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"SEEDS OF LOVE": ENHANCING THE *EX-SITU* CONSERVATION OF SEVEN PRECIOUS PLANT SPECIES OF TUSCANY

Abstract - M. Calbi, B. Foggi, G. Ferretti, A. Carta, "Seeds of Love"*: enhancing the* ex-situ *conservation of seven precious plant species of Tuscany.*

Tuscany hosts a remarkable botanical biodiversity. In order to preserve and enhance the conservation of species of phytogeographical or ecological value and restricted distribution, *ex-situ* seed banking is one effective tool. In this article we provide an overview of the main methodology employed during the *"Seeds of Love"* project and of the steps that were taken to ensure the *ex-situ* conservation of the target species. Moreover, we lay out a detailed outline of three main seed morphological features for each single accession, that might be a useful baseline for future systematic studies involving our target species.

Key words - conservation, endemic, fruit, seeds, seed banking, Tuscany, Italy

Riassunto - M. Calbi, B. Foggi, G. Ferretti, A. Carta, "Seeds of Love"*: migliorare la conservazione* ex-situ *di sette specie vegetali di pregio della Toscana.*

La Toscana ospita una grande biodiversità vegetale. Per mantenere e migliorare la conservazione si specie di interesse fitogeografico o ecologico e a distribuzione ristretta, la conservazione ex-situ dei semi in apposite banche del germoplasma è uno dei metodi più efficaci. In questo articolo forniamo una visione generale dei metodi impiegati durante il progetto *"Seeds of Love"* e dei passi necessari ad assicurare la conservazione *ex-situ* delle specie target. Inoltre forniamo dati dettagliati sulle tre caratteristiche morfologiche principali dei semi di ogni accessione, che possono essere una base utile per studi sistematici futuri che includano le specie trattate in questo lavoro.

Parole chiave - conservazione, endemico, frutti, semi, banche del germoplasma, Italia, Toscana

Introduction

Tuscany is one of the richest Italian regions on floristic grounds (Bartolucci *et al.*, 2018), hosting about 4372 plant species belonging to 1143 genera and 171 families (Peruzzi *et al.*, 2021). This remarkable species richness is closely linked to the evolutionary history of Tuscany (Carta *et al.*, 2019) and to the extant high environmental heterogeneity, supporting a variety of habitats (Viciani *et al.*, 2014). Unfortunately, many of these natural and semi-natural habitats are threatened by urbanization, pollution and the abandonment of traditional and sustainable farming practices, as reported also for Italy in general (Falcucci *et al.*, 2007; Orsenigo *et al.*, 2021). This, together with a higher frequency of extreme climatic events such as droughts, wildfires, and extreme weather and exotic species introduction, results in habitat loss and growing pressure upon native plant communities (Barga *et al.*, 2020; Millennium Ecosystem Assessment, 2005; Seebens, 2019; Vázquez *et al.*, 2017). Furthermore, rare or endemic species of Tuscany are more exposed to environmental changes and stochastic events driven by human activities (*e.g.* quarrying, eutrophication, land use and land cover changes etc.) due to their naturally fragmented range (*i.e.* Tuscan Archipelago: Foggi *et al.*, 2015). Moreover, a lack of adequate protection regimes for delicate habitats (*i.e.* serpentine flora: Chiarucci, 2009) may trigger further population reductions that are likely to lead to critical demographic thresholds (Selvi, 2007).

Within biodiversity conservation strategies, seed banking in dedicated *ex-situ* facilities (i.e., seed banks) has been recognized as a necessary and cost-effective technologies (FAO, 2014), complementing *in-situ* conservation of wild plants as one of the main task to maintain and restore native ecosystem functioning (Barga *et al.*, 2020). Indeed, *ex-situ* seed banking promotes the conservation of species genetic resources and prevents species or genotype loss until actual or potential threats are removed (Cochrane *et al.*, 2007; Hay & Probert, 2013). While providing a fundamental source of material for ecological restoration of degraded ecosystems (León-Lobos *et al.*, 2012; Maunder *et al.*, 2004) ensuring the functioning of natural ecosystems (Maestre *et al.*, 2012; Schulze & Mooney, 1994), *ex-situ* conservation also includes the protection of the taxonomic and regional variation across the range of a species (Linhart & Grant, 1996;

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Carta, 2015). Nevertheless, at global level, many taxa are underrepresented in seed collections, especially endemics, rare and threatened species (O'Donnel & Sharrock, 2017), thus the importance of seed banking becomes particularly high in the case of species with restricted distributions or peculiar ecological requirements (CBD, 2010). Preserving their inestimable ecological and cultural value is becoming increasingly important in the Anthropocene (Rivière *et al*., 2018).

