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Characterisation of woody *Medicago* (sect. *Dendrotelis*) species, on the basis of seed and seedling morphometry

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Abstract

The three species belonging to the sect. *Dendrotelis* of the genus *Medicago*: *M. arborea* L., *M. citrina* (Font Quer) Greuter, and *M. strasseri* Greuter, Matthäs & Risse, were characterized using 12 morphometrical characters of seeds and 20 of seedlings. These species have interest for the regeneration of degraded lands in semi-arid climates. *M. arborea* presents the largest phenotypic variability and is dispersed throughout the Mediterranean basin, while the other two species are endemic to a very reduced area, *M. citrina*, in Columbretes Islands and Cabrera Islands (Spain) and *M. strasseri* in Crete. Several populations of *M. arborea* were measured, in order to compare the intraspecific variability within *M. arborea* with the differences among all three species. The multivariate analysis of the data used characters that were independent of the size of the studied organs. The three species were clearly differentiated on the basis of seed characters. On the other hand, seedling characters distinguished *M. citrina* from the other two species, but *M. strasseri* and the populations of *M. arborea* were intermingled. In spite of this, we have described relevant morphological differences among *M. arborea*, *M. strasseri* and *M. citrina*, which are consistent with other ecological or bromatological differences. The study supports that the three accessions merit to be considered as three different species.

Introduction

Land degradation is a major problem in temperate regions of the world. Wild fires, unsound cultivation practices, and inadequate or excessive grazing, initiate the process of land degradation, and this is generally followed by soil erosion and decline of vegetation and land productivity. Degraded areas have a serious risk of desertification, which is an irreversible phenomenon (Le Houérou, 1979; Grainger, 1990). One alternative for degraded lands is agroforestry with multiple-use of the land to develop tourism, wildlife, hunting, and sports, combined with extensive grazing by livestock and game, and timber production (Le Houérou, 1993). Legume shrubs play an important role in agroforestry, especially those species providing good quality browsing (Lefroy et al., 1992; Douglas et al., 1996).

Despite high plant diversity in the Mediterranean region, only a few shrubby leguminous species have been evaluated intensively for the above purposes. These have consisted mostly of *Cytisus* and its allies, namely *Cytisus*, *Chamaecytisus*, and *Genista* sect. *Teline* (Townsend & Radcliffe, 1990; Douglas et al., 1996; González-Andrés & Ortíz, 1996 a, b); of *Colutea* sp. (Koller & Negli, 1955; Ceresuela & Pereira, 1993); of *Dorycnium* (Wills et al., 1989; Alegre et al., 1993a; Sancha et al., 1993; Douglas et al., 1996); and of *Medicago arborea sensu stricto* (Papanastasis, 1987; Lambert et al., 1989 a, b, c, d; Olea et al., 1993; Alegre et al., 1993a; Martiniello et al., 1994; Bouzid & Papanastasis, 1996; González-Andrés & Ceresuela, 1998).

The section *Dendrotelis* of the genus *Medicago* (Leguminosae; Trifolieae) consists of three species: *Medicago arborea* L., *Medicago citrina* (Font Quer) Greuter and *Medicago strasseri* Greuter, Matthäs & Risse, that form the so-called *Medicago arborea* aggregate. *M. arborea* ($2n = 4x = 32$) displays the greatest variability and dispersal area, covering Greece, the Aegean Sea Islands, Albany, Crete, Turkey and Italy. As a crop, it was naturalised in Sicily, France, Spain, Portugal, Algeria, Tunisia, and Morocco (Coste, 1906; Villax, 1963; Pignatti, 1982; Bolòs & Vigo, 1984). *M. citrina* is an hexaploid ($2n = 6x = 48$) endemic to the Columbretes Islands (Castellón, Spain) and Cabrera Island (Baleares, Spain). Font Quer (1924) considered it a variety (*Medicago arborea* var. *citrina* Font Quer), and Bolòs & Vigo (1974) as a subspecies (*M. arborea* subsp. *citrina* (Font Quer) O. Bolòs & Vigo). In the Med-Checklist (Greuter et al., 1989) it was classified as a new species. *M. strasseri*, a tetraploid ($2n = 4x = 32$) (data from the authors, not published), is an endemism from Crete (Greece), that was recently described by Greuter et al. (1982).

Medicago arborea has been widely cultivated as a forage plant outside its native areas, in the Mediterranean region (Olives, 1969; Elhamrouni & Sarson, 1976), or other parts of the world, as in Oceania (Lefroy et al., 1992). It has also been tested as a source of biologically fixed nitrogen in N-deficient ecosystems (Douglas et al., 1994). It is able to produce significant dry matter in semi-arid environments. Martiniello et al. (1994) analysed several clones and obtained an average of 199 g of dry matter per plant. The bromatological quality of the herbage is similar to alfalfa (*Medicago sativa* L.) with an average Acid Detergent Fibre (ADF) content under 25%, and high crude protein content, over 18% (Alegre et al., 1993a). Lambert et al. (1989b) analysed the preference of sheep and goats for several forages, and the forage of *M. arborea* belonged to the medium preference class for sheep, and to the high preference class for goats. The digestibility *in vitro* and *in vivo* of the dry matter is even higher than the digestibility of hay or pasture (Lambert et al., 1989c).

However the infraspecific phenotypic variability of *M. arborea* is very high. Martiniello et al. (1994) observed great differences between clones for morphological and yield parameters such as green biomass, dry matter yield, leaf/branch ratio, and plant height. Conversely fewer studies have been carried out on the botanical, agronomical, and ecological characteristics of *M. strasseri* and *M. citrina. M. strasseri* is more tolerant to temperatures below 0 °C than *M. arborea*, and the growth rate is also significantly higher. (Alegre et al., 1993b). It has a similar crude protein and fibres contents to those of *M. arborea* throughout the year (González-Andrés & Ceresuela, 1998). *M. citrina* grows better than *M. arborea* under saline conditions (Sibole et al., 1994), it is more tolerant to summer drought, has lower fibres content in winter and summer than both *M. arborea* and *M. strasseri* (65.1% vs. 39.7%), and consequently it is a better option in summer, when the other two species shed leaves. All the three species produce a good winter forage in winter-cold semi-arid environments (González-Andrés & Ceresuela, 1998).

