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CHROMOSOME COUNTS OF NINETEEN PLANT SPECIES FROM IRAN

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Abstract
Seeds from nineteen native plant species belonging to 6 families (Apocynaceae, Capparaceae, Cleomaceae, Fabaceae, Resedaceae, and Zygophyllaceae) were collected in Bushehr Province, Iran. Chromosome counts revealed the following diploid numbers for all studied species, representing first records for the flora of Iran: *Caralluma edulis* (2n=22), *Pergularia tomentosa* (2n=22), *Capparis cartilaginea* (2n=20), *C. parviflora* (2n=20), *C. spinosa* (2n=20), *Cleome glaucescens* (2n=20), *C. oxypetala* (2n=20), *Astragalus annularis* (2n=16), *A. boeticus* (2n=16), *A. hamosus* (2n=16), *A. meridionalis* (2n=16), *Ochradenus aucheri* (2n=24), *Oligomeris linifolia* (2n=16), *Reseda alba* subsp. *alba* (2n=24), *R. alba* subsp. *decursiva* (2n=24), *R. arabica* (2n=12), *R. aucheri* subsp. *bracteata* (2n=24), *Zygophyllum qatarense* (2n=20) and *Tribulus terrestris* var. *inermis* (2n=24). Notably, three species in our samples (*Capparis spinosa*, *Reseda alba* subsp. *decursiva*, and *R. aucheri* subsp. *bracteata*) were found to be diploid (2n=20, 2n=24, and 2n=24, respectively), in contrast to previous reports that identified them as tetraploids (2n=4x=40 and 2n=4x=48).

Keywords: Chromosome number; flora; Iran; mitosis; Ploidy.

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شمارش کروموزومی نوزده گونه گیاهی از ایران

فاطمه غلامیان: استادیار پژوهش، بخش تحقیقات جنگل‌ها و مراتع، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان بوشهر، سازمان تحقیقات، آموزش و ترویج کشاورزی

چکیده: در این پژوهش، بذور ۱۹ گونه گیاهی بومی متعلق به ۶ تیره *Apocynaceae*، *Capparaceae*، *Cleomaceae*، *Fabaceae*، *Resedaceae* و *Zygophyllaceae*، از استان بوشهر جمع‌آوری شد. شمارش کروموزومی ۱۹ گونه شامل *Caralluma edulis* (Edgew.) Benth. ex Hook.f. (2n=22)، *Pergularia tomentosa* L. (2n=22)، *Capparis cartilaginea* Decne. (2n=20)، *C. parviflora* Boiss. (2n=20)، *C. spinosa* L. (2n=20)، *Cleome glaucescens* DC. (2n=20)، *C. oxypetala* Boiss. (2n=20)، *Astragalus annularis* Forssk. (2n=16)، *A. boeticus* L. (2n=16)، *A. hamosus* L. (2n=16)، *A. meridionalis* Bunge. (2n=16)، *Oligomeris linifolia* (Vahl) Macbr. (2n=24)، *Ochradenus aucheri* Boiss. (2n=16)، *Reseda alba* subsp. *alba* (2n=24)، *R. alba* subsp. *decursiva* (Forssk.) Maire (2n=24)، *R. aucheri* subsp. *bracteate* Boiss. (2n=12)، *R. arabica* Boiss. (2n=24)،

Tribulus terrestris var. *inermis* Boiss.(2n=24) و(2n=20) *Zygophyllum qatarense* (Hadidi) Beier & Thulin ، برای اولین بار در فلور ایران گزارش می‌گردند. نکته قابل توجه این مطالعه، مشاهده حالت دیپلوئید در سه گونه *Capparis spinosa*، *Reseda alba* subsp. *decursiva* و *R. aucheri* subsp. *bracteata* است که در مطالعات پیشین به‌صورت تتراپلوئید به‌ترتیب (2n=4x=40)، (2n=4x=48) و (2n=4x=48) گزارش شده‌است.

INTRODUCTION

The determination of chromosome number and assessment of ploidy levels in plant species have important implications for scientific research, ecological understanding, and evolutionary studies (Goldblatt & Lowry, 2011). These cytogenetic parameters serve as fundamental data not only for systematic and taxonomic studies (Rieseberg & Willis, 2007) but also play pivotal roles in biodiversity conservation, plant breeding programs, and understanding adaptive mechanisms in plants (Iranbakhsh & al., 2020). This study aimed to determine the chromosome numbers of various plant species and their populations through mitotic chromosome analysis, while contributing to the chromosomal index database of the flora of Iran.