In this context, the *"Seeds of Love"* project, hereafter 'SOL', funded by the fashion *maison Ermanno Scervino* and designed and carried out in cooperation with the Botanical Garden of the University of Florence *Giardino dei Semplici*, aims at enhancing the *ex-situ* representation of seven wild endemic or precious plant species of Tuscany, selected for both their ecological, cultural and aesthetical value. The project comprises several tasks including: *i)* visiting, mapping and evaluating the overall extent of several of the known populations of the seven target species; *ii)* collecting, processing and conserving the seeds of an adequate number of specimens, in compliance with the European native seed conservation network (ENSCONET, 2009).

In this article, we outline the main steps that were taken in order to collect and store the seeds of the target species, that included visiting and sampling selected populations in Tuscany and surrounding areas, and to address the *ex-situ* conservation of the target species, creating seed lot duplicates of each species by storing them in three independent seed banks. Additionally, since the selected species lacked so far a detailed morphological characterization of seeds, we present the morphological data and microphotographs obtained with a scanning electron microscope (SEM) of a sample of seeds for each of the accessions, which could provide useful baseline knowledge for future comparative and systematic studies involving the SOL target species and beyond.

Materials and methods

The seven target species of the SOL project were: *Centaurea arrigonii* Greuter, *Globularia incanescens* Viv., *Leontodon anomalus* Ball, *Odontarrhena bertolonii* (Desv.) Jord. & Fourr., *Santolina etrusca* (Lacaita) Marchi & D'Amato, *Stipa etrusca* Moraldo, and *Viola etrusca* Erben (Fig. 1 and Tab. 1). Nomenclature and regional distribution data follow the Portale della Flora d'Italia (http://dryades.units.it/floritaly/index.php).

Figure 1. Sampling the seven target species. a) *Viola etrusca*; b) *Centaurea arrigonii*; c) *Leontodon anomalus*, d) *Globularia incanescens*; e) *Santolina etrusca*; f) *Odontarrhena bertolonii*; g and h) Moments of the monitoring and sampling process; i) *Stipa etrusca*. Photo: Mariasole Calbi and Andrea Grigioni.

Seed material collection

To assess the number of individuals of each population, several field trips were carried out between May and June 2021 during the peak of flowering time. This allowed to evaluate which populations could be sampled during the fruiting time. Each population was located following floristic information retrieved from Wikiplantbase #Toscana v2.1 (Peruzzi & Bedini, 2015-) or based on local experts knowledge. Overall, 74 populations were visited. Only half of these were considered suitable (*i.e.* the number of individual was higher than 100 or in exceptional cases of 50, sufficient to ensure that no damage would have been done to the population) for seeds sampling, which took place between July and September 2021 (Fig. 2). To encompass sufficient genetic variability and ensure seed lots representativeness for each species, four to six populations for each target species were sampled. Populations were selected based on their geographical distances and when possible to encompass habitat variation. An herbarium specimen was also collected for each sampled population and stored at the Italian Central Herbarium (FI) or at the Herbarium of the Biology department of the University of Firenze. For each suitable population, a minimum number of 50 individuals was sampled, collecting only about the 10% of available seeds or dispersal units (in some cases the whole fruit) as recommended by the ENSCONET

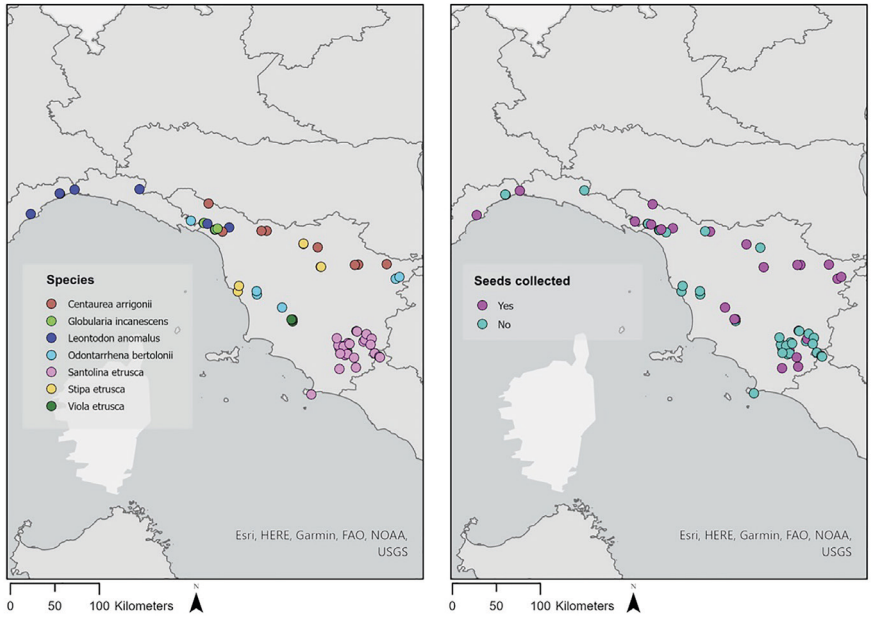

Figure 2. Distribution of visited and sampled populations of the target species (left panel). Visited populations are represented by circles that can be distinguished according to their color (right panel) into sampled (violet) and not sampled (light blue).