According to Greuter et al. (1982), *M. arborea* is distinguished from *M. strasseri* in its larger seeds, much larger pods with less numerous coils, a shorter stipe seldom exceeding the calyx tube, and a central opening at least 1 mm across, but usually much wider. On the other hand the hexaploid *M. citrina* is distinguished from *M. arborea* and *M. strasseri* by larger leaves, fruits and seeds (Robledo et al., 1993) and lemon-yellow flowers (in the other two species they are orange). However, *M. arborea* is itself rather variable, so it is necessary to compare several populations of *M. arborea* with *M. strasseri* and *M. citrina*. Chebby et al. (1995) characterised one population of *M. arborea, M. strasseri*, and *M. citrina* using morphological characters and molecular markers. They concluded that large differences between the three taxa exist, but infra-populational variability was evident.

The objective of the present study was to characterise the three species in order to compare infraspecific vs. interspecific variability, but including several populations of *M. arborea* because of its high intrinsic variability. The study was carried out using 12 morphometrical characters of seeds and 20 of seedlings.

Material and methods

Table 1 lists the studied accessions and their origins. Plant material was obtained from seeds maintained at the germplasm bank of the Departamento de Biología Vegetal, Escuela Técnica Superior de Ingenieros Agrónomos, Madrid.

Table 1. Plant material

Accession	Origin
Medicago arborea 6081	Murcia (Spain)
Medicago arborea 7391	Sunion Cape (Greece)
Medicago arborea 8262	Nursery located at Madrid (Spain)
Medicago arborea 8265	Llafranc (Gerona, Spain)
Medicago arborea 8273	Corse (France)
Medicago arborea 9504	Valencia (Spain)
Medicago arborea 9505	Niza Botanical Garden
Medicago strasseri	Crete
Medicago citrina	Columbretes Islands (Castellón, Spain)

Twelve morphometrical characters of seeds and 20 of seedlings (Figure 1) were selected following the recommendations of Sneath & Sokal (1973). To obtain the seedlings, seeds were scarified with concentrated sulphuric acid (96%) for 30 min, sowed in pots (3 cm diameter, 20 cm deep) with a substrate consisting of 50% washed river sand and 50% commercial loam, and placed in a greenhouse. When seedlings had developed the two cotyledons, the eophyll and three metaphylls, we collected one cotyledon, the eophyll and the first metaphyl from 30 seedlings per accession, following drying in a press. Measurements $\pm 10^{-3}$ mm were carried out using an image analysis system Summagraphics, model MM 1103, connected to an ADI DM computer with a VIDS III program (Analytical Measuring Systems). The weight of 100 seeds was determined to an accuracy of ± 0.1 mg. The Operational Taxonomical Units (OTU) were the accessions. Measurements were taken of 20 seeds and 30 seedlings per accession. Within each accession, the standard deviation was under 12% of the mean.

With all the sample means obtained, two data matrices were prepared for seed data and one for seedling data. The first seed data matrix included all the measured characters, and the second one excluded those characters based on absolute size of seeds, namely area, seed length, thickness, and 100-seed weight. For seedling data only one data matrix, not including characters based on absolute size, was prepared. Matrices were standardized and a multivariate analysis based both on Cluster Analysis (CA) and Principal Component Analysis (PCA) was carried out. In the first case, the similarity matrix was calculated using the average taxonomic distance, Unweighted Pair-Group Method Arithmetic Average (UPGMA) was used for clustering. In the second case, the similarity matrix was based on the product-moment correlation. From this correlation matrix, eigenvalue and eigenvector matrices were obtained. The multivariate analysis was carried out with the assistance of the software package NTSYS-pc (Rohlf, 1990).

Results and discussion

Table 2 shows the results obtained for all the measurements on seeds. The first 4 characters – seed area, seed length, thickness, and 100 seed weight – reached maximum values in *M. citrina* and minimum in *M. strasseri*, while in the *M. arborea* accessions they were intermediate. These four characters depended on the absolute size of the seeds, while the rest of the characters were relations between two measurements, thus not depending on the absolute size. *M. citrina* is hexaploid, whereas the other two species are tetraploid, and the first has larger leaves, fruits, and seeds. Conversely one of the characteristics that differentiates *M. strasseri* from *M. arborea* is that the former has smaller seeds (Greuter et al., 1982). For the remaining characters, the ratio of seed width/seed length was higher in *M. citrina* than in the other two species. The hilum angle was greatest in *M. citrina* and least in *M. strasseri*, whereas for *M. arborea* it was intermediate. The ratio of hilum length/seed length was maximum in *M. strasseri*, whereas the ratio of hilum width/hilum length reached maximum values both for *M. citrina* and *M. strasseri*. Finally, the ratio of hilum area/seed area was highest in *M. citrina*, lowest in *M. strasseri*, and intermediate in *M. arborea*.

Two different multivariate analyses were carried out from seed data, one of them including the 12 metrical characters (Figures 2, 3 and Tables 3, 4), and the

Table 2. Characters scored in seeds. Values are the average of 20 seeds

Character	*M. citrina*	*M. strasseri*	*M. arborea* 9504	*M. arborea* 9505	*M. arborea* 6081	*M. arborea* 7391	*M. arborea* 8262	*M. arborea* 8273	*M. arborea* 8265
Seed area[(a)] (mm^2)	16.74	6.12	8.74	8.19	9.91	9.50	7.77	7.24	8.85
Seed length (mm)	5.39	3.71	4.15	3.85	4.54	4.21	3.95	4.08	4.44
Thickness (mm)	1.74	0.96	1.14	1.19	1.16	1.28	1.03	1.00	0.95
100 seed weight (g)	2.30	0.4457	0.8337	0.779	0.7974	0.937	0.7337	0.5995	0.6268
Form factor[(b)]	0.81	0.75	0.80	0.82	0.77	0.82	0.79	0.73	0.71
Seed width/seed length	0.68	0.51	0.55	0.58	0.52	0.60	0.55	0.49	0.48
Distance hilum-radicle/seed length	0.53	0.54	0.58	0.60	0.56	0.56	0.53	0.55	0.57
Distance lens-radicle/seed length	0.29	0.22	0.25	0.29	0.24	0.25	0.25	0.23	0.3
Hilum angle (°)	133.0	104.6	117.5	126.8	119.4	131.9	117.4	119	118
Hilum length/seed length	0.0631	0.0744	0.0648	0.0631	0.0592	0.0691	0.0640	0.0596	0.0557
Hilum width/hilum length	0.86	0.84	0.75	0.80	0.80	0.69	0.78	0.76	0.81
Hilum area/seed area	0.0062	0.0032	0.0052	0.0043	0.0051	00055	0.0061	0.0053	0.0049

[a] Seed area: Maximum projected area.
[b] Form factor: Form factor of the maximum projected area (4π area)/($perimeter^2$).

