Development and Availability of a Melon Differential Set for Determination of Virulence Variation of Cucurbit Powdery Mildews (*Podosphaera xanthii* and *Golovinomyces orontii*)

Aleš Lebeda*, Božena Sedláková, Eva Křístková
Palacký University, Faculty of Science, Department of Botany, Šlechtitelů 27, 783 71 Olomouc-Holice, Czech Republic.
**Corresponding author E-mail: ales.lebeda@upol.cz*

James D. McCreight
U.S. Department of Agriculture, Agricultural Research Service, 1636 E. Alisal St., Salinas, CA 93905, U.S.A.

Maarten den Hertog
Rijk Zwaan Breeding B.V., Burgemeester Crezéelaan 40, P.O. Box 40, 2678 ZG De Lier, The Netherlands.

Kathleen Reitsma
North Central Regional Plant Introduction Station, Department of Agronomy, Iowa State University and the U.S. Department of Agriculture, 1305 State Avenue, Ames, Iowa 50014, U.S.A.

Introduction

Cucurbit powdery mildew (CPM), a disease of field and greenhouse cucurbit crops, is caused most frequently by the two obligate erysiphaceous ectoparasites *Golovinomyces orontii* (Go) and *Podosphaera xanthii* (Px) that greatly vary in their ecology, specificity of host-pathogen interactions and virulence (Lebeda *et al*., 2021). Go and Px are distributed worldwide (Braun and Cook, 2012), and differ in their spatio-temporal and geographic distribution (Křístková *et al*., 2009). Changes in species spectrum are substantially influenced by ecological factors (Trecate *et al*., 2019) and climate change (Lebeda *et al*., 2009, 2021). Economically important cucurbit crops (*Cucumis sativus*, *C. melo*, *Cucurbita* spp., *Citrullus lanatus*, *Momordica charantia* and many others) host Go and Px (Lebeda *et al*., 2007, 2021).

The first attempts to breed melon (*Cucumis melo*) for resistance to CPM (likely Px) took place in California starting in the 1920s (for a review see Pryor *et al*., 1946). Similar attempts were made later for other cucurbits (Jahn *et al*., 2002; Sitterly, 1972). Success of these efforts was complicated by the presence of different pathogen biotypes (races) (Lebeda *et al*., 2008; McCreight, 2006), later confirmed by the enormous variation in virulence at the population level of both CPM species (Lebeda *et al*., 2018a, 2021). Characterization of virulence variation is basic to understanding host-pathogen interactions and developing strategies for CPM resistance breeding (Lebeda *et al*., 2021).

Melon CPM (MCPM) races 1 and 2 have been commonly observed since the 1920s. Race 3 was observed in 1976 (Thomas, 1978). New MCPM-melon interaction patterns were observed in pathogen populations from the beginning and in the mid-1980s and continuing through the early 2000s (Lebeda *et al*., 2011; McCreight, 2006; Pitrat et al., 1998). Investigators used various host differentials (mostly *C. melo*) and different race denomination systems, which confounded understanding and communication within the CPM research, and melon and cucurbit production communities (Lebeda *et al*., 2011). Lack of a standard set of differentials and clear and uniform descriptions of the genetic variation in the virulence of the CPM pathogens on melon and other cucurbits limit study of the genetics of resistance, resistance breeding, plant protection and production (Lebeda *et al*., 2018b, 2021). Recognition of these obstacles thus led to the development of an international differential set and a methodology of race determination, description, and denomination (Lebeda *et al*., 2008, 2011, 2016a), supported by study of virulence variation at the population level (Lebeda *et al*., 2021).

Research and Initiative Leading to Differential Set

The process of developing a set of differentials was initiated at the end of the 1970s by carrying out some basic studies related to CPM (Lebeda, 1983, 1984, 1986; Lebeda *et al*., 2008; Lebeda and Sedláková, 2010). Discussions at the IXth Eucarpia Meeting on Cucurbitaceae in Avignon, France in 2008, and the collaboration between the Czech Republic (A. Lebeda *et al*.) and the U.S. (J. D. McCreight) led to the International Cucurbit Powdery Mildew Initiative (ICPMI) (Lebeda *et al*., 2011, 2016a,b, 2018a,b). Discussion at Cucurbitaceae 2016, XIth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae (Warsaw, Poland) by the authors and representatives of nine international melon breeding companies led to the unanimous agreement to implement the proposed system for uniform virulence variation and race determination, and denomination of MCPM, as well as CPM on other cucurbits, as summarized in recent publications (Lebeda *et al*., 2016a,b, 2018a,b, 2021).

