Cucurbit Powdery Mildew-resistant Bitter Gourd Breeding Lines Reveal Four Races of *Podosphaera xanthii* in Asia

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Additional index words. Momordica charantia, Podosphaera xanthii, physiological races, resistance breeding, virulence variation

**Abstract.** Bitter gourd (*Momordica charantia* L.) is a commercially and nutritionally important market vegetable in Asia cultivated mainly by smallholder farmers. Cucurbit powdery mildew (CPM) caused by *Podosphaera xanthii* (Px) is a nearly ubiquitous and serious fungal disease of bitter gourd. Five bitter gourd breeding lines (THMC 113, THMC 143, THMC 153, THMC 167, and THMC 170) were selected at the World Vegetable Center for resistance to a local isolate of *Px* in Kamphaeng Saen, Thailand. We evaluated the resistance potential of these five inbred lines against local isolates of *Px* at 12 locations in five Asian countries. Plants were inoculated with the respective local *Px* isolate 15 and 30 days after transplanting and additional *Px*-infected plants of the inoculated control were interplanted throughout each test. Plants were rated 60 days after transplanting for CPM reaction using a 0 (no evidence of infection) to 5 (>75% infection evident on individual leaves) disease severity scale. THMC 153 and THMC 167 were resistant to the local race of *Px* in all locations, whereas THMC 143 was observed resistant in all test locations except one in China. THMC 113 was resistant in each location except one in India. THMC 170 was susceptible in three locations in India. The multilocation tests revealed four unique *Px* races on bitter gourd in different Asian countries and sources of resistance for breeding CPM-resistant bitter gourd cultivars. Six strains of *Px* isolated from other cucurbits (*Cucumis* and *Cucurbita*) and representing five melon CPM races were unable to infect the susceptible *M. charantia* accession THMC 144 and the five resistant breeding lines, indicating pathotype differences between them and an isolate of *M. charantia* origin typed as race 1 on melon. THMC 143 and THMC 167, which originated from India, exhibited good yield potential in trials conducted in Thailand, Myanmar, Vietnam, and Bangladesh.
Bitter gourd (M. charantia L.) is an important cucurbitaceous market vegetable in Asia, where more than 340,000 ha are devoted to its cultivation annually (McCreight et al., 2013). Its cultivation is gaining popularity in some African countries such as Ghana, Zambia, Congo, and Madagascar for local consumption or for export to Europe and the Middle East to cater the demand of emigrant Asian communities. It is also cultivated to a lesser extent in the southern United States and Australia (Northern Territory, Queensland, New South Wales, and Victoria), where popular Asian hybrid cultivars are cultivated for consumption mainly by ethnic communities from Asia (Morgan and Midmore, 2002).

Bitter gourd fruit is a rich source of betacarotene, vitamin C, folic acid, magnesium, phosphorus, and potassium (Dhillon et al., 2017; Yuwai et al., 1991). The health and pharmacological properties of bitter gourd have been well documented (Tan et al., 2016). Currently, 422 million people worldwide have diabetes (World Health Organization, 2016) and Type 2 diabetes accounts for around 90% (379 million). Bitter gourd fruit is used in folk medicine to manage Type 2 diabetes (Abascal and Yarnell, 2005; Grover and Yadav, 2004; Lams, 2006).

Cucurbit powdery mildew (CPM) caused by *P. xanthii* is a serious fungal foliar disease of cucurbit production in open fields and greenhouses. Disease outbreak brings reduction in plant growth, premature foliage loss, and reduction in yield and fruit quality (Keinath and DuBois, 2004). CPM on bitter gourd is currently controlled by fungicides, although fungicide resistance has developed in some areas (Lebeda et al., 2010; McGrath, 2006). The pathogen is highly variable in virulence and represented by many pathotypes (Lebeda et al., 2011) and races (Lebeda et al., 2016). The use of disease-resistant varieties is an economical and safe approach for disease management. Accessions resistant to CPM have been identified in melon (*Cucumis melo*; Dhillon et al., 2012), watermelon (*Citrullus lanatus*; Thomas et al., 2005), cucumber (*Cucumis sativus*; Block and Reitsma, 2005), squash (*Cucurbita pepo*; Lebeda and Krístková, 1996), pumpkin (*Cucurbita moschata*; Wessel-Beaver, 1993), and bottle gourd (*Lagenaria siceraria*; Kousik et al., 2008). Resistance to CPM is, however, often race-specific and not durable (Lebeda et al., 2008, 2016).

