

## GENERATIVE PROPAGATION IN MAMMILLARIA IN ORDER TO OBTAIN ORNAMENTAL PLANTS

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### Abstract

This research evaluated the different products as growth media influence on some *Mammillaria* species propagation, in order to ornamental plants obtained. Some *Mammillaria* species were tested: *Mammillaria elongata* - *M.el*; *Mammillaria mazatlanensis* - *M.ma*; *Mammillaria neomystax* - *M.ne*; *Mammillaria obconella* - *M.ob*; *Mammillaria prolifera* - *M.pr*; *Mammillaria spinigemmatus* - *M.sp*; *Mammillaria blanckii* - *M.bl*. Different substances, as growth media, were used: sand - *San*; garden soil - *GS*; perlite - *Per*; peat - *Pea*; hydroculture - *HydC*; agar-based medium - *InVitro*. Based on these, resulted the experimental variants: *San+GS* - *V1*; *Per+GS* - *V2*; *Pea* - *V3*; *San+Pea* - *V4*; *HydC* - *V5*; *InVitro* - *V6*. *In Vitro* method (*V6*) provided the best spread rate for most *Mammillaria* species tested, but *Mammillaria neomystax* recorded the best result in the *V5* variant. Some *Mammillaria* species had good propagation rate in more growth media (eg. *Mammillaria neomystax* in *V1* - *San+GS*; *V4* - *San+Pea*; *V5* - *HydC*), and others only in a growth media (eg. *Mammillaria spinigemmatus* in *V6* - *In Vitro*). Based on Principal Component Analysis, 56.171% of variance was explained by *PC1*, and 25.00% of variance was explained by *PC2*. Cluster analysis, based on Euclidean distances, facilitated the grouping of the studied *Mammillaria* species, in conditions of statistical safety (*Coph.corr.* = 0.857). *Mammillaria mazatlanensis* (*M.ma*) and *Mammillaria prolifera* (*M.pr*) species, showed the highest degree of similarity, *SDI* = 2.027.

**Key words:** Cluster analysis, *Mammillaria*, PCA, propagation, similarity indices

### INTRODUCTION

Cactaceae include different xerophytes and epiphytic plants species (more than 125 genera, and about 2000 species), and many species presented interest in being studied [20], [35], [34].

In the cactus group, *Mammillaria* represent a rich and representative genre. The *Mammillaria* group includes a large number of species (it is estimated between 140 - 180 species, or even more), in relation to the analysis and evaluation level [41], [23], [58], [6]. Various studies and researches have contributed to the identification, classification and analysis of new species of cacti, as well as to the evaluation of the ecophysiology of some species, or of their niche locations [22], [25], [31].

Cacti, as plants with specific ecophysiology, grow in different places in terms of ecological conditions, in different microhabitats and have

the capability to colonize highly diverse areas [15], [30], [32], [52]. Cacti have an important functional role in natural ecosystems remediation [53].

The economical importance of cacti can be appreciated by their potential and use in the pharmaceutical industry and medicine [51], [14], food industry [11], [10], [57], as animal feed [2], [3], energy source [12], [5], as friendly dye [1], [18], and possibly others [24].

Cacti are a group of plants with a very high potential for arid and semi-arid, non-irrigated areas, and with a large number of uses, cacti have a huge potential to be a plant resource and even the food of the future [51].

At the same time, it is appreciated that there are areas around the world that are sensitive and prone to invasive species of cacti, such as Central Africa, East Asia and China [35], [36]. Techniques based on imaging and remote sensing, have facilitated studies at

different scales, to approach plants and the vegetal cover, in terms of individual and spatiotemporal variability [49], [50], different areas that included cactaceae being also the subject of such studies [9], [17], [29].

Cacti, however, are also important in the decorative aspect, being a special group of plants within the ornamental horticultural plants [4], [56], [13], [8], [35], [37]. *Mammillaria sp.* has a high weight in the group of ornamental cacti for the market, but also interest for the pharmaceutical industry [45]. Cacti have also been studied in the context of strategic management plans, including the tourist potential [26], tourism being approached in different studies from socioeconomic and cultural perspectives [42], [43], [44].