The proposed triple septet of 21 MCPM differentials (Lebeda *et al*., 2016a,b, 2018a,b) was the next logical step in facilitating communication within and among researchers, commercial breeders, plant pathologists, extension specialists and crop consultant communities (Table 1). During this process, a fourth septet was established with the addition of melon accession SVI105, designed as 4.1 (Table 1). Additional melon accessions deemed crucial for characterization of CPM virulence variation in the future may be added to the fourth septet.

Establishment of a uniform system for MCPM virulence characterization, and race determination and denomination is based upon four components: (1) a standard set of race differentials, (2) a uniform screening methodology, (3) a uniform code for the host-CPM interactions/scores (Lebeda *et al*., 2016a,b; 2018a,b), and (4) a system that is open to the addition of new differential accessions. The proposed set of 22 melon differentials was acceptable to commercial melon breeders and pathologists attending Eucarpia Cucurbitaceae 2016 (Warsaw, Poland) (Lebeda *et al*., 2016a). Rijk Zwaan Breeding B.V. (De Lier, The Netherlands) took on the task of increasing the entire differential set for distribution to the international melon CPM community. The seeds have recently been deposited in the cucurbit collection of the United States Department of Agriculture (USDA), National Plant Germplasm System (NPGS), North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, U.S.A., which will be responsible for distribution of the seed material to requestors. Ales Lebeda coordinated the process of purification, multiplication, and deposition from 2016 through 2021. Details about this activity and results are summarized below.

Genetic Homogenization and Multiplication of Differential Set

Seed multiplication of differentials was done by Rijk Zwaan at its Breeding Support Location, Arusha, Tanzania. The materials were grown in strict quarantine conditions from the seedling stage through fruit harvest; seedlings and mature plants were checked for presence of seed-transmissible diseases. Seventy-five to 150 plants per accession per project cycle were placed in a greenhouse compartment. Leaf disk samples of all plants in the greenhouse were taken and sent to De Lier and stored in a freezer for DNA extraction and SNP-marker analysis. Well-trained personnel performed the required controlled, hand-pollinations. Plants were pruned, prepared and female flowers emasculated the day before pollination. The different origins (Table 1) and backgrounds of the accessions ensured highly variable flowering and fruit setting patterns between and within the differentials and required extended pollination periods to obtain fruit from all differentials. Fruits were harvested when maturity signs were showing. Seeds were threshed and washed the same day, followed by drying in dedicated cabinets.

Clear phenotypic variation for leaf, plant and fruit characters was observed in many of the differentials in the initial planting; this was expected based on their origins. Self-pollinations were, therefore, used in order to achieve phenotypic homogeneity within each of the accessions.

DNA samples were analyzed using a dedicated set of markers spread evenly over the chromosomes in order to assess the genetic homozygosity and used to select the seeds of each differential for the next cycle of seed multiplication. It was also used to check on similarity of all individuals within the same accession. The set of markers used, specifically designed for this project, were evenly spread over the chromosomes.

Once the individuals in each accession were similar in phenotype and exhibited the same genetic marker profile, the decision was taken to finalize purification and proceed with the multiplication to produce 10,000 to 15,000 seeds. The plants and seed in the final multiplication were checked in order to certify freedom from important seed-transmissible pathogens, e.g., melon necrotic spot virus (MNSV).

Seed Deposition, Maintenance, Availability and Distribution

USDA, NPGS, NRCPIS received seeds of 21 of the 22 differentials (Table 1), designated by ICPMI in November 2021. NCRPIS which maintains the NPGS *Cucumis* collection will distribute, but not maintain, the differential set. The

missing differential, PI 414723, will be increased in 2022 and deposited in the near future as pollination and quarantine procedures are completed.

The Rijk Zwaan-generated seed lots were shipped to the USDA-APHIS (Animal and Plant Health Inspection Service) for quarantine inspection prior to being shipped to NCRPIS. Accession passport data including source, donor, identifiers, inventory, etc., have been uploaded to the GRIN-Global database. The 100-seed and total seed weights were determined for each differential inventory lot and the total quantity of seeds calculated (total seed weight divided by the 100-seed weight × 100). Prepacks of the ICPMI differential set, which consists of individually packaged, 25-seed lots of each differential, have been placed in -20 °C storage to facilitate order processing. A 100-seed backup sample of each accession will be sent to the National Laboratory for Germplasm Resources Preservation in Fort Collins, Colorado, USA.