guidelines (ENSCONET, 2009). Seeds (or fruits) were collected exclusively from the plants, except for two accessions (SOL_17 and SOL_39) where they were partially collected from the ground, and stored in paper bags. The seeds were kept at stable conditions (approx. 20 °C and 40% relative humidity [RH]) for 2 weeks before being cleaned, measured and stored at international accepted standards for genebanks (see below; FAO, 2014).

Seeds measuring, photographing and data analysis

For each accession, six samples consisting of 50 seeds were weighted with a precision Radwag AS 60/220.R2 scale (Radwag, Poland). In addition, 30 seeds for each accessions were photographed on millimeter paper, and height and width were measured with the software ImageJ 1.53k (Schneider *et al.*, 2012). An overall percentage of healthy seeds was also visually estimated by counting the number of intact, filled-in seeds.
The obtained data were analysed in R v 4.1.2 (R Core Team, 2021), and, either averaged for each accession to perform a PCA (*prcomp* function, *stats* package), or kept separate to test for significant differences between populations of the same species (by means of an ANOVA, *aov* function, *stats* package). Lastly, Electronic Scanning Microscope (ZEISS EVO 40, Carl Zeiss, Germany) pictures were taken of at least one seed for each species at the Centro di Servizi di Microscopia Elettronica e Microanalisi (MEMA) of the University of Florence.

Selected seed banks

After the measurements were taken, the seeds were delivered to three Seed banks: the Pisa Seed Bank (University of Pisa), the Tuscia Seed Bank (University of Tuscia) and the Millennium Seed Bank (Royal Botanic Gardens, Kew), to be kept at controlled temperature and humidity conditions (equilibration of seeds at 15% RH and 15 °C, and then storage in sealed aluminum banks at -20 °C; FAO, 2014). The selected three scientific facilities are dedicated to the conservation of wild plant species. The Seed Bank of Pisa, is an important center for conservation of Tuscan biodiversity, as recognized officially by the law 56/2000 (Regione Toscana, 2000). Its main goal is the conservation of rare, threatened, endemic or phytogeographically relevant plant taxa in Tuscany and more generally in the Mediterranean (Carta & Bedini, 2015). The seed Bank of Tuscia is an important center that deals with *ex-situ* conservation of germplasm of endemic, rare or endangered species of the Italian wild flora, especially from Northern Lazio (Magrini *et al.*, 2012). Both the Pisa and the Tuscia Seed Banks form part of the RIBES (Rete Italiana delle Banche del Germoplasma) and the ENSCONET networks, together with the Millennium Seed Bank. ENSCONET coordinates native seed conservation initiatives aiming for the long-term conservation in seed banks of representative samples of the genetic diversity of European flora, prioritizing threatened and endangered species (ENSCONET, 2009). The Millennium Seed Bank, the world's largest *ex-situ* repository of native seeds, run by the Royal Botanic Gardens Kew that hosts a remarkable collection of more than 92,500 accessions of over 40,000 plant species from around the world (Liu *et al.*, 2018).

Table 1: The seven target species in the SOL (*Seeds of love*) project.

Family	Species	Life form	Regions	IUCN	Habitat	Flowers
Asteraceae	*Centaurea arrigonii*	Scapose hemicryptophyte	Emilia-Romagna; Marche; Tuscany; Umbria	DD	Nutrient-poor rocky or detritic substrates	May-July
Plantaginaceae	*Globularia incanescens*	Scapose hemicryptophyte	Emilia-Romagna; Liguria; Tuscany	LC	Calcareous rocky slopes	April-June
Asteraceae	*Leontodon anomalus*	Rosulate hemicryptophyte	Emilia-Romagna; Liguria; Piemonte; Tuscany	LC	Calcareous or serpentinic rocky walls	May-August
Brassicaceae	*Odontarrhena bertolonii*	Suffruticose camaephyte	Liguria; Tuscany	LC	Serpentine rock outcrops	April-June
Asteraceae	*Santolina etrusca*	Nano-Phanerophyte	Lazio; Tuscany; Umbria	NT	Loose sandy/gravelly substrates, especially river beds	May-September
Poaceae	*Stipa etrusca*	Caespitose hemicryptophyte	Emilia-Romagna; Lazio: historical record; Lombardy; Tuscany	LC	Serpentine rock outcrops or grasslands	May-July
Violaceae	*Viola etrusca*	Scapose hemicryptophyte	Tuscany	EN	At the margins of meadows or pastures	March-July

