

Variability of Seed Traits in Interspecific and Intergeneric Combinations between Different Genotypes of *Cactaceae*

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Abstract

Based on aesthetic consideration of cacti plants and germination capacity of the pollen grains (more than 30%), 19 genotypes of cacti (including *Aylostera narvaecensis*, *A. buiningiana*, *Rebutia kupperiana* var. *spiniflorum*, *R. donaldiana*) have been used in cyclic cross-pollination pattern. Fruits and seeds obtained from 24 interspecific and intergeneric combinations were analyzed by studying their main traits. The highest weight was registered for fruit belonging to *R. pseudodeminuta* var. *schumaniana* x *R. senilis* (23.3 mg) and *A. buiningiana* x *A. vallegardensis* (20.4 mg). Coefficient of variability for fruit weight had large amplitude, between 12.3% (*A. spinosissima* x *A. albiflora*) and 98.4% (*A. favistyla* x *A. archibuinigiana*). The fruits belonging to *A. muscula* x *A. vallegardensis* were registered with the highest value of number of seeds/fruit (93.2). In the cross between genera, the greatest value of fruits weight's mean was registered on *R. senilis* x *A. archibuinigiana* (29.2 mg) and *R. cajasensis* x *A. muscula* (23.1 mg). Peculiarities of the fruits and seeds resulted from intergeneric combinations presented closer connections than interspecific ones. The phenotypic traits of fruits and seeds did not influence the seeds germination, but germination was clearly influenced by compatibility between genitors.

Keywords: *Cactaceae*, interspecific hybridization, intergeneric hybridization, fruits, seeds

Introduction

The *Cactaceae* family contains 1500-1800 species, distributed in both Americas, from southern Patagonia to Canada (Barthlott and Hunt, 1993). This family was traditionally divided into three subfamilies: the *Pereskioideae*, the *Opuntioideae*, and the *Cactoideae*, but Wallace (1995), Nyffeler (2002), and Anderson (2005) suggested a fourth monogeneric subfamily, *Maihuenioideae*.

The monophyletic subfamily, *Cactoideae* contains 80% of the described species (Nyffeler, 2002).

The greatest diversity of the *Cactaceae* family has been recorded in Mexico, with 586 species, followed by Brazil, Argentina, Bolivia, and Peru (Ramawat, 2010).

The goals of cacti breeding research are to understand their interesting and useful traits, to use this understanding in order to develop new varieties that are better suited for human needs. Improvements of cacti varieties fall into three main categories: expansion of the production area on new types of environments, improvement of cacti quality and productivity for expansion into new markets, and addition of new traits to allow the development of new uses for cacti (Nobel, 2002).

Cacti are well adapted to the arid and semiarid regions where food and fodder crops are limited, although minimum temperatures substantially limit the growing range

in some areas (Wang *et al.*, 1997). Thus, a primary breeding goal should be the expansion of the production area for cacti by producing cold-tolerant cultivars. Current high-yielding fruit, vegetable, and forage varieties do not survive between temperatures of -5°C and -8°C (Loik and Nobel, 1991; Parsih and Felker, 1995; Wang *et al.*, 1997).

Cacti are threatened by loss and degradation of habitat, and illegal collection (Oldfield, 1997; Boyle and Anderson, 2002). The most important factor, causing biodiversity loss, is the intensive land-use (Zak *et al.*, 2004). The “Red List” of threatened species of the International Union for the Conservation of Nature (IUCN) provides a categorization of species based on the relative risk of extinction at global scale. It includes at least 104 cacti species (7% of all species) as vulnerable to extinction (IUCN, 2008). Therefore, this study has the aim to identify potential genitors for breeding programs and to provoke the artificial variability, which will permit an efficient selection of new cultivars.

