

Cytotaxonomic Diversity of some Medicinal Species of Hyacinthaceae from Algeria

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ABSTRACT

Introduction: The Hyacinthaceae family is a large group of attractive petaloid species which synthesize specialized metabolites involved in their pharmacological usefulness. In Algeria, bulbous plants have long been one of the most trusted sources of traditional medicines. The aim of this work is to highlight the significance of systematic and karyological data to evaluate the diversity of species used in traditional pharmacopeia, particularly those endemics or isolated populations which can produce adapted chemotypes.

Materials and Methods: Analyses were performed on natural populations of four endemic and rare Hyacinthaceae, *Battandiera amoena*, *Drimia noctiflora*, *D. fugax* and *D. undata* sampled in contrasted biogeographic areas of Algeria, in coastal hills and in the Saharan border. Chromosomes number, karyotype formulas and asymmetry indices were established for all taxa. **Results:** The three species of the genus *Drimia* were diploid with $2n=2x=20$ chromosomes. In *Battandiera amoena* chromosome number is $2n=2x=18$. Karyotype analyses reveal marked variation in length and chromosomes morphology and in the chromosomal asymmetry indices.

Conclusion: Cytotaxonomical data are discussed in relation to the endemism and the biogeographical distribution of the species and in the context of the conservation of the biodiversity in Algeria.

Key words: Biodiversity, *Battandiera*, *Drimia*, Karyotype, Endemic medicinal plants.

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INTRODUCTION

The family of Hyacinthaceae is a large group of bulbous plants, divided in four monophyletic subfamilies: Hyacinthoideae, Ornithogaloideae, Urginoideae and Oziroeoideae.¹⁻⁴ Currently, this family is considered as a subfamily Scilloideae in the expanded Asparagaceae *sensu* APGIII.^{5,6}

Within the Hyacinthaceae, the species synthesize specialized secondary metabolites associated to each subfamily: Homoisoflavanones and triterpenoids (Hyacinthoideae), bufadienolides (Urginoideae) and cardenolides and steroidal glycosides (Ornithogaloideae).⁷⁻⁹ These metabolites are involved in the pharmacological properties of treatment of various infections, inflammation, disorders associated with the central nervous system.^{9,10}

In North-Africa and in Algeria, bulbous plants are traditionally used either for the prevention, or for the curative treatment of several diseases.^{11,12} The most widespread bulbous species and the most noteworthy used by traditional healers, is undoubtedly *Drimia maritima* (L.) Stearn (= *Urginea maritima*) also called red squill. Other species of the genus *Drimia* Jacq. (ex Willd.) and those of the genus *Battandiera* Maire, are sometimes used by Algerian populations especially in the Sahara desert.^{13,14} The huge importance of these species resides not only in their biochemical components but also in their endemism and narrow distribution under extreme ecological conditions. Unfortunately, these species are poorly-known and challenges us on their correct systematic identification and their karyological knowledge which is an important step to understand the morphological and genecological variation.¹⁵

The genus *Drimia* is one of the most important among the 9-11 genera of the subfamily Urginoideae¹ which includes a hundred species characterized by a stable chromosome base number $x=10$. However, phylogenetic studies,³ have retained only three genera *Igidia* Speta, *Bowiea* Harv. ex Hook. and *Drimia* Jacq., the latter including the genus *Urginea* Steinh.

The genus *Battandiera* belongs to the subfamily Ornithogaloideae which comprises about 250-300 taxa.^{4,16} The complexity of Ornithogaloideae is

due to a varying chromosome numbers from $2n=2$ to $2n=108$.¹⁷⁻¹⁹ Genus *Battandiera* was first typified by Maire²⁰ on the basis of a single species endemic to Algeria, *B. amoena* (Batt.) Maire, considered as a homotypic synonym to *Ornithogalum amoenum* Batt.²¹ In fact, this genus includes eight other species occurring in desert or arid environments with a highest diversity in southern Africa and Namibia.⁴

Considering the frequent changes in the nomenclature of taxa of the Ornithogaloideae and the Urginoideae,^{3,16,22} the names given to plants, used in the pharmacological studies, are unstable and differ radically from the currently accepted names.⁹ That is noteworthy in the case of the Algerian flora, which was poorly reviewed since the last synthetic flora in 1962.²³ Algerian flora lags behind in recent taxonomic and nomenclature updating, leading to controversies about status and circumscriptions of many taxa, particularly the Asparagales.^{24,25}

A renewed interest by the Algerian people for natural substances generates an increase in the use of plants as remedies. Therefore, programs of scientific research tend increasingly towards phytochemical studies.^{14,26,27} However, many species, particularly the bulbous plants, are also a source of poisons. Therefore, misidentification as well as inappropriate use, causes numerous cases of life-threatening poisoning.²⁸ Furthermore, many of the medicinal species are over-exploited and endangered; most of them are not yet taxonomically studied.