Species nomenclature and regional distribution data retrieved from: Portale della Flora d'Italia (https://dryades.units.it/floritaly/); life form from Pignatti *et al.*, 2017; Habitat from: Repertorio Naturalistico Toscano - (RE.NA.TO.); flowering time from ACTA PLANTARUM (2020). IUCN categories were retrieved from Orsenigo *et al.* (2021): DD = Data Deficient; LC = Least Concern; NT = Near Thretened, EN = Endangered.

RESULTS

Ex-situ *conservation*

In total, 37 accessions were collected from suitable populations (Tab. 2). In order to equally represent species among the institutions but maintaining a higher number of accession in the 'local' seed bank for a more potential usage, 21 accessions were handed in to the Pisa Seed bank (PSB), 10 were sent to the Tuscia seed bank (TSB), and 7 to the Millennium Seed Bank (MSB). One accession (SOL_09) was divided into two duplicates, one handed in to the Pisa Seed Bank, the other to MSB. The number of seeds of each accession ranged from 200 (SOL_11, *Stipa etrusca*) to about 14,530 for SOL_21 (*Globularia incanescens*).

Sampled populations characteristics

Seeds from each target species were different in weight, size and shape (Tab. 3, Fig. 3, Fig. 4). Seed weight (mean ± SD) ranged from 0.200 ± 0.018 mg or 0.222 ± 0.013 mg in the case of *Santolina etrusca* (SOL_31) and *Odontarrhena bertolonii* (SOL_34) respectively, to 29.415 ± 0.939 mg for *Stipa etrusca* (SOL_05), followed by 2.004 ± 0.088 mg for *Centaurea arrigonii* (SOL_37). Seed length ranged from 1.702 ± 0.181 mm and 1.712 ± 0.154 mm, for *Viola etrusca* (SOL_09) and *Odontarrhena bertolonii* (SOL_33), respectively to 20.200 ± 1.581 mm for *Stipa etrusca* (SOL_11), followed by 5.954 ± 1.486 mm of *Leontodon anomalus* (SOL_23). Finally, seed width values ranged from 0.494 ± 0.105 mm for *Santolina etrusca* (SOL_27) and 0.546 ± 0.094 mm for *Leontodon anomalus* (SOL_26) to 1.990 ± 0.178 mm for *Stipa etrusca* (SOL_03), followed by *Centaurea arrigonii* (SOL_39; 1.491 ± 0.155 mm).

Moreover, in terms of the percentage of healthy seeds, *Globularia incanescens, Leontodon anomalus* and *Santolina etrusca* had the highest values, whereas the lowest values were observed for *Odontarrhena bertolonii* and *Centaurea arrigonii.*

The PCA plot showed a marked separation of *Stipa etrusca* seeds, the only species located on the left side of the ordination plot, away from all of the other species that were found in the right part of the graph (Fig. 4). In addition, according to the ANOVA results, different accessions of the same species were found to significantly differ ($p < 0.005$) in weight (except for *Globularia incanescens* [F value = 3.177, p = 0.0306] and *Santolina etrusca* populations [F value = 4.799, p = 0.00519]), length, (except for *Globularia incanescens* populations [F = 1.179, p = 0.323]) and width (except for *Leontondon anomalus* populations [F = 1.263, p = 0.282]).

Table 2. Details of collected accessions. PSB, Pisa Seed Bank; TSB, Tuscia seed bank; MSB, Millennium Seed Bank; Tot. ind, number of total individuals in each population; Area, estimated area covered by the sampled population.

