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Original article

# Influence of Pollen Germination Capacity on a Successful Artificial Hybridization in *Cyclamen* sp [1]

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**Abstract**

The artificial hybridization is one of the most important methods to create variability in breeding programs. The success of artificial hybridization very often depends on the fertility of pollen grains which require a minimum 30% germination level. The aim of the present study was to identify the correlation between the level of pollen grain fertility and success of hybridization in several cyclamen cultivars. There were taken under study seven cultivars of *Cyclamen* sp. very different from phenotypic point of view (maxi, midi, mini). In the first step it was determinated the germination capacity of pollen grains trough viability and germinability percentage and the correlation between these two determinations. The results show a higher ratio of viability then germinability in all cultivars. The best results of artificial hybridization were registered in hybrid combinations in which there were used cultivars with the highest percentage of pollen fertility. Out of seven cyclamen cultivars tested in 18 hybrid combinations, three cultivars showed the highest level of fertility when used as male genitor (Curly White; Curly Magenta Picotee and Allure Salmon Flamed)for a further study on phenol biodegradation especially areas with phenol contamination containing some heavy metals.

**Keywords:** Breeding, Pollen grain germination, Pollen grain viability, *Cyclamen*.

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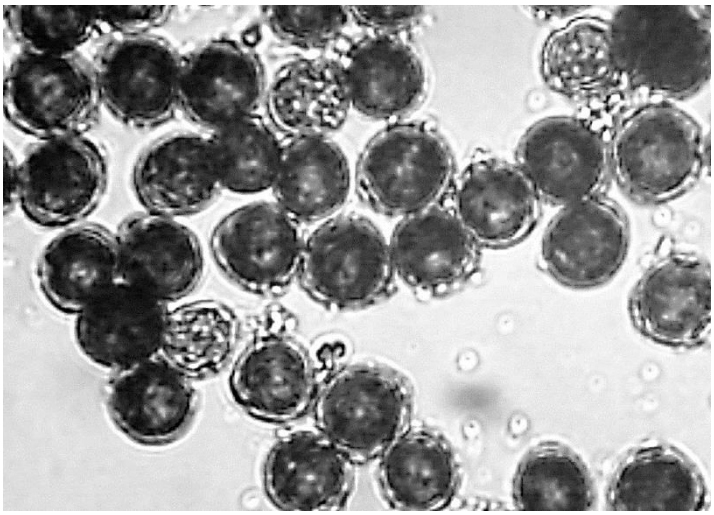
## INTRODUCTION

In contrast to other ornamentals which multiply both vegetative and by seeds, the cyclamen varieties multiply mostly by seeds. This makes important to obtain seeds for propagation. Another important aspect is that most plants in the market are hybrids and need manual castration and cross-pollination. Propagation by seeds presents some difficulties, because cyclamen is sensitive to inbreeding depression (Winkelmann, 2006) and the hybrids are heterogenous. For hybrid production, the germination capacity of pollen grains is very important for a successful artificial hybridization, where its level should be at least 30% (Cordea, 2014). According to Ewald (1996) pollen viability varies between 0.3- 34%. There are two ways for testing the germination capacity of pollen grains: indirectly by testing the pollen viability (by colouring tests with potassium iodide or carmine staining method) and directly, by *in vitro* pollen germination on solid medium. Between viability and germination of pollen grains there is a very strong, direct and positive correlation (Cordea, 2014).

The aim of this study was to evaluate the influence of pollen germination capacity on a successful artificial hybridization in several cyclamen cultivars.



### Material and Methods

In our research there were used seven cultivars of cyclamen belonging to different series of plant height: mini series (<15 cm) with two cultivars Mini Wine and Compact Dark Violet; midi series (15-25 cm) with two cultivars Allure Salmon Flamed and Latinia Succes Light Purple; maxi series (25-35 cm) with three cultivars Mammoth Red, Curly White and Curly Magenta Picotee.

The germination capacity and viability of pollen grains tests have been performed in the Plant breeding laboratory of the Advanced Horticultural Research Institute of Transylvania, UASVM Cluj-Napoca.

The cultivars of cyclamen were involved in a breeding programme resulting 18 hybrid combinations (Table 1). The artificial hybridization was performed as described by Cordea (2014).

***Determination of pollen grains viability***

For testing the viability of pollen grains we chose the potassium iodide colouring test. In the presence of the potassium iodide the fertile pollen grains coloured in dark blue to black, while the sterile ones remained colourless (Fig. 1).

