Anales del Jardín Botánico de Madrid
Vol. 65(1): 149-155
enero-junio 2008
ISSN: 0211-1322

Germplasm image analysis of *Astragalus maritimus* and *A. verrucosus* of Sardinia (subgen. *Trimeniaeus,* Fabaceae)

by

Efisio Mattana[1], Oscar Grillo[2], Gianfranco Venora[2] & Gianluigi Bacchetta[1]

[1] Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze Botaniche, Università degli Studi di Cagliari, v.le S. Ignazio da Laconi 13, 09123 Cagliari, Italy. bgsar@ccb-sardegna.it
[2] Stazione Consorziale Sperimentale di Granicoltura per la Sicilia, via Bouganvillea 20, 95041 Caltagirone (CT), Italy. lab.biologia@granicoltura.it

Abstract

Mattana, E., Grillo, O., Venora, G. & Bacchetta, G. 2008. Germplasm image analysis of Astragalus maritimus and A. verrucosus (subgen. Trimeniaeus, Fabaceae). *Anales Jard. Bot. Madrid* 65(1): 149-155.

The relationships between *A. verrucosus* Moris and *A. maritimus* Moris, exclusively endemics of Sardinia, are studied with a germplasm image analysis system. Morphometric and colorimetric features of the seeds and fruits of the two taxa have been studied and statistically elaborated to verify and confirm the validity of these species and to improve the performance of the correct classification of the image analysis system, previously elaborated to be a tool for taxonomic studies.

Keywords: morphometric and colorimetric analysis, Sardinian endemics, ex situ conservation, seed morphology.

Resumen

Mattana, E., Grillo, O., Venora, G. & Bacchetta, G. 2008. Análisis de imagen de germoplasma de Astragalus maritimus y A. verrucosus de Sardinia (subgen. Trimeniaeus, Fabaceae). *Anales Jard. Bot. Madrid* 65(1): 149-155 (en inglés).

Se presentan las relaciones entre *A. verrucosus* Moris y *A. maritimus* Moris, endemismos exclusivos de Cerdeña, que se han estudiado a través de un sistema de análisis de imagen del germoplasma. Para las dos especies se han tomado las medidas morfométricas y colorimétricas de las semillas y de los frutos, que luego se han analizado desde el punto de vista estadístico. Todo esto con la finalidad de averiguar y confirmar la distinción a nivel sistemático y mejorar el porcentaje de correcta clasificación del sistema de análisis de imagen elaborado con finalidades de carácter taxonómico.

Palabras clave: análisis morfométrico y colorimétrico, endemismos de Cerdeña, conservación ex situ, morfología de semillas.

Introduction

Astragalus L. genus (Fabaceae) is corporate by more of 3000 species (Lock & Simpson, 1991) and it represents the type of the Tribu Astragaleae Bercht. & J. Presl and of the Subtribu Astragalinae DC. (Podlech, 1999a). The principal speciation center of this genus is located in Middle Est Asia, in Iran precisely, where were discovered till now 532 species (Roskov & al., 2005).

Goncharov (1965) and Chater (1968) groups Eurasian taxa belonging to this genus in 9 subgenus. The subgenus *Trimeniaeus* Bunge is constituted generally by annual taxa, mainly with simple hair and imparipinnate leaves (Chater, 1968). Inside this subgenus, Bunge (1868, 1869) set up section *Platyglottis* to which Podlech (1990) assign 8 Middle East species (Libya, Egypt and West Asia) except *Astragalus verrucosus* Moris (Sardinia), *A. peregrinus* Vahl (its areal extend till Algeria) and *A. nitidiflorus* Jiménez Mun. & Pau (SE Spain) (Talavera, 1999).

In this work the relationships between the two exclusively endemic taxa of Sulcis-Iglesiente (Fig. 1): *A. verrucosus* Moris and *A. maritimus* Moris are analysed. The two species, described by Moris (1827, 1837), have been considered doubtful until to 1977, when De Marco & al. (1977) re-evaluate *A. maritimus*, they also considered this species independent from *A. verrucosus,* considered synonymous with *A. tuberculosus* DC. until to 1978 (Corrias, 1978). Validity of the two species was confirmed afterwards by many other botanists (Pignatti, 1982; Bacchetta, 2001; Conti & al., 2005) and Podlech (1999b) assigns *A. maritimus* to the section *Drepanodes* Bunge.