Accessions in the ICPMI differential set will be distributed only as a set, not individually, and can be ordered via the Public GRIN-Global website at https://npgsweb.ars-grin.gov/gringlobal/ search. Requestors must first create a log-in account in order to submit a request *via* the website. To query the ICPMI set, first select the "Advanced Search" tab. Under "Additional search criteria", select "Accession Group" from the drop-down list, then select "Melon Differential Powdery Mildew (International)" from the list of group names, and select the search button. Requestors can select the individual links for each accession to see additional information and they can add the accessions to a shopping cart and submit an order.

In accordance with the NPGS plant germplasm distribution guidelines, germplasm will be supplied to scientists, educators, producers and other *bona fide* research and education entities. There is no charge for the germplasm, though requestors may be asked to provide shipping costs, especially when expedited domestic or international services are requested. All germplasm provided to cooperators outside the U.S. must follow phytosanitary regulations specific to the samples transferred between the U.S. and the importing country. APHIS is contacted before such orders are filled for information regarding the importing country's phytosanitary regulations. APHIS provides, as required, a phytosanitary certificate to accompany seed samples attesting to freedom from specified pests and pathogens. These seed lots were prepared in The Netherlands by Rijk Zwaan which provided seed analysis certificates indicating a representative sample of seeds was tested and found to be negative for: cucumber green mottle mosaic virus (CGMMV), squash mosaic virus (SqMV), MNSV, *Didymella bryoniae*, and *Acidovorax citrulli*. Though we can include electronic copies of these documents for an order, the requestor may need to seek a waiver if the importing country does not accept them or if additional declarations are indicated on the import permit.

Utilization of Differential Set

This differential set was composed with the main idea that it could be used internationally and by everyone, i.e., researchers, academics, plant breeders, seed producers, growers, agricultural testing institutions, etc., who need valid, understandable and internationally comparable information on pathogenic variation of CPM species Go and Px occurring on melon as well as other cucurbits (Lebeda *et al*., 2016a,b). This strategy and approach enable comparisons and understanding of CPM variation in pathogenicity and virulence among CPM isolates and populations across countries and continents. This is the main difference between our system and the system developed by the International Seed Federation Disease Resistance Terminology Working Group (ISF DRT WG), and which had the objectives of 1) defining a more manageable subset of differentiating melon hosts, 2) assembling commercially relevant Px races, and 3) developing a uniform testing protocol for routine disease resistance testing in order to support commercial claims of CPM resistance (Grimault *et al*., 2020).

The ICPMI approach was developed because race denominations of Go and Px (Lebeda *et al*., 2011; McCreight, 2006) over the past century often hampered direct comparisons of results obtained by different research groups (Lebeda *et al*., 2016a,b, 2021). This system enables objective and uniform description of Go and Px virulence variation at the individual (virulence-factors /v-factors/, v-phenotypes and races) and population level (frequencies of v-factors and v-phenotypes) (Lebeda *et al*., 2021), thus allowing a thorough understanding of the prevalence and dynamics of race-specific v-factors, which play a crucial role in deployment of resistance sources and/or specific R-genes (resistance genes) in plant breeding, as demonstrated in long-term population studies (Lebeda *et al*., 2018a, 2021).

Conclusions

After nearly a century of research of CPM species virulence variation, the contributions of generations of scientists and plant breeders have been critically analyzed, organized, and tested in long-term virulence studies. This research yielded the first comprehensive and internationally (globally) applicable differential set and system for CPM virulence description and denomination as a background for better communication and breeding of melon and other cucurbits for

resistance to CPM. The differential set is publically available and open for future enlargement and development.

Acknowledgements

This research was funded by the Ministry of Education, Youth and Sports, Czech Republic, Project No. MSM 6198959215; Czech Ministry of Agriculture, Projects No. QD 1357 and QH 71229; National Programme of Conservation and Utilization of Genetic Resources of Plants and Microorganisms; and by the Internal Grant Agency of Palacký University in Olomouc, Czech Republic, grants IGA_PrF_2018_001; IGA_PrF_2019_004; IGA_PrF_2020_003; IGA_PrF_2021_001; IGA_PrF_2022_002).