Hybridization experiments by Hentzschel and Hentzschel (2001) with *Sulcorebutia*, *Weingartia*, *Rebutia* and *Echinopsis* have produced the following results: most *Sulcorebutia* species can hybridize with each other; hybridization failed between *Sulcorebutia* and *Rebutia* of the *Rebutia padcayensis* group, which closely resembles *Sulcorebutia*, and with several other *Rebutia* species it was also not pos-

sible to produce an F₁ generation. Also hybridization between species like *Aylostera densiseta*, *Aylostera roseiaurata*, *Aylostera brunescens*, *Aylostera fiebrigii*, *Aylostera muscula*, *Aylostera pseudodeminuta*, *Aylostera kupperiana*, *Aylostera kieslingii* and species from *Rebutia* genus can succeed because they are very close genetically (Mihalte et al., 2008).

The majority of the studies regarding cacti hybridization have been restricted to the *Opuntioideae* subfamily. Little is known about the role of hybridization on the more diverse subfamily, *Cactoideae* (Machado, 2008). This is the aim which enlivens the present research.

In this experiment the *Aylostera* and *Rebutia* genera have been chosen, because these are popular ones. Often they are the first cacti plants that bloom in spring, and at an early age.

Materials and methods

The *Cactaceae* species used in the present research were located in the greenhouse complex from the “Alexandru Borza” Botanical Garden of Cluj-Napoca, Romania. This *Cactaceae* collection counts more than 4100 specimens, belonging to 115 genera, from the 241 total known, after the Backeberg system (Backeberg, 1958).

Based on aesthetic consideration (shape of plant, flower diameter, color of flowers) there have been chosen as genitors in interspecific and intergeneric hybridization the following genotypes: *Aylostera muscula*, *Aylostera flavistyla*, *Aylostera fiebrigii*, *Aylostera fiebrigii* var. *densiseta*, *Aylostera spinosissima*, *Aylostera narvaecensis*, *Aylostera archibuiningiana*, *Aylostera buiningiana*, *Aylostera vallegardensis*, *Aylostera albiflora*, *Rebutia senilis*, *Rebutia senilis* var. *hyalacantha*, *Rebutia tarvitaensis*, *Rebutia cajasensis*, *Rebutia spegazziniana*, *Rebutia pseudominuta* var. *schumaniana*, *Rebutia kupperiana*, *Rebutia kupperiana* var. *spiniflora*, *Rebutia donaldiana* (Tab. 1).

Using a cyclic hybridization pattern, 12 interspecific combinations were realized, and other 12 intergeneric ones.

To isolate a cross-pollinated flower from the possibility of contamination by other plant's pollen, individual sticky paper insulation and different colour of thread per insulation has been used for each flower, to distinguish the differences between hybrid fruits and the other fruits.

Differences regarding the peculiarities of fruit weight, number of seeds/fruit, seeds weight were analysed statistically using ANOVA “t” test, using the mean of experiment as control, and coefficient of variability (CV%) for traits were computed.

Generally, the parental genotypes are globular plants with large flowers (flower diameter between 2.5 cm and 4 cm) and with large palette of colours (Tab. 1).

For each combination ten plants were pollinated, every plant having about ten flowers. Hybrid seeds have been obtained from 12 interspecific and 12 intergeneric combinations.

It is important to know the pollen germination ability (Oyiga et al., 2010) because this allows to know the value of a pollinator genotype in artificial hybridization (the pollen must have at least 30% germination ability to ensure pollination success).

The pollen viability has been achieved through iodine staining method of potassium iodide. In the presence of the dye, fertile pollen was coloured black, while the sterile pollen remained colourless or slightly coloured.

The anthers were harvested at the stage of advanced floral button from 10 genotypes (collection of the “Alexandru Borza” Botanical Garden, Cluj-Napoca, Romania). Anthers were fixed in Carnoy reagent.