Morphometric and colorimetric features of the seeds and fruits of the two *taxa* have been measured and statistically analyzed, to confirm the validity of the two species and to carry out a first approach to investigate their taxonomic position inside the subgenus *Trimeniaeus* Bunge.

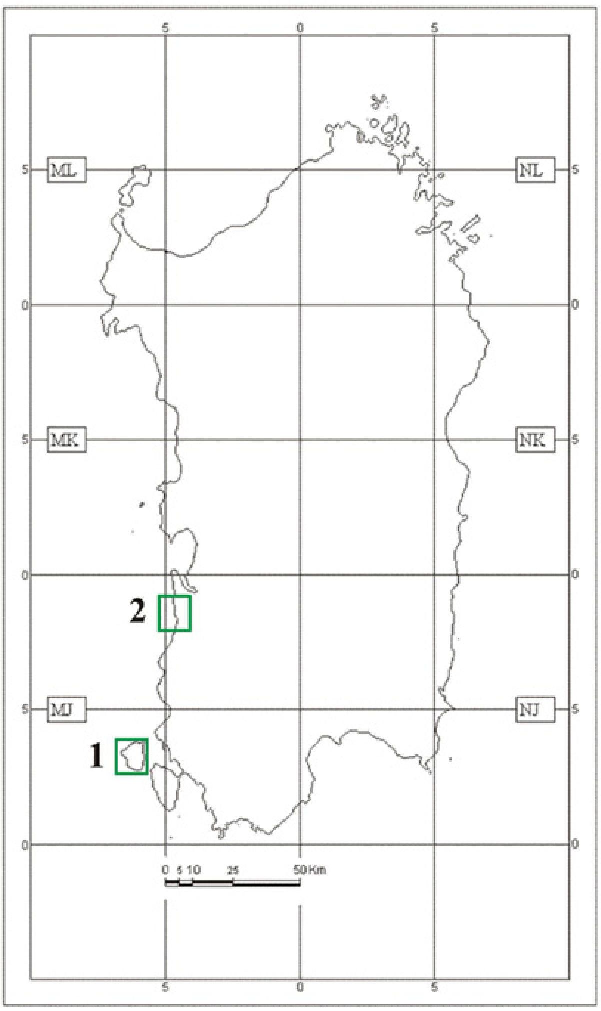

Fig. 1. Geographic location of *Astragalus maritimus* (1) and *A. verrucosus* (2) populations (Sardinia).

Materials and Methods

The fruits of the two species, were collected in the field (Tab. 1) or obtained from *ex situ* cultivation in the Botanical Garden of Cagliari, and the seeds were stored at the Sardinian Germplasm Bank, BG-SAR (Mattana & al., 2005).

The image of the samples was acquired by a flatbed scanner (Epson GT-15000), with a resolution of 200 dpi, with a scanning area not exceeding 1024 × 1024 pixel, before their entrance into the dehydration room (15 °C at the 15% of R.H.), in order to avoid any possible variation in dimension, shape and colour. The acquisition method was performed according to Bacchetta & al. (2008). For the analysis of every accession, a sample constituted by 100 seeds and 20 fruits (Tab. 1) was randomly prepared and sent by e-mail at the Stazione Sperimentale di Granicoltura per la Sicilia fot the image processing. The image analysis system was KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany). The KS-400 can be customized for specific applications by editing appropriate image analysis algorithms in 'macros', that allow to make the analysis automatically. In order to achieve the relative dimensions, shapes and colour (RGB - Red Green Blue and HLS - Hue Light Saturation channels) measures of the individual objects (seeds and fruits) a macro, formerly developed for identify diaspores of wild plants species (Bacchetta & al., 2008), was specially modified, adding 20 new seed features. The 34 measured features for each accession are shown in Table 2, 33 were recorded on seeds and 1 specific on fruits.

