Effect of different ovule isolation times on the embryo development of *Campanula* hybrids

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CONCLUSION

- Ovule isolation time is an important factor for a successful embryo rescue
- Ovule isolation time affected both the number of germinating embryos and the rate of their development *in vitro*
- Successful interspecific hybridisation: here obtained hybrids *in vitro* might not be prevented through prefertilisation or postfertilisation barriers

OBJECTIVES

In this study cultivars of three *Campanula* species from southeastern Europe have been selected:

- To develop new interspecific hybrids
- To investigate the number of ovules and their subsequent germination rates *in vitro* from intra- and interspecific hybrids
- To determine the optimal ovule harvest period after *in vivo* pollination

C. carpatica 'White'

C. medium 'Pink' and *C. formanekiana*

RESULTS

Isolation of ovules

The number of isolated ovules varied between the intraspecific cross and the interspecific reciprocal crosses and depended on the ovule isolation time after pollination.

Table 1. The number of ovules: Self-pollination, intra- and interspecific hybridisation of selected *Campanula* species (from 15 pollinated flowers) isolated after different weeks after pollination

Cross	Number of ovules			
	1 week	2 weeks	3 weeks	4 weeks
<i>C. carpatica</i> 'White' (Intraspecific cross)	372	2250	1	158
<i>C. medium</i> 'Pink' (Self-pollination)	75	25	324	550
<i>C. medium</i> 'Pink' x <i>C. formanekiana</i>	1	25	121	474
<i>C. formanekiana</i> x <i>C. medium</i> 'Pink'	50	270	350	3150

- A later isolation time after pollination from the intraspecific cross did not result in an increased number of isolated ovules (Table 1)
- An increased number of ovules as an effect of a longer *in vivo* ovule development from self-pollination and the reciprocal interspecific crosses was observed (Table 1)
- The highest number of ovules was isolated when *C. formanekiana* was used as maternal parent

Germination

- Germination rate for ovules from *C. formanekiana* x *C. medium* 'Pink' increased from week 2 to week 3 (Figure 1)
- Ovules isolated 3 weeks after pollination germinated faster than after 2 weeks

Fig. 1. Germination, determined as percentage of ovules with a root longer than 1 mm, for *C. formanekiana* x *C. medium* 'Pink' after ovule isolation 2 and 3 weeks after pollination

- The ovules collected 3 weeks after pollination showed a 50 % germination after 38 days whereas a 50% germination after 2 weeks took 55 days (Figure 1).
- Ovule isolation 2 weeks after pollination resulted in approx. 14 % germinated ovules in total, compared to a germination efficiency of 93 % when ovules were isolated 3 weeks after pollination

MATERIALS AND METHODS

For the experiment two *Campanula* species with difficulties to hybridise were selected. Intraspecific cross and self-pollination were used as controls. The ovules were allowed to mature *in vivo* for 1-4 weeks after pollination, sterilised and placed on a half strength MS medium.

Analyses

Number of collectable ovules were recorded each week. The germination was monitored for 80 days and defined as emergence of the first visible root (> 1mm).

Statistical analysed as time to event data (Ritz *et al.*, 2010) by using the statistic software program R (R Development Core Team, 2011).

References

- Ritz, C., Pipper, C., Yndgaard, F., Fredlund, K. and Steinbrücken, G. 2010 Modelling flowering of plants using time-to-event methods. *Europ. J. Agronomy* 32: 155-161
- R Development Core Team 2011 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>. ISBN 3-900051-07-0