For both, ornamental plants and for interest in the pharmaceutical field, to different extracts obtaining, cacti proliferation in controlled conditions is of actuality. Different plant propagation techniques have also been used in the case of cactus multiplications [19], [28], [7], [27]. "In vitro" multiplication techniques generally have a higher success rate on plant propagation, aspects also reported in cacti by some studies [40], [27]. Various other studies and research have been conducted in order to

improve techniques and methods of vegetative or generative plant propagation, through the use of nanoparticle treatments [48], by chemical or physical scarification of seeds [16], microwave treatments [54 ], or with certain bioactive substances [39].

In the presented context, this study evaluated the influence of different growth methods and substrates on the process of generative multiplication in several *Mammillaria* species, in order to obtain decorative plants.

## MATERIALS AND METHODS

The study analyzed and compared the influence of some growth substrates and propagation methods, on generative multiplication in several *Mammillaria* species. Seven *Mammillaria* species represented the biological material: *Mammillaria elongata* - M.el; *Mammillaria mazatlanensis* - M.ma; *Mammillaria neomystax* - M.ne; *Mammillaria obconella* - M.ob; *Mammillaria prolifera* - M.pr; *Mammillaria spinigemmatum* - M.sp; *Mammillaria blanckii* - M.bl, Figure 1 (the abbreviations: M.el, M.ma, M.ne, M.ob, M.pr, M.sp, and M.bl, have only experimental significance in this study).



*Mammillaria elongata*



*Mammillaria neomystax*



*Mammillaria obconella*



*Mammillaria blanckii*

Fig. 1. Some images with *Mammillaria* species  
Sources: selective photos for species studied.

Different components, alone or in a mixture, represented the growth substrates: sand - San; garden soil - GS; perlite - Per; peat - Pea; sand and nutrient solution for hydroculture - HydC; "In Vitro" technique with specific media - InVitro. From the combination of components, the following experimental variants resulted: San+GS - V1, Per+GS, Pea - V3, San+Pea - V4, HydC - V5, InVitro - V6. A control variant (Ct) as experience average for each species was considered. The number of seedlings, resulting for each species studied, was analyzed in accordance with the growth substrate considered.

The ANOVA test was used for the general analysis of the experimental data. Additionally, Variance analysis, Principal Component Analysis, and Cluster analysis were used. To assess and quantify the significance of the differences recorded, the limits of significance of differences (LSD) were calculated for 5%, 1% and 0.1% respectively. In addition, the Cophenetic coefficient as well as the Similarity and Distances Indices (SDI) were used in order to evaluate and interpret the safety of the results. PAST software [21], was used for the statistical processing of experimental data.

## RESULTS AND DISCUSSIONS

The multiplication of each species of *Mammillaria* was tested under the same conditions as the growing substrates.

In the case of *Mammillaria elongata* (M.el) the V6 variant provided the best multiplication rate (13.00 plants, average value), compared to V2 variant, that provided 9.66 plants (average value), Table 1. The analysis of the experimental data, in the case of this species, confirmed the statistical safety of the differences (LSD<sub>1%</sub>), in the case of the San+GS (V1), HydC (V5) and InVitro (V6) variants.

In the case of *Mammillaria mazatlanensis* (M.ma) were obtained 9.00 plants on the San+Pea substrate (V4), and 14.00 plants on the InVitro conditions (V6), Table 2. Obtained results analysis, evidenced the differences between variants, in conditions of statistical safety for variant V5 - HydC (LSD<sub>5%</sub>), for

variant V3 - Pea (LSD<sub>1%</sub>), and for variant V6 - InVitro (LSD<sub>0.1%</sub>).

Table 1. Number of plants resulting on experimental variants at *Mammillaria elongata*

Trial	Experimental variant	Average value	Relative value	Differences
V1	San+GS	12.66	129.71	2.90**
V2	Per+GS	9.66	98.97	-0.10
V3	Pea	11.00	112.70	1.24
V4	San+Pea	9.33	95.59	-0.43
V5	HydC	12.66	129.71	2.90**
V6	InVitro	13.00	133.19	3.24**
V7	Ct	9.76	100.00	-
LSD	LSD <sub>5%</sub> = 1.67; LSD <sub>1%</sub> = 2.34; LSD <sub>0.1%</sub> = 3.31			

Source: original data, resulting from own experiences.