Commercial cultivars of bitter gourd resistant to CPM are not currently available. We developed five inbred lines resistant to CPM after screening 150 accessions of a global collection of bitter gourd in the World Vegetable Cucurbit Genebank against the local CPM population at Kamphaeng Saen (Thailand). A single resistant plant was identified in each of five segregating populations derived from five genebank accessions that originated from India, Thailand, Taiwan, and Belize. Multiple cycles of inbreeding and selection led to the development of the five CPM-resistant inbred lines. We evaluated four of these inbred lines in 2011 against local isolates of *P. xanthii* in Thailand, Taiwan, and the United States (South Carolina, Florida, California) (Dhillon et al., 2015). We report here the reactions of the five bitter gourd CPM-resistant inbred lines against local isolates of *P. xanthii* at 12 locations in five Asian countries (China, India, Thailand, Vietnam, and Philippines) in 2013 and 2014. In addition, we sought to relate Asian CPM-bitter gourd interactions to the more developed body of knowledge of CPM-melon interactions, first by challenging these lines with European and the Mediterranean CPM isolates, and second by challenging a set of melon CPM race differentials with a single-spore strain isolated from a local isolate of *P. xanthii* on bitter gourd from Kamphaeng Saen, Thailand. The latter test also challenged representatives of cucumber, summer squash, and watermelon, cucurbit species on which few CPM races have been identified, with the exception of watermelon where four CPM races have been defined (Davis et al., 2007; Kousik et al., 2011; Mercier et al., 2014; Zhang et al., 2011). Furthermore, we evaluated horticultural fruit characters of the CPM-resistant bitter gourd breeding lines in the field test at Kamphaeng Saen, Thailand in 2014, and assessed the yield potential of two of the lines in Thailand, Myanmar, Vietnam, and Bangladesh in 2016, to assess their horticultural value as sources of CPM resistance.

**Materials and Methods**

**Germlaspm and field test sites.** Five bitter gourd CPM-resistant inbred lines and a susceptible bitter gourd check line were evaluated against the respective local *P. xanthii* isolates at 12 locations in five countries in 2013 and 2014 (Table 1). Each field test was planted in a randomized complete block design with three replications of five plants per plot. Entries were planted on raised, 1.6-m wide beds covered with black plastic mulch. Plots were 5-m long on a single bed; each consisted of five transplants spaced 1-m apart. Plants were trellised on the plastic net erected on vertical bamboo poles.

**Field test.** At each location, a spore suspension was prepared by detaching heavily sporulating leaves of susceptible THMC 144 and washing them with a spray of 100 mL of water and filtering through a double layer of cheesecloth. The suspension was diluted to a concentration of 4 × 10⁸ conidia/mL of water as determined by a hemocytometer. This was freshly prepared as required for each inoculation. Seedlings were inoculated 15 and 30 d after transplanting, at the three-leaf stage of growth, at each location. The spore suspension was sprayed over the plants until runoff, by using a pressurized sprayer. THMC 144 plants with abundantly sporulating CPM were used as spreader plants, placed between rows as additional sources of powdery mildew inoculum.

**Growth chamber tests.** There were two growth chamber tests. The first evaluated the five breeding lines against European and Mediterranean CPM isolates, whereas the second evaluated various cucurbit species with a single-spore CPM strain from Kamphaeng Saen, Thailand. Five plants, each of the five bitter gourd CPM-resistant inbred lines and a susceptible check (Table 2) were inoculated at two-leaf stage, similarly as explained previously, with six CPM strains isolated from cucurbits in Europe and the Mediterranean area and typed for race on melon: Sm3 (race 1), S87-7 (race 2F), 005m39 (race 3), 985m65 (race 5), and 045m2 and 085m9 (race 3.5). A single-spore CPM strain isolated from *M. charantia* grown in an open field in Kamphaeng Saen, Thailand, was inoculated on five plants each of ‘Market’ cucumber, ‘Diamant’ summer squash, ‘Sugar Baby’ watermelon, and melon CPM race differentials (‘Védantais’, ‘PMR 45’, ‘PMR 5’, WMR 29, PI 124112, 90625 (PI 313970), and AR Hale’s Best Jumbo). Plants were raised in a greenhouse until the second leaf stage and then inoculated after inoculation in a growth chamber (16 h day 26°C/8 h night 20°C) at GAFL, INRA, Montfavet, France. Disease severity was rated on leaves of individual plants after 10–14 d of inoculation, using 0–3 visual rating scale, where 0 = no visible

Received for publication 2 Oct. 2017. Accepted for publication 11 Jan. 2018.