In this work, we aim to highlight the significance of systematic and karyological data to interpret and to evaluate the infraspecific diversity of plants, particularly those used in traditional pharmacopeia, endemics or isolated populations which can able to produce adapted chemotypes. It focuses on two endemic species to the Sahara, *Battandiera amoena* which is narrowly located in the South-West of Algeria, and *Drimia noctiflora* which is more broadly distributed throughout the Central Sahara.^{29,23,13} Cytotaxonomic analyzes were performed in comparison with two others closely related taxa, *Drimia undata* and *Drimia fugax*.

Table 1: Geographic origins and bioclimatic characteristics of studied species

Species	Locality	Latitude	Longitude	Altitude (m)	Bioclimate
<i>Battandiera amoena</i>	Taghit 1	30°54'43.0"N	02°01'40.0"W	580	Arid
	Taghit 2	30°48'17.6"N	02°03'21.3"W	615	Arid
<i>Drimia noctiflora</i>	Béchar	31°31'12.6"N	02°15'40.6"W	747	Arid
	Taghit 2	30°48'17.6"N	02°03'21.3"W	615	Arid
<i>Drimia fugax</i>	Staouéli (Algiers)	36°45'24"N	02°53'08"E	20	Subhumid
<i>Drimia undata</i>	Staouéli (Algiers)	36°45'24"N	02°53'08"E	20	Subhumid

Table 2: Chromosome numbers, karyotypic formulae and asymmetry indices of studied species

Species	2n	Karyotypic formulae	CLR (μm)	THL (μm)	MCA (%)	CV _{CL} (%)
<i>Battandiera amoena</i>	18	10st+2st-sat+6sm	2.56-10.38	61.96	57.96	44.51
<i>Drimia noctiflora</i>	20	4t+10st+4sm+2m	3.04-11.46	62.06	52.81	45.27
<i>Drimia fugax</i>	20	6st+6sm+8m	2.69-9.12	47.84	40.64	46.43
<i>Drimia undata</i>	20	2t+14st+4sm	2.86-10.62	54.45	60.16	47.26

CLR: Chromosome length range; THL: Total haploid length; M_{CA} : Mean centromeric asymmetry; CV_{CL} : Coefficient of variation of chromosome length.

The results may be of predictive value for the inventory of taxa and populations which must be integrated as a priority in the programs of the valorization of the medicinal plants and of the biodiversity conservation.

MATERIALS AND METHODS

Sampling and taxonomic determinations

Analysis were performed on natural populations collected in contrasted biogeographic areas in Algeria (September 2011-March 2013) on sand dunes in the Saharan border for *D. noctiflora* and *B. amoena* and in Mediterranean coastal hills for *D. fugax* and *D. undata* (Table 1). The species are named in this work following the last taxonomic and nomenclatural updates.^{1,16,20-22}

Chromosome observations

For mitotic chromosomes examination, root-tips were pretreated in 0.25 % aqueous colchicine at room temperature for five hours, fixed in Carnoy (ethanol-acetic acid 3:1) for 48h, and stored in ethanol 70° at 4°C until use. The chromosome staining was made using Feulgen's method.³⁰ The root-tips were briefly washed in distilled water to remove the fixative, hydrolyzed in 1N HCl for 12 min at 60°C, washed again, and stained with Schiff's reagent in darkness for 2-3 hours. Squash preparation was made under the coverslip in a drop of 45 % acetic acid. Observations were made using a light microscope Carl Zeiss, equipped with a digital camera.

Karyotype and idiogram construction

Measurements of chromosomes were made by using Micro Measure Software version 3.3.³¹ At least, five well-spread metaphase plates from different individuals were utilized. Karyotypes and idiograms were constructed on the basis of the arm ratio of chromosomes (r =long arm/short arm) and centromeric index ($CI\%$ =short arm/long arm+short arm $\times 100$). Chromosomes are described following the nomenclature of Levan *et al.*³²: metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t). Karyotype asymmetry was estimated by statistical parameters based on the proposal of Peruzzi and Eroglu.³³ The mean centromeric asymmetry $M_{CA}=A\times 100^{33}$ is the intrachromosomal asymmetry,

where A is the average ratio of long arm–short arm/long arm+short arm, according to Watanabe *et al.*³⁴ The coefficient of variation of chromosome length $CV_{CL}=A_2\times 100^{35}$ is the interchromosomal asymmetry where A_2 is the standard deviation of chromosome length/mean chromosome length.³⁶

RESULTS AND DISCUSSION

The karyotypes of the four investigated taxa were established, using conventional measurements of chromosomes. Chromosome number, ploidy level, karyotypic formula and asymmetry indices are presented in Table 2.

