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METHODS TO IMPROVE SEED GERMINATION OF *CACTACEAE* SPECIES

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Abstract

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Responses of seed germination to different chemical treatments were investigated for species belonging to *Rebutia, Aylostera, Mediolobivia* and *Sulcorebutia* genera (*Cactaceae*). Germination was optimal when the seeds immersed for 8 hours in aqueous solution, which contained sodium nitrofenolate (1.8%), and the germination percentage decreased when just tap water has been used. Maximal germination obtained for seeds that immersed for 8 hours in an aqueous solution, which contained 9% naphthaleneacetic acid (NAA). The germination was completely blocked when the seeds were treated with salicylic acid and acetylsalicylic acid. Whatever treatment option has applied, the species *A. kieslingii* recorded the lowest percentage of germination energy and germination capacity. The species *M. orurensis* recorded the highest percentage of germination energy, and *R.* var. *spiniflorum kupperiana* the highest percentage of germination capacity. In addition, high values of germination capacity were present in *A. buniningiana, A. narvacensis, A. pseudodeminuta, R.* var. *luteispina violaciflora*. As the process to obtain new plants in cacti is a difficult one, a database designed to improve cacti seed germination using different chemical treatments can be extremely useful for seed production industry.

Key words: Auxin, *Cactaceae* species, germination capacity, germination energy

Introduction

The life cycle of any plant is divided into different phases and seed germination is the basic stage to start the growth of the plant. Seed propagation is a reproduction method of special importance because through this method, the populations' genetic diversity is maintained. However, at *Cactaceae,* studies regarding the seeds viability, the seeds longevity and germination stimulation techniques are scarce (Rojas-Arehchiga and Vahzquez-Yanes, 2000). In arid and semiarid areas, in addition to other risk factors, seed germination is hard to achieve because of the lack of water. Rainy periods are short and the soil surface accumulates large amounts of salts (Kigel, 1995). Cacti seeds present several morphological, physiological and biochemical peculiarities to improve germination in arid conditions. Thus, Bregman and Gravena (1997) report about the presence of protein complexes developed in the cuticle of the seed, which have a great capacity to absorb water.

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Foliar organs, flowers, reproductive organs, glochids, and roots develop from the areoles (Booke, 1980), which is considered homologous to a lateral (axillary) bud (Buxbaum, 1950). Cactus flowers are sessile and solitary, and commonly only one flower is produce per areole (Ramirez and Berry, 1995). The time from flower opening to fruit maturation is relatively short, 40 to 50 days for *Pilosocereus lanuginosus*, *Stenocereus griseus*, and *Subpilocereus repandus* (Petit, 1995). In general, mature cactus seeds have the following parts: testa, embryo, endosperm, perisperm (in most primitive groups), arillus cover (typically for subfamily *Opuntioideae*) (Buxbaum, 1951), funicle (*Opuntia*), and hilum (Elizondo-Elizondo et al., 1994). Some species have a caruncle (*Pereskia*) (Dau and Labouriau, 1974) and a strophiole (*Mammillaria erectacantha*) (Fittkau, 1968). The number of seeds produced by a single fruit can be enormous, sometimes more than 1000 seeds per fruit (*Pilosocereus chrysacanthus*), or just a few (1 to 5 seeds per fruit in *Epithelantha* and *Pereskia aculeata*) (Lode, 1995 and Pedroni and Sanchez, 1997).

Most studies regarding the cacti propagation through seeds only suggest recommendations for different soil mixtures (Grandet, 1995; Bach, 1998; Peters, 1998 and Kohlschreiber, 1998). Based on these considerations, this study designed a database with improved techniques of germination seeds of species belonging to *Aylostera*, *Mediolobivia*, *Rebutia* and *Sulcorebutia* genera.

The results of this approach can be extremely useful in seed production, to improve the germination and to improve the development of the seedling, which are difficult issues to cacti.

Materials and Methods

Plant material and experimental conditions

The biological material investigated was represente by 34 species of cacti belonging to four genera from the *Chaetolobiviae* subgroup*: Rebutia*, *Aylostera, Mediolobivia*, and *Sulcorebutia*.