Collection number	Collectors	Date	Institution	n of seeds	Sampled ind.	Tot. ind.	Area (m^2)	Herbarium voucher
SOL_01	M. Calbi	09/06/2021	PSB	370	50	100	2500	MSC(f) 1
SOL_02	M. Calbi; A. Grigioni	11/06/2021	PSB	715	100	300	1500	MSC(f) 15
SOL_03	M. Calbi; A. Grigioni	11/06/2021	MSB	1050	260	2000	1500	MSC(f) 17
SOL_04	M. Calbi	15/06/2021	PSB	240	35	50	4000	MSC(f) 23
SOL_05	M. Calbi	15/06/2021	TSB	1200	286	400	15000	MSC(f) 20
SOL_06	M. Calbi; M. Calbi; R. Martinero; F. Fajardo	18/06/2021	PSB	4700	100	1000+	3500	MSC(f) 4; 31
SOL_07	M. Calbi; M. Calbi; R. Martinero; F. Fajardo	18/06/2021	PSB	2790	50	500+	40	MSC(f) 3
SOL_08	M. Calbi; M. Calbi; R. Martinero; F. Fajardo	19/06/2021	TSB	2000	50	1000+	50	MSC (f) 35
SOL_09	M. Calbi; M. Calbi; R. Martinero; F. Fajardo	19/06/2021	PSB/MSB	2180	50	1000+	1000	MSC(f) 34
SOL_11	M. Calbi; F. Fajardo	22/06/2021	TSB	200	30	100		MSC(f) 20
SOL_12	M. Calbi; F. Fajardo	30/06/2021	PSB	7500	57	1000+	1500	MSC(f) 2
SOL_13	M. Calbi	03/07/2021	PSB	2750	51	100	3000	MSC(f) 41; 42
SOL_14	M. Calbi	06/07/2021	PSB	2000	28	50	100	MSC (f) 43
SOL_15	M. Calbi	07/07/2021	PSB	3100	50	100	2400	MSC (f) 21
SOL_16	M. Calbi	09/07/2021	MSB	13800	35	50	600	MSC (f) 6
SOL_17	M. Calbi	12/07/2021	TSB	560	62	100+	10000	MSC (f) 5
SOL_18	M. Calbi	18/07/2021	PSB	9790	51	100	900	MSC(f) 11
SOL_19	M. Calbi	18/07/2021	PSB	4950	51	80	900	none
SOL_20	M. Calbi	18/07/2021	PSB	5900	51	100	1000	MSC (f) 45
SOL_21	M. Calbi	18/07/2021	PSB	14530	54	100+	1000	MSC(f) 46
SOL_22	M. Calbi	18/07/2021	PSB	6400	57	100+	1000	MSC(f) 7
SOL_23	M. Calbi	18/07/2021	TSB	1230	51	200+	1000	MSC(f) 13
SOL_24	M. Calbi	18/07/2021	MSB	13400	80	200+	1000	MSC(f) 12
SOL_25	M. Calbi	18/07/2021	TSB	8630	54	200+	1000	MSC(f) 9
SOL_26	M. Calbi	18/07/2021	MSB	1670	50	200+	1000	MSC (f) 10
SOL_27	M. Calbi; B. Foggi	21/07/2021	PSB	2660	55	100	250	MSC(f)47
SOL_28	M. Calbi; B. Foggi	21/07/2021	MSB	4280	50	1000+	10000	MSC(f) 48
SOL_29	M. Calbi; B. Foggi	21/07/2021	TSB	2880	50	1000+	10000	MSC(f) 49
SOL_30	M. Calbi; B. Foggi	21/07/2021	PSB	2200	51	1000+	10000	MSC(f) 50
SOL_31	M. Calbi; B. Foggi	21/07/2021	PSB	6970	53	200+	10000	MSC (f) 51
SOL_33	M. Calbi; G. Ferretti	03/08/2021	TSB	7670	58	200+	200	MSC(f) 18
SOL_34	M. Calbi; G. Ferretti	03/08/2021	TSB	3140	40	50+	100	MSC(f) 16
SOL_35	M. Calbi; G. Ferretti	03/08/2021	PSB	2840	52	100+	500	MSC(f) 19
SOL_36	M. Calbi; G. Ferretti	03/08/2021	PSB	1270	52	100+	500	MSC(f) 54
SOL_37	M. Calbi; G. Ferretti	03/08/2021	MSB	2140	95	300+	500	MSC(f) 55
SOL_38	M. Calbi; B. Foggi	04/08/2021	TSB	1620	34	100+	150	MSC(f) 56
SOL_39	M. Calbi; C. Silvestri; A. Carta	15/08/2021	PSB	410	50	200	500	MSC(f) 25

Table 3. Seeds morphological features and percentage of healthy seeds by accession (mean ± SD). Accessions are ordered based on the collection number.

