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Morphological differentiation within the diploid cytotypes of *Urginea maritima* s.l. (*Hyacinthaceae*)

Abstract

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Caryological studies were performed on 22 populations of *Urginea maritima* s.l. from central and western Mediterranean region and three levels of ploidy, 2x (2n = 20), 4x (2n = 40) and 6x (2n = 60) were identified. Diploids were found in Sicily (Italy) and Minorca (Balearic Islands, Spain), tetraploids in Sardinia (Italy), Mallorca and Ibiza (Balearic Islands, Spain) and Algeria, whereas hexaploids were identified only from the SE Spanish mainland (provinces of Almería, Alicante and Castellón). The morphological analyses of fourteen of the studied populations showed that the diploid plants from Minorca clearly differ by several characters from all other studied plants, including the diploids from Sicily. The diploids from Minorca are characterised by superficial and green bulbs, their leaves of light green colour are leaned on the ground and have a peculiar shape with almost parallel margins. On the contrary, it was impossible to distinguish on the basis of the morphological characters considered the diploids from Sicily from the tetraploids and hexaploids, which are all quite similar. The results obtained suggest that specific category should be assigned to the plants from Minorca.

Introduction

Urginea maritima (L.) Baker is a bulbous perennial of the Mediterranean region, extensively used in pharmacology for its well known cardiotonic, stimulating and diuretic properties. Even though it was the object of many phytochemical and caryological studies, the taxonomy of the maritime squill still remained problematic. Linnaeus (1753) described this plant as *Scilla maritima*, then it was considered as *Urginea maritima* aggregate (Steinheil 1834), and recently it was ascribed to a new genus, *Charybdis* (Speta 1998). Molecular phylogeny based on plastid DNA sequences (Pfosser & Speta 1999) differentiates an *Urgineoideae* clade, where this complex of species are grouped in the vicinity of a true member of *Urginea*, namely *U. undulata* (Desf.) Steinh.

Independently of the systematic treatment accorded, *U. maritima* shows a great variability of morphologic and anatomic characters. Different levels of ploidy ranging from diploids (2n = 20) to hexaploids (2n = 60) were described. The geographical distribution of the cytopyes, the relation of different ploidy levels with morphological or anatomical characters, or comparative analyses of karyotypes were studied by Martinoli

(1949), Battaglia (1957a, 1957b, 1964), Maugini (1953, 1956, 1960), and Maugini & Bini Maleci (1974) among others. However, a detailed taxonomy of this complex was carried out only later by Speta (1980), who assigned different taxa to the different ploidy levels. He classified diploid plants from Italy, Sicily, Corsica, Malta and Mallorca under *U. pancration* (Steinh.) Philippe, and 2x-plants from Central Atlas (Morocco) under *U. maura* Maire. Tetraploids from Italy (including Sardinia), Balearic Islands, North of Africa and Greece were ascribed to *U.* cf. *numidica* (Jord. & Fourr.) Grey, those from the Canary Islands to *U. hesperia* Webb & Berth, and 4x from Israel and Turkey to *U. aphylla* (Forssk.) Speta, whereas hexaploids were maintained as *U. maritima* (L.) Baker. More recently Talavera & al. (1995) reported new chromosome counts and revised the previous ones.

The present study started with the observation of clear morphological differences of plants collected from Minorca (Balearic Islands, Spain) with respect to other plants of *U. maritima* from different geographical regions. The object of this preliminary study was to identify the ploidy level of the populations of *U. maritima* s.l. from Minorca, and to compare their morphological characters with those of plants from other localities with the same chromosome number, or with other cytotypes. For comparison material was collected from other localities in the Balearic Islands, Sardinia, Iberian Peninsula, Sicily and Algeria.