Literature Cited

1. Braun, U., and R.T.A. Cook. 2012. Taxonomic Manual of the Erysiphales (Powdery Mildews), CBS Biodiversity Series No. 11. CBS-KNAW Fungal Diversity Centre, Utrecht, The Netherlands.
2. Grimault, V., S. Houdault, and P. Himmel. 2020. Development of differential hosts to identify commercially relevant races of melon *Podosphaera xanthii* against which vegetable seed companies make claims of resistance. Cucurbit Genet. Coop. Rpt. 43:1-3.
3. Jahn, M., H.M. Munger, and J.D. McCreight. 2002. Breeding cucurbit crops for powdery mildew resistance, pp. 239-248. In: Bélanger R.R., W.R. Bushnell, A.J. Dik, and T.L.W. Carver. (eds.), The Powdery Mildews. A Comprehensive Treatise. American Phytopathological Society, St. Paul, MN, USA.
4. Křístková, E., A. Lebeda, and B. Sedláková. 2009. Species spectra, distribution and host range of cucurbit powdery mildews in the Czech Republic and some other European and Middle Eastern countries. Phytoparasitica 37:337-350.
5. Lebeda, A. 1983. The genera and species spectrum of cucumber powdery mildew in Czechoslovakia. Phytopath. Z. 108:71-79.
6. Lebeda, A. 1984. Screening of wild *Cucumis* species for resistance to cucumber powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). Sci. Hortic. 24:241-249.
7. Lebeda, A. 1986. Padlí okurkové (Cucumber powdery mildew), *Erysiphe cichoracearum, Sphaerotheca fuliginea*, pp. 87-91. In: A. Lebeda (ed.), Metody testování rezistence zelenin vůči rostlinným patogenům (Methods of testing vegetable crops for resistance to plant pathogens). VHJ Sempra, VŠÚZ Olomouc, Czechoslovakia.
8. Lebeda, A., E. Křístková, B. Sedláková, M.D. Coffey, and J.D. McCreight. 2011. Gaps and perspectives of pathotype and race determination in *Golovinomyces cichoracearum* and *Podosphaera xanthii*. Mycoscience 52:159-164.
9. Lebeda, A., E. Křístková, B. Sedláková, and J.D. McCreight. 2016b. Initiative for international cooperation of researchers and breeders related to determination and denomination of cucurbit powdery mildew races, pp. 148-152. In: E.U. Kozik, and H.S. Paris (eds.), Cucurbitaceae 2016, XIth Eucarpia Meeting on Genetics and Breeding of Cucurbitaceae, Warsaw, Poland.
10. Lebeda, A., E. Křístková, B. Sedláková, and J.D. McCreight. 2018b. Initiative for uniform cucurbit powdery mildew race determination and denomination: status of race differentials. Cucurbit Genet. Coop. Rpt. 41:17-19.
11. Lebeda, A., E. Křístková, B. Sedláková, J.D. McCreight, and M.D. Coffey. 2008. New concept for determination and denomination of pathotypes and races of cucurbit powdery mildew, pp. 125-134. In: M. Pitrat (ed.), Proceedings of Cucurbitaceae 2008, IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae. INRA, Avignon, France.
12. Lebeda, A., E. Křístková, B. Sedláková, J.D. McCreight, and M.D. Coffey. 2016a. Cucurbit powdery mildews: methodology for objective determination and denomination of races. Europ. J. Plant Pathol. 144:399–410.
13. Lebeda, A., E. Křístková, B. Sedláková, J.D. McCreight, and E. Kosman. 2018a. Virulence variation of cucurbit powdery mildews in the Czech Republic – population approach. Europ. J. Plant Pathol. 152:309-326.
14. Lebeda, A., and B. Sedláková. 2010. Screening for resistance to cucurbit powdery mildew (*Golovinomyces cichoracearum, Podosphaera xanthii*), Chapter 19, pp. 295-307. In: M.M. Spencer, and A. Lebeda, (eds.), Mass Screening Techniques for Selecting Crops Resistant to Diseases. IAEA, Vienna, Austria.
15. Lebeda, A., B. Sedláková, E. Křístková, and M. Vysoudil. 2009. Long-lasting changes in the species spectrum of cucurbit powdery mildew in the Czech Republic – influence of climate changes or random effect? Plant Protect. Sci. 45 (Special Issue):S41–47.
16. Lebeda, A., B. Sedláková, E. Křístková, M.P. Widrlechner, and E. Kosman. 2021. Understanding pathogen population structure and virulence variation for efficient resistance breeding to control cucurbit powdery mildews. Plant Pathol. 70:1364-1377.
17. Lebeda, A., M.P. Widrlechner, J. Staub, H. Ezura, J. Zalapa, and E. Křístková. 2007. Cucurbits (Cucurbitaceae; *Cucumis*

spp., *Cucurbita* spp., *Citrullus* spp.), Chapter 8, pp. 271-376. In: R. Singh (ed.), Genetic Resources, Chromosome Engineering, and Crop Improvement Series, Volume 3 – Vegetable Crops. CRC Press, Boca Raton, FL, USA.