Notably, this study reports chromosome counts for nineteen native plant species from Bushehr province for the first time in the flora of Iran, including three species that had been previously documented as tetraploids in other geographical regions. These findings provide valuable baseline data for future research in plant systematics, evolutionary biology, and conservation genetics.

MATERIALS AND METHODS

In this comprehensive cytogenetic study, seeds from nineteen species were collected from their natural habitats during 2021-2023. Voucher specimens, authenticated using botanical references (Assadi & al. 1989-2015, Ghahraman 1999-2003, Rechinger 1963-2015), are preserved at the Herbarium of Bushehr Agricultural and Natural Resources Research and Education Center. Seed germination was conducted under controlled laboratory conditions (25-30°C) using sterile Petri dishes containing moist filter paper. Actively growing root tips (0.5-1.5 cm) were excised and subjected to a rigorous cytological protocol: pretreatment with 0.5% saturated α-bromonaphthalene at 4°C for 2 hours, followed by fixation in 10% Levitsky solution (4°C, 16 hours). After thorough rinsing with distilled water, samples underwent hydrolysis in 1N NaOH at 60°C for 6 minutes, followed by staining with 4% hematoxylin-iron solution (1 hour, room temperature). Chromosome preparations were made using an optimized squash technique with 45% acetic acid-lactic acid solution.

High-resolution metaphase spreads were examined using an Olympus BH-2 microscope with a 100× objective lens and advanced imaging systems. Chromosome morphology was assessed following the standardized criteria of Levan & al. (1964) to ensure consistent and accurate karyotypic descriptions. This meticulous protocol enabled precise determination of both chromosome numbers and structural features across all studied taxa.

RESULTS

This study defined the optimal time window for chromosomal analysis in the examined plant species as occurring between 08:00 and 13:00 hours, when root tip cells exhibit peak frequencies of somatic metaphases. Successful chromosome counts were obtained for nineteen plant species representing six distinct families for the first time for the flora of Iran, with the following results:

Apocynaceae

Caralluma edulis (Edgew.) Benth. ex Hook.f. (2n=22), (Fig. 1, A).

Specimen examined: Iran, Bushehr province, Tangestan, Bojikdon, 0 m, Gholamian 732.

C. edulis, a succulent species, was found to be diploid with a chromosome number of 2n=2x=22 in our study. This result aligns with previous cytological reports from Pakistan's Balochistan populations, where both Albers & Meve (2002) and Khan & al. (2014) consistently documented the same diploid count (2n=22).

Pergularia tomentosa L. (2n=22), (Fig. 1, B).

Specimen examined: Iran, Bushehr province, Tangestan, Konary village, 75 m, Gholamian 323.

P. tomentosa is a scrambling or twining subshrub characterized by cordate leaves and axillary umbellate inflorescences. Cytogenetic analysis revealed a diploid chromosome number of 2n=2x=22, consistent with previous reports from Egyptian populations (Soliman & al., 1994).

Capparaceae

Capparis cartilaginea Decne. (2n=20), (Fig. 1, C).

Specimen examined: Iran, Bushehr province,

Dashtestan, Bushkan, 650 m, Gholamian 806.

Cytogenetic analysis of *C. cartilaginea* confirmed a diploid chromosome number of 2n=2x=20. This result is consistent with previous reports from Red Sea coastal populations in Saudi Arabia (Al-Hemaid & Al-Turki, 2011), but contrasts with the chromosome count of 2n=40 documented in Egyptian populations by Soliman & al. (2002).

Capparis parviflora Boiss., (2n=20), (Fig. 1, D).

Specimen examined: Iran, Bushehr province, Dashtestan, Bushkan, 610 m, Gholamian 580.

Cytogenetic analysis of *C. parviflora* confirmed a diploid chromosome complement of 2n=2x=20. This result is consistent with previous reports from Balochistan, Pakistan (2n=20; Khatoon & Ali, 1993). However, contrasting ploidy levels have been documented in Egyptian populations, with reports of tetraploid (2n=4x=40) and hexaploid (2n=6x= 60) cytotypes by Soliman & al. (2002).

Capparis spinosa L. (2n=20), (Fig. 1, E).