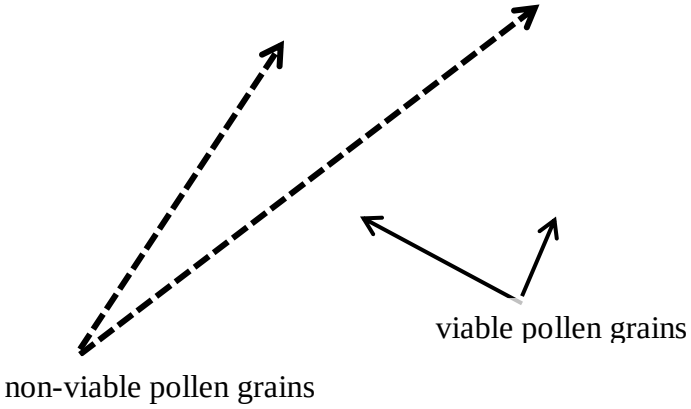



**Figure 1.** Pollen grain viability in cyclamen: filled arrow - viable pollen grains; dashed arrow - non-viable pollen grains (source: original)

To determinate the viability of pollen grains, the anthers were collected in early bud floral phase, before their maturation. After harvesting, immediately anthers were soak in the Carnoy solution for a 1.5 hours and then passed into alcohol until the colouring test was performed. For each cultivar there were counted approximately 300 pollen grains in several microscopic fields. Based on these data it has been calculated the rate (%) of pollen grain viability for each cultivar.

***Determination of in vitro pollen grains germination***

The fertile pollen grains with viable cytoplasm and spermatia germinated and released pollen tube on an artificial medium in presence of different sucrose concentrations (10-30%), with 70-90% humidity at a temperature between 20-22°C. There are similar researches about components of the medium for *in vitro* pollen grain germination, Soares et al., (2013) obtaining in *Passiflora* sp. best results with 15% sucrose.

In order to determine *in vitro* the pollen grain germination, the anthers were collected from flower buds before anthesis and put on Petri dishes at room temperature (22°C) until they opened (48 h) and released the pollen grains. The medium for pollen germination has been made of 1.5% agar and 15% sucrose. The dried pollen grains have been sown on medium and maintained at 22$^{o}$C in semi dark lab

conditions. After 48 hours the germinated grains were counted in different microscopic fields so that in the end there were analysed around 300 pollen grains (Fig.2).

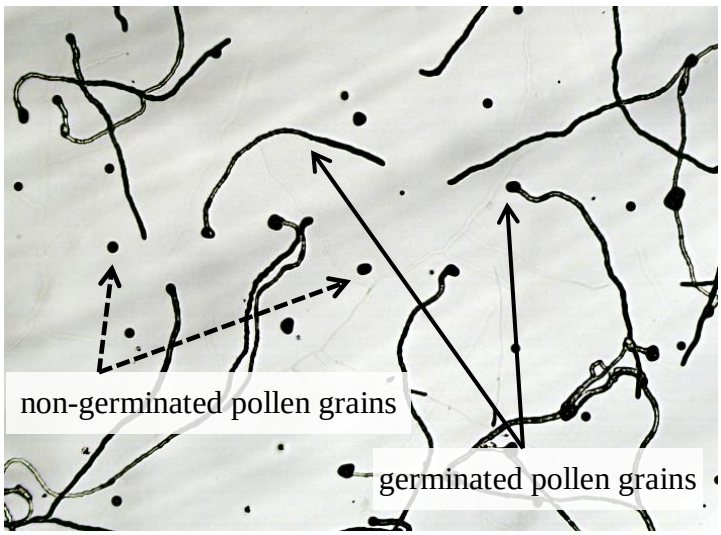



**Figure 2.** Pollen grain germination in cyclamen: filled arrow – germinated pollen grains; dashed arrow - non-germinated grains (source:original)

**Artificial hybridization**

The artificial hybridization was performed between all seven cultivars: Curly White (C1), Compact Dark Violet (C2), Curly Magenta Picotee (C3), Mammoth Red (C4), Allure Salmon Flamed (C5), Latinia Succes Light Purple (C6), Mini Wine (C7) as shown in table 1. For each hybrid combination there were cross-pollinated about 5-8 flowers (table 1). The technique of artificial hybridization was performed as described by Cordea (2014).

**Results**

The cross pollination it is very important to create variability which will be exploited by selection to obtained new cultivars and on the other hand to obtain seeds for $F_1$ hybrids. On the market most of the cyclamen plants are hybrids and are multiplied by seeds. In *Cyclamen* species there are many incompatibility barriers especially in interspecific hybridization. Embryo rescue and *in vitro* chromosome doubling are methods to overcome these barriers (Ishizaka, 2008).

In plant breeding program, when choosing the genitors an important aspect is the germination capacity of pollen grains. To achieve a successful cross pollination, pollen grains should have a minimum 30% germination rate (Cordea, 2014). In our research the analysis of pollen grains of seven cultivars of *Cyclamen* are presented in Figure 3.

