxa was also confirmed in morphological and anatomical studies (Abivardi et al., 2010). Chromosomes are of the metacentric (m) and sub-metacentric (sm) types. The amount of metacentric chromosomes in the cytotypes studied suggests that the karyotype of these species shows a trend to be stable. *Aeluropus macrostachyus* with 3A Stebbins's symmetry class had more symmetrical chromosomes than two other species. Among species studied, *A. macrostachyus* had the lowest inter-chromosomal (CV_{CL}) and intra-chromosomal asymmetry (M_{CA}) while *A. littoralis* had the highest values of these parameters.

To have a better understanding of the variation in this genus, a cytogenetic study is suggested to consider the behaviour of the chromosomes during meiosis. There are some hybrid populations of *Aeluropus* species in Iran (Abivardi et al., 2010). Studying the chromosome counts of these populations is highly recommended.

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