Specimen examined: Iran, Bushehr province, Dashtestan, Bushkan, 650 m, Gholamian 506.

Cytogenetic analysis of *C. spinosa* revealed a diploid chromosome complement of 2n=2x=20. These results show complete consistency with the previous report by Magulaev (1979) from populations in the Northern Caucasus (Russia), while contradicting the chromosomal count of 2n=34 documented by Al-Turki & al. (2000) from the Riyadh region (Saudi Arabia).

Cleomaceae

Cleome glaucescens DC. (2n=20), (Fig. 1, F).

Specimen examined: Iran, Bushehr province, Dashtestan, Rahdar village to Dalakey, 70 m, Gholamian 738.

Cytogenetic analysis of *C. glaucescens* confirmed a diploid chromosome complement of 2n=2x=20. These findings exhibit complete cytological congruence with diploid populations in Egypt (Soliman & El-Saidi, 1999), and the Red Sea Hills, Sudan (Khalik & al., 2001), all maintaining the conserved diploid state (2n=20).

Cleome oxypetala Boiss. (2n=20), (Fig. 1, G)

Specimen examined: Iran, Bushehr province, Dashtestan, Dalaki to Ghalesefid village, 95 m, Gholamian 1209.

Cytogenetic examination of *C. oxypetala* established a diploid chromosome complement of 2n=2x=20. This result aligns with previous reports from the populations in Pakistan (2n=20; Khatoon & Ali, 2013).

Fabaceae

Astragalus annularis Forssk. (2n=16), (Fig. 1, H).

Specimen examined: Iran, Bushehr province, Bushehr, Helyleh, 0 m, Gholamian 630.

Our cytogenetic analysis of *A. annularis* populations revealed a diploid chromosome number of 2n=16, consistent with one cytotype reported from Kuwait by Malallah & al. (2001). However, this species exhibits notable karyological variation, with different studies reporting 2n=14 in Egyptian populations (Badr & al., 1996) and both 2n=16 and 2n=24 in Kuwaiti populations (Malallah & al., 2001).

Astragalus boeticus L. (2n=16), (Fig. 1, I).

Specimen examined: Iran, Bushehr province, Kangan, Bandar Tahery to Asaluyeh, 0 m, Gholamian 827.

Chromosomal analysis of *A. boeticus* revealed a somatic chromosome number of 2n=16, corresponding to the diploid level (2n=2x=16). This result aligns with previous reports from Mediterranean populations, including Portuguese specimens from the Iberian Peninsula (2n=16; Benedí & Montserrat, 2000). However, these results contrast with the chromosome count of 2n=30 reported from both Egyptian (Badr & al., 1996) and Portuguese populations (Fernandes & Queirós, 1978).

Astragalus hamosus L. (2n=16), (Fig. 1, J).

Specimen examined: Iran, Bushehr province, Kangan, Bandar Tahery to Asaluyeh, 0 m, Gholamian 763.

Cytogenetic analysis of *A. hamosus* revealed a diploid chromosome complement of 2n=2x=16. This finding contrasts with previous reports, including 2n=32 in Egyptian populations (Badr & al., 1996), 2n=44 in North African accessions (Oberprieler & Vogt, 1996), and 2n=42 in Portuguese populations (Fernandes & Queirós, 1978).

Astragalus meridionalis Bunge. (2n=16), (Fig. 1, K).

Specimen examined: Iran, Bushehr province, Tangestan, Delvar to Hadakan, 5 m, Gholamian 755.

Cytogenetic analysis of *A. meridionalis* confirmed a diploid chromosome complement of 2n=2x=16. This finding aligns with previous cytological reports from geographically proximate regions, including Armenian populations in Russia (Avetisian, 1980) and Dagestani populations (Mammadova, 2007).

Resedaceae

Ochradenus aucheri Boiss. (2n=24), (Fig. 1, L).

Specimen examined: Iran, Bushehr province, Kangan, Bandar Tahery to Asaluyeh, 0 m, Gholamian 805.

Cytogenetic analysis of *O. aucheri* revealed a stable diploid chromosome complement of 2n=2x=24. This finding demonstrates complete cytological congruence with reports from both documented populations across the Arabian Peninsula, including those from the Asir Mountains in Saudi Arabia (2n=24; Al-Harbi & al., 2018) and the broader peninsula (Alzahrani & Albokhari, 2017).