18. McCreight, J.D. 2006. Melon–powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. J. Amer. Soc. Hort. Sci. 131:59-65.
19. Pitrat, M., C. Dogimont, and M. Bardin. 1998. Resistance to fungal diseases of foliage in melon, pp. 167-173. In: J.D. McCreight (ed.), Cucurbitaceae '98: Evaluation and Enhancement of Cucurbit Germplasm. ASHS Press, Alexandria, VA, USA.
20. Pryor, D.E., T.W. Whitaker, and G.N. Davis. 1946. The development of powdery mildew resistant cantaloupes. Proc. Amer. Soc. Hort. Sci. 47:347-356.
21. Sitterly, R.W. 1972. Breeding for disease resistance in cucurbits. Ann. Rev. Phytopathology 10:471-490.
22. Thomas, C.E. 1978. A new biological race of powdery mildew of cantaloups. Plant Disease Rpt. 62:223.
23. Trecate, L., B. Sedláková, B. Mieslerová, V. Manstretta, V. Rossi, and A. Lebeda. 2019. Effect of temperature on infection and development of powdery mildew on cucumber. Plant Pathol. 68:1165-1178.

Table 1. GRIN accession numbers and septet group identifications of melon cucurbit powdery mildew (CPM) race differentials designated by the International Cucurbit Powdery Mildew Initiative (ICPMI) (Lebeda *et al.*, 2016a,b, 2018a,b, unpubl.).[z]

GRIN no.	Septet no.	Differential	Other designation(s)	Source[y]	Country
ICPMI 1 01	1.1	Iran H	–	INRA	Iran
ICPMI 1 02	1.2	Védrantais	M 319[y]	INRA	France
ICPMI 1 03	1.3	PI 179901	Teti	USDA	India
ICPMI 1 04	1.4	PI 234607	Sweet Melon	USDA	South Africa
ICPMI 1 05	1.5	AR HBJ	AR Hale's Best Jumbo	USDA	USA
ICPMI 1 06	1.6	PMR 45	M 321[x]	USDA	USA
ICPMI 1 07	1.7	PMR 6	Ames 26810	USDA	USA
ICPMI 1 08	2.1	WMR 29	M 322[x]	USDA	USA
ICPMI 1 09	2.2	Edisto 47	NSL 34600	Clemson Univ.	USA
ICPMI 1 10	2.3	PI 414723	LJ 90234	USDA	India
ICPMI 1 11	2.4	PMR 5	Ames 26809	USDA	USA
ICPMI 1 12	2.5	PI 124112	Koelz 2564	USDA	India
ICPMI 1 13	2.6	MR-1	Ames 8578	USDA	USA
ICPMI 1 14	2.7	PI 124111	Koelz 2563	USDA	India
ICPMI 1 15	3.1	PI 313970	PI 315410; VIR 5682	USDA	India
ICPMI 1 16	3.2	Noy Yizre´el	–	Bar Ilan Univ.	Israel
ICPMI 1 17	3.3	PI 236355	–	USDA	England
ICPMI 1 18	3.4	Negro	–	Univ. Zaragoza	Spain
ICPMI 1 19	3.5	Amarillo	–	Univ. Zaragoza	Spain
ICPMI 1 20	3.6	Nantais Oblong	M 320[x]	INRA	France
ICPMI 1 21	3.7	Ames 31282	–	USDA	China
ICPMI 1 22	4.1	SVI105	–	INRA	France

[z]The complete set of differentials is available by request: https://npgsweb.ars-grin.gov/gringlobal/search

[y]INRA = L'Institut National de la Recherche Agronomique, Montfavet (France); USDA = United States Department of Agriculture, Agricultural Research Service.

[x]designation by M. Pitrat, INRA, Montfavet (France) of seed provided to A. Lebeda in 1997.