The fruits were collected in August 2009 in the Botanical Garden Cluj-Napoca (Romania) and were dried at room temperature and seeds were separated by dissection and washed with tap water to remove any remaining pulp. Seeds were dried on absorbent paper before placing them into the germination dishes (Linhardt dishes). All germination experiments were performed at laboratory room temperatures (16-25ºC) and natural daylight conditions.

Investigated parameters

Two parameters were taken in consideration: germination energy and germination capacity.

The germination energy represents the percent of seeds, which germinate within a given period (defined as the energy period) under optimum or stated conditions, also called the minimum time required for the seeds to start germinating. The energy period for cacti was defined as being 8-10 days (De la Barrera and Nobel, 2003). The germination capacity refers to the percentage of seeds capable of germinating in 17-20 days (De la Barrera and Nobel, 2003). The criterion used to consider a seed as germinated was radicle emergence from the testa.

Statistical analyses

One hundred seeds per dish at a distance of 0.5-1.5 cm between them were used and three replicates were established for each treatment. In order to compare the effect of interaction treatment *x* genotype, the influence of the genotype and the effect of the treatment on germination energy and capacity ANOVA has been used, having the mean of the experiment as control.

Five experiments were conducted to study germination energy and germination capacity: V_1-seeds were sown in Linhardt dishes and soaked with tap water; V_2-seeds were immersed for 8 hours in aqueous solution which contained sodium nitrofenolate (1.8%); V_3-seeds were immersed for 8 hours in aqueous solution which contained 9%. naphthaleneacetic acid (NAA); V_4-seeds were

immersed for 8 hours in aqueous solution which contained salicylic acid (0.1 μM); V_5-seeds were immersed for 8 hours in aqueous solution which contained acetyl salicylic acid (0.1 μM).

Results and Discussion

The acetyl salicylic acid and the salicylic acid are considered plant hormones, which stimulate cell proliferation at low doses and stimulate the plant's resistance system, while higher doses induce cell death (Clark et al., 2001).

The results showed that treatments with salicylic acid (V_4) and acetylsalicylic acid (V_5) completely blocked the germination of seeds. Following these treatments, no seed germinated, probably because the salicylic acid is plant hormone, which in certain doses induces cell death (Tenhaken, 1997).

Germination energy

Analyzing the influence of genotype *x* treatment interaction (Table 1), the highest values of germination energy were recorded in the following genotypes: *R. marsoneri* (21.5%), *A. fiebrigii* (21.8), *R. krainziana* (21.8) treated with ANA (V_3) and *M. orurensis* (22.5%) treated with V_2.

Analyzing the influence of the genotype on the germination energy of seeds (Table 2), the lowest percentage of germination energy was recorded in *A. kieslingii* (14.1%) and the highest in *M. orurensis* (20.8%).

Comparing the treatment variants (Table 3) with the mean of experience, the values of germination energy were significant with the auxins treatment and with sodium solutions. When the seeds were treated just with water, the percentage of germination energy was much lower (14.9%) than the mean of the experiment.

Germination capacity

The interaction genotype *x* treatment (Table 1) regarding the germination capacity has the amplitude of variation between 25.0% (*M. pygmaea*, treated with water) and 34.3% (*A. narvacensis* treated with NAA), compared with the mean of experience (29.9%). Species like *A. buniningiana, A. narvacensis, A. pseudodeminuta, R.* var. *luteispina violaciflora* showed the highest values of germination capacity, both in the treatment with sodium and with NAA.

Analyzing the influence of the genotype on seed germination capacity (Table 2), it can be stated that the lowest percentage of germination capacity was recorded by *M. pygmaea* (27.3%) and the highest by *A. brunescens* (32.2%).

Comparing treatment variants (Table 3) assured statistic values were obtained in the experimental variants V_1 and V_3. In the case of treated seeds with NAA, the germination capacity values were significant.

The positive result obtained after the seed's treating with auxins was expected, knowing all the positive roles that auxins have in a number of plant activities, including: development of the embryo, leaf formation, apical dominance, fruit development, root initiation and development (Ni et al., 2001; Thakur, 2008).