Statistical analysis of the seeds images variables was performed by Stepwise Linear Discriminant analysis method, it was used to find the best features to classify the two species, an algorithm included in the SPSS software package, release 14 (SPSS Inc. 1989-2006). The selection for enter a new variable in the model is based on the higher value of *F* and least values of Wilks' Lambda. For each variable in the model, the *F* to remove and Wilks' Lambda statistics are useful for describing what happens if the variables is removed from current model (given that others variable remain).

Table 1. Stored accessions (BG-SAR) used for morphometric and colorimetric analysis.

Taxon	Code	Locality	Amount of seeds	N seeds	N fruits	Seed analysis	Fruit analysis
A. maritimus	312/05	Cala dello Spalmatore (Carloforte, CI)	36,881	100	20	X	X
A. maritimus	ROC 12/06	Botanic Gardens (Cagliari, CA)	3,661	100		X	
A. maritimus	200/06	Cala dello Spalmatore (Carloforte, CI)	13,120	100		X	
A. verrucosus	305/05	Case Puxeddu (Arbus, CI)	26,704	100	20	X	X

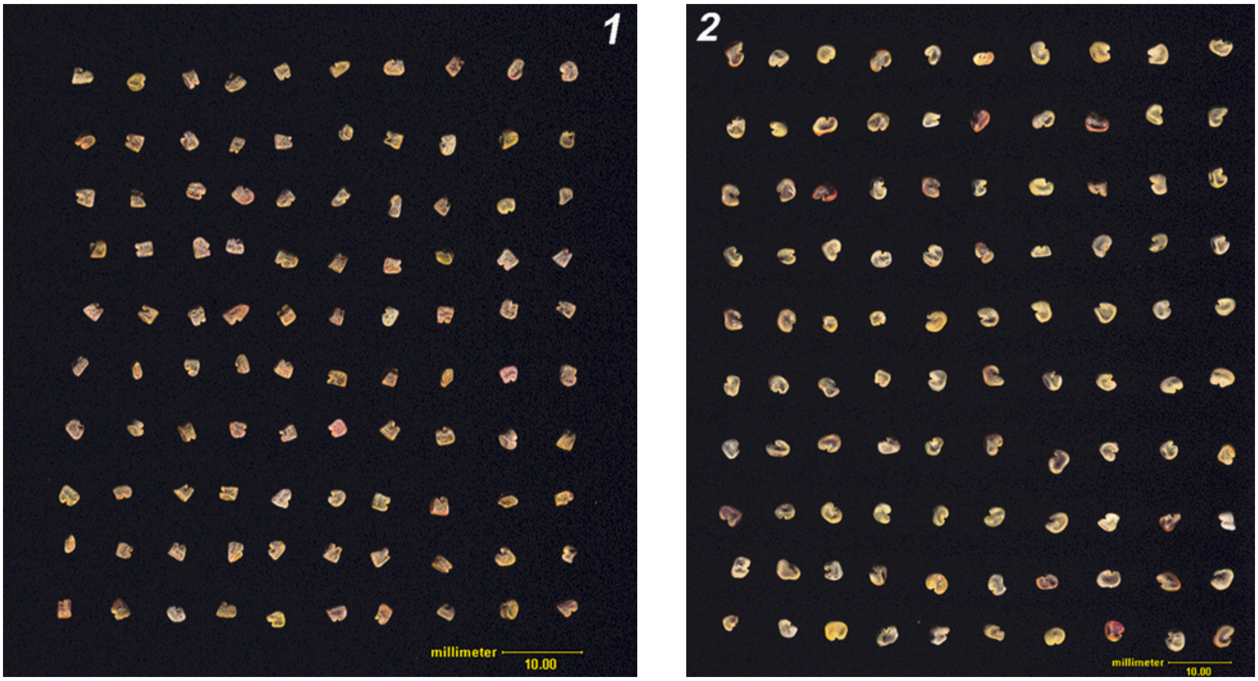

Fig. 2. Seeds of *Astragalus maritimus* (1) and *A. verrucosus* (2). The yellow mark corresponds to 10 mm.