Table 2. Number of plants resulting on experimental variants at *Mammillaria mazatlanensis*

Trial	Experimental variant	Average value	Relative value	Differences
V1	San+GS	10.00	105.04	0.48
V2	Per+GS	9.66	101.47	0.14
V3	Pea	12.33	129.52	2.81**
V4	San+Pea	9.00	94.54	-0.52
V5	HydC	11.66	122.48	2.14*
V6	InVitro	14.00	147.06	4.48***
V7	Ct	9.52	100.00	-
LSD	LSD <sub>5%</sub> = 1.91; LSD <sub>1%</sub> = 2.68; LSD <sub>0.1%</sub> = 3.79			

Source: original data, resulting from own experiences.

For the *Mammillaria neomystax* specie (M.ne) the number of plants obtained varied depending on the variants, between 9.66 plants in San+Pea (V4), and 13.66 plants at the HydC variant (V5), Table 3. The analysis of the experimental results evidenced the presence of differences and statistical safety, in the case of Pea (V3) variant (LSD<sub>5%</sub>), at the San+GS (V1), San+Pea (V4) and InVitro (V6) variants (LSD<sub>1%</sub>), and in the HydC variant (V5) for LSD<sub>0.1%</sub>.

Table 3. Number of plants resulting on experimental variants at *Mammillaria neomystax*

Trial	Experimental variant	Average value	Relative value	Differences
V1	San+GS	13.33	123.89	2.57**
V2	Per+GS	9.66	89.78	-1.10
V3	Pea	12.66	117.66	1.90*
V4	San+Pea	13.00	120.82	2.24**
V5	HydC	13.66	126.95	2.90***
V6	InVitro	13.00	120.82	2.24**
V7	Ct	10.76	100.00	-
LSD	LSD <sub>5%</sub> = 1.44; LSD <sub>1%</sub> = 2.02; LSD <sub>0.1%</sub> = 2.86			

Source: original data, resulting from own experiences.

For the *Mammillaria obconella* specie (M.ob), were obtained 7.33 plants at San+Pea (V4)

variant, and 14.00 plants at InVitro (V6) variant, with intermediate values for the other variants, Table 4. Analysis of the obtained results, confirmed the existence of negative differences, with statistical significance in conditions of LSD<sub>1%</sub> (San+Pea, V4), and of positive differences, with statistical significance in conditions of LSD<sub>5%</sub> (HydC, V5), and for LSD<sub>0.1%</sub> (Pea, V3; InVitro, V6).

Table 4. Number of plants resulting on experimental variants at *Mammillaria obconella*

Trial	Experimental variant	Average value	Relative value	Differences
V1	San+GS	10.00	106.16	-0.58
V2	Per+GS	10.33	109.66	0.91
V3	Pea	13.33	141.51	3.91***
V4	San+Pea	7.33	77.81	-2.09 <sup>oo</sup>
V5	HydC	11.00	116.77	1.58*
V6	InVitro	14.00	148.62	4.58***
V7	Ct	9.42	100.00	-
LSD	LSD <sub>5%</sub> = 1.51; LSD <sub>1%</sub> = 1.61; LSD <sub>0.1%</sub> = 2.28			

Source: original data, resulting from own experiences.

In the *Mammillaria prolifera* specie (M.pr), the values recorded were 9.00 plants at San + GS (V1), and 13.00 plants at InVitro (V6), and for the other variants in this interval, Table 5. Analysis of the experimental data, evidenced positive differences, in condition of statistical safety, for LSD<sub>1%</sub> (Pea, V3), and for LSD<sub>0.1%</sub> (InVitro, V6).