Battandiera amoena (Batt.) Maire (Synonym *Ornithogalum amoenum* Batt.)

B. amoena is endemic to the north-western Algerian Sahara (South of Oran, Beni-Abbes, and Béchar) and Moroccan neighboring areas.^{13,23,29} It grows in sandy and stony desert pastures along the Zousfana and Saoura wadis and in the plain of Abadla. Because of its narrow endemism, *B. amoena* has a low reputation in the traditional medicine except some use against leishmaniasis.³⁷ However, local population easily recognize this species with its large and highly toxic bulbs.^{11,28} Its toxicity should be due to alkaloids, as in the case for example, of the Colchicaceae although this is yet to be adequately tested.¹³ *B. amoena* is also diploid with $2n=18$ and a chromosome base number of $x=9$. This number is in agreement with a previous study by Reese³⁸ which examined the geographical distribution of polyploidy in the Northern Sahara. The karyotype formula was determined to be 10st+2st-sat+6sm, with evident satellites on the short arms of the second subtelocentric pairs (Figure 1, Table 2). Chromosome length varied from 2.56 μm to 10.38 μm . The total haploid length was 61.96 with a mean centromeric asymmetry (M_{CA})=57.96 % and a coefficient of variation (CV_{CL})=44.51 %.

Drimia noctiflora (Batt. & Trab.) Stearn (Synonym *Urginea noctiflora* (Batt. & Trab.) Steinh.)

This species is endemic to the northern Algerian Sahara. It is used by the local population in the same way as *D. maritima*^{11,12} and was reported to be highly toxic.¹⁴ Glycosides may be involved in this toxicity as in the

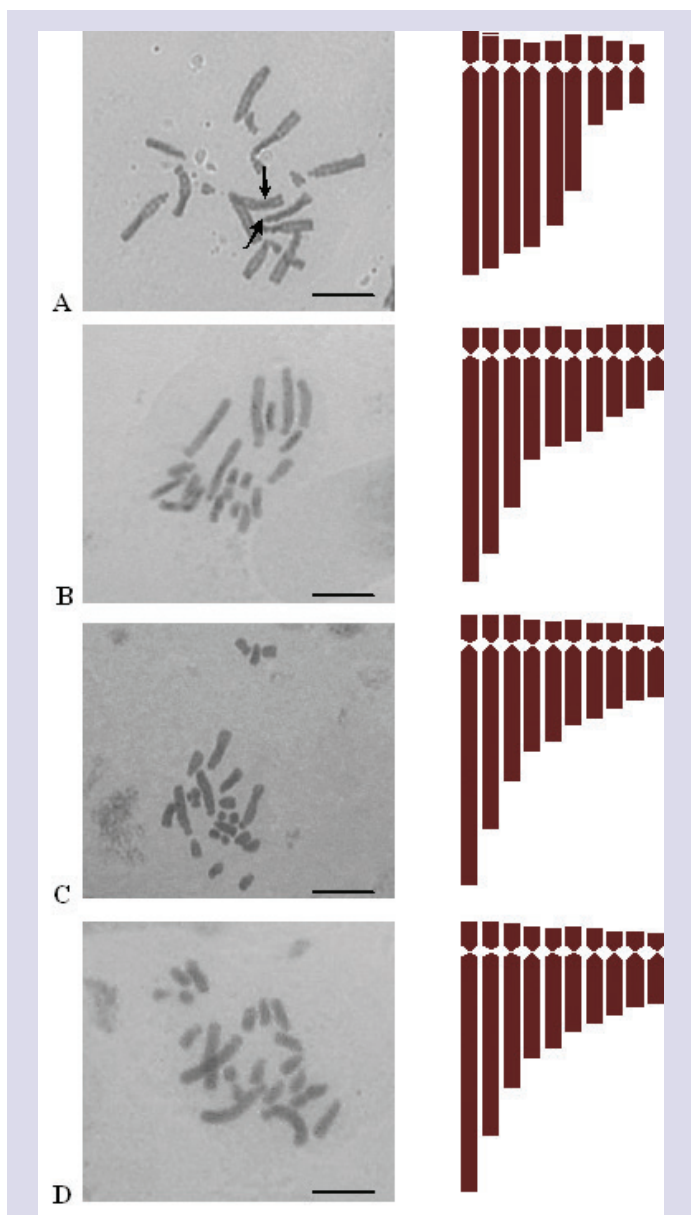


Figure 1: Mitotic chromosomes and haploid idiograms. *Battandiera amoena* (A) with $2n=2x=18$; *Drimia noctiflora* (B), *Drimia fugax* (C) and *Drimia undata* (D) with $2n=2x=20$: Scale bars 10 μm