Table 3. Eigenvalues, percentages and cumulative variance in Principal Component Analysis of complete seed data matrix

Component	Eigenvalue	% Variance	Cumulative variance
First	6.98	58.14	58.14
Second	2.03	16.91	75.04
Third	1.39	11.58	86.62
Fourth	1.00	8.33	94.95
Fifth	0.34	2.80	97.75
Sixth	0.17	1.38	99.13
Seventh	0.07	0.54	99.68
Eighth	0.04	0.32	100.00
Ninth	>0.00	0.00	100.00
Tenth	>0.00	0.00	100.00
Eleventh	>0.00	0.00	100.00
Twelfth	>0.00	0.00	100.00

Table 4. Eigenvector values on the first three axes in Principal Component Analysis of complete seed data matrix

Character	1st component	2nd component	3rd component
Seed area	0.941	−0.294	0.056
Seed length	0.824	−0.418	0.029
Thickness	0.959	−0.083	0.012
100 seeds weight	0.953	−0.267	−0.010
Form factor	0.619	0.546	−0.034
Seed width/seed length	0.908	0.107	−0.022
Distance hilum-radicle/seed length	−0.180	0.600	0.728
Distance lens-radicle/seed length	0.537	−0.022	0.711
Hilum angle	0.858	0.383	0.106
Hilum length/seed length	0.904	0.360	−0.136
Hilum width/hilum length	0.116	−0.853	0.367
Hilum area/seed area	0.701	0.122	−0.428

Table 5. Eigenvalues, percentages and cumulative variance in Principle Component Analysis of seed data matrix excluding seed area, seed length, thickness and 100 seed weight

Component	Eigenvalue	% Variance	Cumulative variance
First	4.03	50.42	50.42
Second	1.44	17.99	68.41
Third	1.35	16.85	85.26
Fourth	0.87	10.85	96.11
Fifth	0.18	2.21	98.32
Sixth	0.10	1.29	99.61
Seventh	0.03	0.37	99.99
Eighth	0.00	0.01	100.00

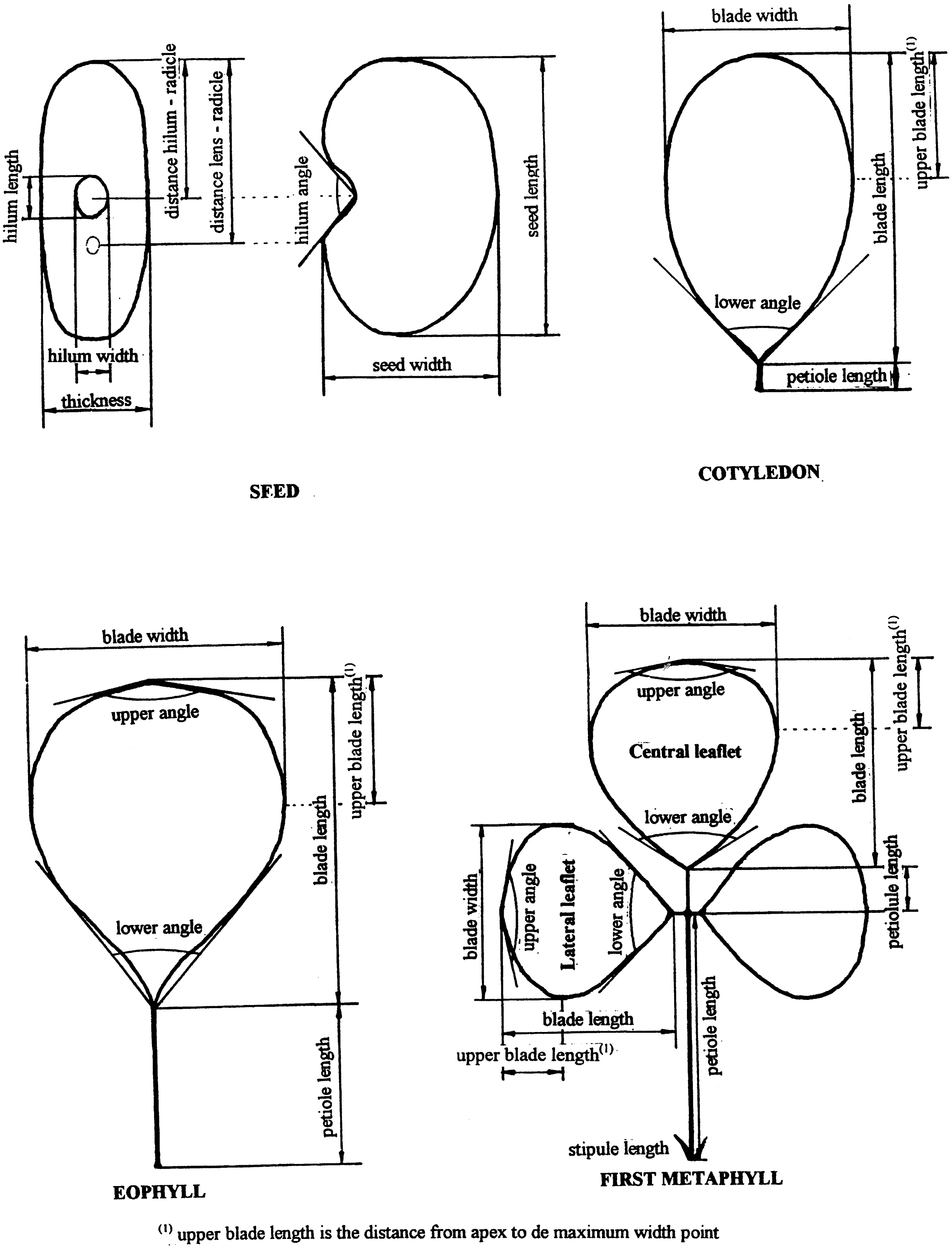

Figure 1. Characters measured.

second excluding the first four characters that depend on absolute size of the seeds and could bias the results (Figures 4, 5 and Tables 5, 6). In the dendrogram obtained after cluster analysis of the complete seed data matrix (Figure 2) all the *M. arborea* accessions were grouped together, with a maximum dissimilarity among the different accession of 1.10. *M. strasseri* was located on a different branch with a dissimilarity of 1.43. Finally *M. citrina* had a dissimilarity of 2.13 with the other two species. The results of the

