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Embryo rescue of cucumber (*Cucumis sativus*), muskmelon (*C. melo*) and some wild *Cucumis* species (*C. anguria, C. zeyheri,* and *C. metuliferus*)

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Summary

Cucumis sativus is one of the most economically important crops of the Cucurbitaceae. Recent cucumber cultivars are susceptible to some serious diseases and pests, including downy mildew, powdery mildew, nematodes, and spider mites. Sources of resistance to these pathogens and pests were identified in some accessions of wild *Cucumis* species. One possible way of introducing these resistances into cucumber germplasm is interspecific hybridization. However, *C. sativus* is sexually incompatible with nearly all other *Cucumis* species, because of substantially different chromosome numbers, n = 7 in *C. sativus* versus n = 12 in *C. melo* and most wild *Cucumis* species. Overcoming this obstacle can be accomplished through the use of embryo rescue and/or ovule culture. Results of experiments using these methods, especially of embryo rescue of cucumber and selected wild *Cucumis* species after intra- and interspecific hybridization, are summarized in this paper. Various culture media and selected genotypes were tested in our experiments. Successful regeneration of mature embryos of some *Cucumis* spp. was observed on all types of media, and callus or sporadic plant formation from immature embryos and seeds occurred on media with coconut water and gibberellic acid.

Abbreviations

BA – benzyladenin; IBA – indole-3-butyric acid; MS – Murashige and Skoog medium (1962).

Introduction

The Cucurbitaceae family consist of approximately 118 genera and 825 species almost equally divided between the New and Old World, with an emphasis on tropical regions. One of the most important genera is *Cucumis* (Jeffrey, 2001), which has two subgenera. Subgenus *Cucumis* considered to be of Asiatic origin (n = 7), and subgenus *Melo* Miller of African origin (n = 12). Subgenus *Cucumis* includes *Cucumis sativus* L. and *C. hystrix* Chakrav. Although *Cucumis hystrix* resembles *C. sativus* morphologically and biochemically, its base chromosome number (n = 12) is the same as *C. melo*, which is included with wild *Cucumis* species in subgenus *Melo* (Lebeda et al., 2007).

Cucumber cultivars are susceptible to some serious diseases and pests. Genes for resistance to several pathogens and pests that are not known to occur in cucumber have been found in accessions of wild *Cucumis* species (Leppik, 1966; Chen and Adelberg, 2000; Chen et al., 2003; Lebeda et al., 2007). Accessions of *Cucumis anguria* L., *C. metuliferus* E. Mey ex Naud. and *C. zeyheri* Sond. have high levels of resistance to root-knot nematode (den Nijs and Custers, 1990; Wehner et al., 1990). Very high levels of resistance to *Tetranychus urticae* were found in some accessions of *C. zeyheri* (Lebeda, 1996). *Cucumis metuliferus* also displays resistance to Squash mosaic virus (SqMV) and Watermelon mosaic virus (WMV) (McCarthy et al., 2001). *Cucumis melo* line MR-1 and some other accessions have some resistance to cucumber downy mildew (*Pseudoperonospora cubensis*) (Lebeda et al., 1996, 1999, 2007). One possible method of introducing these resistance genes to cucumber cultivars is via interspecific hybridization (Lebeda et al., 2007). To this end, it is necessary to use unconventional techniques, including various methods of *in vitro* culture, due to hybridization barriers and embryo abortion in the early globular stage of embryo development (Ondřej et al., 2001). One approach to overcome this crossability barrier, which is primarily caused by different chromosome numbers, is to use embryo culture, a method of embryo rescue (Chen and Adelberg, 2000; Lebeda et al., 1996; Skálová et al., 2004). The composition of the culture media has the major influence on the successful regeneration of isolated and cultivated embryos (Skálová et al., 2004).

Successful embryo rescue techniques for the genus *Cucumis* is considered important for future efforts toward interspecific hybridization (Lebeda et al., 2007; Skálová et al., 2004). The main purpose of this paper is to report our recent results in the research of *Cucumis* spp. embryo rescue. Selected wild species and new types of media were tested for supporting regeneration, especially of immature embryos.

Materials and methods

Plant material

Selected *Cucumis* species were used for embryo rescue experiments (Tab. 1). The plant material originated from the vegetable germplasm collection of the Research Institute of Crop Production (Prague), Gene Bank Department, Olomouc Workplace, Czech Republic (Web site: <www.vurv.cz/>, EVIGEZ) and the USDA-ARS North Central Regional Plant Introduction Station, Iowa State University, Ames, IA. The plants were cultivated in a glasshouse at the Department of Botany, Palacký University in Olomouc, Czech Republic (25°C/15°C day/night; with daily watering and weekly fertilization by Kristalon Start (Hydro Agri, Rotterdam, Netherlands), 10 ml of fertilizer (19:6:20 N:P:K) /10 l of H_2O), in organic substrate (Florcom SP, BBcom s.r.o, Letohrad, Czech Republic), and without pest control.