Funding for this research was provided by the Federal Ministry for Economic Cooperation and Development, Germany (BMZ), Japan Ministry of Agriculture, Forestry and Fisheries (Japan MAFF), and core donors to the World Vegetable Centre: Republic of China (ROC), UK Department for International Development (UK aid), United Nations Food and Agriculture Organization (FAO), International Atomic Energy Agency for International Development (USAID), Australian Center for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan. A. Lebeda was supported by the following grants: MSM 6198959215 (Czech Ministry of Education); QH 7129 (NAVF, National Agency for Agricultural Research of the Czech Republic); Internal Grant Agency of Palacky University in Olomouc (PrF_2017_001). Technical support of Sorawit Limsirivat is acknowledged.

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symptoms; 1 = very light sporulation; 2 = clear sporulation but not abundant; and 3 = abundant sporulation. A plant rating of 0 was considered resistant and the others as susceptible.

**Fruit trait evaluation.** The five CPM-resistant inbred lines were evaluated at Kamphaeng Saen, Thailand, for seven horticultural fruit traits: color, shape, skin pattern, fruit bitterness, fruit length, fruit width, and fruit weight (Table 3). The market class was designated as described in Dhillon et al. (2016). The experimental design was a randomized complete block design with three replications. Row spacing was 1.6 m, within-row spacing was 1.0 m, with ten transplants per plot. Plants were trellised similar to the trial explained previously. Transplant date was 25 May 2014. Ten marketable fruit of each entry were harvested in each replication for assessment of fruit traits. Fruit bitterness was evaluated using fresh marketable fruit of each line, washed and cut into small (3.0 g) pieces, and assessed by a five-person taste panel. The evaluators rinsed their mouth with water after each sample. Two classes of bitterness were recorded: low and high.

**Yield trials of two of the CPM-resistant breeding lines, THMC 143 and THMC 167** (along with CPM susceptible check THMC 144 and local cultivars), were performed at four locations: Kamphaeng Saen, Thailand; Nay Pyi Taw, Myanmar; Hanoi, Vietnam; and Dhaka, Bangladesh. The transplant dates at these four locations were 25 May 2014, 30 Aug. 2015, 11 Sept. 2015, and 10 Dec. 2014, respectively. The experimental design, row and plant spacing, and trellising were similar to the trial described previously. Plants were furrow irrigated, fertilized, and protected from pathogens and pests as per standard horticultural practices in each location. Fruit were harvested every 4 d for 7 weeks, for a total of 11 harvests per line at each location.

### Table 1. Summary disease ratings and mean disease reactions of six bitter gourd breeding lines and a susceptible control (THMC 144) to cucurbit powdery mildew incited by *Podosphaera xanthii* in field tests at 12 locations in five countries, and tentative bitter gourd powdery mildew race designations based on the reaction patterns of the breeding lines.

| Country | Location | Transplant date | Susceptible control | Breeding line | THMC 113 | THMC 143 | THMC 153 | THMC 167 | THMC 170 |
|---------|----------|-----------------|---------------------|---------------|----------|----------|----------|----------|----------|
| Vietnam | Fruit and Vegetable Research Institute, Hanoi | 12 Sept. 2013 | S (5a) | R (0c) | R (0c) | R (0b) | R (0b) | R (0b) | Mc-1 |
| Philippines | East-West Seed, Bukidnon | 6 Sept. 2014 | S (5a) | R (0c) | R (0c) | R (0b) | R (0b) | R (0b) | Mc-1 |
| India | Rasi Seeds, Bangalore | 6 July 2013 | S (5a) | R (1b) | R (1a) | R (1a) | R (1a) | R (1a) | S (3a) |
| India | Rasi Seeds, Pune | 7 Aug. 2013 | S (5a) | R (1b) | R (1a) | R (1a) | R (1a) | R (1a) | S (3a) |
| India | VNR Seeds, Hyderabad | 8 Dec. 2013 | S (5a) | R (0c) | R (0c) | R (0b) | R (0b) | R (0b) | S (3a) |
| India | VNR Seeds, Raipur | 12 Aug. 2013 | S (5a) | R (0c) | R (0c) | R (0b) | R (0b) | R (0b) | S (3a) |
| China | Enza Zaden, Qingyuan | 2 Apr. 2014 | S (5a) | R (0c) | R (0c) | R (0c) | R (0c) | R (0c) | Mc-1 |
| China | Clover Seeds, Kaiping City | 18 Mar. 2013 | S (5a) | R (0c) | R (0c) | R (0c) | R (0c) | R (0c) | Mc-1 |

*Inoculated with spore suspensions 15 and 30 d after transplanting, evaluated for disease reaction 60 d after transplanting; summary disease rating R = resistant; S = susceptible. Means of disease reaction in the same column followed by the same letter do not differ significantly at P ≤ 0.05 according to Fisher’s least significant difference test.

