Determination of the Crossing Barriers in Hybridization of *Cucumis sativus* and *Cucumis melo*

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Introduction: Serious crossing barriers prevent the successful hybridization of *Cucumis sativus* L. and *Cucumis melo* L. (5,6). However, such hybridization would be important for transferring several resistances from *C. melo* or other wild *Cucumis* spp. to *C. sativus* (1,4,5). The determination of crossing barriers can help in selecting potentionally successful methods for overcoming obstacles to fertilization. For example, *in vitro* pollination followed by *in vitro* cultivation of rescued hybrid embryos without challenge to extirpation shocks *in ovulo* (9, 13) could be used as a methodology.

Interspecific crossing barriers can be classified into two groups: (a) prezygotic (including all factors hindering effective fertilization), and; (b) postzygotic (occurring during or after syngamy) (14). Experiments were designed to overcome these barriers in *C. sativus* x *C. melo* mating by *in situ* and *in vitro* pollination. The first stages of embryos development were observed to investigate the responses to treatments for overcoming fertility barriers.

Material and Methods: Plants of *Cucumis sativus* (line SM 6514) and *Cucumis melo* (cv. Solartur) were grown in a glasshouse. The seeds originated from the Vegetable Germplasm Collection of the Research Institute of Crop Production, Prague, Gene Bank Division, Workplace Olomouc, Czech Republic. Female flowers at the stage of anthesis were self-pollinated or pollinated with pollen of the opposite *Cucumis* species.

The observation of *in situ* fertilization was made by cutting of pollinated pistils and staining in aniline blue. The stained slides were observed by fluorescence microscopy. The observations of pistils were made 6, 24 and 48 hours after hand-pollination.

The pollen grains and ovules for *in vitro* observation were aseptically isolated onto a YS culture medium (10) (Table 1) for use in fertilization experiments. The process of *in vitro* fertilization was observed via inverted microscopy. After 20 hours the ovules were transferred onto DI1a culture medium (Table 1) (2) in Petri dishes. They were cultivated in a growth chamber under light intensity 32 to 36 μmol (PAR) $m^{-2}s^{-1}$, with light/dark cycles being 16/8 hrs and temperature 22 $\pm$ 2 °C.

Seven days after *in situ* and *in vitro* pollination, the immature seeds were taken for embryological analyses. Seeds were fixed in Carnoy solution, cutted in paraffin and stained in hematoxyline.

Results and Discussion: *Differences in fertilization of C. sativus, C. melo and interspecific hybrids.* Cucumber and melon ovules were of the anatropous type and contained a monosporic, Polygonum-type embryo sac (3). The pollen grains of both species were triporate and contained vegetative and generative nuclei during cell maturation. The size of *C. sativus* grains was about 60 μm, and *C. melo* was about 50 μm (11).

Twenty to 30 minutes after *C. sativus* self-pollination, pollen grains began to germinate on the stigma. Six hours after pollination, pollen tubes were observed on the stigma-style border. Twenty-four hours after pollination the pollen tubes were localized on the style – ovary border. The pollination process in *C. melo* was slower than in *C. sativus.* During the first 6 hours the pollen tubes were still in stigma, and at 24 hours in style. The penetration of ovules and fertilization occurred 48 hours after pollination.

During hybridization of *C. sativus* (male) x *C. melo* (female) and *C. melo* (male) x *C. sativus* (female), abnormalities were not found in pollen tubes, and during their germination and development. Likewise, no comparative differences in the growth and speed of tube maturation were observed after self-pollination of parental stocks.

Table 1. The composition of culture medium used *in vitro* pollination and ovule cultivation of interspecific hybrid progeny between *C. melo* and *C. sativus*.

Composition	Type of medium	
	DI1a	YS
Macro and micronutrients (mg/l)	MS	600 $Ca(NO_3)_2xH_2O$ & 100 H_3BO_3
Vitamins (mg/ml)	MS & 5 vit. PP	none
Amino acids (mg/ml)	MS	none
Protein hydrolysates (g/l)	0,4 CH	none
Sucrose (g/l)	30	80
Agar (g/l)	8	10
Growth regulators (mg/l)	4 IAA	none
	2,5 Kin	
	0,4 2,4-D	

MS - Murashige and Skoog medium (7)