Embryo culture

Fruits of selected *Cucumis* species (Tab. 1) were harvested after three days or one, two and three weeks, and in the case of *Cucumis sativus* and *C. anguria*, also after six weeks post hand self-pollination. For cross-pollination, *C. sativus* was used as a maternal parent and other *Cucumis* species (Tab. 1) as paternal parents, and the fruits were harvested two weeks after hand cross-pollination. They were surface sterilized with 70% ethanol (fruits were rinsed and then flamed) and seeds (S) were dissected. Immature seeds (originating from the 3 day fruits after self-pollination and from 2 week fruits after cross-pollination) were cultured intact. Zygotic embryos (E) were excised from the seeds of 1, 2, 3 and 6 week fruits while viewed using a stereoscopic binocular microscope under sterile conditions.

Embryos or seeds were cultivated on various media (Tab. 2) in test tubes for six weeks at 25°C in the dark (5 ml of medium per tube). One of these was OK-medium, which acted as a control, and the others, ON-, CW-, and GA-medium, were supplemented with components which are thought to have a positive influence on embryogenesis: casein hydrolysate, coconut water, and gibberellic acid. MS-medium (Murashige and Skoog, 1962) was used as a basis for macroelements,

Tab. 1: *Cucumis* species used for embryo rescue.

Cucumis species	Abbreviation	Accession number
C. sativus (line SM-6514)	CS	CZ 09H3900768
C. anguria var. *longipes*	CA	PI 249896
C. zeyheri	CZ	PI 364473
C. melo (line MR-1)	CM1	PI 124111
C. melo var. Charentais	CM2	PI 261778
C. metuliferus	CME	PI 292190

Tab. 2: Composition of culture media.

Culture medium	Basic medium	Other components
OK	MS	20mg/l ascorbic acid, 0.01mg/l IBA, 0.01mg/l BA, 20mg/l sucrose, 8g/l agar (control medium)
ON	MS	1g/l casein hydrolysate, 0.01mg/l IBA, 0.01mg/l BA, 20g/l sucrose, 6g/l agar
CW	MS	5% coconut water, 200mg/l α-glutamin, 0.01mg/l IBA, 0.01mg/l BA, 60g/l sucrose, 6g/l agar
GA	MS	0.3mg/l gibberellic acid, 0.01mg/l IBA, 0.01mg/l BA, 20g/l sucrose, 8g/l agar

Explanatory notes: MS = Murashige and Skoog (1962); OK, ON, CW, GA = abbreviations for MS-media supplemented with different type of additions supporting embryogenesis; IBA = indole-3-butyric acid; BA = benzyladenin.

microelements and vitamins. Other components were supplemented depending on the type of medium (ascorbic acid, casein hydrolysate, α- glutamin, IBA, BA, sucrose, and agar), and the pH was adjusted to 5.8 before autoclaving. Solutions with coconut water and gibberellic acid were filter-sterilized (Steritope, Millipore, Billerica, MA) and added to the autoclaved part of the medium.

After germination, the embryos or seeds were transferred to a culture room (22±2°C and 16h-day/8h-night photoperiod, 32 - 36 μmol · m^{-2} · s^{-1}) and then the type and frequency (%) of regeneration of embryos of various ages and seeds on the media were evaluated and compared. We describe three types of regeneration in our experiments – 1. callus formation (C; no organogenesis was observed from isolated embryos and seeds; only callus without stems, roots or leaves); 2. abnormal plant formation (AP; indirect organogenesis from isolated embryos and seeds was observed, such as the formation of stems, roots or leaves on callus; vitrificated plants also occurred – callus formation on developed stems, roots or leaves) and 3. normal plant formation (P; direct organogenesis from isolated embryos and seeds was observed, with the development of entire plants) (Fig. 1).

The experiments were repeated. The frequency of regeneration was expressed in percent (average percentages were presented). In graphs, Y-error bars were used to represent the standard deviation of means.