Table 2. List of the measured seed morphometric and colorimetric features; in bold the new features not considered in Bacchetta & al. (2008).

Feature	***Description and units of measurement***
Area	Area of the object (mm^2)
Perimeter	Perimeter of the object (mm)
Convex Perimeter	**Convex Perimeter of the object (mm)**
Crofton Perimeter	**Crofton Perimeter of the object (mm)**
Ratio Co. P./Cr.P	**Ratio between Convex and Crofton Perimeters**
Max. diameter	Maximum diameter of the object (mm)
Min. diameter	Minimum diameter of the object (mm)
Ratio between ends distances[b]	**Ratio between ends distances**
Diameter ratio	**Ratio between diameters**
Shape factor	$= (4 \times \pi \times \text{area})/\text{perimeter}^2$
Diameter Eq. Ellipse	**Ellipse diameter of Equivalent area**
Ellipse Eq. Max. and Min.	**Ellipse of equivalent area, Maximun and Minimum axis**
Roundness factor	$= (4 \times \text{area})/(\pi \times \text{max. diameter}^2)$
Red colour[a]	Channel Red model RGB
Std. dev. Red	**Standard deviation Red channel**
Green colour[a]	Channel Green model RGB
Std. dev. Green	**Standard deviation Green channel**
Blue colour[a]	Channel Blue model RGB
Std. dev. Blue	**Standard deviation Blue channel**
Hue[a]	Channel Hue model HLS
Std. dev. Hue	**Standard deviation Hue channel**
Light[a]	Channel Light model HLS
Std. dev. Light	**Standard deviation Light channel**
Saturation[a]	Channel Saturation model HLS
Std. dev. Saturation	**Standard deviation Saturation channel**
Mean Density	**Mean grey level Density**
Std. dev. Density	**Standard deviation Density**
Skew	**Grey level asymmetry**
Kurtosis	**Grey level dispersion**
Energy	**Power of increasing intensity**
Entropy	**Power of dispersion**
Sum density	**Sum of density grey level**
Sum square density	**Sum square density grey level**

[a] Grey levels (Range 0 – 255). [b] For fruits

Moreover the procedure of cross validation was applied to verify the performance of the classifier developed.

For SEM pictures, seeds were dried with silica-gel and mounted on metal stubs using double-stick tape and taken by a FEI ESEM QUANTA 200.

Results

Seeds of the two taxa (Fig. 2) show a relative shape uniformity. They are flat, reniform shaped and they appear smooth, without sculptures and ornaments. In Table 3 the comparison among all morphometric and colorimetric parameters measured is presented. The Shape factor (0.87 ± 0.04 and 0.89 ± 0.04) and the Roundness factor (0.69 ± 0.06 and 0.72 ± 0.06) values confirm how the shape of these seeds is similar, but *A. maritimus* seeds are smaller (1.37) 2.22 × 2.89 (3.90) mm than *A. verrucosus* (2.26) 3.07 × 3.96 (4.74)

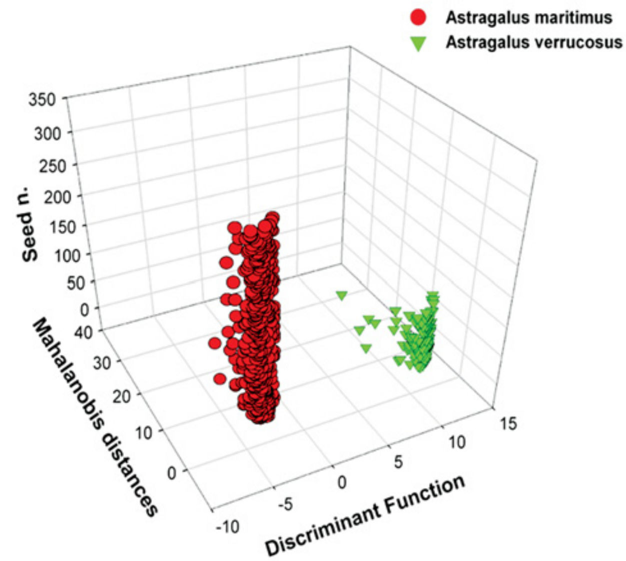

Fig. 3. Plot of the discriminating analysis. The Linear Discriminant Analysis verified by cross validation shows a performance of 100%.

