

Chromosome numbers for the Italian flora: 10

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Abstract

In this contribution, new chromosome data regarding two taxonomically critical genera of the Italian flora, namely *Plantago* and *Sesleria*, are presented. All the specimens analysed in this paper were collected in the Italian territory and include three chromosome counts for *Plantago* (*P. albicans*, *P. crassifolia*, and *P. subulata*) and two counts for *Sesleria* (*S. caerulea* and *S. nitida*).

Keywords

Cytogeography, cytotaxonomy, chromosome number instability, *Plantago*, *Sesleria*

How to contribute

Texts concerning new chromosome data should be submitted electronically to Giovanni Astuti (gastuti@biologia.unipi.it), including indications on voucher specimens and methods used.

Chromosome counts

Plantago albicans L. (Plantaginaceae)

Chromosome number. $2n = 20$ (Fig. 1)

Voucher specimen. ITALY. Basilicata. Madonna delle Grazie (Pisticci, Matera) (WGS84: 40.374528N, 16.557667E), stenomediterranean dry grasslands developed on quaternary marine sands, 150 m, 28 May 2001, R. Di Pietro (HFLA).

Method. Squash preparations were made on root tips obtained from living plants. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. In the last ten years, narrow leaved plantains have been the subject of accurate taxonomic and nomenclatural revisions which led to numerous changes in their classification (Di Pietro et al. 2013; Di Pietro and Iamónico 2014a, b; Hassemer et al. 2017; Iamónico et al. 2017). On the contrary, the karyological data available at present for the Italian territory are few. *Plantago albicans* is a steno-Mediterranean species which in Italy occurs in the following administrative regions: Puglia, Basilicata, Calabria, Sicilia, Sardegna, and Liguria (Bartolucci et al. 2018). Fedorov (1969) listed a number of chromosome counts from north Africa: $2n = 10, 12, 20, 24$ and 30. Badr and El-Kholy (1987), found solely a chromosome number of $2n = 30+3B$ for Egyptian populations, whereas Puech (1987, 1988) found different chromosomal numbers between Tunisian populations from the north ($2n = 20$) and south ($2n = 10$) of the country, and Vogt and Oberprieler in Marhold (2009) reported $2n = 10$ for plants from Morocco. Furthermore, Maamri et al. (2016), for Algeria, found a different chromosome number associated to different altitudinal belts. In fact, they recorded $2n = 10$ and $2n = 20$ from *P. albicans* populations collected at medium-altitude and high-altitude sites, respectively. In addition, they found various intermediate chromosome numbers ($2n = 6, 8, 9, 12, 14, 15, 17, 18$) interpreted as aneuploid cytotypes. As far as Europe is concerned, Runemark (1967) reported $2n = 30$ for Aegean populations. In Italy, hexaploid karyotypes ($2n = 30$) were reported by Bartolo et al. (1978) and Brullo et al. (1985) for Sicilian populations and by Peruzzi and Cesca (2002) for Calabrian populations. In our study, we have analysed 13 plates and we have always counted $2n = 20$ chromosomes. This result is interesting as this number has never been reported so far for Italian populations, whereas it was already found in populations from Spain (Lorenzo-Andreu 1951) and France (Rahn 1957). Although *P. albicans* exhibits a high karyological and phenotypic variability, it may be possible to highlight a geographical separation between a south-eastern area (North Africa, Greece, Sicily, and southern Calabria) with $2n = 30$ chromosomes and a south-western area (continental Italy, Portugal, Spain, and France) with $2n = 20$ chromosomes. Puech et al. (1998) pointed out that the two groups are also differentiated from a functional point of view due to the fact that the $2n = 30$ cytotype exhibits a



Figure 1. *Plantago albicans* L. from Madonna delle Grazie (Pisticci, Matera), $2n = 20$. Scale bar: 10 µm.

much shorter seed germination period than the $2n = 20$ one. According to the same author, this feature could represent an evolutionary advantage in places where the wet season is usually very short.