Results and discussion

Embryo rescue after hand self-pollination in *Cucumis* species

The most important aspects to consider regarding embryo development in cultures are the age of embryos (seeds), the genotype, a suitable

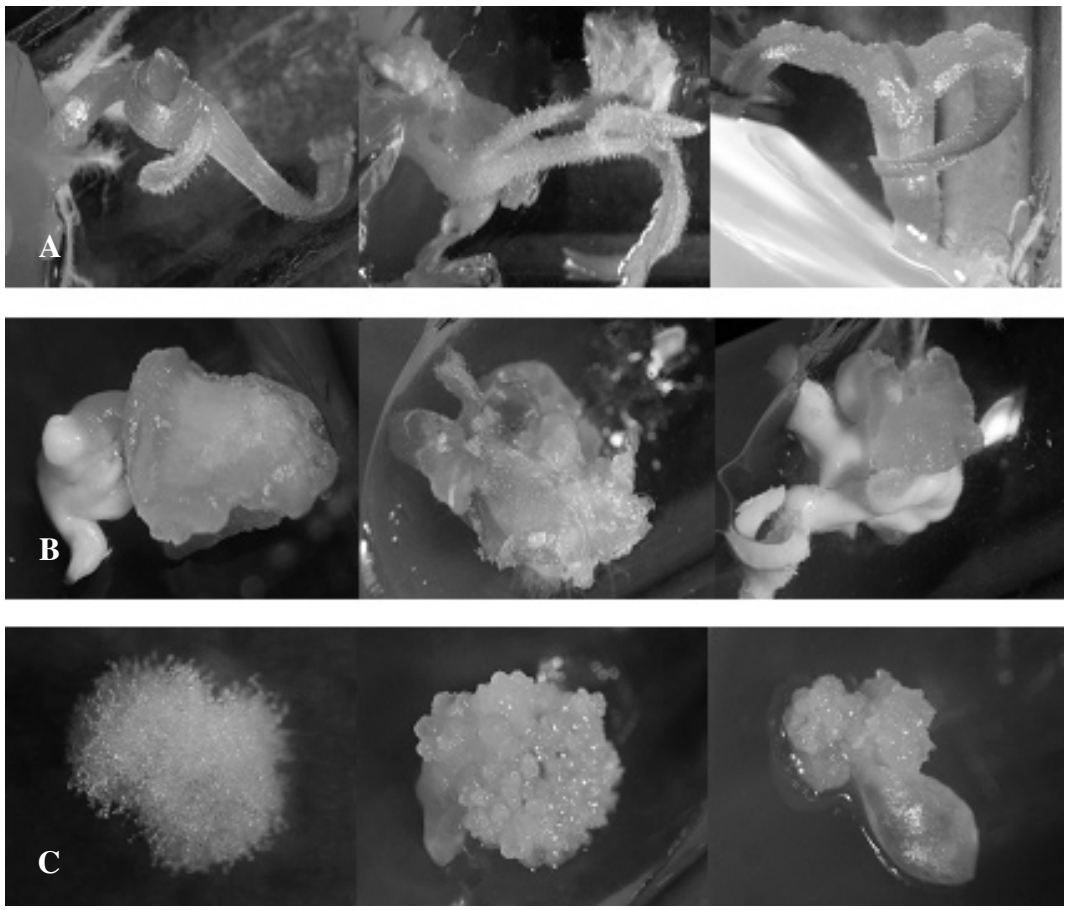

Fig. 1: Various types of regeneration from isolated embryos or seeds of *Cucumis* species (A – normal plant formation; B – abnormal plant formation; C – callus formation).

culture medium, the concentration of sucrose and growth regulators and the influence of light (Ondřej and Navrátilová, 1999). Our recent experiments focused on the selection of the most suitable genotypes and media for embryo rescue in the genus *Cucumis*. Embryos and seeds isolated from fruits in different time periods after hand self-pollination were cultivated on various types of media, and the type and frequency (%) of regeneration was compared (Tabs. 3 and 4; Fig. 4).

For cucumber (*C. sativus*), the *in vitro* response of one genotype, SM 6514, was studied. We observed normal development of mature embryos (6 week) to entire plants on the OK- and ON-media (100% regeneration; Fig. 1). On the other hand, coconut water in CW-medium often supported indirect regeneration with callus development on mature isolated embryos (Fig. 2). A similar effect of coconut water was noted by Ondřej et al. (2000). Callus formation and abnormal plant formation were noted during the development of the 1, 2 and 3 week embryos (especially on CW-medium; Fig. 1). The 3 day seeds developed to plants on ON-medium (61%), and callus development and abnormal plant development were recorded on GA-medium (72%).

Regeneration of 3 and 6 week embryos of *C. anguria* resembled results from *C. sativus*. The most suitable media for normal plant development were OK (90%; 100%) and ON (80%; 100%), and abnormal plant formation was again noted on the CW-medium. Immature embryos and seeds were regenerated to entire plants on OK-medium (Fig. 2).

For 3 week embryos of *C. zeyheri*, OK- and ON-media (80%; 90%) were suitable, but for the 2 week embryos it was CW-medium (70%). Ondřej et al. (2000) also found these media to be suitable for *C. zeyheri*. Nuñez-Palenius et al. (2006) reported a beneficial effect of coconut water on embryo resuce of *Cucumis melo*, and Goralski and Przywara (1998) observed its positive effects on regeneration of immature embryos generally. Regeneration of immature 1 week embryos and the 3 day seeds was the most efficient on GA-medium (60%). The positive influence of GA_3 (but at a different concentration) was recorded in previous experiments (Ondřej et al., 2002).