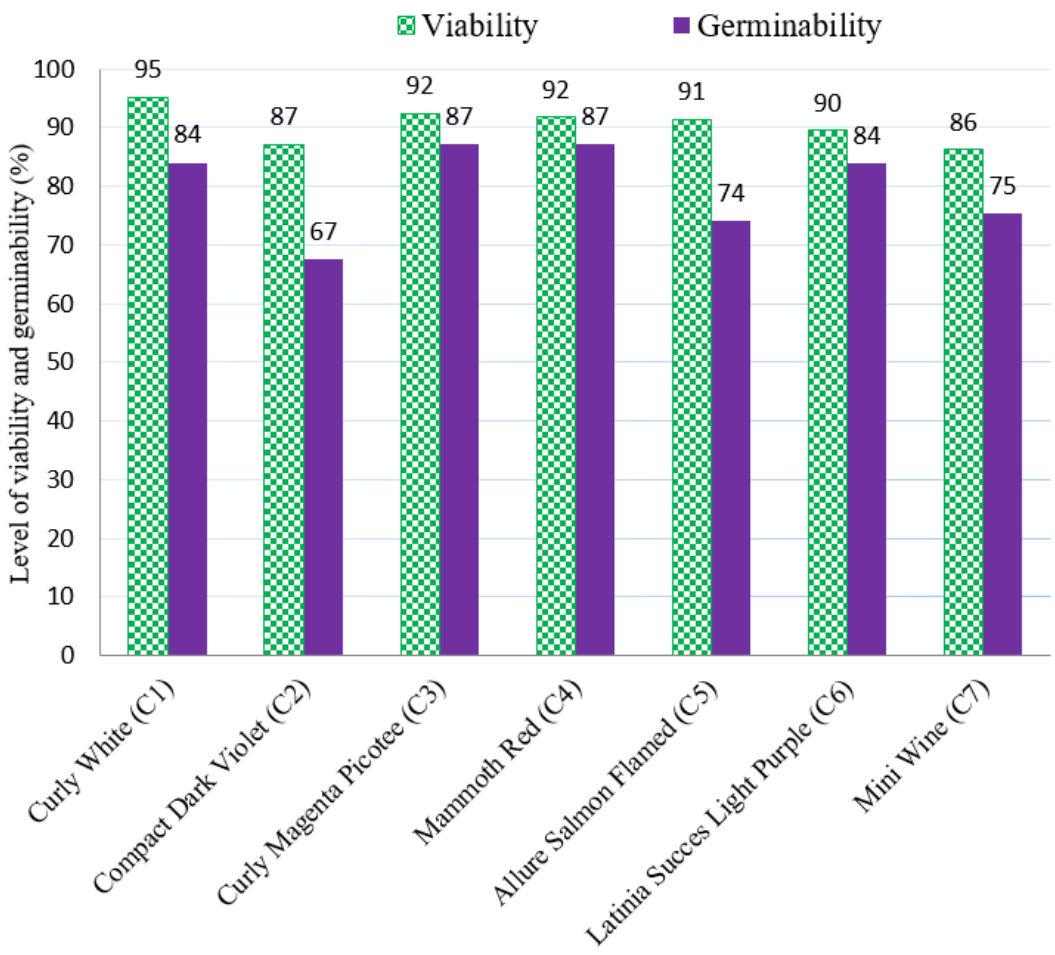



**Figure 3.** Pollen grain viability and germinability in seven Cyclamen cultivars

In the first step we analysed the viability of pollen grain and five out of seven cultivars registered over 90% of viability (Curly White – 95%; Curly Magenta Picotee – 92%, Mammoth Red – 92%, Allure Salmon Flamed – 91% and Latinia Succes Light Purple- 90%).

*In vitro* germinability of pollen grains presented almost the same results. The cultivars with a high percentage of viability registered also high values in germinability (Curly White – 84%; Curly Magenta Picotee – 87%, Mammoth Red – 87% and Latinia Succes Light Purple- 84%). Only one cultivar (Compact Dark Violet) registered 67% germination capacity.

In terms of the artificial hybridization results, 11 out of 18 hybrid combinations were fertilized and formed seeds, but these didn't germinate (Table 1). In seven hybrid combinations, all emasculated and hand-pollinated, fertilization didn't succeed, flowers were aborted before seed set. Out of 18 hybrid combinations, only six resulted capsules-seeds and plants.