Plantago crassifolia Forssk. (Plantaginaceae)

Chromosome number. $2n = 20$ (Fig. 2)

Voucher specimen. ITALY. Puglia. Saline di Punta della Contessa (Brindisi) (WGS84: 40.615361N, 18.024000E), temporarily inundated sands characterizing retrodunal areas (*Schoeno nigricantis-Plantaginetum crassifoliae* Br.-Bl. 1931), 1 m, 20 May 2008, R. Di Pietro (HFLA).

Method. Squash preparations were made on root tips obtained from living plants. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. *Plantago crassifolia* is a Mediterranean species which occurs throughout southern Italy with the exception of Campania, and in Emilia-Romagna, Veneto, and Friuli-Venezia Giulia (Bartolucci et al. 2018). In the studied population we found chromosome number $2n = 20$, which confirms the karyological literature for Sicily (Brullo et al. 1985) and for Puglia itself on the Gargano Promontory (Snogerup 1985), Porto Cesareo (Peruzzi 2003) and for unspecified localities (Tornadore and Marcucci 1988). The number $2n = 20$ was also found by Böcher et al. (1955) from southern France and confirmed by Chater and Cartier (1976).

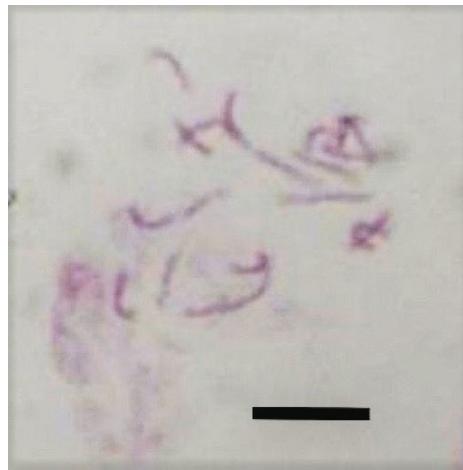


Figure 2. *Plantago crassifolia* Forssk. from Saline di Punta della Contessa (San Godenzo, Brindisi), $2n = 20$. Scale bar: 10 μm .



Figure 3. *Plantago subulata* L. from Isola di San Domino (Isole Tremiti, Foggia), $2n = 12$. Scale bar: 10 μm .

Plantago subulata L. (Plantaginaceae)

Chromosome number. $2n = 12$ (Figs 3, 4)

Voucher specimens. ITALY. Puglia. Torre dell'Orso (Melendugno, Lecce) (WGS84: 40.268722N, 18.439611E), rocky coastal areas on limestone, 1 m a.s.l., 20 May 2008, R. Di Pietro (HFLA); Isola di San Domino (Isole Tremiti, Foggia) (WGS84: 42.120972N, 15.495389E), rocky coastal side 12 May 2002, M. Cutini (HFLA).

Method. Squash preparations were made on root tips obtained from living plants. Root tips were pre-treated with 0.4% colchicine for 3 hours and then fixed in Carnoy

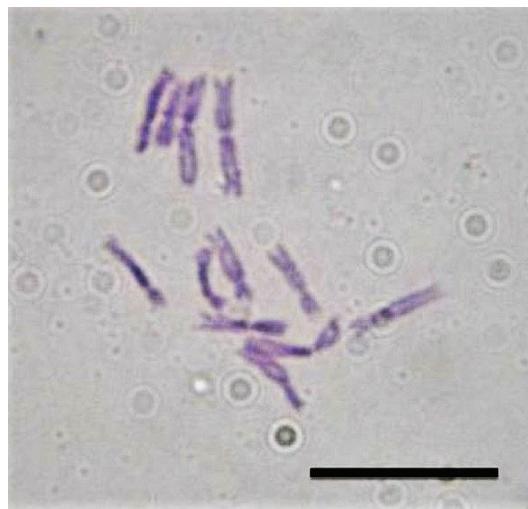


Figure 4. *Plantago subulata* L. from Torre dell'Orso (Melendugno, Lecce), $2n = 12$. Scale bar: 10 µm.

fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C, the tips were stained in leuco-basic fuchsine for 7–8 minutes.