The most important species for interspecific hybridization of cucumber is considered to be muskmelon (*C. melo* (line MR-1)). We observed 100% regeneration of its 2 week embryos on CW- and GA-media; however, the type of regeneration was only callus or abnormal plants (Fig. 1). Normal plants were obtained from embryos cultivated on OK- and ON-media (89%). Ondřej et al. (2000) reported that the most suitable medium for *C. melo* (line MR-1) was generally the OK-medium. Gibberellic acid (GA-medium) was suitable for the 3 day seeds and 1 week embryos (60%; 80%).

Embryos and seeds of a second genotype of muskmelon, *C. melo* var. Charentais, regenerated less frequently than did line MR-1. The most suitable media for the 3 week embroys were ON- and CW- (only callus formation; 100%). Gibberellic acid and coconut water were suitable for all immature embryos (2 and 1 week) and seeds (3 day) (callus formation predominated) (Fig. 2).

The most suitable medium for *C. metuliferus* embryos was OK-medium, but lower reduced concentrations of sucrose and growth regulators (Ondřej et al., 2000). The 3 week embryos of *C. metuliferus* expressed the best results on OK- and ON-media (100%). No regeneration of plants was observed on media with coconut water and gibberellic acid. Nevertheless, these media were the best for the cultivation of the 2 week, 1 week and the 3 day seeds (mostly callus formation, rarely abnormal plant production was observed).

It is clear from these experiments that each species responds differently to these *in vitro* culture conditions. Our results confirm the fact that the successful cultivation (= obtaining the entire plants through direct organogenesis) of immature embryos and seeds is more complicated than is regeneration from mature embryos and seeds (Preťová, 1995).

Tab. 3: Frequency (%) of successful regeneration of *Cucumis* genotypes on various culture media.

Cucumis spp.		CS	CA	CZ	CM1	CM2	CME
Age	Medium						
3 days	OK	22	35	6	6	0	20
	ON	61	7	8	11	5	30
	CW	72	27	12	6	10	30
	GA	72	–	4	17	0	23
1 week	OK	56	10	10	60	40	10
	ON	50	20	10	25	0	10
	CW	50	20	20	60	20	25
	GA	60	-	60	80	40	28
2 weeks	OK	20	50	55	89	30	0
	ON	78	20	40	89	20	5
	CW	78	50	70	100	23	20
	GA	40	40	40	100	30	15
3 weeks	OK	100	90	80	50	43	100
	ON	86	80	90	45	100	100
	CW	86	80	75	55	100	50
	GA	–	–	60	40	83	75
6 weeks	OK	100	100	–	–	–	–
	ON	100	100	–	–	–	–
	CW	100	100	–	–	–	–
	GA	–	–	–	–	–	–

Explanatory notes: CS, CA, CZ, CM1, CM2, CME – selected genotypes of *Cucumis* (Tab. 1); OK, ON, CW, GA – various types of media (Tab. 2); – - variant was not tested.

Tab. 4: Types of regeneration of *Cucumis* genotypes on various culture media.

Cucumis species		CS	CA	CZ	CM1	CM2	CME
Age	Medium						
3 days	OK	P, AP	P	P, AP	C	0	C
	ON	P	AP	AP	C	C	C
	CW	AP	AP	P	C	C	C
	GA	C, AP	–	P	C	0	C
1 week	OK	P	P	P, AP	P	C	C
	ON	P	AP	AP	AP	C, AP	C
	CW	AP	AP	P	C, AP	C, AP	C
	GA	AP	–	P	AP	C	C
2 weeks	OK	P	P	P	P	C	C
	ON	P	P	P	P	C	C
	CW	AP	AP	P, AP	AP	AP	AP, C
	GA	AP	–	P, AP	AP	AP	AP, C
3 weeks	OK	P	P	P	P	C	P
	ON	P	P	P	P	AP	P
	CW	P, AP	P, AP	P, AP	AP	AP	C
	GA	–	–	–	AP	C	C
6 weeks	OK	P	P	–	–	–	–
	ON	P	P	–	–	–	–
	CW	P, AP	P, AP	–	–	–	–
	GA	–	–	–	–	–	–

Explanatory notes: CS, CA, CZ, CM1, CM2, CME – selected genotypes of *Cucumis* (Tab. 1); OK, ON, CW, GA – various types of media (Tab. 2); C – callus formation, AP – abnormal plant formation, P – normal plant formation; – - variant was not tested.