case of species of *Urginea* from South African such as *Sekanama burkei* (synonym *Urginea burkei*) and *Urgineopsis modesta* (synonym *Urginea rubella*) that produce glycosides with activities on either the heart, or on the central nervous system and respiration of animals.⁹ The chromosome number for *D. noctiflora* was determined here for the first time. This number indicates a diploid level with $x=10$ with a following karyotype formula: $4t+10st+4sm+2m$. Chromosome length varied from 3.04 μm to 11.46 μm . The total haploid length was 62.06 μm . The mean centromeric asymmetry (M_{CA}) and the coefficient of variation of chromosome length (CV_{CL}), are 52.81 % and 45.27 % respectively (Figure 1, Table 2). The cytotypes appear to be stable for our specimen, however, a previous study on a closely related species, *Urginea aurantiaca* Lindb. (= *Drimia aurantiaca*), endemic to Morocco, had showed different cytotypes with one or two B chromosomes.^{39,40}

Drimia fugax (Moris) Stearn (Synonym *Urginea fugax* (Moris) Steinh.)

D. fugax is a polymorphic species that grows in northern Algeria in pastures and open forest of humid areas of the coastal and low mountains of the Tellian Atlas.^{23,29} This species has medicinal properties and various cardenolides have been identified.⁸ It is also diploid with $2n=2x=20$ and karyotype formula $6st+6sm+8m$. This chromosome number is in accordance with that quoted in Morocco by Talavera *et al.*⁴¹ Chromosome lengths varied from 2.69 μm to 9.12 μm , with a total haploid length=47.84 μm . The mean centromeric asymmetry (M_{CA}) and the coefficient of variation of the chromosome length (CV_{CL}) were 40.64 % and 46.43 % respectively (Table 2). These values indicate a slightly asymmetrical karyotype. No supernumerary chromosomes were observed in our samples, while B chromosomes were recorded among populations from Morocco and Tunisia^{40,42} and from Sardinia.⁴³

Drimia undata Stearn (Synonyms *Scilla undulata* Desf.; *Urginea undulata* (Desf.) Steinh.; *Charybdis undulata* (Desf.) Speta)

Drimia undata is a South-West Mediterranean species mainly encountered in North Africa, which has undergone frequent changes and taxonomic revisions. In Algeria, populations of this species are distributed on the coastal plains and on the mountains of the Tellian Atlas. All are diploids with $2n=2x=20$ chromosomes with a karyotype formula $2t+14st+4sm$. The chromosome lengths varied from 2.86 μm to 10.62 μm with a mean centromeric asymmetry (M_{CA}) and a coefficient of variation of chromosome length (CV_{CL}) 60.16 % and 47.26 % respectively. This species has the most asymmetrical karyotype, compared to the four studied species. All specimens of *D. undata* are diploids in accordance with previous reports.^{42,44}

CONCLUSION

Most of the chromosome numbers of the studied species are reported here for the first time. All species are diploid, with base number $x=10$ for *Drimia noctiflora*, *D. undata* and *D. fugax*, and $x=9$ for *Battandiera amoena*. As for the majority of Hyacinthaceae, the karyotypes were asymmetric. The endemism and the diploid level argue for a primitivity of these species, whereas the karyotype asymmetry indicates an evolutionary trend in process. Together, with other tools, such information can help assess genotypic diversity and thus aid in predicting the potential of endemic and rare plants as resource of natural substances in pharmacopoeia and medicine.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

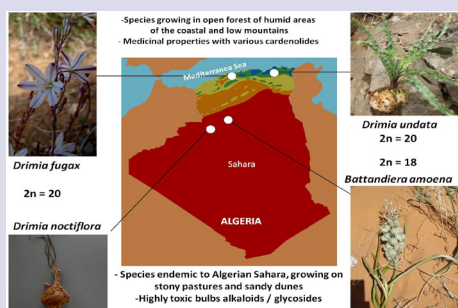
ABBREVIATION USED

CLR: Chromosome length range; THL: Total haploid length; MCA: Mean centromeric asymmetry; CVCL: Coefficient of variation of chromosome length.

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PICTORIAL ABSTRACT



SUMMARY

- In this study we investigate the genetic diversity of endemic Hyacinthaceae of Algeria used in traditional pharmacopoeia : *Drimia noctiflora*, *D. undata*, *D. fugax* and *Battandiera amoena*.
- All species are diploids with base number $x=10$ or $x=9$.
- Endemism, diploid level and karyotype asymmetry argue for genotypic diversity in predicting the potential of these species as resource of natural substances in pharmacopoeia and medicine.

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