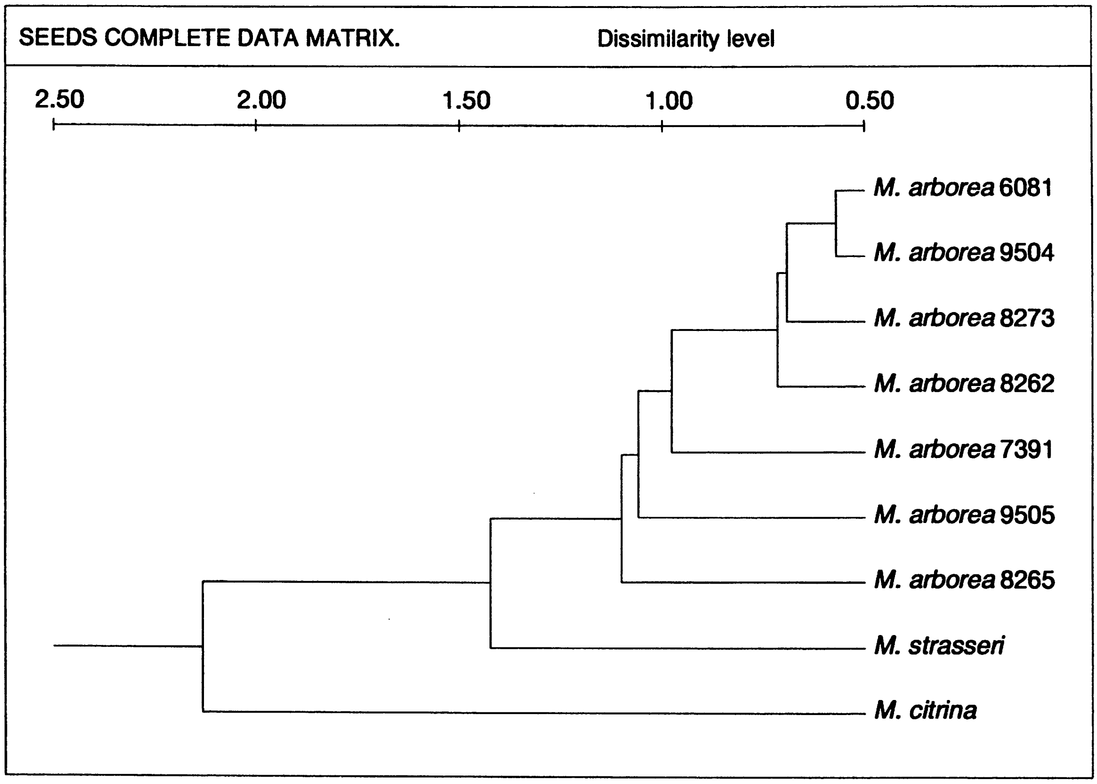

Figure 2. Dendrogram obtained after cluster analysis of the seed complete data matrix (UPGMA method).

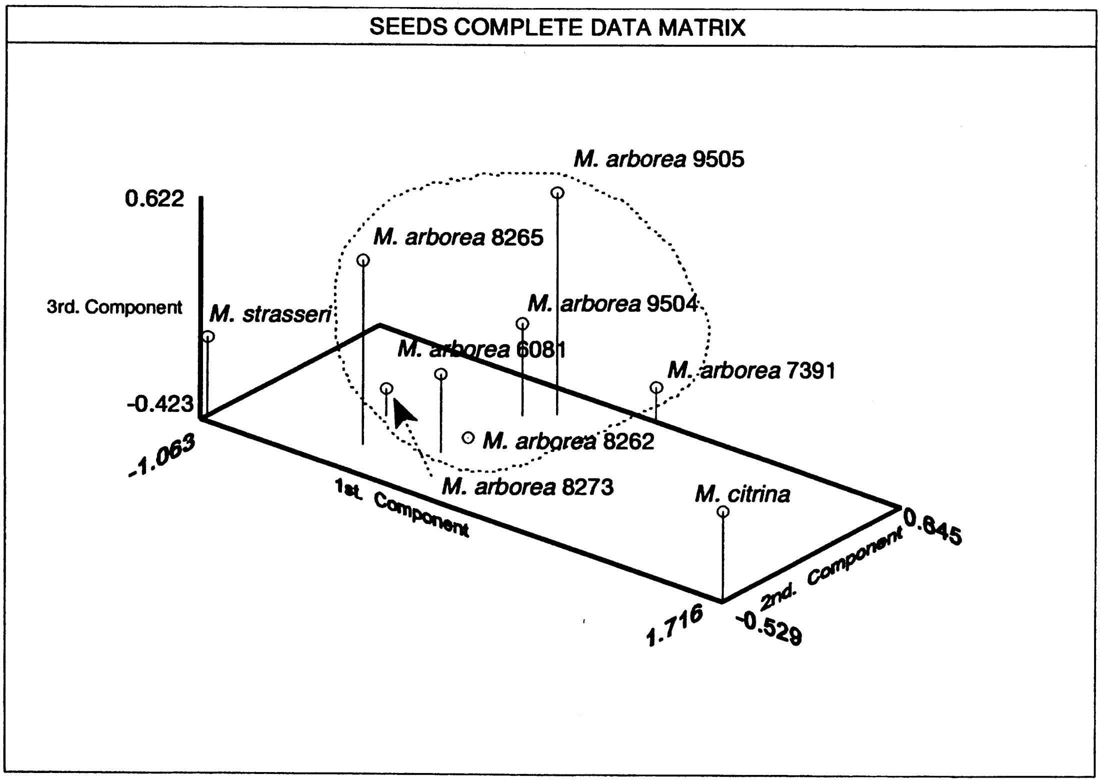

Figure 3. Scatter diagram obtained from the first three principal components after Principal Component Analysis (PCA) of the seed complete data matrix.