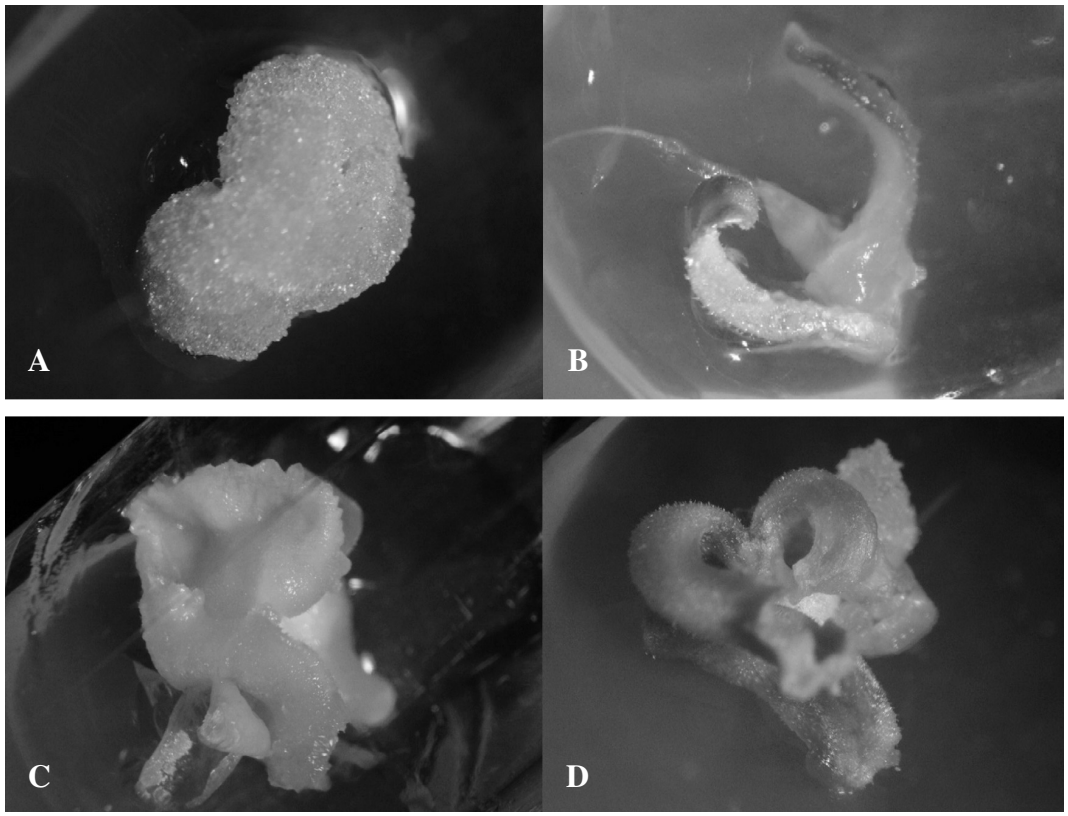

Fig. 2: Regeneration of embryos isolated at different time after a hand self-pollination from various *Cucumis* genotypes (A – 1 week *C. sativus* embryo on CW-medium; B – 2 week *C. anguria* embryo on OK-medium; C – 2 week *C. melo* (line MR-1) embryo on CW-medium; D – 2 week *C. melo* (var. Charentais) embryo on GA-medium).

Embryo rescue after hand cross-pollination in *Cucumis* species

Cucumis sativus as a maternal parent and *C. melo* and selected wild *Cucumis* species (Tab. 1) as paternal parents were used in interspecific hybridization. Embryos and seeds isolated from fruits after two weeks of hand cross-pollination were cultivated on several types of media. Because of the different embryos responses on various media that we observed from previous hand self-pollination, we focused on frequency of successful pollination (= formation of fruits; Fig. 5) and on the type and frequency of regeneration after hand cross-pollination with various interspecific partners (Tab. 5).

The regeneration of embryos after interspecific hybridization of *Cucumis* has been infrequently accomplished. In total, the regeneration of seven embryos from interspecific crosses of *C. sativus* × *C. melo* was observed (Lebeda et al., 1996, 1999). Five embryos developed small roots or a shoot meristem, however callus formation predominated. Two embryos developed into entire flowering plants and their hybrid origin was confirmed using isozyme analyses and flow cytometry. Only callus formation was observed in our experiments with classic hybridization of cucumber with other *Cucumis* species. With respect to the evaluation of the success of cross-pollination, the highest frequency of fruits was obtained with *C. metuliferus* as an paternal parent (74%; Fig. 5). On the other hand, the worst partner for cucumber was *C. anguria* (19 % successful pollination; Fig. 5). Only callus formation was observed after hybridization with *C. melo* (line MR-1) or *C. metuliferus*. The frequency of regeneration was very low (1.5% for *C. melo* and 1.7% for *C. metuliferus*). Ondřej et al. (2001) had similar results with interspecific hybridization in the genus *Cucumis*. As suitable media for obtained hybrid embryos were