Tab.1.The main traits of parental genotypes

Species	Plant shape	Throne		Flower diameter (cm)	Colours of flower
		Number	Length (mm)		
<i>Aylostera muscula</i>	oval	23	2.4	3.5	orange
<i>Aylostera flavistyla</i>	globular	15-22	5-10	3	red
<i>Aylostera fiebrigii</i>	globular	30	40	3.5	orange
<i>Aylostera fiebrigii</i> var. <i>densiseta</i>	globular	40	50	3.5	orange
<i>Aylostera spinosissima</i>	globular	20-30	15	3	red
<i>Aylostera narvaecensis</i>	globular	10-20	2-3	4	pink
<i>Aylostera archibuiningiana</i>	globular	12	2-3	4	red
<i>Aylostera buiningiana</i>	globular	14-16	6-10	3	pink
<i>Aylostera vallegardensis</i>	globular	20	30	3.5	pink
<i>Aylostera albiflora</i>	globular	15	5	2.5	white
<i>Rebutia senilis</i>	globular	25	>30	3.5	red
<i>Rebutia senilis</i> var. <i>hyalacantha</i>	globular	25	20	3	red
<i>Rebutia tarvitaensis</i>	globular	7-9	2-5	6	red
<i>Rebutia cajasensis</i>	globular	13	15,5	3.5	red
<i>Rebutia spegazziniana</i>	cylindrical	23	4	4	dark red
<i>Rebutia pseudodeminuta</i> var. <i>schumaniana</i>	globular	27	12	2.5	orange
<i>Rebutia kupperiana</i>	globular	13	15,5	3.5	red
<i>Rebutia kupperiana</i> var. <i>spiniflorum</i>	globular	13	15,8	3	red
<i>Rebutia donaldiana</i>	globular	25	20	3.5	pink

The samples (10 anthers from all the genotypes) were examined using Aigo Digital LCD microscope and photographs were taken with the LDM EV 5610 camera. The diameter, expressed in μm , of the pollen grains (20 measurements/genotypes) was analyzed using CellB program.

Results and discussion

At cacti the number of pollen grains vary from 160,000 at *Opuntia rastrera* (Mandujano et al., 1996) to 330,000 for male flowers of *Opuntia robusta* (Del Castillo and Argueta, 2009).

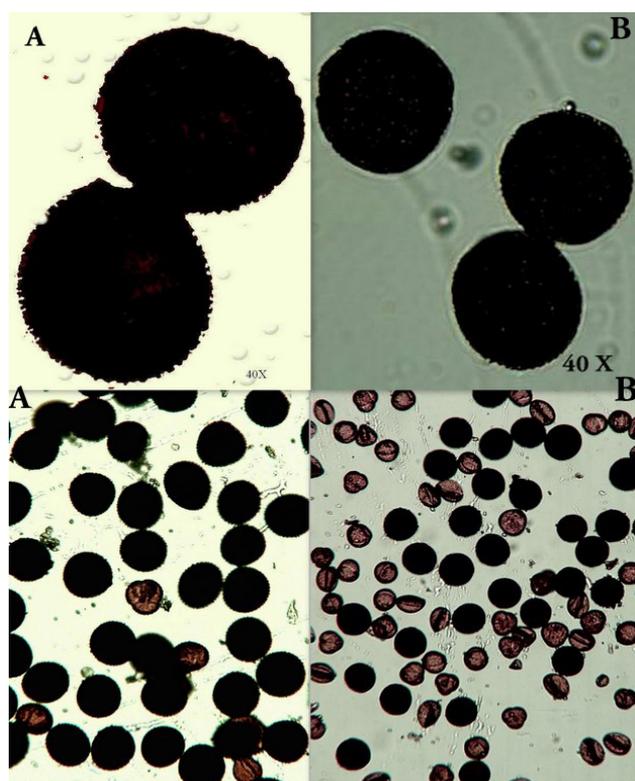


Fig. 1. The pollen grain at *Aylostera buiningiana* (A) and *Rebutia kupperiana* var. *spiniflorum* (B)

Tab. 2. The number of pollen grains at different cacti species

The species	Total number counted	Viable grains*	Sterile grains**	Percentage of viable grains	The mean of diameter (μm)
<i>R. kupperiana</i> var. <i>spiniflorum</i>	430	380	50	88,1	123
<i>R. krainziana</i>	352	303	49	85,4	89,3
<i>A. buiningiana</i>	329	233	96	70,2	56,7
<i>R. spegazziniana</i>	311	245	66	78,9	79
<i>R. senilis</i>	452	394	58	87,1	93,6
<i>R. kupperiana</i>	347	288	59	83,0	67
<i>A. flavistyla</i>	359	296	63	82,3	73
<i>A. narvaecensis</i>	396	333	63	83,5	87,4
<i>A. brunescens</i>	427	363	64	84,3	89
<i>R. donaldiana</i>	417	358	59	85,3	92