Principal Component Analysis (PCA) (Figure 3 and Tables 3, 4) confirmed the results of the dendrogram. The first three components accounted for 58.14%, 16.91% and 11.58% of the variance respectively, and

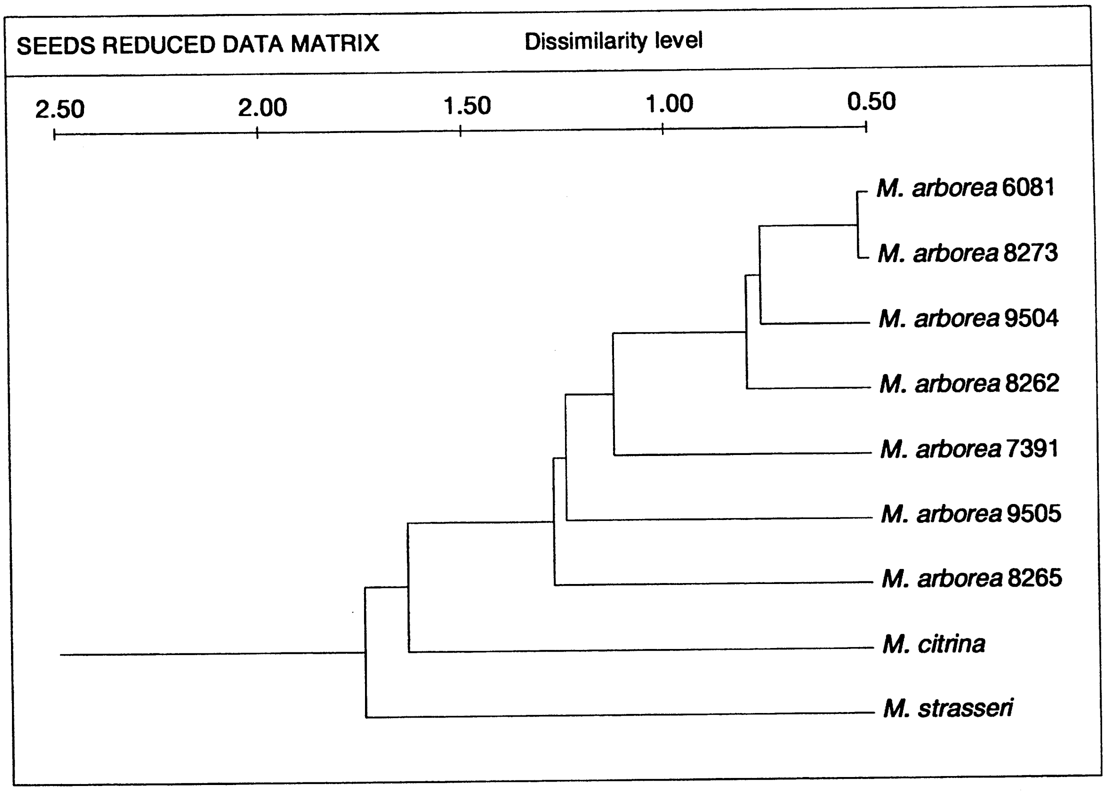

Figure 4. Dendrogram obtained after cluster analysis of the seed data matrix excluding seed area, seed length, thickness, and 100 seeds weight (UPGMA method).

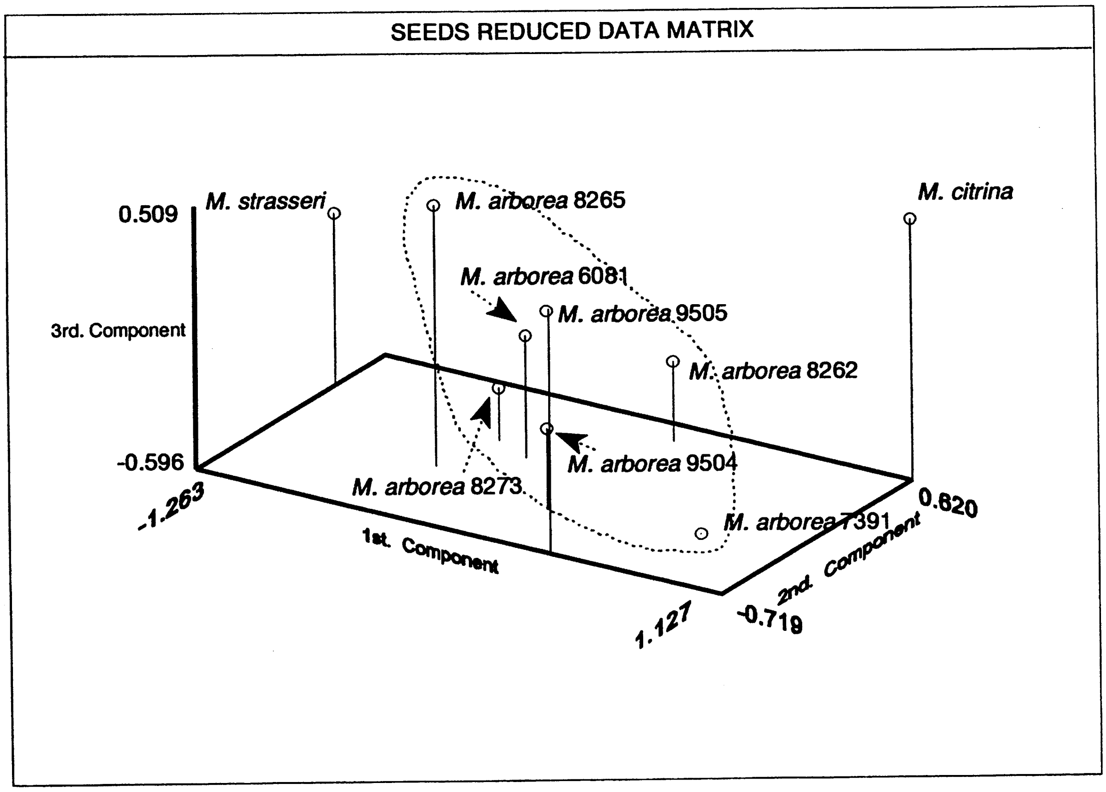

Figure 5. Scatter diagram obtained from the first three principal components after Principal Component Analysis (PCA) of the seed data matrix excluding seed area, seed length, thickness, and 100 seeds weight.