### Results

**Disease evaluation.** The combined ANOVA revealed highly significant differences (P ≤ 0.01) among bitter gourd lines for mean disease rating (Data not shown). Location × line interaction was highly significant (P ≤ 0.01), indicating that bitter gourd lines performed differently among locations for reaction to CPM, thus indicating different *P.x* races among test locations. THMC 144 was susceptible to CPM at all 12 locations (mean rating = 5) (Table 1). In contrast, THMC 153 and THMC 167 were resistant to CPM at all 12 locations (mean rating = 0–1). THMC 113 and THMC 143 were resistant at 11 locations (mean rating = 0–1) and were susceptible at one location in India and China, respectively. THMC 170 exhibited susceptible reactions at three (Bangalore, Pune, and Raipur) of the five locations in India and resistant reactions at nine locations in Thailand, Vietnam, Philippines, India, and China (with a mean rating = 0–1). The 12 CPM populations differed in pathogenicity based on the pattern of resistance reactions among the six bitter gourd lines, and four *P.x* races were tentatively identified (Table 1). All lines were resistant to race 1. THMC 170 was susceptible to races 2 and 3. THMC 113 and THMC 143 were susceptible to races 3 and 4, respectively. Race 1 was observed in all five countries. Races 2 and 3 were observed only in India. Race 4 was only observed at one location in China.

All the bitter gourd lines, including the susceptible control, THMC 144, were resistant (no visible sporulation) in the growth chamber test to the six CPM strains isolated from cucurbits in Europe and the Mediterranean area that represented five CPM races among test locations. THMC 144 was susceptible to CPM at all 12 locations (mean rating = 5) (Table 1). In contrast, THMC 153 and THMC 167 were resistant to CPM at all 12 locations (mean rating = 0–1). THMC 113 and THMC 143 were resistant at 11 locations (mean rating = 0–1) and were susceptible at one location in India and China, respectively. THMC 170 exhibited susceptible reactions at three (Bangalore, Pune, and Raipur) of the five locations in India and resistant reactions at nine locations in Thailand, Vietnam, Philippines, India, and China (with a mean rating = 0–1). The 12 CPM populations differed in pathogenicity based on the pattern of resistance reactions among the six bitter gourd lines, and four *P.x* races were tentatively identified (Table 1). All lines were resistant to race 1. THMC 170 was susceptible to races 2 and 3. THMC 113 and THMC 143 were susceptible to races 3 and 4, respectively. Race 1 was observed in all five countries. Races 2 and 3 were observed only in India. Race 4 was only observed at one location in China.

All the bitter gourd lines, including the susceptible control, THMC 144, were resistant (no visible sporulation) in the growth chamber test to the six CPM strains isolated from cucurbits in Europe and the Mediterranean area that represented five races defined on melon CPM race differentials, where ‘Védranitsa’ and AR Hale’s Best Jumbo were susceptible, and the other five were resistant with mean disease reactions = 0.

**Horticultural characterization and yield potential.** The five CPM-resistant inbred lines produced spindle-shaped fruit with spiny skin surface (Table 3). Three fruit skin colors were observed in these lines: green (THMC 143), light green (THMC 113, THMC 153, and THMC 170), and dark green (THMC 167). The lines were categorized into two market segments based on the fruit length: short (THMC 113, THMC 153, and THMC 170) and medium (THMC 143 and THMC 167). Bitterness ratings of THMC 167 fruit were high, whereas those of the other four lines were low. Yields (t/ha) of THMC 143 and THMC 167 were comparable at each of the four locations (Table 4). Yields (t/ha) of CPM susceptible checks were significantly (P ≤ 0.05) less than these two CPM-resistant lines in the trials conducted in Thailand, Myanmar, and Vietnam. BARI Koralla 1 is a popular CPM moderately resistant cultivar in Bangladesh, and its yield was comparable with the yields of THMC 143 and THMC 167.