Oligomeris linifolia (Vahl) Macbr. (2n=16), (Fig. 1, M).

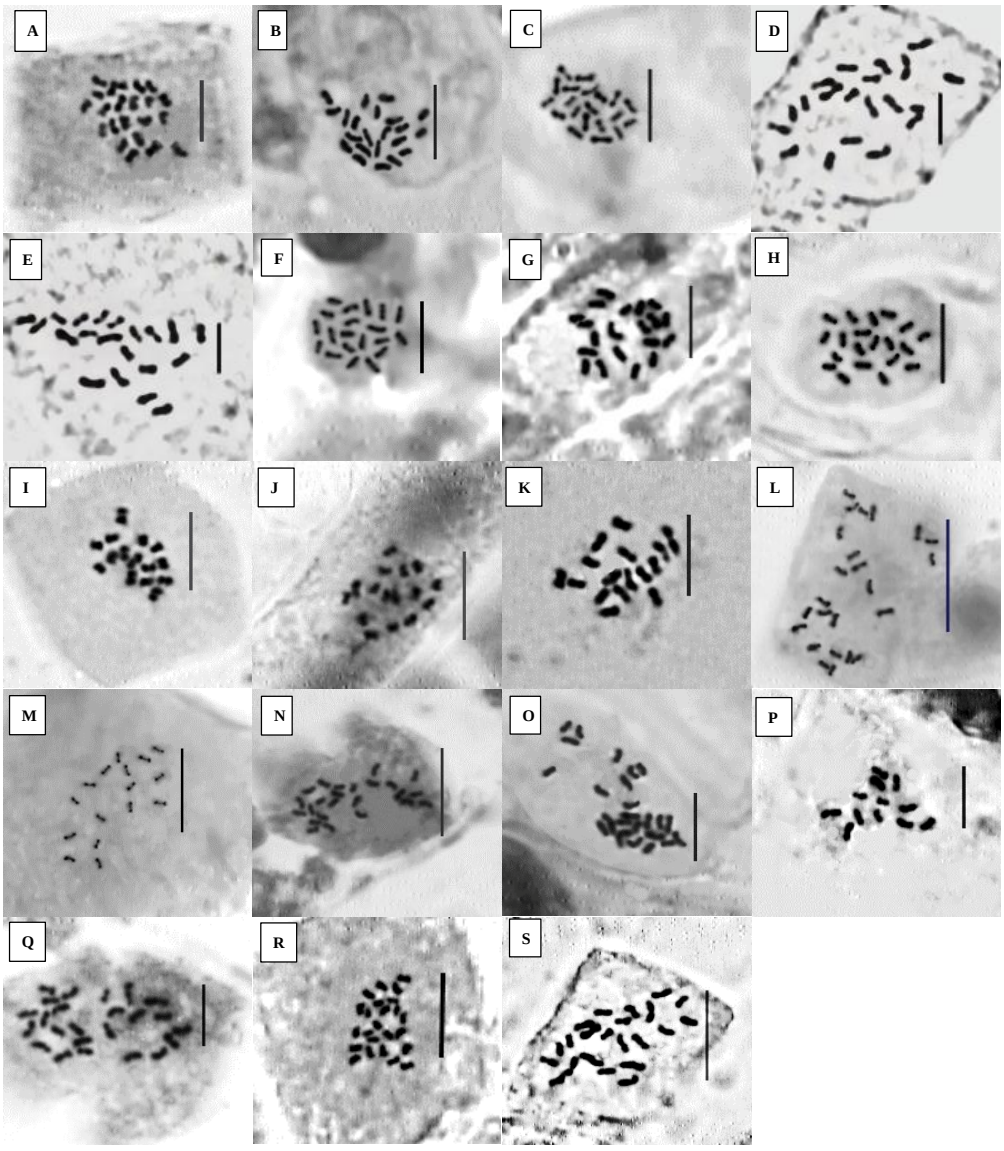

Fig. 1. A-S, Somatic metaphases of the studied species. A, *Caralluma edulis* (2n=2x=22); B, *Pergularia tomentosa* (2n=2x=22); C, *Capparis cartilaginea* (2n=2x=20); D, *Capparis parviflora* (2n=2x=20); E, *Capparis spinosa* (2n=2x=20); F, *Cleome glaucescens* (2n=2x=20); G, *Cleome oxypetala* (2n=2x=20); H, *Astragalus annularis* (2n=2x=16); I, *Astragalus boeticus* (2n=2x=16); J, *Astragalus hamosus* (2n=2x=16); K, *Astragalus meridionalis* (2n=2x=16); L, *Ochradenus aucheri* (2n=2x=24); M, *Oligomeris linifolia* (2n=2x=16); N, *Reseda alba* subsp. *alba* (2n=2x=24); O, *Reseda alba* subsp. *decursiva* (2n=2x=24); P, *Reseda arabica* (2n=2x=12); Q, *Reseda aucheri* subsp. *bracteate* (2n=2x=24); R, *Tribulus terrestris* var. *inermis* (2n=2x=24); S, *Zygophyllum qatarense* (2n=2x=22); Scale bars=5µm.

Specimen examined: Iran, Bushehr province, Tangestan, Konary village, 65 m, Gholamian 776

Cytogenetic analysis of *O. linifolia* confirmed a diploid chromosome number of 2n=2x=16. This finding aligns with previous reports from geographically distinct populations across Saudi Arabia (El-Mekkawy & al., 2020) and the Saharo-Sindian region (Podlech, 1986).

Reseda alba subsp. ***alba*** (2n=24), (Fig. 1, N).

Specimen examined: Iran, Bushehr province,

Gezderaz to Abtavil village, 70 m, Gholamian 845. Cytogenetic analysis of *R. alba* subsp. *alba* revealed a diploid chromosome number of 2n=2x=24. This finding aligns with previous reports from Red Sea coastal populations in Saudi Arabia (Al-Hemaid & Al-Turki, 2011), but contrasts with the chromosome count of 2n=40 documented in Iberian Peninsula populations by Martín-Bravo & al. (2010).

Reseda alba subsp. ***decursiva*** (Forssk.) Maire (2n=24), (Fig. 1, O).

Specimen examined: Iran, Bushehr province, Tangestan, Konary village, 65 m, Gholamian 286.