Whatever the genotype, treating the *Cactaceae* seeds with a minimum concentration (9%) of auxins, it can be observed that this treatment significantly improves both the germination energy and germination capacity. Probably the same positive results could be achieved if the seeds were treated with abscisic acid, cytokinins, ethylene, gibberellins, compounds that are classified as plant hormones or growth regulators (Moreno et al., 1992; Swarup, 2007 and Wang, 2009).

Whatever treatment option was applied, the species *A. kieslingii* recorded the lowest percentage of germination energy and germination capacity. The species *M. orurensis* recorded the highest percentage of germination energy, and *R.* var. *spiniflorum kupperiana* highest percentage of germination capacity. The species *Aylostera fiebrigii* presented a small capacity of germination in the present research, but also had a small germination capacity when it was used as parental genotype in different hybrid combinations (Mihalte et al.,

Table 1
The influence of treatment x genotype interaction on germination energy and germination capacity of several *Cactaceae* species

No of entry	The genotype	Germination energy ± s_x, %			Germination capacity ± s_x, %		
		V_1	V_2	V_3	V_1	V_2	V_3
1.	*A. pulvinosa*	17.8 ± 0.2	19.5 ± 2.0	18.5 ± 0.9	30.3 ± 0.3	27.3 ± 2.7 ^{oo}	27.8 ± 2.2 ^{o}
2.	*A. brunescens*	16.5 ± 1.1	18.8 ± 1.2	19.8 ± 2.2	31.0 ± 1.1	32.0 ± 2.1	33.5 ± 3.6 **
3.	*A. deminuta*	15.0 ± 2.6	19.8 ± 2.2	20.0 ± 2.5	31.3 ± 1.3	30.5 ± 0.6	32.3 ± 2.3 *
4.	*A. buniningiana*	16.8 ± 0.8	19.5 ± 2.0	19.0 ± 1.5	29.8 ± 0.2	32.5 ± 2.6 *	33.0 ± 3.1 **
5.	*A. fiebrigii* var. *densiseta*	16.0 ± 1.6	15.8 ± 1.8	17.5 ± 0.1	28.8 ± 1.2	28.5 ± 1.4	30.8 ± 0.8
6.	*A. narvacensis*	13.5 ± 4.1 ^{oo}	15.8 ± 1.8	16.0 ± 1.6	28.3 ± 1.7	33.8 ± 3.8 $^{**(*)}$	34.3 ± 4.3 ***
7.	*A. pseudodeminuta*	14.5 ± 3.1 ^{o}	19.0 ± 1.5	19.3 ± 1.7	29.8 ± 0.2	34.3 ± 4.3 ***	32.3 ± 2.3 *
8.	*A. robustispina*	13.8 ± 3.8 ^{oo}	16.0 ± 1.6	17.3 ± 0.3	27.8 ± 2.2 ^{o}	31.5 ± 1.6	32.8 ± 2.8 *
9.	*R. violaciflora* var. *luteispina*	14.3 ± 3.3 ^{o}	19.0 ± 1.5	20.3 ± 2.7 $^{(*)}$	27.3 ± 2.7 ^{o}	33.3 ± 3.3 **	33.5 ± 3.6 **
10.	*R. marsoneri*	14.8 ± 2.8 ^{o}	20.0 ± 2.5	21.5 ± 4.0 **	27.0 ± 2.9 ^{oo}	29.3 ± 0.7	32.5 ± 2.6 **
11.	*A. ithyacantha*	14.8 ± 2.8 ^{o}	18.3 ± 0.7	17.8 ± 0.2	29.0 ± 0.9	30.0 ± 0.1	33.3 ± 3.3 **