*means coloured polen grains; **means colourless polen grains

In the present study, the number of viable pollen grains was high (more than 30%), which means the success of pollination could be ensured, using as father genitor one of the following species: *A. flavistyla*, *A. narvaecensis*, *A. buiningiana*, *R. kupperiana* var. *spiniflorum*, *R. donaldiana*. The highest percentage of viable pollen grains was recorded at *R. kupperiana* var. *spiniflorum* genotype, while the lowest percentage was presented at the *Aylostera buiningiana* (Tab. 2).

The shape (Fig. 1) and the size of pollen grains (Tab. 2) showed relatively small differences between species, which

Tab. 3. Hybrid combinations and results of hybridizations

No.	Mother genotype (♀)	Father genotype (♂)	Results of hybridization (fruits and seeds)
Interspecific hybridizations			
1	<i>R. spegazziana</i>	<i>R. senilis</i>	Yes
2	<i>R. pseudodeminuta</i> var. <i>schumaniana</i>	<i>R. senilis</i>	Yes
3	<i>R. cajasensis</i>	<i>R. senilis</i>	Yes
4	<i>R. senilis</i>	<i>R. kupperiana</i> var. <i>spiniflora</i>	Yes
5	<i>R. senilis</i>	<i>R. spegazziana</i>	Yes
6	<i>R. senilis</i> var. <i>hyalacantha</i>	<i>R. spegazziana</i>	Yes
7	<i>A. flavistyla</i>	<i>A. archibuiningiana</i>	Yes
8	<i>A. buiningiana</i>	<i>A. vallegardensis</i>	Yes
9	<i>A. muscula</i>	<i>A. vallegardensis</i>	Yes
10	<i>A. fiebrigii</i>	<i>A. vallegardensis</i>	Yes
11	<i>A. muscula</i>	<i>A. buiningiana</i>	Yes
12	<i>A. buinigiana</i>	<i>A. flavistyla</i>	Yes
13	<i>A. muscula</i>	<i>A. albiflora</i>	No
14	<i>A. spinosissima</i>	<i>A. albiflora</i>	Yes
Intergeneric hybridizations			
15	<i>R. senilis</i>	<i>A. muscular</i>	No
16	<i>R. tarvitaensis</i>	<i>A. muscula</i>	No
17	<i>R. cajasensis</i>	<i>A. muscula</i>	Yes
18	<i>A. flavistyla</i>	<i>R. senilis</i>	No
19	<i>A. fiebrigii</i> var. <i>densiseta</i>	<i>R. senilis</i>	Yes
20	<i>A. spinosissima</i>	<i>R. senilis</i>	Yes
21	<i>A. muscula</i>	<i>R. kupperiana</i> var. <i>spiniflora</i>	Yes
22	<i>A. narvaecensis</i>	<i>R. kupperiana</i> var. <i>spiniflora</i>	Yes
23	<i>A. spinosissima</i>	<i>R. spegazziniana</i>	Yes
24	<i>R. cajasensis</i>	<i>A. archibuiningiana</i>	Yes
25	<i>R. senilis</i>	<i>A. archibuiningiana</i>	Yes
26	<i>R. donaldiana</i>	<i>A. buiningiana</i>	Yes
27	<i>R. kupperiana</i> var. <i>spiniflora</i>	<i>A. buiningiana</i>	Yes
28	<i>R. cajasensis</i>	<i>A. flavistyla</i>	Yes
29	<i>R. senilis</i>	<i>A. flavistyla</i>	No
30	<i>R. senilis</i>	<i>A. albiflora</i>	No

can provide a taxonomic key for different species' determination, but only if this is accompanied by other characters of pollen grain, such as pore number, the exine and intine morphology (Garralla and Cuadrado, 2007; Ferguson and Kiesling, 1997; Punt et al., 1994). The greatest diameter value has been observed at *R. kupperiana* var. *spiniflorum*, and the lowest at *Aylostera buiningiana*. The results obtained are in accordance with the Gottelli et al. (2009) findings, which affirmed that pollen grain size varied between 50 and 132 μm in four species of *Pterocactus*.