Table 3. Morphometric and colorimetric measures of *Astragalus verrucosus* and *A. maritimus*.

Parameter	*A. verrucosus*		*A. maritimus*	
	Seeds	Fruits	Seeds	Fruits
Mean Red colour	145.90 ± 10.77	186.42 ± 13.55	158.72 ± 11.89	174.71 ± 24.16
Std. Dev. Red	37.91 ± 4.44	48.84 ± 3.79	34.22 ± 4.82	48.15 ± 6.23
Mean Green colour	115.53 ± 12.43	154.04 ± 14.56	125.52 ± 11.31	134.96 ± 21.29
Std. Dev. Green	32.55 ± 4.11	41.14 ± 5.29	26.42 ± 3.81	38.42 ± 7.67
Mean Blue colour	73.16 ± 11.33	123.13 ± 11.27	100.44 ± 10.76	108.14 ± 11.23
Std. Dev. Blue	25.03 ± 3.31	26.98 ± 5.02	15.85 ± 3.42	23.30 ± 4.94
Mean Hue	24.37 ± 1.86	29.67 ± 2.01	28.32 ± 5.24	39.33 ± 17.90
Std. Dev. Hue	5.50 ± 3.86	41.67 ± 5.28	43.30 ± 12.74	59.82 ± 20.83
Mean Light	109.28 ± 10.40	154.41 ± 12.28	129.18 ± 10.99	140.84 ± 17.55
Std. Dev. Light	30.80 ± 3.66	37.59 ± 4.21	24.70 ± 3.88	35.08 ± 5.17
Mean Saturation	95.26 ± 11.54	108.04 ± 21.98	70.64 ± 8.89	103.18 ± 32.01
Std. Dev. Saturation	21.43 ± 5.89	57.75 ± 13.29	26.79 ± 6.81	55.87 ± 16.54
Mean Density	76.30 ± 3.98	97.37 ± 11.29	76.05 ± 5.65	94.45 ± 12.36
Std. Dev. Density	43.97 ± 3.68	70.03 ± 7.84	53.57 ± 5.15	70.51 ± 6.36
Skew	4.76 ± 8.69	4.14 ± 0.51	4.82 ± 2.25	3.03 ± 1.18
Kurtosis	113.05 ± 181.52	16.32 ± 4.30	27.97 ± 34.50	9.07 ± 6.51
Energy	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Entropy	5.79 ± 0.15	7.21 ± 0.05	5.19 ± 0.30	7.28 ± 0.08
Sum Density	42,108.93 ± 6,370.05	956,532.32 ± 217,863.31	21,471.12 ± 3,735.84	452,955.07 ± 90,131.17
Sum square Density	4,293,328.23 ± 772,713.71	144,454,893.95 ± 47,109,687.29	2,44,.817.74 ± 504.274.51	67,627,344.73 ± 19,112,492.35
Area	8.84 ± 1.25	155.80 ± 22.18	4.54 ± 0.82	76.53 ± 9.67
Max. diameter	3.96 ± 0.32	27.21 ± 2.78	2.89 ± 0.25	28.98 ± 2.41
Min. diameter	3.07 ± 0.25	8.25 ± 0.98	2.22 ± 0.24	3.56 ± 0.39
Diameter ratio	0.78 ± 0.06	3.32 ± 0.37	0.77 ± 0.07	8.22 ± 1.10
Ratio between ends distances	n.a.	20.90 ± 2.91	n.a.	7.76 ± 3.06
Perimeter	11.80 ± 0.88	65.30 ± 5.84	8.50 ± 0.76	63.38 ± 4.73
Convex Perimeter	11.20 ± 0.79	57.09 ± 5.27	8.18 ± 0.69	43.66 ± 3.21
Crofton Perimeter	11.18 ± 0.84	61.91 ± 5.54	8.06 ± 0.72	60.09 ± 4.49
Ratio Co. P./Cr. P.	1.00 ± 0.02	0.92 ± 0.03	1.02 ± 0.02	0.73 ± 0.05
Shape factor	0.89 ± 0.04	0.51 ± 0.05	0.87 ± 0.04	0.27 ± 0.02
Diameter Eq. Ellipse	3.35 ± 0.24	14.05 ± 1.03	2.39 ± 0.21	9.85 ± 0.64
Ellipse Max. diameter	1.91 ± 0.16	10.95 ± 1.08	1.35 ± 0.14	7.33 ± 0.56
Ellipse Min. diameter	1.48 ± 0.12	5.03 ± 0.37	1.08 ± 0.12	4.92 ± 0.48
Roundness factor	0.72 ± 0.06	0.27 ± 0.03	0.69 ± 0.06	0.12 ± 0.01