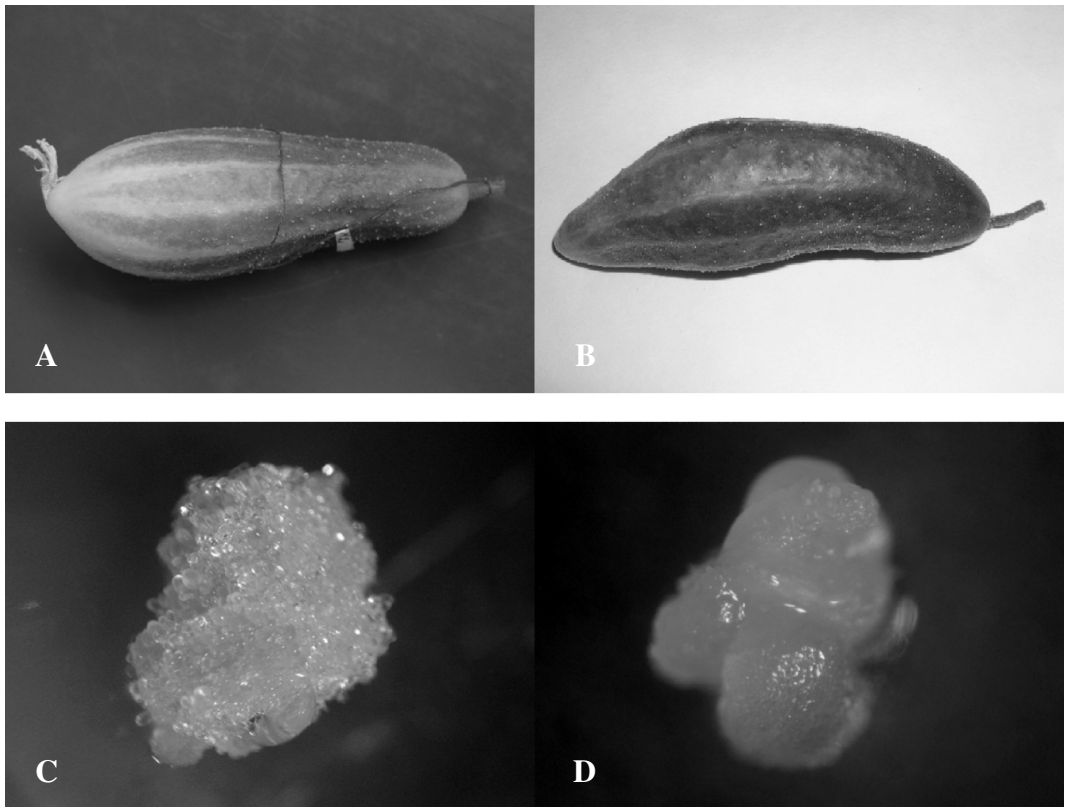

Fig. 3: Regeneration of embryos isolated two weeks after a hand cross-pollination of *C. sativus* with other *Cucumis* species (A – fruit developing from *C. sativus* × *C. melo*; B – fruit developing from *C. sativus* × *C. zeyheri*; C, D – callus formation on embryos from *C. sativus* × *C. melo* on GA-medium).

Tab. 5: Results of interspecific hybridization of *C. sativus* with *Cucumis* species.

Partner	No. of pollinations	No. of obtained fruits	No. of isolated seeds	No. of isolated embryos	No. and type of regener.	Successful media
CM1	27	13	260	260	8 (C)	OK (3C), CW (2C), GA (3C)
CM2	18	8	120	160	0	-
CA	11	2	80	0	0	-
CZ	16	7	280	0	0	-
CME	8	6	240	0	4 (C)	CW (2C), GA (2C)

Explanatory notes: Partner for IH – partner for interspecific hybridization with *C. sativus*; CM1, CM2, CA, CZ, CME – selected genotypes of *Cucumis* (Tab. 1); OK, ON, CW, GA – various types of media (Tab. 2); C – callus formation.