**Table 1.** The results of artificial hybridization in seven cyclamen cultivars

| No. | Hybrid combination | Code of hybrid combination | Pollinated flower no./ hybrid combination | Flower no. fertilize/aborted | % fertility | No. of hybrid seeds | % of germina-ted seeds | No. of plants F1 |
|---|---|---|---|---|---|---|---|---|
| 1 | Curly White x Compact Dark Violet | 1x2 | 7 | 0/7 | 0 | 0 | 0.0 | 0 |
| 2 | Curly White x Curly Magenta Picotee | **1x3** | 7 | 6/1 | 85.7 | 47 | 17.0 | 8 |
| 3 | Curly White x Mammoth Red | 1x4 | 7 | 0/7 | 0 | 0 | 0.0 | 0 |
| 4 | Compact Dark Violet x Curly White | **2x1** | 9 | 7/2 | 77.7 | 32 | 15.6 | 5 |
| 5 | Compact Dark Violet x Curly Magenta Picotee | 2x3 | 6 | 0/6 | 0 | 0 | 0.0 | 0 |
| 6 | Compact Dark Violet x Mammoth Red | 2x4 | 8 | 0/8 | 0 | 0 | 0.0 | 0 |
| 7 | Compact Dark Violet x Allure Salmon Flamed | **2x5** | 9 | 5/4 | 55.5 | 13 | 46.2 | 6 |
| 8 | Curly Magenta Picotee x Curly White | **3x1** | 8 | 7/1 | 87.5 | 6 | 33.3 | 2 |
| 9 | Curly Magenta Picotee x Compact Dark Violet | 3x2 | 7 | 0/7 | 0.0 | 0 | 0.0 | 0 |
| 10 | Curly Magenta Picotee x Mammoth Red | 3x4 | 8 | 1/7 | 12.5 | 4 | 0.0 | 0 |
| 11 | Curly Magenta Picotee x Allure Salmon Flamed | **3x5** | 9 | 7/2 | 77.7 | 7 | 85.7 | 6 |
| 12 | Mammoth Red x Curly White | 4x1 | 5 | 0/5 | 0.0 | 0 | 0.0 | 0 |
| 13 | Allure Salmon Flamed x Curly White | 5x1 | 7 | 1/6 | 14.3 | 3 | 0.0 | 0 |
| 14 | Allure Salmon Flamed x Compact Dark Violet | 5x2 | 6 | 1/5 | 16.7 | 4 | 0.0 | 0 |
| 15 | Allure Salmon Flamed x Curly Magenta Picotee | **5x3** | 8 | 5/3 | 62.5 | 2 | 50.0 | 1 |
| 16 | Allure Salmon Flamed x Mammoth Red | 5x4 | 5 | 1/4 | 20.0 | 4 | 0.0 | 0 |
| 17 | Allure Salmon Flamed x Mini Wine | 5x7 | 7 | 2/5 | 28.6 | 6 | 0.0 | 0 |
| 18 | Mini Wine x Allure Salmon Flamed | 7x5 | 8 | 0/8 | 0.0 | 0 | 0.0 | 0 |

**Discussion**

The success of artificial cross pollination depends on many factors: level of ploidy, genetic compatibility, pollen fertility of genitors, environmental conditions etc. Kermanshahani (2014) observed that genotypes with 30% higher pollen germination led to 10% increase in fruit set.

According to the data presented in Figure 3 it can be noticed that the viability of pollen grains in all tested cultivars reached higher levels than their germination rate. This situation can be explained by the fact that for *in vitro* germination capacity the pollen grain has to be dried 48 hours before testing while for viability testing the anthers with pollen grains are collected just before maturation.

The results of the presented study emphasize three cultivars (Curly White; Curly Magenta Picotee and Allure Salmon Flamed) both being recommended as male and female parents. Best results were obtained with Compact Dark Violet cultivar used as the female parent in combination with Curly White cultivar from maxi category and Allure Salmon Flamed from midi category. Two of seven cultivars (Mammoth Red and Mini Wine) tested in our study presented the poorest results even they were used like a female or male parent. The best result of fertility (over 85%) and most seeds were obtained in direct and reciprocal cross pollination of Curly White x Curly Magenta Picotee cultivars, both of them belonging to maxi category. We cannot say the same thing about direct and reciprocal cross pollination between Curly White x Mammoth Red even they are in the same category.

Another interesting result of our study presented the direct and reciprocal cross pollination of Curly Magenta Picotee (maxi) x Allure Salmon Flamed (midi). Even they belong to different categories we obtained a high percent of fertility (77.7% – 62.5%) and germinated seeds (85.7%).

**Conclusions**

Based on these results a higher viability than germination rate might be due to the length of the drying and preservation period of pollen grains between collecting and analysis (3-4 days). This suggests that the fertile period of *Cyclamen* pollen is rather short, only a few days. The cultivars (Curly White; Curly Magenta Picotee and Allure Salmon Flamed) with high rate of viability and germinability pollen grain give the best results in artificial cross pollination. These results obtained in artificial hybridization offer useful information for selecting the best genitors for *Cyclamen* breeding programs.

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