CH - casein hydrolysate

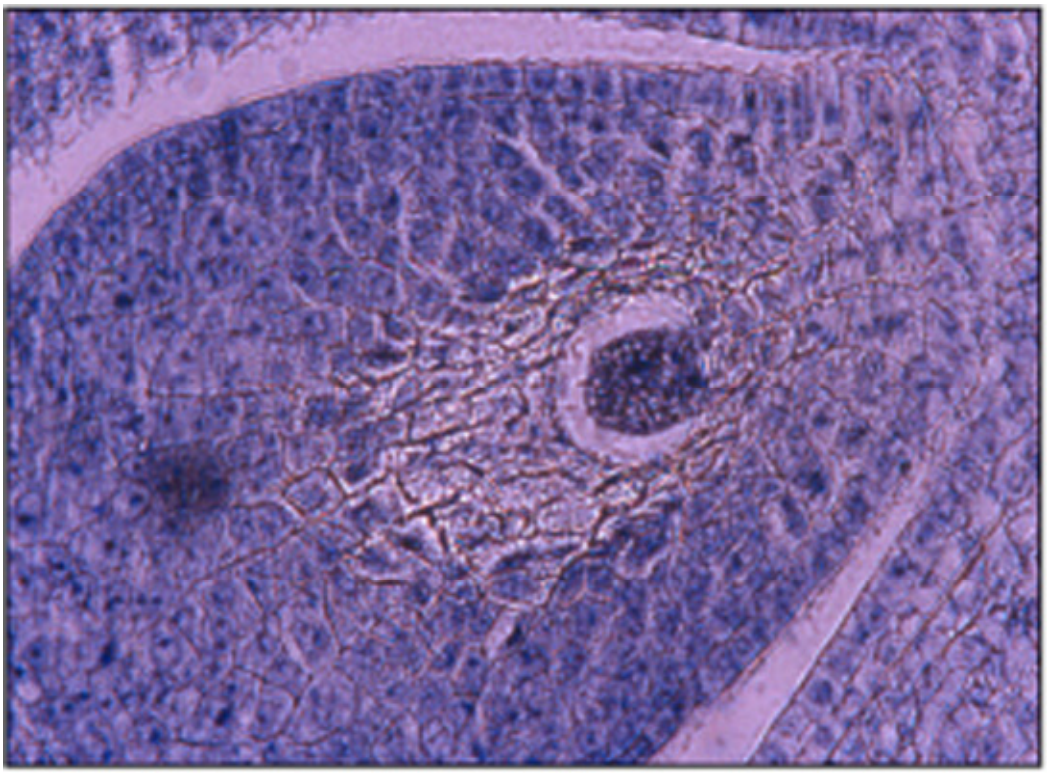

Figure 1. Globular embryo of *C. sativus* seven days after *in situ* pollination.

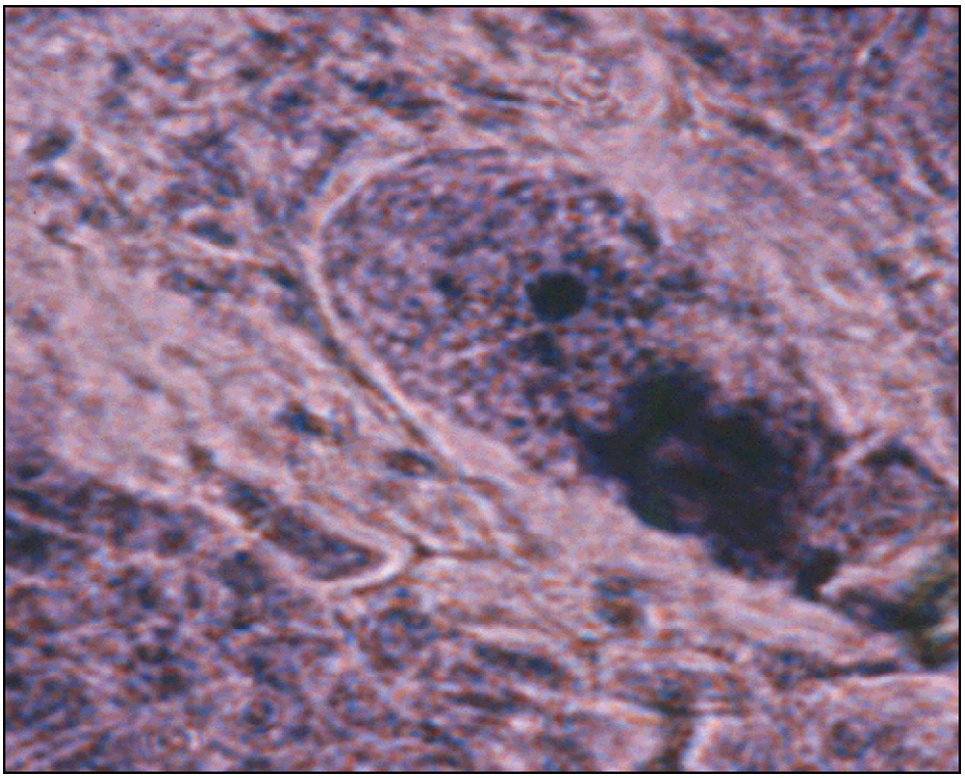

Figure 2. Hybrid embryo of *C. sativus x C. melo* seven days after *in situ* pollination.

Prezygotic barriers have previously been found during interspecific crosses *C. melo* x *C. metuliferus* (1), or by crosses of *C. melo* (2n) x *C. melo* (4n) (10). However, in our experiments, these kinds of barriers were not observed. Seven days after self-pollination of *C. sativus* (Fig. 1) and *C. melo* plants, globular embryos developed. Thereafter, normal development of embryos, seeds and fruits was recorded.

The hybrid immature seeds contained globular embryos seven days after pollination (Fig. 2). However, the development of embryos and fruits stopped at this stage. Embryos aborted and immature fruits became yellow in color. This developmental sequence is in agreement with previously published data (8). The abortion of hybrid embryos (postzygotic barrier) could be considered as a main factor in the inability of these *Cucumis* species to cross fertilize.

Differences in fertilization in situ and in vitro. The pollen germination *in vitro* started 10 min after transfer to the culture medium. This was a shorter time than that demonstrated by germination *in situ*. The suitability of medium for germination *in vitro* was demonstrated by the absence of cracked pollen tubes and calloses in tubes. The highest concentration of boric acid and sucrose stimulated pollen germination and pollen tube growth. The tubes length was around 450 μm one hour after germination in both species, and 1350 μm for *C. sativus* and 1100 μm for *C. melo* 24 hours after cultivation. At that time, the tubes growth stopped. In contrast to pollination *in situ*, no taxis of tubes were observed; except for a very small area near the ovules.

Penetration of ovules by pollent tubes was noted and globular embryos were detected in both species and their hybrid seven days after cultivation. These results showed that the complete process of sexual reproduction can be accomplished in *C. sativus* x *C. melon* matings, and that embryos can be obtained for *in vitro* cultivation at an early stage of development after fertilization.

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