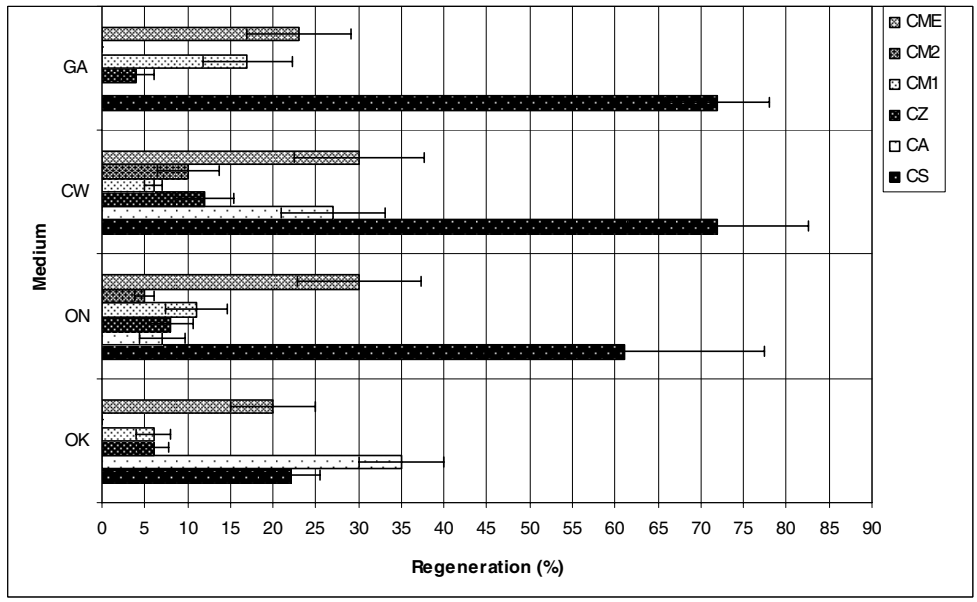

Fig. 4: Frequency (%) of regeneration 3 day seeds of tested *Cucumis* species (CS, CA, CZ, CM1, CM2, CME – selected genotypes of *Cucumis* species (Tab. 1); OK, ON, CW, GA – various types of media (Tab. 2); Y-error bars represent standard deviations).

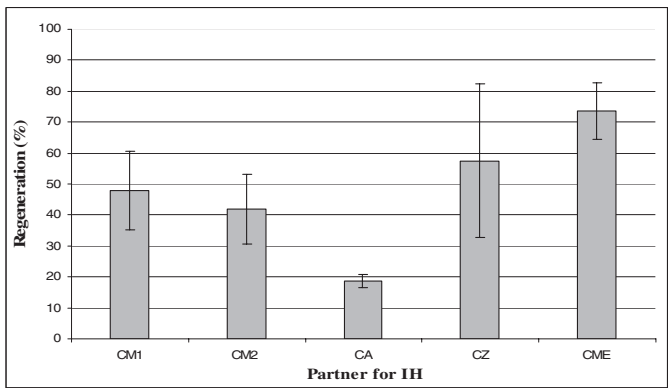

Fig. 5: Frequency (%) of successful hand cross-pollination (formation of fruits) *C. sativus* with *Cucumis* species (CS, CA, CZ, CM1, CM2, CME – selected genotypes of *Cucumis* species (Tab. 1); IH partner – partner for interspecific hybridization with *C. sativus*; Y-error bars represent standard deviations).

appeared CW- and GA-. There was observed a positive influence of coconut water and gibberellic acid again on immature embryos (Goralski and Przywara, 1998; Ondřej et al., 2002; Nuñez-Palenius et al., 2006). However, no direct or indirect organogenesis was noted from these hybridizations. It is necessary to use other strategies to obtain hybrids from crossing *C. sativus* with other *Cucumis* species. For example the polyploidization of cucumber maternal plant was used and entire plants were developed after cross *C. sativus* × *C. melo* (Skálová et al., 2006).

Conclusions

From this study, it is evident that methods of embryo rescue in the genus *Cucumis* were successful. Direct organogenesis was observed in selected *Cucumis* species cultivated on media supporting embryogenesis. An important hybridization partner for cucumber, *C. melo* line MR-1, regenerated with 10 % frequency but only formed callus. Among wild *Cucumis* species, immature *C. metuliferus* embryos (3 day), showed the highest frequency of regeneration (26%). These two genotypes were characterized by callus formation from interspecific hybridization with cucumber. The addition of coconut water (CW-medium) and gibberellic acid (GA-medium) to culture media had positive effects on the regeneration of immature embryos (27%; 29%). These same media supported callus formation, the only degree of regeneration, in the case of interspecific hybrids between *C. sativus* and *C. melo* or *C. metuliferus*. Future work will concentrate on optimizing methods to increase the frequency of regeneration and make it possible to recover entire plants from the interspecific hybridization of cucumber with muskmelon.

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References

Chen, J.F., Adelberg, J., 2000: Interspecific hybridization in *Cucumis* – progress, problem, and perspectives. HortSci. 35, 11-15.

Chen, J.F., Staub, J., Qian, Ch., Jiang, J., Luo, X., Zhuang, F., 2003: Reproduction and cytogenetic characterization of interspecific hybrids derived from *Cucumis hystrix* Chakr. *Cucumis sativus* L. Theor. Appl. Genet. 106, 688-695.