Table 1. New chromosome counts in *U. maritima.*

No.	Origin of material	2n
1	Spain, Balearic Islands, Minorca, Cabo de Cavalleria, *J, Güemes*, 1998	20
2	Spain, Balearic Islands, Minorca, Punta Nati, *J, Güemes*, 1998	20
3	Spain, Balearic Islands, Minorca, *P, Fraga*, 1999	20
4	Italy, Sicily, Siracusa, Monte Láuro, *J, Güemes*, 2000	20
5	Italy, Sicily, Trápani, Monte Cofane, *J, Güemes*, 2000	20
6	Italy, Sicily, Caltagirone, Borgo St, Pietro, *M, Marchese*, 2001	20
7	Spain, Balearic Islands, Mallorca, near Soller, *J, Güemes*, 1999	40
8	Spain, Balearic Islands, Minorca, Cabo de Cavalleria, *J, Güemes*, 1998	40
9	Spain, Balearic Islands Ibiza, Cala Salada, *J, Güemes*, 2000	40
10	Spain, Balearic Islands, Ibiza, Santa Ines, *J, Güemes*, 2000	40
11	Spain, Balearic Islands, Ibiza, Cala Albarca, *J,A, Rosselló,* 2000	40
12	Italy, Sardinia, Cagliari, Giorgino, *G, Bacchetta*, 1998	40
13	Italy, Sardinia, Sarroch, Dispensa de Porcileddu, *G, Bacchetta*, 1998	40
14	Italy, Sardinia, Capoterra, S, Gerolamo, *G, Bacchetta*, 1999	40
15	Italy, Sardinia, Alghero, Capo Caccia, *G, Bacchetta,* 1999	40
16	Italy, Sardinia, Uta, Perdu-Melis, *G, Bacchetta*, 1998	40
17	Algeria, Tiaret, *A, Chehda,* 2000	40
18	Spain, Castellón, Peñiscola, *J,Riera & E, Estrelles,* 1998	60
19	Spain, Alicante, Cabo de San Antonio, *J, Güemes & M, Boscaiu*, 1999	60
20	Spain, Alicante, Castillo de Benirrama, *J, Güemes & M, Boscaiu*, 1999	60
21	Spain, Castellon, Torreblanca, *J, Riera & E, Estrelles,* 1998	60
22	Spain, Almería Cuevas del Almanzora, *M, Soler*, 2000	60

Material and methods

The material used for this study is listed in Table 1. The study was carried out on plants of wild origin, cultivated in the Botanical Garden of the University of Valencia (Spain) and Cagliari (Italy). For each locality 3 to 5 individuals were examined. The voucher specimens are deposited in the herbaria VAL and CAG.

Mitotic chromosomes were studied from root tip squashes. The root tips were fixed in glacial acetic acid: ethanol (1:3) after a pre-treatment of 2 hours in 0.2% colchicine. For staining the Feulgen standard method was used. After a 5-10 min wash in distilled water, the root tips were hydrolised for 30 min in 5M HCl at room temperature, then transferred to distilled water and stained for 30 min to 1h in Schiff's reagent. Finally, squash preparations were carried out in a drop of carmine acetic acid.

For karyotype analyses the arm ratio (r = l/s, where l is the length of the long arm and s the length of the short arm) was used to classify the chromosomes according to Levan & al. (1964) into m = metacentric (r = 1,05-1,69), sm = submetacentric (r = 1,70-2,99) st = subtelocentric (r = 3,00-6,99) and t = telocentric (r = 7,00-39,00).

Morphological observations regarded the size, the shape and the colour of bulbs and leaves, and characters of inflorescences and flowers. Four morphological characters of the leaves were analysed in detail in 14 of the studied populations: the length, the maximal width, the position of the maximal width (calculated as L_1/L_2, where L_1 is the length from the leaf apex to the maximal width and L_2 the length from the leaf base to the maximal width), and the angle of the leaf apex (Table 2).

Table 2. Morphometric analyses of four characters of the leaves. Mean values followed by SE. L1 = length of the leaf from the apex to the max. width, L2 = length of the leaf from the base to the max. width.

Origin and population no.	2n	Mean leaf length (cm)	Mean max. leaf width (cm)	Position of max. width (L1/L2)	Angle of leaf apex (°)
Minorca P.1	2x	32,58 ±1,90	6,88±0,46	0,99±0,12	85±13,4
Minorca P.2	2x	33,51±0,80	4,95±0,29	1,78±0,24	77,16±12,86
Minorca P.3	2x	39,13±1,44	5,95±0,28	1,74±0,26	75±4,87
Sicily P.4	2x	32,83±1,16	4,56±0,40	2,26±0,13	61±2,30
Sicily P.5	2x	46,52±3,14	5,26±0,70	2,84±2,86	61,66±8,01
Mallorca P.7	4x	62,2±1,69	6,9±0,36	2,21±0,14	59,33±5,51
Minorca P.8	4x	33,26±0,74	4,8±0,21	1,90±0,08	69±4,49
Ibiza P.9	4x	50,07±1,55	5,57±0,64	1,83±0,16	52,75±7,96
Ibiza P.10	4x	47,8±5,49	4,6±0,64	1,75±0,26	49±6,90
Ibiza P.11	4x	37,12±0,86	3,9±0,16	1,78±0,15	48,2±1,85
Sardinia P.12	4x	56,14±0,85	5,65±0,55	2,63±0,17	41,5±3,5
Sardinia P.13	4x	55,8±1,19	8,07±0,61	1,9±0,16	69,25±4,71
Sardinia P.14	4x	55,77±2,62	5,12±0,37	2,21±0,26	40±4,16
Peñiscola P.18	6x	46,06±0,79	3,9±0,44	2,22±0,15	42±4,84