Observations. Prior to Hassemer's review (2018), the following narrow-leaved plantains were considered to occur in Puglia: *Plantago grovesii* Brullo (a local Apulian endemic taxon whose distribution area is restricted to a narrow strip of rocky Adriatic coast of the southern Salento Peninsula from Torre dell'Orso to Otranto), *P. holosteum* Scop. subsp. *holosteum* (submontane and montane belt of Mount Gargano), and *P. holosteum* Scop. subsp. *scopulorum* (Degen) H-ic' restricted to the Tremiti Archipelago. In his recent revision, Hassemer (2018) synonymised all these species, together with other southern European narrow-leaved plantains occurring in Italy, such as *P. insularis* Gren. & Godr. and *P. humilis* Guss., to *P. subulata* L. and this classification was also followed in Bartolucci et al. (2018). From a karyological point of view, however, the literature referring to *P. subulata* s.l. is very variable. If we consider only the samples referring to *P. subulata* L. s.str., these show a chromosome number of $2n = 2x = 12$ (Contandriopoulos 1962). On the other hand, samples from Sardinia (= *P. insularis*), Sicily (= *P. humilis*) and North Africa (= *P. subulata* subsp. *atlantis* (Emb. & Maire) Greuter & Burdet) show $2n = 4x = 24$ chromosomes (Contandriopoulos 1962; Corrias 1980). Currently, the most relevant hypothesis (Contandriopoulos 1962; Brullo et al. 1985) consider the tetraploid taxa as derived from a diploid *P. subulata*. In our karyological investigation, the Apulian specimens of *P. subulata* analysed (formerly attributed to *P. grovesii* and *P. holosteum* subsp. *scopulorum*) were quite similar and both provided a chromosome number $2n = 12$. However, the three aforementioned taxa occurring in Puglia seem to be morphologically quite dissimilar from one another (personal observations), besides being clearly separated geographically. For this reason, further investigations are necessary to clarify their taxonomic status.

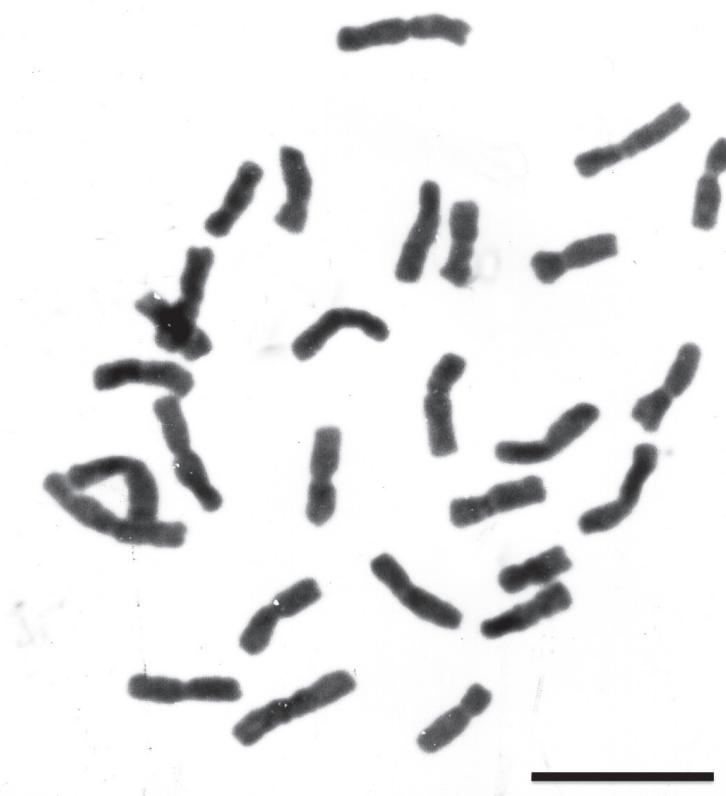


Figure 5. *Sesleria caerulea* (L.) Ard. from Bosco delle Tagliate (Capo di Ponte, Brescia), $2n = 28$. Scale bar: 10 μm .