Collection number	Species	Seed weight (mg)	Seed length (mm)	Seed width (mm)	% of healthy seeds
SOL_01	*Stipa etrusca*	22.108 ± 0.398	18.385 ± 1.662	1.702 ± 0.263	80
SOL_02	*Stipa etrusca*	25.952 ± 0.969	17.860 ± 0.994	1.673 ± 0.211	85
SOL_03	*Stipa etrusca*	26.413 ± 0.475	18.200 ± 1.149	1.990 ± 0.178	80
SOL_04	*Stipa etrusca*	21.492 ± 0.681	16.227 ± 0.797	1.744 ± 0.219	80
SOL_05	*Stipa etrusca*	29.415 ± 0.939	19.295 ± 0.955	1.810 ± 0.205	90
SOL_06	*Viola etrusca*	0.771 ± 0.041	1.796 ± 0.180	0.868 ± 0.119	80
SOL_07	*Viola etrusca*	0.791 ± 0.062	2.001 ± 0.212	0.976 ± 0.113	80
SOL_08	*Viola etrusca*	0.968 ± 0.038	1.740 ± 0.143	0.920 ± 0.076	90
SOL_09	*Viola etrusca*	0.814 ± 0.055	1.702 ± 0.181	0.876 ± 0.117	90
SOL_11	*Stipa etrusca*	28.843 ± 0.758	20.200 ± 1.581	1.783 ± 0.226	90
SOL_12	*Odontarrhena bertolonii*	0.424 ± 0.011	1.864 ± 0.142	1.280 ± 0.129	60
SOL_13	*Leontodon anomalus*	0.668 ± 0.022	5.583 ± 0.672	0.593 ± 0.077	70
SOL_14	*Odontarrhena bertolonii*	0.408 ± 0.021	2.092 ± 0.197	1.382 ± 0.123	80
SOL_15	*Odontarrhena bertolonii*	0.328 ± 0.020	1.999 ± 0.148	1.335 ± 0.138	75
SOL_16	*Odontarrhena bertolonii*	0.302 ± 0.022	1.845 ± 0.164	1.272 ± 0.082	80
SOL_17	*Leontodon anomalus*	0.626 ± 0.043	4.683 ± 1.171	0.561 ± 0.108	80
SOL_18	*Globularia incanescens*	0.265 ± 0.025	2.056 ± 0.270	1.126 ± 0.137	95
SOL_19	*Leontodon anomalus*	0.693 ± 0.069	5.215 ± 0.580	0.604 ± 0.109	80
SOL_20	*Globularia incanescens*	0.282 ± 0.016	1.998 ± 0.264	1.103 ± 0.143	85
SOL_21	*Globularia incanescens*	0.281 ± 0.021	1.961 ± 0.232	0.973 ± 0.205	90
SOL_22	*Leontodon anomalus*	0.833 ± 0.038	5.706 ± 0.618	0.572 ± 0.095	90
SOL_23	*Leontodon anomalus*	0.825 ± 0.048	5.954 ± 1.486	0.592 ± 0.145	85
SOL_24	*Globularia incanescens*	0.267 ± 0.017	1.905 ± 0.278	1.067 ± 0.147	95
SOL_25	*Globularia incanescens*	0.245 ± 0.024	1.990 ± 0.336	0.928 ± 0.158	95
SOL_26	*Leontodon anomalus*	0.839 ± 0.057	5.272 ± 0.889	0.546 ± 0.094	90
SOL_27	*Santolina etrusca*	0.254 ± 0.019	1.894 ± 0.179	0.494 ± 0.105	90
SOL_28	*Santolina etrusca*	0.241 ± 0.021	1.926 ± 0.271	0.555 ± 0.117	90
SOL_29	*Santolina etrusca*	0.240 ± 0.014	2.211 ± 0.223	0.659 ± 0.098	90
SOL_30	*Santolina etrusca*	0.233 ± 0.033	2.077 ± 0.311	0.554 ± 0.103	90
SOL_31	*Santolina etrusca*	0.200 ± 0.018	2.103 ± 0.291	0.524 ± 0.086	90
SOL_33	*Odontarrhena bertolonii*	0.261 ± 0.019	1.712 ± 0.154	1.169 ± 0.124	80
SOL_34	*Odontarrhena bertolonii*	0.222 ± 0.013	1.919 ± 0.241	1.256 ± 0.157	85
SOL_35	*Centaurea arrigonii*	1.723 ± 0.141	3.587 ± 0.288	1.384 ± 0.170	80
SOL_36	*Centaurea arrigonii*	1.548 ± 0.090	3.149 ± 0.258	1.263 ± 0.127	85
SOL_37	*Centaurea arrigonii*	2.004 ± 0.088	3.219 ± 0.332	1.302 ± 0.136	85
SOL_38	*Centaurea arrigonii*	1.914 ± 0.077	3.043 ± 0.292	1.256 ± 0.171	85
SOL_39	*Centaurea arrigonii*	1.096 ± 0.026	3.010 ± 0.238	1.491 ± 0.155	70