Table 4. Ranking of selected features after fourteen cycles of stepwise selection (SPSS Linear Discriminant Analysis - Stepwise method).

	Tolerance	*F* to remove	Wilks' Lambda
Std. Dev. Hue	0.154	190.960	0.038
Std. Dev. Blue	0.133	147.097	0.035
Std. Dev. Saturation	0.199	121.112	0.033
Mean Hue	0.158	53.024	0.029
Mean Saturation	0.122	49.270	0.029
Kurtosis	0.031	40.997	0.028
Skew	0.029	32.617	0.028
Std. Dev. Red	0.059	15.855	0.027
Std. Dev. Green	0.038	10.249	0.026
Area	0.012	7.835	0.026
Sum Density	0.012	4.446	0.026
Mean Green colour	0.112	3.424	0.026

mm with an area of 4.54 ± 0.82 mm² and 8.84 ± 1.25 mm² respectively.

The Stepwise Linear Discriminant analysis carried out for all the features measured on this work, increase the performance of the correct classification between the seeds of the two taxa at the 100% (Fig. 3), on respect of the 98.5% obtained in Bacchetta & al. (2008).

The best features selected by Stepwise method and its power in contributing to the discriminating process are reported in Table 4. The discrimination was carried out principally by colorimetric features (RGB and HLS channels) and the only one morphometric is the Area at the tenth position.

The SEM images (Fig. 4) of *A. maritimus* seeds highlight how they are angular and have more bor-