Even the pollen germination ability was higher, cross-pollination among *Rebutia senilis* x *Aylostera muscula*, *Rebutia tarvitaensis* x *Aylostera muscula*, *Aylostera flavistyla* x *Rebutia senilis*, *Rebutia senilis* x *Aylostera flavistyla*, *Aylostera muscula* x *Aylostera albiflora*, *Rebutia senilis* x *Aylostera albiflora*, did not succeed (Tab. 3). Species like *Aylostera flavistyla*, *Rebutia senilis* can be incompatible, considering that manual cross-pollination did not succeeded neither using *Aylostera flavistyla* as mother genitor, nor using it as father genitor. In addition, manual cross-pollination, studied in wild, at *Stenocereus stellatus* failed between some domesticated and wild phenotypes, suggesting the pollen incompatibility (Casas et al., 1999).

In the interspecific combinations (Tab. 4) fruits weight and seeds weight presented large values, especially in *R. pseudodeminuta* var. *schumaniana* x *R. senilis* and *A. buiningiana* x *A. vallegardensis*.

It is possible that these results may be due mainly to the inherited maternal genitors dowry, but not exclude possible effects of the father genitor, and, of course, those caused by environmental conditions, culture, and interactions between genotype and ecotype.

In combinations *A. muscula* x *A. vallegardensis* and *R. pseudodeminuta* var. *schumaniana* x *R. senilis* resulted the

biggest number of seeds/fruit, with significant positive difference. A small number of seeds per fruit were registered when *A. flavistyla* was pollinated with *A. archibuinigiana*, *R. senilis* var. *hyalacantha* with *R. spegaziana*, and *A. fiebrigii* with *A. vallegardensis* crosses (Tab. 4).

Possible interspecific hybrid combinations in new works of cacti breeding, in order to obtain a large number of seeds and relatively good germination percentage, could be recommended: *R. senilis* x *R. spegaziana*, *A. spinosissima* x *A. albiflora*, *R. cajasensis* x *R. senilis*, *A. fiebrigii* x *A. vallegardensis*.

Germination percentage of seeds has generally a small value, only seeds belonging to several combinations were being registered with a relative better germination: *A. spinosissima* x *A. albiflora* combination (3.40%); *R. senilis* x *R. spegaziana* (3.24%). In the following combinations: *R. spegaziana* x *R. senilis*, *R. pseudodeminuta* var. *schumaniana* x *R. senilis*, *R. senilis* var. *hyalacantha* x *R. spegaziana*, *A. flavistyla* x *A. archibuinigiana* the germination percentage was 0.00%.

The extreme values of the coefficient of variability for fruits weight (Tab. 4) were registered at the *A. spinosissima* x *A. albiflora* (with the lowest level of CV%, 12.3%) respectively *A. flavistyla* x *A. archibuinigiana* (CV%=98.4) combinations.

The seeds weight and the number of seeds/fruit (Tab. 4) presented a small variability that suggested a great uniformity.

In the intergeneric combinations *R. senilis* x *A. archibuinigiana* and *R. cajasensis* x *A. muscula* cross (Tab. 5) were registered the highest value of fruits weight, compared to the mean of the experiment, considered as control (14.54 mg). For seeds weight, assured statistically values were registered for the following hybrids: *R. senilis*

Tab. 4. The fruits weight*, seeds weight* and the number of seeds/fruit* of hybrid interspecific combinations, coefficient of variability and germination percentage of seeds