Table 5. Number of plants resulting on experimental variants at *Mammillaria prolifera*

Trial	Experimental variant	Average value	Relative value	Differences
V1	SanGS	9.00	97.94	-0.19
V2	PerGS	9.66	105.19	0.47
V3	Pea	12.00	130.58	2.81**
V4	SanPea	10.00	108.81	0.81
V5	HydC	10.66	115.99	1.47
V6	InVitro	13.00	141.46	3.81***
V7	Ct	9.19	100.00	-
LSD	LSD <sub>5%</sub> = 1.50; LSD <sub>1%</sub> = 2.11; LSD <sub>0.1%</sub> = 2.98			

Source: original data, resulting from own experiences.

At *Mammillaria spinigemmatum* specie, was registered 8.66 plants at San+Pea (V4) variant, and 14.33 plants at InVitro (V6) variant; for the others variants the results ranged between these values, table 6. Statistical analysis of the data, showed differences compared to the experience average, statistically assured for LSD<sub>0.1%</sub> for San+GS (V1) and InVitro (V6) variants.

Table 6. Number of plants resulting on experimental variants at *Mammillaria spinigemmatum*

Trial	Experimental variant	Average value	Relative value	Differences
V1	San+GS	12.66	135.69	3.33***
V2	Per+GS	10.00	107.18	0.67
V3	Pea	9.66	103.54	0.33
V4	San+Pea	8.66	92.82	-0.67
V5	HydC	10.00	107.18	0.67
V6	InVitro	14.33	153.59	5.00***
V7	Ct	9.33	100.00	-
LSD	LSD <sub>5%</sub> = 1.56; LSD <sub>1%</sub> = 2.19; LSD <sub>0.1%</sub> = 3.09			

Source: original data, resulting from own experiences.

At *Mammillaria blanckii* specie (M.bl), was recorded 6.66 plants in San+Pea (V4) variant and 14.00 plants in InVitro (V6) variant, and for other variants the plants number obtained were between these values, Table 7. The statistical analysis of the experimental data showed negative differences, which presented statistical safety for LSD<sub>0.1%</sub>, San+Pea (V4) variant. There were also positive differences who presented safety for LSD<sub>5%</sub> in Per+GS (V2) variant, for LSD<sub>1%</sub> in San+GS (V1) variant, and for LSD<sub>0.1%</sub> in Pea (V3) variant, and InVitro (V6) variant respectively.

Table 7. Number of plants resulting on experimental variants at *Mammillaria blanckii*

Trial	Experimental variant	Average value	Relative value	Differences
V1	SanGS	12.00	125.00	2.40**
V2	PerGS	11.00	114.50	1.40*
V3	Pea	13.00	135.42	3.40***
V4	SanPea	6.66	69.37	-2.94 <sup>oo</sup>
V5	HydC	10.66	111.04	1.06
V6	InVitro	14.00	145.83	4.40***
V7	Ct	9.60	100.00	-
LSD	LSD <sub>5%</sub> = 1.38; LSD <sub>1%</sub> = 1.93; LSD <sub>0.1%</sub> = 2.73			

Source: original data, resulting from own experiences.

The analysis of the whole experimental data set by the ANOVA test (Alpha = 0.001), confirmed the presence of variance and data safety (Fcrit < F; p < 0.001).

The overall analysis of the data showed that the InVitro variant (V6) was the best method of propagation to the tested *Mammillaria* species, except for the species *Mammillaria neomystax* (M.ne), in which case, the HydC variant (V5) gave the better results. Analyzing the response of each species of *Mammillaria* to the method and substrate for propagation, it was found that the species *Mammillaria neomystax* (M.ne) recorded the best values in three of the growth substrates, San+GS (V1),

San+Pea (V4), and HydC (V5).

The species *Mammillaria obconella* (M.ob) had very good results on two growth substrates Per+GS (V2) and Pea (V3), and

followed the *Mammillaria spinigemmatum* specie (M.sp), which had very good results only in InVitro variant (V6), Figure 2.