Table 6. Eigenvector values on the first three axes after Principal Component Analysis of seed data matrix excluding seed area, seed length, thickness and 100 seeds weight

Character	1st component	2nd component	3rd component
Form factor	0.772	−0.165	−0.141
Seed width/seed length	0.877	0.196	0.165
Distance hilum-radicle/seed length	0.016	−0.974	0.140
Distance lens-radicle/seed length	0.510	−0.265	0.716
Hilum angle	0.932	−0.139	0.044
Hilum length/seed length	0.979	0.058	−0.103
Hilum width/hilum length	−0.203	0.444	0.836
Hilum area/seed area	0.735	0.367	−0.238

their cumulative variance was 86.62% (Table 3). A scatter diagram representing these three components (Figure 3) showed an intermediate group of *M. arborea* accessions, between those of *M. strasseri* and *M. citrina.* On the basis of the character eigenvector values along the first three factors (Table 4), the three attributes responsible for maximum separation along the first component (with values in parentheses) were the seed area (0.941), the thickness (0.959) and the 100-seed weight (0.953). The three depended on the absolute size of the seed. Along the second component they were the form factor (0.546), the relation distance hilum-radicle/seed length (0.600) and the relation hilum width/hilum length (−0.853); and along the third the relation distance hilum-radicle/seed length (0.728), the relation distance lens-radicle/seed length (0.711), and the relation hilum area/seed area (−0.428).

The dendrogram obtained when the first four seed characters were excluded (Figure 4) differed from the previous one in the relative position of *M. citrina* and *M. strasseri.* In this case *M. citrina* was closer to *M. arborea* (dissimilarity of 1.64) than *M. strasseri* (dissimilarity of 1.75). Again the different accessions of *M. arborea* were grouped together, with a maximum dissimilarity of 1.28 among them. The three-dimensional diagram (Figure 5) was consistent with the dendrogram with *M. arborea* accessions, intermediate between those of *M. strasseri* and *M. citrina.* The first three components accounted for 50.42%, 17.99% and 16.85% respectively of the variance (Table 5). The cumulative variance of the three components was 85.26%. According to the character eigenvector values along the first three factors (Table 6), the three attributes responsible for maximum separation along the first component (with values in parentheses) were the ratio of seed width/seed length (0.877), the hilum angle (0.932) and the ratio of hilum length/seed length (0.979); along the second component the ratio of hilum radicle/seed length (−0.974), the ratio of hilum width/hilum length (0.444) and the ratio of hilum area/seed area (0.367); and along the third the ratio of distance lens-radicle/seed length (0.716), the ratio of hilum width/hilum length (0.836), and the ratio of hilum area/seed area (−0.238).

The multivariate analyses of seed data indicated clear differences among the three taxa. These differences were also described by Chebbi et al. (1995) on the basis of other morphological and molecular characters, but they studied only one population of *M. arborea.* In spite of the great infraspecific variability described for *M. arborea*, in the present study the variability among the different populations of *M. arborea* was always smaller than the variability among the different species. This result did not change when size characters of the seeds were deleted. One important difference among the three species of section *Dendrotelis* is the size of fruits and seeds, larger in *M. citrina*, intermediate in *M. arborea* and smaller in *M. strasseri* (Robledo et al., 1993; Greuter et al., 1982), but the present work indicates other seed differences. When size characters were excluded form the analysis, the results indicated that the seeds of *M. citrina* are more rounded (higher relation seed width/seed length), the seeds of *M. strasseri* have a longer hilum compared with the total length of the seed, and the hilum angle is higher in *M. citrina*, lower in *M. strasseri* and intermediate in *M. arborea.*

Table 7. Characters scored in seedlings. Values are the average of 30 seedlings

Characters	*M. citrina*	*M. strasseri*	*M. arborea* 9504	*M. arborea* 9505	*M. arborea* 6081	*M. arborea* 7391	*M. arborea* 8262	*M. arborea* 8273	*M. arborea* 8265
Cotyledon									
blade width/blade length	0.61	0.50	0.54	0.57	0.53	0.57	0.54	0.50	0.53
petiole length/blade length	0.14	0.20	0.24	0.19	0.21	0.21	0.20	0.16	0.19
upper blade length/blade length	0.39	0.32	0.31	0.33	0.33	0.34	0.33	0.32	0.30
lower angle	58.36	39.47	42.98	47.34	47.96	50.79	50.03	45.73	44.91
Eophyll									
blade width/blade length	0.75	0.80	0.77	0.76	0.78	0.80	0.78	0.88	0.79
petiole length/blade length	0.60	1.03	1.13	1.31	1.03	1.20	1.16	1.05	1.04
upper blade length/blade length	0.34	0.40	0.48	0.46	0.40	0.42	0.48	0.48	0.43
lower angle	71.65	77.69	90.59	96.55	86.04	87.34	94.40	92.15	90.31
upper angle	101.45	79.74	75.70	73.86	86.66	88.54	80.66	72.13	77.97
First metaphyll									
stipule length/petiole length	0.57	0.33	0.33	0.30	0.34	0.34	0.25	0.32	0.31
Central leaflet									
blade width/blade length	0.85	0.88	0.73	0.76	0.77	0.74	0.77	0.84	0.76
petiole length/blade length	0.89	2.11	1.85	2.00	1.84	1.57	2.08	1.84	2.31
petiolule length/petiole length	0.24	0.16	0.18	0.16	0.17	0.19	0.16	0.17	0.19
upper blade length/blade length	0.94	0.94	0.96	0.95	0.91	0.93	0.96	0.95	0.93
lower angle	52.53	54.74	66.40	68.52	61.42	68.17	68.55	64.71	62.82
upper angle	104.65	103.18	98.06	96.51	107.31	105.89	98.04	105.53	107.04
Lateral leaflet									
blade width/blade length	0.91	0.88	0.83	0.89	0.90	0.90	0.85	0.84	0.94
upper blade length/blade length	0.92	0.95	0.96	0.96	0.94	0.96	0.95	0.98	0.96
lower angle	47.47	48.44	63.21	63.56	55.79	60.71	66.16	52.66	57.40
upper angle	105.98	100.29	96.28	94.77	98.93	96.12	95.04	99.93	99.16

Table 7 shows the results obtained for all the measurements in seedlings. None of them depended on absolute size, as they were either angles or relations between two measurements. For most characters, *M. citrina* differed from the other two species. Cotyledon measures were greatest in *M. citrina*, with the lower angle at a minimum in *M. strasseri*. In the eophyll, the ratios of petiole length/blade length and upper length/blade length and the lower angle were minimum in *M. citrina*. The lower angle was maximum in *M. arborea*, whereas in *M. strasseri* it was intermediate. The upper angle was maximum in *M. citrina*. In the first metaphyll, the ratio between stipule length and petiole length was maximum in *M. citrina*, as well as the ratio of petiolule of the central leaflet/petiole length, and the upper angle of the lateral leaflet. Conversely, the ratio of upper length/blade length for the lateral leaflet was minimum in *M. citrina*. The lower angle of the central and lateral leaflets was minimum both for *M. citrina* and *M. strasseri*. Conversely, the relation between blade width and blade length for the central leaflet was maximum in those two species, and finally the relation petiole length/blade length for the central leaflet was minimum in *M. citrina* and maximum in *M. strasseri*.