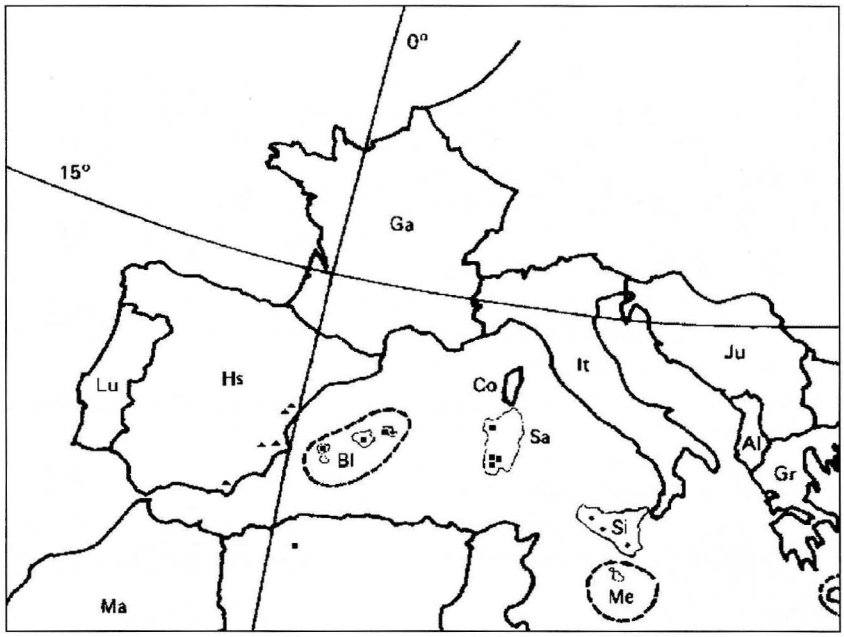

Fig. 1. The geographical distribution of the populations studied (circles = 2x, squares = 4x, triangles = 6x).

Results

The geographical distribution of the populations studied is presented in Fig. 1.

Three ploidy levels (2x = 20, 40, and 60) were identified in the 22 populations studied (Table 1). The plants with peculiar morphology from three localities in Minorca were found to be diplod, as the plants from three populations from Sicily. Tetraploids were identified in material from the Balearic Islands, Sardinia and Algeria, whereas all tetraploids were collected from the Spanish mainland. Chromosome morphology is very stable, and no variation was detected within populations or cytotypes.

The karyotypes of the diploid plants from Minorca (Fig. 2) were identical to those of plants from Sicily (Fig. 3). The centromer is located mostly subterminal; the longest pairs of chromosomes (1 and 2) are telocentric, pairs 3 and 4 are subtelocentric and only two pairs (5 and 6) are submetacentric. The shorter pairs (7-10) are also subtelocentric.

The karyotypes of the tetraploid (Fig. 4) and hexaploid plants (Fig. 5) apparently consist of a duplication and a triplication, respectively, of the diploid chromosome complement.

Morphologically *U. maritima* is very variable, but we could distinguish some traits specific for the diploid plants from Minorca, or specific for all diploids examined.

The bulbs of the tetraploids and hexaploids are covered by dry, reddish foliage tunics, and at the surface the bases of the green leaves are not apparent, on the contrary to the two diploid cytotypes studied, which have this peculiarity.

All leaf characters studied are highly variable (Table 2). The mean values for leaf length

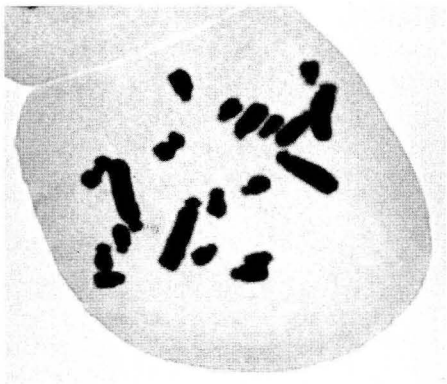

Fig. 2. Karyotype of diploid from Minorca (Spain).

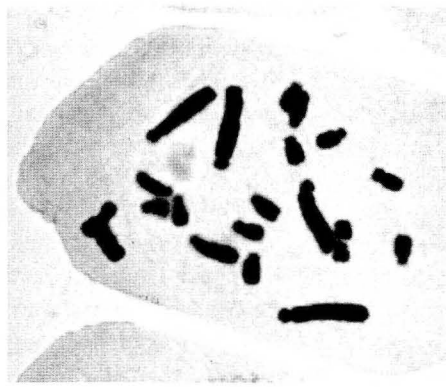

Fig. 3. Karyotype of diploid from Sicily (Italy).