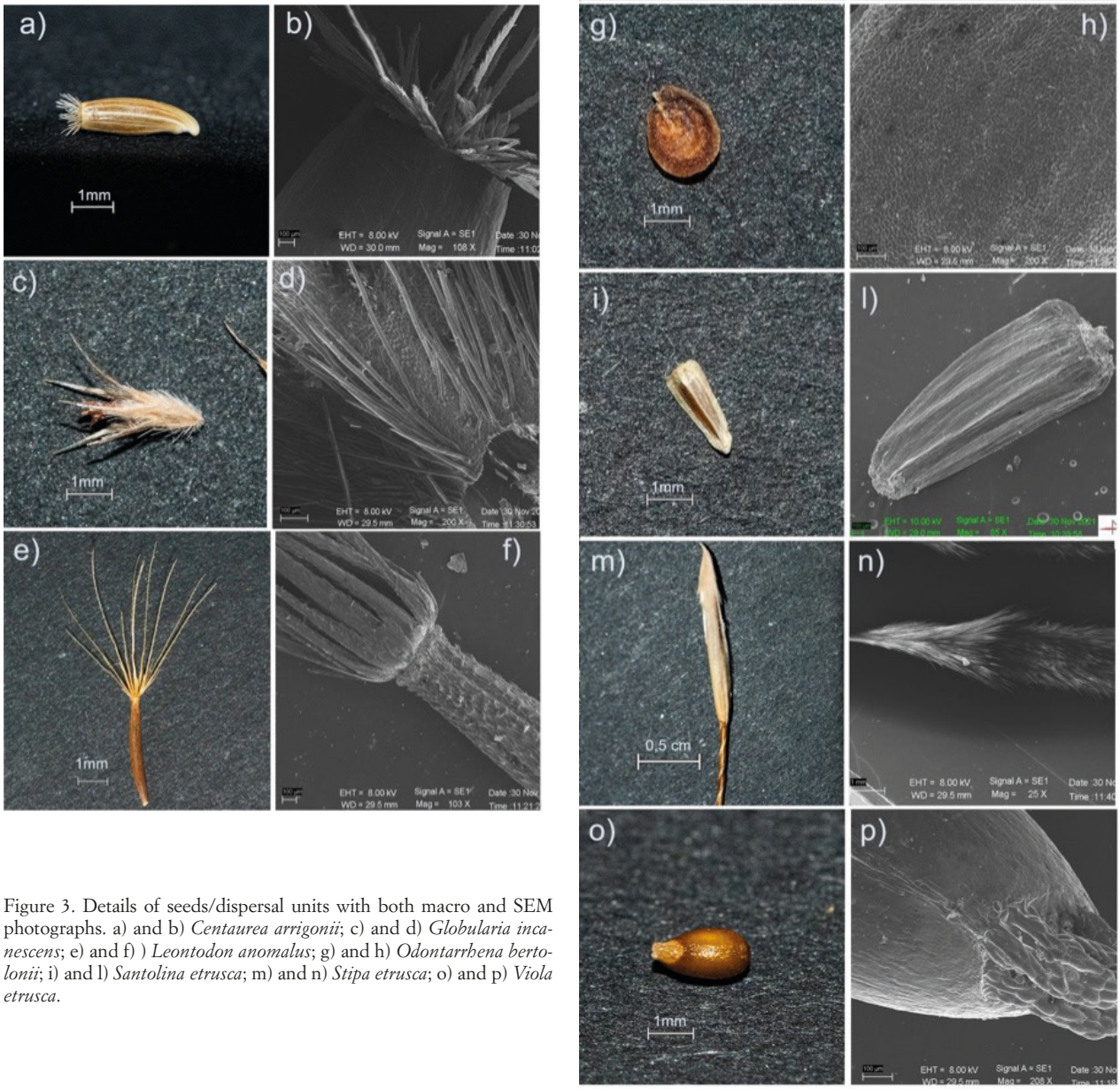

Figure 3. Details of seeds/dispersal units with both macro and SEM photographs. a) and b) *Centaurea arrigonii*; c) and d) *Globularia incanescens*; e) and f)) *Leontodon anomalus*; g) and h) *Odontarrhena bertolonii*; i) and l) *Santolina etrusca*; m) and n) *Stipa etrusca*; o) and p) *Viola etrusca*.

Discussion

In this paper we provided an overview of the main methodology employed during the SOL project and of the steps that were taken to ensure the *ex-situ* conservation of the target species. Moreover, we laid out a detailed outline of three main morphological features for each single accession, that might be a useful baseline for future comparative studies involving our target species.

During this work, we carried out a thorough population sampling and seed processing, visiting overall 74 populations, collecting seeds from more than four populations per species scattered across their native range, thus reaching adequate coverage. Regarding seed morphology, we observed marked differences among seeds from different species, especially between the ones belonging to *Stipa etrusca*, the only monocot investigated in this study, and the ones that belonged to the other six species. The PCA further confirmed the separation of the *Stipa etrusca* seeds for all of the three measured morphological characters. Regarding the in-between population differences for each species, a certain degree of variation in term of

seed weight, length and width was apparent for all the species except for *Santolina etrusca,* whose accessions were tightly grouped together in the ordination diagram, and to a lesser extent for *Globularia incanescens* that showed a similar pattern (Fig. 4).