In the dendrogram obtained after cluster analysis of the seedlings data matrix (Figure 6) *M. strasseri* and the *M. arborea* accessions were intermingled within the same branch, indicating that the intraspecific variability among *M. arborea* seedlings was greater than the interespecific variability with *M. strasseri*. Conversely *M. citrina* had a dissimilarity of 2.2 with the other two species and was located in a different branch. The results of PCA (Figure 7 and Tables 8, 9) confirmed the results of the dendrogram. The first three components accounted for 54.98%, 18.34% and 10.84% of the variance respectively. The cumulative variance of the three components was 84.16% (Table 8). The three-dimensional diagram (Figure 7) showed a group that included all the *M. arborea* accessions plus *M. strasseri*, whereas *M. citrina* was clearly apart. Character eigenvector values along the first three factors (Table 9) showed that the three attributes responsible for maximum separation along the first component (with values in parentheses) were the ratio of petiole length/blade length (−0.912), and the ratio of upper length/blade length (−0.923) for the eophyll, and ratio of stipules length/petiole length (0.951) for the first metaphyll; along the second component the ratio of width/length for the cotyledon blade (0.788), the same ratio for the eophyll (−0.704) and the lower angle in the lateral leaflet of the first metaphyll (0.655); along the third the ratio of upper length/blade length (−0.786), and the upper semiangle (0.442) for the central leaflet of the first metaphylle, and the ratio of width/length for the lateral leaflet blade of the first metaphyll.

The multivariate analysis of seedlings characters indicated large differences between *M. citrina* and *M. strasseri–M. arborea*. The characters responsible for this separation were the shorter petiole of the eophyll relative to the blade length, the longer stipules of the first metaphyll in relation to the petiole, and the shape of the eophyll. In *M. citrina* the maximum width is nearer the apex than in the other two species. On the other hand, it was not possible to differentiate the populations of *M. aborea* from *M. strasseri*.

As a conclusion of this work, the three species were clearly differentiated using morphometrical characters of seeds independent of absolute size. For the seed characters, the intraspecific variability of *M. arborea* was always smaller than the interspecific variability. On the other hand, seedling characters distinguished between *M. citrina* and the other two species, but they were not able to differentiate *M. arborea* from *M. strasseri*. However seeds are better than seedlings for characterization, since the latest show high adaptability to ecological conditions (Duke & Polhill, 1981). In spite of the great variability of *M. arborea*, morphological differences in seeds and seedlings exist among *M. arborea*, *M. strasseri* and *M. citrina*, which are consistent with agronomical, ecological and bromatological differences (Alegre et al., 1993b; Sibole et al., 1994; González-Andrés & Ceresuela, 1998). The study supports that the three accessions merit to be considered as three different species.

The three species could be identified on the basis of the following seeds and seedlings characters:

Based on seed characters

1.1 Large seeds (area over 16 mm^2; 100 seed weight over 2 g) = *Medicago citrina*

1.2 Smaller seeds (area under 10 mm^2; 100 seed weight under 1 g.)

1.2.1 Longer hilum compared with the total length of the seed (ratio of hilum length/seed length around 0.0744) and lower hilum angle (around 105°) = *Medicago strasseri*

1.2.2 Shorter hilum compared with the total length of the seed (ratio of hilum length/seed length un-

Table 8. Eigenvalues, percentages and cumulative variance in Principal Component Analysis of seedling data matrix

Component	Eigenvalue	% Variance	Cumulative variance
First	11.00	54.98	54.98
Second	3.67	18.34	73.32
Third	2.17	10.84	84.16
Fourth	1.44	7.19	91.35
Fifth	0.72	3.61	94.96
Sixth	0.58	2.89	97.85
Seventh	0.26	1.31	99.16
Eighth	0.17	0.84	100.00
Ninth	>0.00	0.00	100.00
Tenth	>0.00	0.00	100.00
Eleventh	>0.00	0.00	100.00
Twelfth	>0.00	0.00	100.00
Thirteenth	>0.00	0.00	100.00
Fourteenth	>0.00	0.00	100.00
Eighteenth	>0.00	0.00	100.00
Nineteenth	>0.00	0.00	100.00
Twentieth	>0.00	0.00	100.00

Table 9. Eignevector values on the first three axes in Principal Component Analysis of seedling data matrix

Character	1st component	2nd component	3rd component
Cotyledon: Blade width/blade length	0.567	0.788	−0.007
Cotyledon: Petiole length/blade length	−0.636	0.205	0.423
Cotyledon: Upper length/blade length	0.835	0.371	−0.243
Cotyledon: Lower semi-angle	0.659	0.564	−0.130
Eophyll: Blade width/blade length	−0.311	−0.704	−0.313
Eophyll: Petiole length/blade length	−0.912	0.175	0.174
Eophyll: Upper blade length/blade length	−0.923	0.078	−0.329
Eophyll: Lower angle	−0.893	0.231	−0.031
Eophyll: Upper angle	0.892	0.291	0.167
First metaphyll: Stipules length/petiole length	0.951	0.110	−0.154
First metaphyll central leaflet: Blade width/blade length	0.520	−0.651	−0.404
First metaphyll central leaflet: Petiole length/blade length	−0.812	−0.326	0.278
First metaphyll central leaflet: Petiolule length/petiole length	0.850	0.250	−0.058
First metaphyll central leaflet: Upper blade length/blade length	−0.318	0.342	−0.786
First metaphyll central leaflet: Lower angle	−0.818	0.438	−0.001
First metaphyll central leaflet: Upper angle	0.462	−0.547	0.442
First metaphyll lateral leaflet: Blade width/ blade length	0.488	0.005	0.698
First metaphyll lateral leaflet: Upper blade length/blade length	−0.810	−0.310	−0.219
First metaphyll lateral leaflet: Lower angle	−0.736	0.655	0.086
First metaphyll lateral leaflet: Upper angle	0.890	−0.378	−0.152