12.	*A. muscula*	14.8 ± 2.8 ^{o}	17.3 ± 0.3	18.3 ± 0.7	28.5 ± 1.4	29.5 ± 0.4	32.5 ± 2.6 *
13.	*A. kieslingii*	12.3 ± 5.3 ^{ooo}	15.0 ± 2.6	15.3 ± 2.3	26.0 ± 3.9 ^{ooo}	28.3 ± 1.7	30.3 ± 0.3
14.	*A. fiebrigii*	14.0 ± 3.6 ^{o}	17.3 ± 0.3	21.8 ± 4.2 **	27.0 ± 2.9 ^{oo}	30.3 ± 0.3	31.8 ± 1.8
15.	*R. kupperiana* var. *spiniflorum*	14.3 ± 3.3 ^{o}	16.5 ± 1.1	17.3 ± 0.3	31.0 ± 1.1	30.8 ± 0.8	34.0 ± 4.1 ***
16.	*R. horstii*	15.3 ± 2.3	19.3 ± 1.7	20.3 ± 2.7	29.0 ± 0.9	31.0 ± 1.1	33.3 ± 3.3 **
17.	*M. pygmaea* var. *densiseta*	12.0 ± 5.6 ^{ooo}	18.3 ± 0.7	18.8 ± 1.2	26.5 ± 3.4 ^{oo}	28.3 ± 1.7	30.0 ± 0.1
18.	*M. pygmaea*	13.0 ± 4.6 ^{oo}	14.3 ± 3.3 ^{o}	16.3 ± 1.3	25.0 ± 4.9 ^{ooo}	27.8 ± 2.2 ^{o}	29.0 ± 0.9
19.	*M. nigricans*	13.0 ± 4.6 ^{oo}	17.0 ± 0.6	18.8 ± 1.2	25.8 ± 4.2 ^{ooo}	31.3 ± 1.3	31.5 ± 1.6
20.	*M. orurensis*	16.0 ± 1.6	22.5 ± 5.0 **	23.8 ± 6.2 ***	26.5 ± 3.4 ^{oo}	28.0 ± 1.9	30.0 ± 0.1
21.	*M. rosalbiflora*	15.0 ± 2.6	19.3 ± 1.7	20.8 ± 3.2	25.5 ± 4.4 ^{ooo}	32.0 ± 2.1	32.0 ± 2.1
22.	*M. ritteri*	17.0 ± 0.6	17.8 ± 0.2	19.0 ± 1.5	28.3 ± 1.7	29.3 ± 0.7	30.0 ± 0.1
23.	*R. tarvitaensis*	16.5 ± 1.1	18.0 ± 0.4	20.25 ± 2.7	27.0 ± 2.9 ^{oo}	28.8 ± 1.2	29.8 ± 0.2
24.	*R. cajasensis*	15.5 ± 2.1	18.8 ± 1.2	20.5 ± 3.0	27.8 ± 2.2 ^{o}	29.0 ± 0.9	30.0 ± 0.1
25.	*R. donaldiana*	15.8 ± 1.8	15.5 ± 2.1	18.5 ± 0.9	28.0 ± 1.9	29.8 ± 0.2	32.3 ± 2.3 *
26.	*R. flavistyla*	16.3 ± 1.3	20.0 ± 2.5	19.8 ± 2.2	26.8 ± 3.2 ^{oo}	29.0 ± 0.9	31.8 ± 1.8
27.	*R. violaciflora*	15.0 ± 2.6	18.8 ± 1.2	21.0 ± 3.5 *	28.5 ± 1.4	28.3 ± 1.7	31.3 ± 1.3 *
28.	*R. krainziana*	17.8 ± 0.2	19.8 ± 2.2	21.8 ± 4.2 **	27.8 ± 2.2 ^{o}	31.0 ± 1.1	34.3 ± 4.3
29.	*R. senilis* var. *kesselringii*	14.3 ± 3.3 ^{o}	22.5 ± 5.0 **	21.5 ± 4.0 **	28.5 ± 1.4	30.3±0.3	32.3 ± 2.3 *
30.	*R. graciliflora*	15.5 ± 2.1	21.5 ± 4.0 **	21.5 ± 4.0 **	28.0 ± 1.9	30.5±0.6	31.5 ± 1.6
31.	*R. xanthocarpa* var. *violaciflora*	13.75 ± 3.8 ^{oo}	15.5 ± 2.1	17.3 ± 0.3	26.8 ± 3.2 ^{oo}	28.8±-1.2	32.5 ± 2.6 *
32.	*R. senilis* var. *breviseta*	14.8 ± 2.8 ^{o}	17.8 ± 0.2	18.8 ± 1.2	26.8 ± 3.2 ^{oo}	30.3±0.3	32.0 ± 2.1
33.	*A. pulchella*	14.8 ± 2.8 ^{o}	20.5 ± 3.0 *	20.0 ± 2.5	27.5 ± 2.4 ^{o}	27.3±-2.7 ^{o}	32.3 ± 2.3 *
34.	*R. albipilosa*	14.3 ± 3.3 ^{o}	19.8 ± 2.2	21.0 ± 3.5 *	27.8 ± 2.2 ^{o}	30.3±0.3	31.8 ± 1.8
Mean of experiment (Control)			17.16			29.9	
LSD 5%			2.89			2.17	
LSD 1%			3.88			2.9	
LSD 0.1%			5.12			3.83	