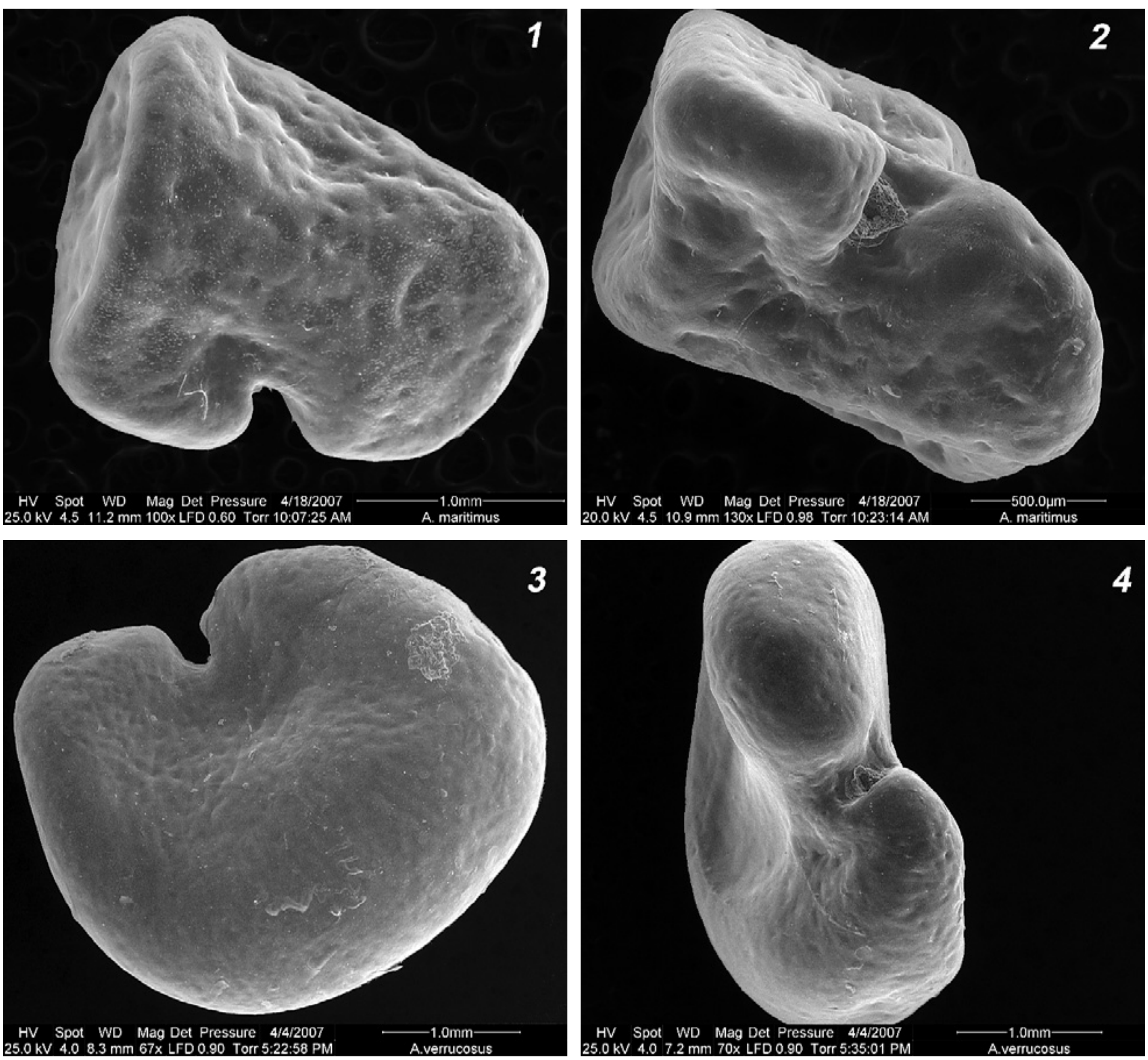

Fig. 4. SEM pictures on secondary electrons aquired by "low vacuum" modality of *Astragalus maritimus* (1-2) and *A. verrucosus* (3-4) seeds. The mark corresponds to 1 mm, except for picture 2 (0,5 mm).

Fig. 5. Fruits of *Astragalus verrucosus* (1) and *A. maritimus* (2). The yellow mark corresponds to 10 mm.

ders irregularities and widespread sags and swellings while *A. verrucosus* seeds are flatter, smoother and rounder.

Fruits of the two *taxa* (Fig. 5), conforming to the literature, look very differents. *A. maritimus* pods are brown-coloured, C-shaped and smooth; *A. verrucosus* pods are cream-coloured, oblongs with tubercles and with two hooks at an extremity (Corrias, 1978; Bacchetta, 2001). The image analysis carried out on the fruits (Table 3), confirm this high dissimilarity on size (Area 155.80 ± 22.18 mm^2 for *A. verrucosus* and 76.53 ± 9.67 mm^2 for *A. maritimus*), shape (Shape factor 0.51 ± 0.05 and 0.27 ± 0.02 respectively) and colour (see values for RGB and HLS channels).

Discussion

The germplasm image analysis system carried out to identify diaspores of wild plant species (see Bacchetta & al., 2008) and here modified with the adding of new 20 features and also applied on fruits, confirm to be a helpful tool on taxonomic studies with high performances of correct classification and the comparison with SEM images show also how this system is able to discriminate very similar seeds.

In fact, how in this case, the shape and dimensions are not significantly different, the integration of morfometric with colorimetric features in the analysis, assure in any case high percentages of correct classification.

The morphometric and colorimetric characterization carried out by image analysis of the seeds confirm the taxonomic distance between these taxa and the validity of the two species. The results of this work represents also a first approach to investigate the taxonomic relationship of these two species inside the subgenus *Trimeniaeus*.

Studies on the life form and other plant traits of these two species are in progress to verify their position on the sections *Platyglottys* and *Drepanodes*.