Sesleria caerulea (L.) Ard. (Poaceae)

Chromosome number. $2n = 28$ (Fig. 5)

Voucher specimen. ITALY. Lombardia. Val Camonica, Bosco delle Tagliate, Pes-carzo (Capo di Ponte, Brescia) (WGS84: 46.032639N, 10.318400E), undergrowth of *Ostrya carpinifolia* woods 25 October 2005, R. Di Pietro, G. Ciaschetti, S. Armiraglio, M. Caccianiga, E. Bona (HFLA); **Trentino-Alto Adige.** Mezzolombardo (Trento) (WGS84: 46.219833N, 11.083031E), chasmophytic vegetation on limestone cliffs in the hilly belt, 15 March 2006, R. Di Pietro & P. Fortini (HFLA).

Method. Squash preparations were made on root tips obtained from living plants. Root tips were pre-treated with 8-hydroxyquinoline 0.002M for 24 hours at 4 °C temperature and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 2–3 hours. Subsequently an enzymatic treatment of approximately 10–20 minutes with 10% pectinase solution and powdered cellulase in 5% solution was carried out.



Figure 6. *Sesleria nitida* Ten. from Pian de Valli (Vazia, Rieti), $2n = 28$. Scale bar: 10 μm .

Observations. The genus *Sesleria* in Italy has been the object of an accurate taxonomic-nomenclatural revision (Brullo and Giusso Del Galdo 2006; Foggi et al. 2007; Di Pietro et al. 2013, 2015; Alonso et al. 2015; Di Pietro et al. 2017). It is well-known that in *Sesleria*, the ploidy level has a great discriminatory power for taxonomic classifications (Ujhelyi and Felföldy 1948; Strgar 1979; Di Pietro et al. 2005; Trombetta et al. 2005; Di Pietro 2007; Lazarević et al. 2015). *Sesleria caerulea* (= *S. varia* (Jacq.) Wettst.; *S. albicans* Kit ex Schult) is generitype of *Sesleria* (Foggi et al. 2001). In Italy, this species is widespread throughout the Alps and pre-Alps; it occurs also in a few relic sites in the western side of the northern Apennines. From the karyological point of view there are numerous karyological data available for this species published especially in eastern Europe (Májovský 1976; Lysák et al. 1997; Lysák and Doležel 1998; Petrova 2000; Budzáková et al. 2014), all reporting $2n = 4x = 28$ chromosomes. Recently, Lazarević et al. (2015) found octoploid individuals in two *S. caerulea* populations from the Julian Alps. This is the first count for Italy.

Sesleria nitida Ten. (Poaceae)

Chromosome number. $2n = 28$ (Fig. 6)

Voucher specimen. ITALY. LAZIO. Monte Terminillo, Pian de Valli (Vazia, Rieti) (WGS84: 42.453639N, 12.987333E), dry grasslands on rocky limestone slopes, 25 June 2003, *R. Di Pietro, A. Granati* (HFLA).

Method. Squash preparations were made on root tips obtained from living plants. Root tips were pre-treated with 8-hydroxyquinoline 0.002M for 24 hours at 4 °C

temperature and then fixed in Carnoy fixative solution for 1 hour. After hydrolysis in HCl 1N at 60 °C for 7–8 minutes, the tips were stained in leuco-basic fuchsine for 2–3 hours. Subsequently an enzymatic treatment of approximately 10–20 minutes with 10% pectinase in 10% solution, and powdered cellulase in 5% solution was carried out.

Observations. *Sesleria nitida* Ten. is a species endemic to the central and southern Apennines and Sicily. Our chromosome count confirms what was already reported for this species by Ujhelyi (1960) and by Trombetta et al. (2005).

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