Note: V_1-seeds were sown in Linhardt dishes and soaked with tap water; V_2-seeds were immersed for 8 hours in aqueous solution which contained sodium nitrofenolate (1.8%); V_3-seeds were immersed for 8 hours in aqueous solution which contained 9%. naphthaleneacetic acid (NAA); s_x-standard error of the mean; *, **, ***/ $^{o, oo, ooo}$ significant at P<0.05, 0.01 and 0.001 (positive, respectively negative).

Table 2
The genotype influence on germination energy and germination capacity of several *Cactaceae* species

No. of entry	The genotype	Germination energy ± $s_{x,}$ %	Germination capacity ± $s_{x,}$ %
1.	*A. pulvinosa*	18.6 ± 1.0	28.4 ± 1.52 ^{o}
2.	*A. brunescens*	18.3 ± 0.8	32.2 ± 2.23 ***
3.	*A. deminuta*	18.3 ± 0.7	31.3 ± 1.39 *
4.	*A. buniningiana*	18.4 ± 0.9	31.8 ± 1.81 **
5.	*A. fiebrigii* var. *densiseta*	16.4 ± 1.1	29.3 ± 0.61
6.	*A. narvacensis*	15.0 ± 2.5 ^{ooo}	32.1 ± 2.14 **
7.	*A. pseudodeminuta*	17.6 ± 0.0	32.1 ± 2.14 **
8.	*A. robustispina*	15.6 ± 1.9 ^{oo}	30.7 ± 0.73
9.	*R. violaciflora* var. *luteispina*	17.8 ± 0.3	31.3 ± 1.39 *
10.	*R. marsoneri*	18.7 ± 1.2	29.6 ± 0.36
11.	*A. ithyacantha*	16.9 ± 0.6	30.8 ± 0.81
12.	*A. muscula*	16.8 ± 0.8	30.2 ± 0.23
13.	*A. kieslingii*	14.1 ± 3.4 ^{ooo}	28.2 ± 1.77 ^{oo}
14.	*A. fiebrigii*	17.7 ± 0.1	29.7 ± 0.27
15.	*R. kupperiana* var. *spiniflorum*	16.0 ± 1.6	31.9 ± 1.98 **
16.	*R. horstii*	18.3 ± 0.7	31.1 ± 1.14
17.	*M. pygmaea* var. *densiseta*	16.3 ± 1.2	28.3 ± 1.69 ^{oo}
18.	*M. pygmaea*	14.5 ± 3.0 ^{ooo}	27.3 ± 2.69 ^{ooo}
19.	*M. nigricans*	16.3 ± 1.3	29.5 ± 0.44
20.	*M. orurensis*	20.8 ± 3.2 ***	28.2 ± 1. 77 ^{oo}
21.	*M. rosalbiflora*	18.3 ± 0.8	29.8 ± 0.11
22.	*M. ritteri*	17.9 ± 0.4	29.2 ± 0.77
23.	*R. tarvitaensis*	18.3 ± 0.7	28.5 ± 1.44 ^{o}
24.	*R. cajasensis*	18.2 ± 0.7	28.9 ± 1.02
25.	*R. donaldiana*	16.6 ± 0.9	30.0 ± 0.06
26.	*R. flavistyla*	18.7 ± 1.1	29.2 ± 0.77
27.	*R. violaciflora*	18.3 ± 0.7	29.3 ± 0.61
28.	*R. krainziana*	19.8 ± 2.2 ***	31.0 ± 1.06
29.	*R. senilis* var. *kesselringii*	19.4 ± 1.9 **	30.3 ± 0.39
30.	*R. graciliflora*	19.5 ± 1.9 **	30.0 ± 0.06
31.	*R. xanthocarpa* var. *violaciflora*	15.5 ± 2.0 ^{oo}	29.3 ± 0.61
32.	*R. senilis* var. *breviseta*	17.0 ± 0.5	29.7 ± 0.27
33.	*A. pulchella*	18.4 ± 0.9	29.0 ± 0.94
34.	*R. albipilosa*	18.3 ± 0.8	29.9 ± 0.02
Mean of experiment (Control)		17.60	29.90
LSD 5%		1.70	1.25
LSD 1%		2.20	1.67
LSD 0.1%		3.00	2.21