We successfully enhanced the *ex-situ* representation of the target species by delivering accessions to three major Seed Banks. The collections coordinated by these institutions represent long-term storage germplasm material to fulfill conservation, research, and restoration needs (Rivière *et al.*, 2018). Even though the underlying causes of biodiversity loss need to be addressed fast, as threats to biodiversity rise, the most effective conservation strategies are the ones that combine already available resources to provide the highest degree of protection (Cochrane *et al.*, 2007; Hay & Probert, 2013). In this context, seed banks have a crucial role, not only being the physical facilities where seeds are stored and preserved but also where germination tests and population viability analyses are performed (Carta *et al.*, 2022), providing material for ecological restoration projects (Pedrini *et al.*, 2020). Possible future uses of SOL seeds include morphological and ecophysiological study, and the propagation and use of the target species for restoration ecology projects of degraded habitat or to enhance landscape biodiversity and resilience in response to natural disasters in their native ranges, while ensuring associated ecosystem services (FAO, 2019). Moreover, the here provided microphotographs and morphological information could contribute to a better species identification for the studied genera and as a useful baseline for further morphological or ecological studies (Pinzani *et al.*, 2021).

In the last decades the interest towards the uses of plants has been rising significantly, parallel to the growing attention to sustainability development (Paura *et al.*, 2021). Within Tuscany, to this date the species that have known uses in the region are 732, of which 542 medicinal uses 366 alimentary uses (Martelli *et al.*, 2015). Preserving and drawing the attention to the importance of endemic or narrow ranged species of a territory not only serves for conservation purposes but also helps in transmitting their cultural value to the future generations, which may become important for future food security and other uses (Ulian *et al.*, 2020). Among our target species the only one with reported uses is *Santolina etrusca*, traditionally used as an antiparasitic in its native territories (Tundis & Loizzo, 2018), but also employed as part of a religious ritual involving the production of a scented water to honor St. John the Baptiste (Guarrera *et al.*, 2005). Recently *Santolina etrusca* extracts were used to produce an antibiotic cream (*Il Messaggero*, 2020). Additionally, the use of species belonging to the genus *Viola* to protect the throat from infections and inflammations and of the genus *Leontodon* as alimentary herbs are widely known and studied (Guarrera *et al.*, 2005; Signorini *et al.*, 2008).

In conclusion, we provided useful morphological and photographical data on the sampled species for future comparative studies and to aid restoration efforts. All tasks here presented were possible thanks to the cooperation between institutions, highlighting that to address conservation goals, even when geographically localized, it is necessary to act within a cooperation network.

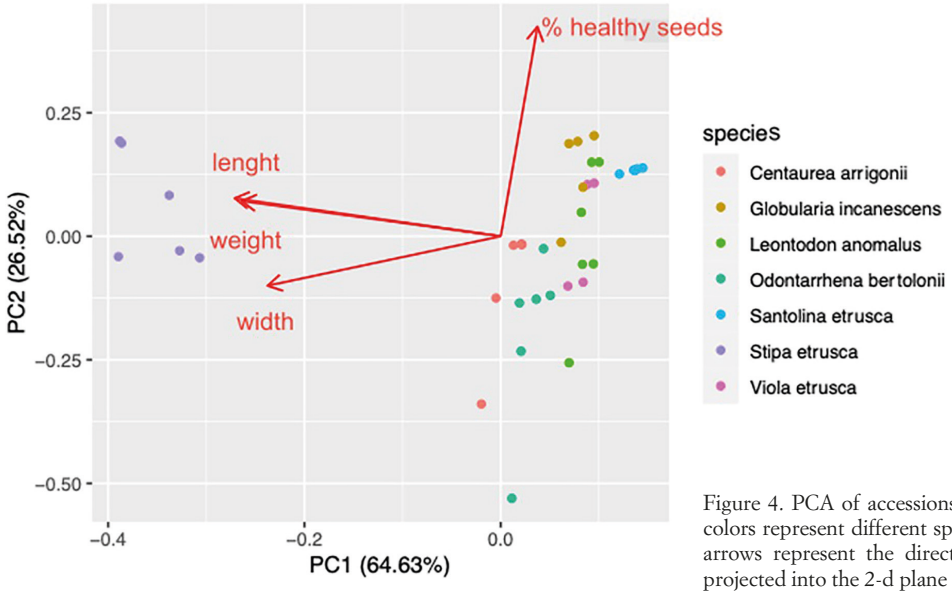

Figure 4. PCA of accessions' morphological features. Different colors represent different species as indicated in the legend and arrows represent the direction of the measured variables, as projected into the 2-d plane of the PCA biplot.

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