Den Nijs, A.P.M., Custers, J.B.M., 1990: Introducing resistances into cucumbers by interspecific hybridization. In: Bates, D. et al. (eds.), Biology and Utilization of the Cucurbitaceae. Cornell University Press, Ithaca, New York, 382-395.

Goralski, G., Przywara, L., 1998: *In vitro* culture of early globular-stage embryos of *Brassica napus* L. Acta Biol. Cracov. 40, 53-60.

Jeffrey, C., 2001: Cucurbitaceae. In: Hanelt, P. (ed.), Mansfeld's Encyclopedia of Agricultural and Horticultural Crops, Vol. 3. Springer-Verlag, Berlin,

Heidelberg, New York, 1510-1557.

Lebeda, A., 1996: Resistance in wild *Cucumis* species to twospotted spider mite (*Tetranychus urticae*). Acta Phytopathol. Entom. Hung. 31, 247-252.

Lebeda, A., Křístková, E., Kubaláková, M., 1996: Interspecific hybridization of *Cucumis sativus* x *Cucumis melo* as a potential way to transfer resistance to *Pseudoperonospora cubensis.* In: Gómez-Guillamón, M.L. et al. (eds.), Cucurbits towards 2000. Proceedings of the VIth Eucarpia Meeting on Cucurbit Genetics and Breeding, Malaga (Spain), 28-30 May, 31-37.

Lebeda, A., Kubaláková, M., Křístková, E., Navrátilová, B., Doležal, K., Doležel, J., Lysák, M., 1999: Morphological and physiological characteristics of plant issued from interspecific hybridization of *Cucumis sativus* x *Cucumis melo*. Acta Hort. 492, 149-155.

Lebeda, A., Widrlechner, M.P., Staub, J., Ezura, H., Zalapa, J., Křístková, E., 2007: Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.), Chapter 8, 271-376. In: Singh, R. (ed.), Genetic Resources, Chromosome Engineering, and Crop Improvement Series, Volume 3 – Vegetable Crops. CRC Press, Boca Raton, FL, USA.

Leppick, E.E., 1966: Searching gene centres of the genus *Cucumis*. Euphytica 15, 323-328.

Mc Carthy, W.H., Wehner, T.C., Xie, J., Daub, M.E., 2001: Isolation and callus production from cotyledon protoplasts of *Cucumis metuliferus*. Cucurbit Genet. Coop. 24, 102-106.

Murashige, T., Skoog, F., 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15, 473-497.

Nuñez-Palenius, H.G., Klee, H.J., Cantliffe, D.J., 2006: Embryo-rescue of „Galia" muskmelon (*Cucumis melo* L. var. *reticulatus* Ser.) male parental line. Plant Cell, Tiss. and Org. Cult. 85, 345-352.

Ondřej, V., Navrátilová, B., 1999: Embryocultures of *Cucumis sativus* L. Acta Biologica Cracoviensa 41, supl. 1, 51.

Ondřej, V., Navrátilová, B., Lebeda, A., 2000: Embryo cultures of wild and cultivated species of the genus *Cucumis* L. Acta Hort. 510, 409-414.

Ondřej, V., Navrátilová, B., Lebeda, A., 2001: Determination of the crossing barriers in hybridization of *Cucumis sativus* and *Cucumis melo*. Cucurbit Gen. Coop. Rep. 24, 1-5.

Ondřej, V., Navrátilová, B., Lebeda, A., 2002: Influence of GA_3 on zygotic embryogenesis of *Cucumis* species *in vitro*. Biologia (Bratislava) 57, 523-525.

Preťová, A., 1995: Embryogenéza vyšších rastlín v in vitro podmienkach. VEDA, Bratislava, Slovak Republic.

Skálová, D., Lebeda, A. , Navrátilová, B., 2004: Embryo and ovule cultures in *Cucumis* species and their utilization in interspecific hybridization. In: Lebeda, A., Paris, H.S. (eds.), Progress in Cucurbit Genetics and Breeding Research. Palacký University in Olomouc, Olomouc, Czech Republic, 415-430.

Skálová, D., Navrátilová, B., Lebeda, A., Gasmanová, N. 2006: Embryo culture as a tool of interspecific hybridization of *Cucumis sativus* and wild *Cucumis* spp. In: Holmes, G. (ed.), Cucurbitaceae 2006. North Carolina State University, Raleigh (NC, USA), 2006, 51-59.

Wehner, T.C., Cade, R.M., Locy, R.D., 1990: Cell, tissue and organ culture techniques for genetic improvement of Cucurbits. In: Bates, D. et al. (eds.), Biology and Utilization of the Cucurbitaceae, Cornell University Press, Ithaca, New York, 367-381.

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