Note: V_1-seeds were sown in Linhardt dishes and soaked with tap water; V_2-seeds were immersed for 8 hours in aqueous solution which contained sodium nitrofenolate (1.8%); V_3-seeds were immersed for 8 hours in aqueous solution which contained 9%. naphthaleneacetic acid (NAA); s_x-standard error of the mean; *, **, ***/ $^{o, oo, ooo}$ significant at P<0.05, 0.01 and 0.001 (positive, respectively negative).

Table 3

The treatment influence on germination energy and germination capacity of several *Cactaceae* species

The treatment	Energy germination average ± $s_{x,}$ %	Energy germination average ± $s_{x,}$ %
V_1	14.9 ± 2.6 ^{ooo}	27.9 ± 2.00 ^{ooo}
V_2	18.3 ± 0.8 **	30.0 ± 0.12
V_3	19.4 ± 1.8 ***	31.8 ± 1.87 ***
Mean of experiment (Control)	17.6	29.9
LSD 5%	0.5	0.37
LSD 1%	0.66	0.50
LSD 0.1%	0.88	0.66

Note: V_1-seeds were sown in Linhardt dishes and soaked with tap water; V_2-seeds were immersed for 8 hours in aqueous solution which contained sodium nitrofenolate (1.8%); V_3-seeds were immersed for 8 hours in aqueous solution which contained 9%. naphthaleneacetic acid (NAA); s_x-standard error of the mean; *, **, ***/ $^{o, oo, ooo}$ significant at P<0.05, 0.01 and 0.001 (positive, respectively negative).

2010). The low proportion of seed germination can be explained though the different growth forms of cacti. Columnar cacti have a greater proportion of seed germination than globular ones (Ortega-Baes et al., 2010) and *Aylostera* species are globular cacti.

In the present experiment, no artificial light and no heat treatment were used for the seeds germination. 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 and Flores et al., 2006). Besides light, the germination of cactus seeds requires wet conditions and high temperature (Rojas-Arechiga and Vazquez-Yanes, 2000). Maiti et al. (1994) suggest that a high germination percentage is associated with a thin test and with the presence of starch granules. The heat treatments of 30°C-38°C did not have any effect on the germination of several cacti species (Sanchez-Soto et al., 2010). Therefore, a great option to improve cactus seeds germination is to use growth regulators like naphthaleneacetic acid (NAA) in a minimum concentration of 9%, or sodium nitrofenolate (1.8%).

Conclusions

From five treatment options studied to improve the germination capacity of cacti species, in three cases positive results were obtained. Treatments with salicylic acid and acetyl salicylic acid completely blocked the germination process. Treating the *Cactaceae* seeds with a minimum concentration (9%) of auxins, or with sodium nitrofenolate (1.8%) significantly improves the germination energy and germination capacity.

Creating a database regarding new techniques to improve cacti germination seeds can be useful and can be applied to propagation projects in the seeds production, to increase the multiplication rate.

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