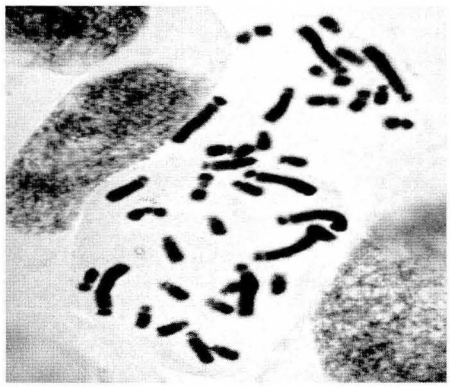

Fig. 4. Karyotype of tetraploid from Sardinia (Italy).

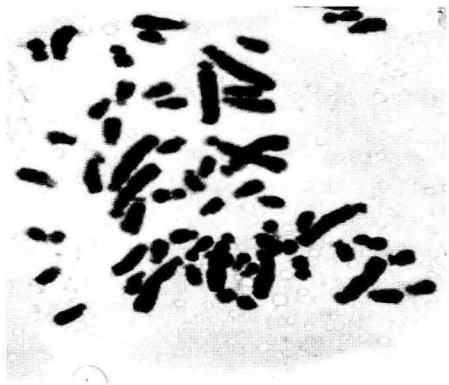

Fig. 5. Karyotype of hexaploid from Spain.

are: 35,63±1,05 cm in the diploid plants from Minorca, 41,77±3,03 cm in diploids from Sicily, 49,27±1,91 cm in tetraploids and 46,06±0,79 cm in hexaploids.

Mean leaf width is also very variable: the Minorcan diploids have 5,86±0,24 cm, the Sicilian diploids 5,03±0,48 cm, the tetraploids 5,57±0,26 cm and the hexaploids 1,99±0,07cm.

The maximal width was registered in the lower half of the leaf in all measured plants, but proximal to the middle of the leaf in the case of the diploid plants from Minorca (L_1/L_2 = 1,55±0,15). For all other individuals studied the maximal width is situated at a lower position: L_1/L_2 is 2,65±0,27 for the diploids from Sicily, 1,99±0,07 for the tetraploids and 2,22±0,28 for the hexaploids.

The angle of the leaf apex varies within populations, but is generally higher in 2x-plants from Minorca, with a mean value of 68,31°±5,46 as compared to that of Sicilian diploids (61,44°±5,22), tetraploids (54,72°±2,52) and hexaploids (42°±4,84).

The leaves are generally straight, sheathing at the base. Only the diploid plants from Minorca have leaves without sheath and leaned on the soil.

The inflorescence is a raceme of variable length. We observed that the length of the raceme is characteristic for each cytotype. The diploid plants from Minorca have the shortest raceme (90-100 cm), with the lowest number of flowers (100-150). The inflorescence of the diploids from Sicily is obviously larger, with a length of 175-190 cm and consisting of about 400-450 flowers. The most developed racemes were found in the tetraploids from Sardinia, which reach 180-200 cm and consist of up to 580-600 flowers. The Iberian hexaploids have inflorescences with less flowers, and their length is shorter than that of the Sicilian diploids, of about 105-115 cm and 370-400 flowers.

The bracts of all diploids examined, both from Minorca and Sicily are greenish, whereas those of the tetraploids and hexaploids have a purple colour.

Tepals are whitish with a central stripe, greenish in the diploids, and reddish in tetra- and hexaploids.

Pedicels after fructification are as long as capsules, or only little longer in the diploid plants from Minorca, but 3-4 fold longer than the fruit in the plants from Sicily, in the tetra- and hexaploids.

Conclusions

We report a new diploid cytotype within the complex *Urginea maritima*, presently known only from the island of Minorca. Its morphological characters differ notably from the tetraploid and hexaploid cytoptypes. It differs also from the Sicilian diploid plants by the following characters: leaves more ovate, with rounded apices, not sheathing at the base, racemes shorter with fewer flowers, and fruit pedicels equal or only slightly longer than the capsule and adpress to the scape.

Even though we consider that a specific name should be assigned to the diploid plants from Minorca, this is rather difficult to be accomplished in a preliminary study. The morphology of the plants from Minorca does not fit to that of *U. maura*, a diploid species from North Africa, with light green, reddish bracts and tepals with purple stripes, characters absent in the plants from Minorca. The Minorcan taxon could be classified to *U. pancration*; both share characteristics of the bulbs, leaves and capsule pedicels, but differ by the purple stripe of the tepals. Moreover, Speta (1980) considered the central Mediterranean diploids as *U. pancration* (Speta 1980), and if this taxonomic treatment is accepted, diploids from Minorca can not be assigned to the same species, requiring a correct identification.

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