### Discussion

CPM caused by *P.x* is a major disease problem of cucurbit production worldwide (Kristkova et al., 2009; Lebeda et al., 2016). CPM and begomoviruses are the chief bitter gourd production constraints encountered by the farmers in India (Dhillon et al., 2015; Khan et al., 2002). About 10% to 15% of the bitter gourd field cultivation cost in China and India is
attributed to the fungicides used to control CPM (J. Fu and V. Chawda, personal communication). Yield losses due to CPM infection are proportional to the severity of the disease and the length of the period that plants have been infected, and yield losses of up to 50% have been observed in bitter gourd fields in Chhattisgarh state of India (N.P.S. Dhillon, unpublished data). The use of genetic resistance provides a more economic and safe approach, but it is also prone to failure. Literature survey by McCreadie et al., (2012) showed 46 races of Px on melon based on sets of CPM race differentials that range in number from two to 28. However, the real number of races is much higher (Lebeda et al., 2016). Four races of Px on watermelon have been reported in the United States (Davis et al., 2007; Kousik et al., 2011; Merrier et al., 2014; Zhang et al., 2011), and race 2W has become more serious in recent years in the United States. The differential reactions of the six lines used in these multilocation tests across Asia revealed four tentative Px races on bitter gourd across the 12 locations (Table 1). Future research emphasis should be on evaluation of a wide array of bitter gourd germplasm for resistance to Px isolates collected from bitter gourd fields in various parts of Asia to identify a set of bitter gourd differentials for identifying bitter gourd Px races. We suggest Px races identified on bitter gourd be given the prefix Mc- to distinguish them from races defined on other cucurbit species.

These results suggest that there are multiple races of Px currently infecting M. charantia in southeast Asia and the Philippines. Pathotypes or forma specialis are defined by their differential pathogenicity among a set of host plant species and genera. The susceptible M. charantia control, THMC 144, was resistant to Px strains isolated from other cucurbit species in Europe and the Mediterranean area; M. charantia could, therefore, be included in the differential set of host species to define pathotypes of CPM (Lebeda et al., 2008).

The sources of resistance to Px in melon, watermelon, cucumber, and bottle gourd originated from the primary centers of diversity of these cucurbit species (reviewed in Dhillon et al., 2012; Kousik et al., 2008; Morishita et al., 2003; Tetteh et al., 2010). Two of the five resistant lines (THMC 143 and THMC 167) used in this study originated in India, the primary center of diversity of bitter gourd. THMC 144, which exhibited susceptible reactions to Px isolates in all the locations across Asia, also originated in India. In a field evaluation conducted in 2016, THMC 143 and THMC 167 exhibited resistance to local Px isolates in Myanmar and Bangladesh (N.P.S. Dhillon, unpublished data). THMC 153 and THMC 167 exhibited uniform resistance to local isolates of Px in all the locations in the present study; it will be interesting to observe the reactions of these lines to Px isolates from other parts of Asia. Elucidation of the M. charantia–Px interaction may be advanced by controlled inoculations (Lebeda and Sedlaková, 2010) with well-defined bitter gourd races of Px (Lebeda et al., 2016) originating from different Asian countries where M. charantia is grown. Such studies will also facilitate research on the genetic control of resistance in bitter gourd to CPM caused by Px.

Fruit traits of THMC 143 and THMC 167 (both belong to South Asian market segment) are appealing to the consumer: green or dark-green fruit color, medium fruit length, and adequate fruit weight (129–144 g), whereas...
fruit of THMC 113, THMC 153, and THMC 170 are short and light-green with low mean weights (8–45 g) and are not preferred by consumers. Moreover, in multiple yield evaluation trials conducted in Thailand, Myanmar, Vietnam, and Bangladesh, THMC 143 exhibited good yield potential (Table 4). THMC 143 and THMC 167 are good candidates for use in South Asian–type bitter gourd–breeding programs for transferring the resistance through backcross breeding, as the desirable fruit color, shape, and weight of these lines will pose less of a challenge for a genetic drag. THMC 143 exhibited good horticultural performance, including resistance to Ps in multiple trials conducted in Bangladesh, and has been released by Bangladesh Agricultural Research Institute for use in home gardens, and, in addition, it has been found resistant to other fungal diseases incited by Alternaria spp., Colletotrichum spp., and Cercospora spp., and it yields fruit over a longer period than varieties commonly grown at present in Bangladesh (M.A.T. Masud, unpublished data).

In conclusion, bitter gourd lines THMC 153 and THMC 167 were rated resistant to local isolates of Ps at 12 locations in five Asian countries. Four tentative Ps races of bitter gourd were also recorded during these multilocation evaluation trials. Lines THMC 143 and THMC 167, resistant to Ps at 11 and 12 locations, respectively, originated from India, possess desirable horticultural fruit traits and demonstrated good yield potential in field trials conducted at four locations in Asia, and would be potential sources of resistance for introgression of resistance into locally adapted bitter gourd cultivars. Genetic control of resistance in bitter gourd to CPM remains to be determined.

Note: Small seed samples of the lines used in this study are available on request, by using release codes, to: seedrequest@worlddev.org. The release codes of THMC 113, THMC 143, THMC 144, THMC 153, THMC 167 and THMC 170 are AVBG1329, AVBG1330, AVBG1331, AVBG1333, AVBG1334 and AVBG1335, respectively.

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