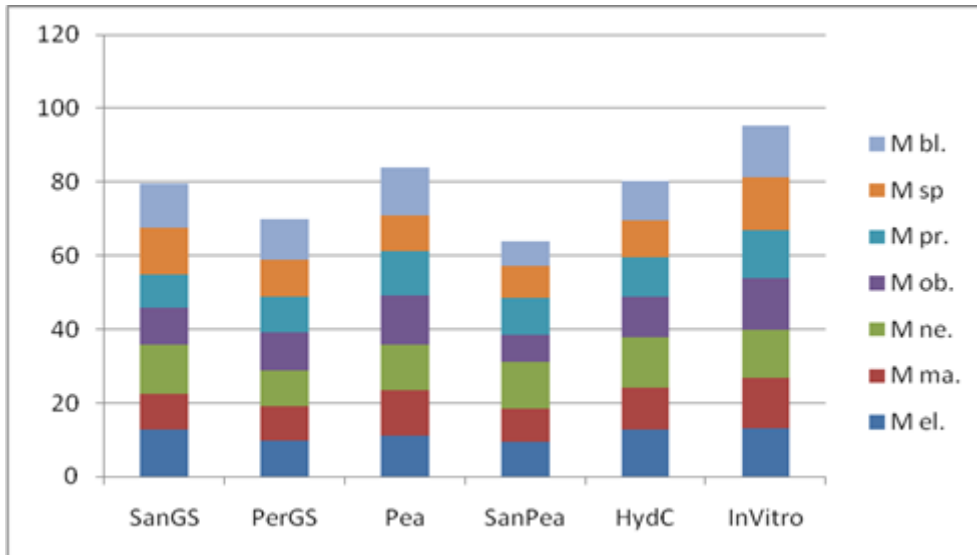


Fig. 2. Graphical distribution of the cumulative effect of propagation substrate at *Mammillaria* species  
 Source: original graph based on own experimental data.

The diagram shows the orientation and placement of the tested species, according to the response generated to the propagation media (San+GS, Per+GS, Pea, San+Pea, HydC, and InVitro). According to the PCA analysis diagram, 56.171% of the variance was explained by PC1, and 25.00% of the variance was explained by PC2.

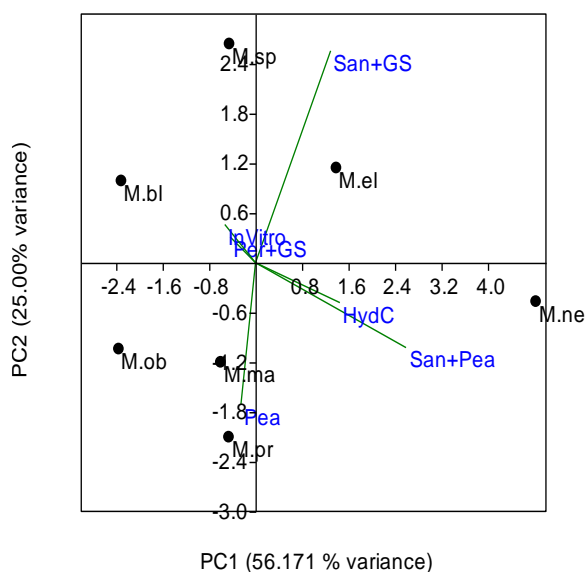


Fig. 3. PCA diagram regarding the spatial distribution of *Mammillaria* species in relation to growth substrate  
 Source: original graph based on own experimental data.

Cluster analysis led to the grouping of *Mammillaria* species, based on Euclidean distances, under statistical assurance conditions (Coph.corr. = 0.857), Figure 4.

The studied *Mammillaria* species (M.el; M.ma; M.ne; M.ob; M.pr; M.sp; M.bl) occupied positions in the dendrogram based on the similarity of the response to the propagation substrates.

The species *Mammillaria neomystax* (M.ne) occupied an independent position (cluster C1), based on the very good response to three propagation substrates, San + GS (V1), San + Pea (V4) and HydC (V5). Cluster C2 comprises three subclusters, C2-1 and C2-2 with common root, and subcluster C2-3.