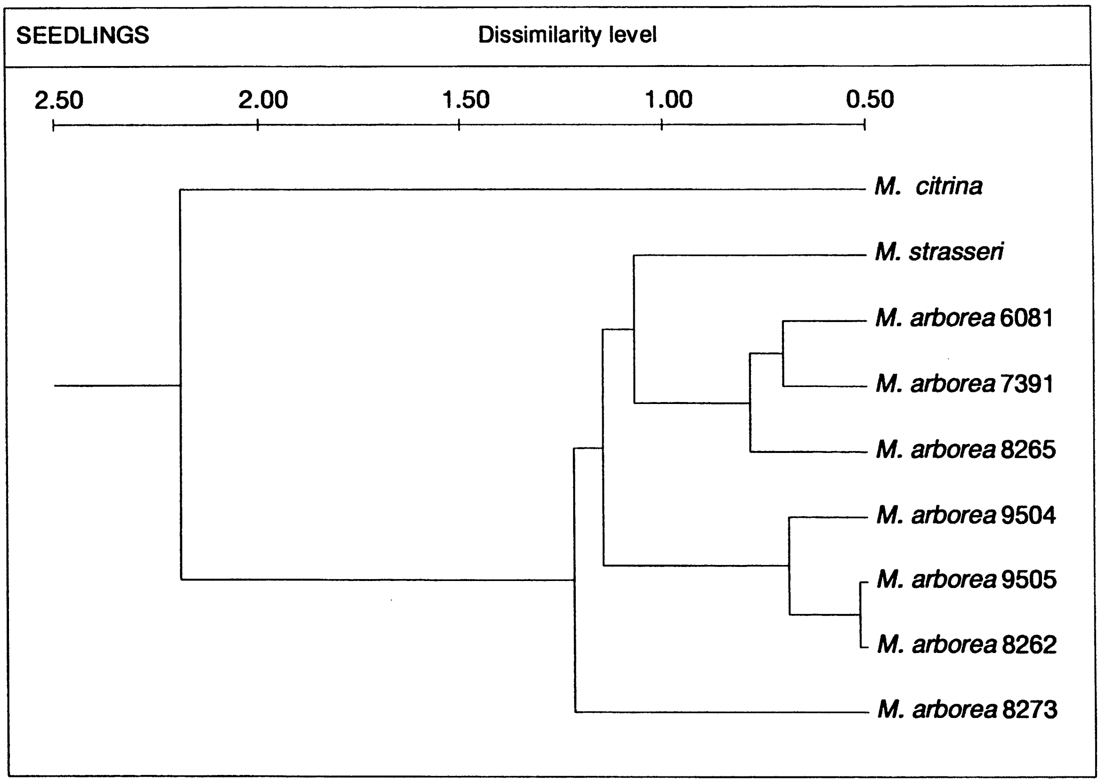

Figure 6. Dendrogram obtained after cluster analysis of the seedling data matrix (UPGMA method).

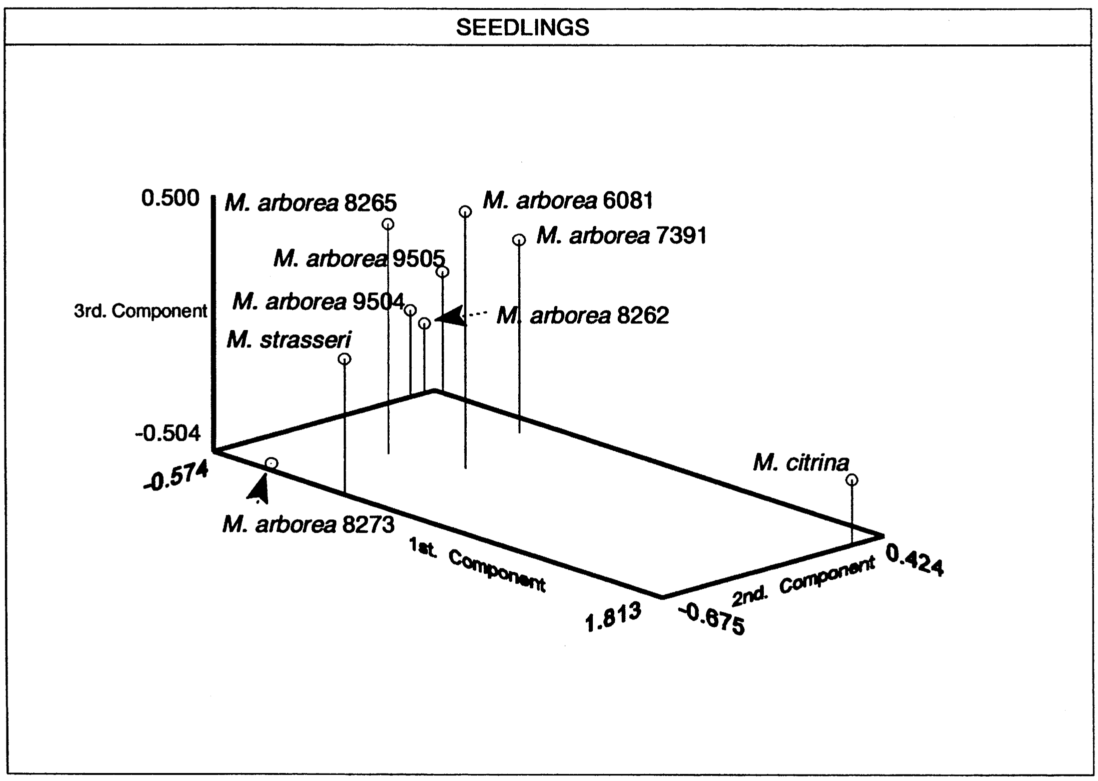

Figure 7. Scatter diagram obtained from the first three principal components after Principal Component Analysis (PCA) of the seedling data matrix.

der 0.0691) and higher hilum angle (over 117°) = *Medicago arborea*

Based on seedlings characters

2.1 The eophyll has a shorter petiole relative to the blade length (ratio of petiole length/blade length considerably lower than 1.00), and its maximum width is near the apex (ratio of upper blade length/blade length considerably lower than 0.50). The first metaphyll has long stipules relative to the petiole (ratio of stipule length/petiole length around 0.50) = *Medicago citrina.*

2.2 The eophyll has a longer petiole relative to the blade length (ratio of petiole length/blade length over 1.00), and its maximum width is farther the apex (ratio of upper blade length/blade around 0.50). The first metaphyll has long stipules relative to the petiole (ratio of stipule length/petiole length considerably lower than 0.50) = *Medicago arborea* or *Medicago strasseri.*

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