No.	Hybrid combination (♀ x ♂)	Fruits weight		Seeds weight		Number of seeds/fruit		Germination percentage
		Mean \pm s _x (mg)	CV%	Mean \pm s _x (mg)	CV%	Mean \pm s _x (mg)	CV%	
1	<i>R. spegaziana</i> x <i>R. senilis</i>	11.54 \pm 4.03	78.1	5.48 ^{ooo} \pm 0.20	8.1	27.0 \pm 0.71	5.9	0.00
2	<i>R. pseudodeminuta</i> var. <i>schumaniana</i> x <i>R. senilis</i>	23.26 \pm 3.59	34.5	22.50 ^{***} \pm 0.49	4.9	67.2 ^{**} \pm 0.37	1.2	0.00
3	<i>R. cajasensis</i> x <i>R. senilis</i>	10.18 \pm 2.12	46.6	7.14 ^{oo} \pm 0.33	10.4	34.8 \pm 0.80	5.1	2.29
4	<i>R. senilis</i> x <i>R. kupperiana</i> var. <i>spiniflorum</i>	12.88 \pm 1.45	25.2	8.38 \pm 0.22	5.8	23.0 ^{ooo} \pm 0.71	6.9	0.86
5	<i>R. senilis</i> x <i>R. spegaziana</i>	9.88 ^o \pm 1.98	44.9	15.26 ^{***} \pm 0.38	5.6	30.8 \pm 0.80	5.8	3.24
6	<i>R. senilis</i> var. <i>hyalacantha</i> x <i>R. spegaziana</i>	12.74 \pm 2.30	40.3	7.98 ^o \pm 0.37	10.3	13.8 ^{ooo} \pm 0.37	6.1	0.00
7	<i>A. flavistyla</i> x <i>A. archibuinigiana</i>	10.54 \pm 4.64	98.4	2.00 ^{ooo} \pm 0.04	5.0	5.0 ^{ooo} \pm 0.45	20.0	0.00
8	<i>A. buiningiana</i> x <i>A. vallegardensis</i>	20.40 ^{***} \pm 1.21	13.2	18.48 ^{***} \pm 0.26	3.1	21.4 ^{ooo} \pm 0.81	8.5	0.93
9	<i>A. muscula</i> x <i>A. vallegardensis</i>	19.76 \pm 3.27	37.0	9.52 \pm 0.23	5.5	93.2 ^{**} \pm 0.86	2.1	0.42
10	<i>A. fiebrigii</i> x <i>A. vallegardensis</i>	8.16 ^{oo} \pm 1.88	51.5	3.36 ^{ooo} \pm 0.27	18.1	14.4 ^{ooo} \pm 0.75	11.6	2.38
11	<i>A. muscula</i> x <i>A. buiningiana</i>	14.14 \pm 1.62	25.6	8.32 \pm 0.30	8.0	30.0 \pm 0.89	6.7	0.66
12	<i>A. spinosissima</i> x <i>A. albiflora</i>	14.02 \pm 0.77	12.3	8.94 \pm 0.19	4.8	47.0 ^{***} \pm 0.45	2.1	3.40
	Control (Mean of experiment)	13.96	42.3	9.78	7.5	33.97	6.8	-

*, **, *** / °, oo, ooo Significant at P < 0.05, 0.01 and 0.001 (positive, respectively negative)

Tab. 5. The fruits weight*, seeds weight* and the number of seeds/fruit* of hybrid intergeneric combinations, coefficient of variability and germination percentage of seeds