Subcluster C2-1 grouped the species *Mammillaria mazatlanensis* (M.ma) and *Mammillaria prolifera* (M.pr), species that, according to similarity and distances indices (SDI), showed the highest degree of similarity, respectively affinity, SDI = 2.027. Subcluster C2-2 grouped the species *Mammillaria obconella* (M.ob) with *Mammillaria blanckii* (M.bl), for which the similarity and distances index (SDI) had the value SDI = 2.2632.

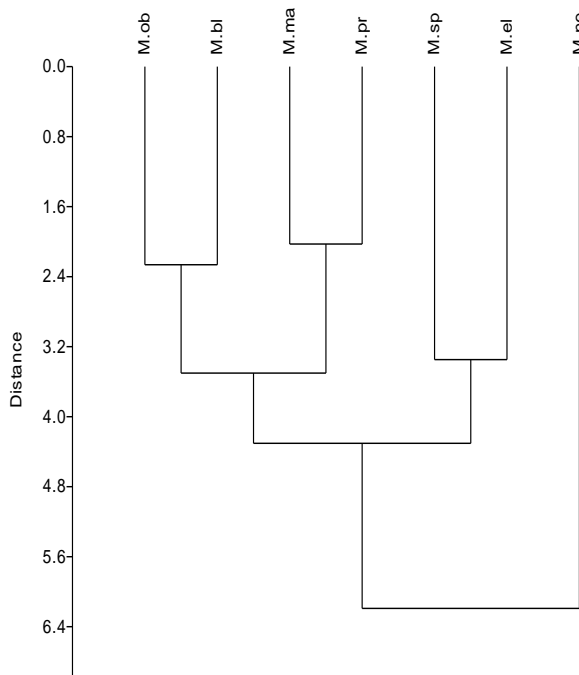


Fig. 4. Clustering of *Mammillaria* species, based on Euclidean distances  
 Source: original graph based on own experimental data.

Subcluster C2-3, grouped the species *Mammillaria spinigemmatum* (M.sp) and *Mammillaria elongata* (M.el), for which the similarity and distances index (SDI) had the value  $SDI = 3.3473$ .

The set of values, for SDI, associated with the values obtained on the experimental variants for *Mammillaria* species, are presented in Table 8.

Table 8. Similarity and distances indices for *Mammillaria* species in relation to propagation media

	M.el	M.ma	M.ne	M.ob	M.pr	M.sp	M.bl
M.el		3.3096	4.2040	4.5507	4.3410	3.3473	4.2849
M.ma	3.3096		5.6743	2.1618	2.0270	4.1594	3.5665
M.ne	4.2040	5.6743		7.2257	6.0979	6.6003	7.3400
M.ob	4.5507	2.1618	7.2257		3.3856	4.8509	2.2632
M.pr	4.3410	2.0270	6.0979	3.3856		4.7945	4.8940
M.sp	3.3473	4.1594	6.6003	4.8509	4.7945		4.1395
M.bl	4.2849	3.5665	7.3400	2.2632	4.8940	4.1395	

Source: original data resulted from our experiments.

In vitro propagation in *Mammillaria* has been used in many studies on MS environments with different supplements (BA, 2iP, NAA, sucrose, etc.), being the method with the highest success rate [38], [45], [46], [19], [33], [27].

Other alternative propagation media, such as sand, perlite, peat or other simple components or in different mixtures, are more accessible, do not require costly techniques and have been used in some *Mammillaria* species propagation studies, and have a good enough success rate [55], [47].

## CONCLUSIONS

The "In Vitro" propagation method (V6 variant) represented the safest multiplication method for the tested *Mammillaria* species. The other propagating variants, based on different substrates, were positioned in descending order as follows: HydC > San+GS > Pea > San+Pea > Per+GS.

*Mammillaria neomystax* (M.ne) was the species with the best response to three of the multiplication variants tested, San+GS (V1), San+Pea (V4), HydC (V5). With good results in two variants Per + GS (V2), and Pea (V3) was placed *Mammillaria obconella* (M.ob), followed by *Mammillaria spinigemmatum* (M.sp) in the InVitro variant (V6).

Principal Component Analysis and Cluster analysis explained the source of variance, in the data set, and facilitated the grouping of variants in relation to specific results.

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