Acknowledgements

The autors thank the "Provincia di Cagliari-Assessorato Tutela Ambiente" for the support to the Sardinian Germplasm Bank and Prof. P. Lattanzi and Dott.ssa E. Musu (CGS-University of Cagliari) for the SEM images. This research has been financed also by the GENMEDOC project (Interreg IIIB Medocc-2003-03-4.1-E-060).

References

Bacchetta, G. 2001. Astragalus maritimus Moris, Astragalus verrucosus Moris. In: Pignatti, S. & al. (eds.), *Liste rosse e blu della flora italiana*: 132-135. ANPA. Roma.

Bacchetta, G., Grillo, O., Mattana, E. & Venora, G. 2008. Morpho-colorimetric characterization by image analysis to identify diaspores of wild plant species. *Flora* (*in press*).

Bunge, A. 1868. Generis Astragali species gerontogeae. Pars prior, claves diagnosticae. *Mémoires de l'Académie Impériale de Sciences de Saint Pétersbourg* 11(16): 1-140.

Bunge, A. 1869. Generis Astragali gerontogeae. Pars altera, speciarum enumeratio. *Mémoires de l'Académie Impériale de Sciences de Saint Pétersbourg* 15(1): 1-254.

Chater, A.O. 1968. Astragalus L. In: Tutin, T.G. & al. (eds.), *Flora Europaea* 2: 108-124. Cambridge.

Conti, F., Abbate, G., Alessandrini, A. & Blasi, C. 2005. *An annotated Checklist of the Italian Vascular Flora*. Palombi Editori. Roma.

Corrias, B. 1978. Le piante endemiche della Sardegna: 24-25. *Bolletino della Società Sarda di Scienze Naturali* 17: 243-252.

De Marco, G., Dinelli, A. & Mossa, L. 1977. Sull'Astragalus maritimus Moris. *Annali di Botanica (Roma)* 35-36: 353-364.

Goncharov, N.F., Borisova, A.G., Gorshkova, S.G., Popov, M.G. & Vasilchenko, I.T. 1965. Leguminosae: Astragalus. In: Komarov, V.L. & Shishkin, B.K. (eds.), *Flora of the U.S.S.R.* 12: 1-918. Israel Program for Scientific Translations, Jerusalem, Smithsonian Institution and the National Science Foundation. Washington, D.C.

Lock, J.M. & Simpson, K. 1991. *Legumes of West Asia, a check-list*. Royal Botanic Gardens. Kew.

Mattana, E., Fenu, G. & Bacchetta, G. 2005. La Banca del Germoplasma della Sardegna (BG-SAR): uno strumento per la conservazione del germoplasma autoctono sardo. *Informatore Botanico Italiano* 37(1): 144-145.

Moris, G.G. 1827. *Stirpium Sardoarum Elenchus*. Ex Regio Typographeo. Caralis.

Moris, G.G. 1837. *Flora Sardoa 1*. Ex Regio Typographeo. Taurini.

Pignatti, S. 1982. *Flora d'Italia* 1. Edagricole. Bologna.

Podlech, D. 1990. Revision von Astragalus L. sect. Platyglottis Bunge (Leguminosae). *Mitteilungen aus der botanischen Staatssammlung München* 29: 541-572.

Podlech, D. 1999a. Astragalus L. In: Talavera, S. & al. (eds.), *Flora Iberica* 7(I): 279-338. Madrid.

Podlech, D. 1999b. *Thesaurus Astragalorum I*. München. [http://www.botanik.biologie.uni-muenchen.de/botsyst/thesau1.html#taxa].

Roskov, Y.R., Bisby, F.A., Zarucchi, J.L., Schrire, B.D. & White, R.J. (eds.). 2005. *ILDIS World Database of Legumes: draft checklist, version 10 (November 2005)*. CD-ROM ILDIS. Reading.

Talavera, S. 1999. Sobre el tratamiento de la tribu Astragaleae Bercht. & J. Presl (Papilioideae, Leguminosae) en "Flora Iberica". *Anales del Jardín Botánico de Madrid* 57(1): 218-220.

Editor asociado: S. Talavera
Recibido: 13-XI-2007
Aceptado: 10-I-2008