No.	Hybrid combination (♀ x ♂)	Fruits weight		Seeds weight		Number of seeds/fruit		Germination percentage
		Mean ± s _x (mg)	CV%	Mean ± s _x (mg)	CV%	Mean ± s _x (mg)	CV%	
1	<i>R. cajasensis</i> x <i>A. muscula</i>	23.10 ^{***} ± 0.92	8.9	16.30 ^{**} ± 0.73	10.1	90.4 ^{***} ± 1.00	2.5	2.21
2	<i>A. fiebrigii</i> var. <i>densiseta</i> x <i>R. senilis</i>	19.40 ^{***} ± 0.68	7.8	14.10 ^{**} ± 0.71	11.2	97.0 ^{***} ± 1.70	3.9	1.44
3	<i>A. spinosissima</i> x <i>R. senilis</i>	1.92 ⁰⁰⁰ ± 0.18	21.0	0.90 ⁰⁰⁰ ± 0.04	11.1	5.0 ⁰⁰⁰ ± 0.40	20.0	0.00
4	<i>A. muscula</i> x <i>R. kupperiana</i> var. <i>spiniflora</i>	8.24 ⁰⁰⁰ ± 0.57	15.5	6.42 ⁰⁰⁰ ± 0.37	13.0	8.4 ⁰⁰⁰ ± 0.40	10.6	2.38
5	<i>A. narvaecensis</i> x <i>R. kupperiana</i> var. <i>spiniflora</i>	9.80 ⁰⁰⁰ ± 0.37	8.4	5.68 ⁰⁰⁰ ± 0.08	3.1	11.6 ⁰⁰⁰ ± 0.20	4.7	5.17
6	<i>A. spinosissima</i> x <i>R. spegaziana</i>	18.44 ^{**} ± 0.54	6.5	5.86 ⁰⁰⁰ ± 0.18	7.0	59.0 ^{**} ± 0.40	1.7	0.00
7	<i>R. cajasensis</i> x <i>A. archibuinigiana</i>	22.70 ^{***} ± 0.73	7.2	19.00 ^{**} ± 0.24	2.9	56.0 ^{**} ± 0.40	1.8	3.92
8	<i>R. senilis</i> x <i>A. archibuinigiana</i>	29.20 ^{**} ± 1.36	10.4	21.30 ^{**} ± 0.37	3.9	75.6 ^{***} ± 0.40	1.2	0.79
9	<i>R. donaldiana</i> x <i>A. buiningiana</i>	15.40 ± 0.84	12.1	12.00 ± 0.45	8.3	58.0 ^{**} ± 0.90	3.4	0.34
10	<i>R. kupperiana</i> v. <i>spiniflorum</i> x <i>A. buiningiana</i>	11.74 ⁰ ± 0.66	12.5	5.64 ⁰⁰⁰ ± 0.24	9.7	19.0 ⁰⁰⁰ ± 0.70	10.5	4.21
11	<i>R. cajasensis</i> x <i>A. flavistyla</i>	8.62 ⁰⁰⁰ ± 0.56	14.6	6.46 ⁰⁰⁰ ± 0.10	3.6	19.2 ⁰⁰⁰ ± 0.40	7.7	4.16
12	<i>R. senilis</i> x <i>A. flavistyla</i>	5.90 ⁰⁰⁰ ± 0.67	25.4	3.02 ⁰⁰⁰ ± 0.34	24.8	8.8 ⁰⁰⁰ ± 0.70	9.5	0.00
Control (Mean of experiment)		14.54	12.5	9.72	9.1	42.33	6.5	-

*, **, ***/ °, °°, °°° Significant at P<0.05, 0.01 and 0.001 (positive, respectively negative)

x *A. archibuinigiana*, *R. cajasensis* x *A. archibuinigiana*, *R. cajasensis* x *A. muscula*.

Large values for number of seeds/fruit presented fruits obtained from *A. fiebrigii* var. *densiseta* pollinated by *R. senilis* (97.00). Also, many seeds per fruit resulted in *R. cajasensis* x *A. muscula* cross (90.40), and *R. senilis* x *A. archibuinigiana* cross (75.60).

The number of seeds/fruit and the seeds weight of intergeneric combinations presented a small variability, compared to the interspecific combinations (Tab. 5). The fruits weight seemed to present a medium variability, the extreme values were observed at the *A. spinosissima* x *R. spegaziana* (with the lowest level of CV=6.5%), respectively *A. buinigiana* x *A. flavistyla* (CV=25.4%) combinations.

Germination percentage of seeds derived from intergeneric hybridizations (Tab. 5) has better values than in case of seeds derived from interspecific hybridizations: *A. narvaecensis* x *R. kupperiana* var. *spiniflorum* (5.17%), *R. kupperiana* var. *spiniflorum* x *A. buiningiana* (4.21%), *R. cajasensis* x *A. flavistyla* (4.16%), *R. cajasensis* x *A. archibuinigiana* (3.92%).