Cytogenetic analysis of *R. alba* subsp. *decursiva* revealed a diploid chromosome complement of 2n=2x=24. This finding demonstrates a significant cytological divergence from the tetraploid cytotype (2n=4x=48) previously reported in Anatolian populations, Turkey (Martin & al., 2011), and the Troodos Mountains, Cyprus (Meikle, 1977).

Reseda arabica Boiss. (2n=12), (Fig. 1, Q).

Specimen examined: Iran, Bushehr province, Dashtestan, Borazjan to Chahkhani village, 60 m, Gholamian 974.

Cytogenetic analysis of *R. arabica* revealed a diploid chromosome number of 2n=2x=12. This finding contrasts with the previously reported count of 2n=40 from Arabian Peninsula populations by Alzahrani & Albokhari (2017).

Reseda aucheri subsp. ***bracteata*** Boiss. (2n=24), (Fig. 1, P).

Specimen examined: Iran, Bushehr province, Dashty, Lar to Khormoj, 60 m, Gholamian 1252.

Cytogenetic analysis of *R. aucheri* subsp. *bracteata* revealed a diploid chromosome complement of 2n=2x=24. This finding demonstrates significant cytogeographic divergence from the tetraploid cytotype (2n=4x=48) reported in Levantine populations, including specimens from Petra, Jordan (Al-Eisawi, 1982), and Egyptian populations (Abd El-Twab & Zahran, 2002).

Zygophyllaceae

Tribulus terrestris var. ***inermis*** Boiss. (2n=24), (Fig. 1, R).

Specimen examined: Iran, Bushehr province, Tangestan, Ahram, Emamzadeh Mir Ahmad, 65 m, Gholamian 429.

Cytological examination of *T. terrestris* var. *inermis* revealed a diploid chromosome number of 2n=2x=24. Our finding aligns with previous reports from the southeastern United States (Porter, 1972).

Zygophyllum qatarense (Hadidi) Beier & Thulin (2n=22), (Fig. 1, S).

Specimen examined: Iran, Bushehr province, Dashty, Lar to Khormoj, 30 m, Gholamian 593.

Our cytological investigation of *Z. qatarense* revealed a diploid chromosome number of 2n=2x=22, aligning with previous reports from Arabian Peninsula populations by Mahmoud & al. (2015) in Qatar and Shehata & Linder (1992) across the region.

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