Among the fruits weights, the number of seeds/fruits and the weight of seeds significant, positive and tight

Tab. 6. Phenotypic correlations among fruit and seed characteristics on the interspecific hybrid combinations

Traits	Number of seeds/fruit	Seeds weight (mg)	Germination percentage
Fruits weight (mg)	0.656 ^{**}	0.756 ^{**}	-0.413
Number of seeds/fruit	-	0.428	-0.027
Seeds weight (mg)	-	-	-0.007
Germination percentage	-	-	-
r5% = 0.532; r1% = 0.661; r0.1%=0.780			

*, **, ***/ °, °°, °°° Significant at r<0.05, 0.01 and 0.001 (positive, respectively negative)

correlations have been recorded (Tab. 6 and Tab.7). Between the weight of fruits and the number of seeds/fruits the coefficient of correlation has the value of 0.656, while between the weight of seeds and fruits this value is 0.756 (Tab. 6). Generally, the coefficients of correlation have large values (0.874, 0.927 and 0.807) at intergeneric combinations (Tab. 7). Between the fruits weight, number of seeds/fruits and germination percentage the coefficients of correlation did not assure statistically remarkable values, neither for interspecific combinations nor for intergeneric ones, that suggests that the analyzed peculiarities of the fruits and seeds, in this experiment, did not have any influence to the germination capacity.

Noticed can be the fact that even if the interspecific and intergeneric hybridizations succeed, the germination percentage remained small. In the present experiment, the light used for the seeds germination was the visible one, and no heat treatment has been used. Anywise the seeds of most *Cactaceae* species can be classified as being positively photoblastic, no seeds germinated under dark conditions (Ortega-Baes et al., 2010; Meiado et al., 2010). The heat treatments of 30°C-38°C did not have any effect on the germination of several species cacti (Sanchez-Soto et al., 2010). In addition, seed germinability of species from *Cac-*

Tab. 7. Phenotypic correlations among fruit and seed characteristics on the intergeneric hybrid combination

Traits	Number of seeds/fruit	Seeds weight (mg)	Germination percentage
Fruits weight (mg)	0.874 ^{***}	0.927 ^{***}	-0.036
Number of seeds/fruit	-	0.807 ^{***}	-0.229
Seeds weight (mg)	-	-	0.055
Germination percentage	-	-	-
r5% = 0.532; r1% = 0.661; r0.1%=0.780			

*, **, ***/ °, °°, °°° Significant at r<0.05, 0.01 and 0.001 (positive, respectively negative)

taceae family was not affected by light intensity or quality (Meiado et al., 2010).

The low proportion of seed germination can be explained through the different growth forms of cacti. Columnar cacti have a greater proportion of seed germination than globular ones (Ortega-Baes et al., 2010). *Aylostera* and *Rebutia* species are globular cacti, and it is possible that their seeds show seed dormancy.

The parental genotype *Aylostera fiebrigii* presented a small capacity of germination, when it has been used in a manner as studying germination percentage at different cacti species (Mihalte et al., 2009). It seems that in this research the low germination percentage was confirmed also in different hybrid combinations.

Conclusions

From a cyclic hybridization of 15 hybrid combinations between species and 15 hybrid combinations between genera, in three interspecific combinations and three intergeneric combinations seeds were not obtained.

Unsuccessful hybridization of cacti may have many causes; one of this may be the incompatibility of the species (eg. *A. muscula* x *A. albiflora*, *R. senilis* x *A. muscula*, *R. tarvitaensis* x *A. muscula*, *R. cajasensis* x *A. muscula*, *R. senilis* x *A. flavistyla*, *R. senilis* x *A. albiflora*).

The other 24 interspecific and intergeneric combinations succeeded, peculiarities of fruits and seeds could be quantified, and a database has been created regarding to different peculiarities of cacti fruits and seeds.

Creating a database regarding the morphological peculiarities of the seeds can be useful in identifying genotypes, as well as in obtaining relevant information concerning the correlations between fruits' and seeds' characteristics.

In this experiment, the traits of fruits and seeds did not influence the germination percentage.

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