Identification of Resistance to Powdery Mildew in Chile Pepper

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Abstract. Powdery mildew [*Leveillula taurica* (Lév.) Arn] is a fungus causing epidemics on chile peppers (*Capsicum* sp.) worldwide. It was first observed in New Mexico in the late 1990s and has been a reoccurring issue. During the 2017 growing season, environmental conditions were highly favorable for powdery mildew development and severe infection was observed. This provided a unique opportunity to identify novel sources of resistance in *Capsicum* to powdery mildew. In the present study, the incidence and severity of powdery mildew was evaluated for 152 chile pepper accessions comprising different cultivars and species. Major differences in disease severity and incidence were observed among the accessions. Of the 152 accessions, 53 were resistant, i.e., received a disease index (DI) score of ≤ 1 . When examining across *Capsicum* species, 16 *Capsicum annuum* accessions, all 8 *Capsicum baccatum*, all 21 *Capsicum chinense*, 5 of 6 *Capsicum frutescens*, the *Capsicum chacoense* accession, and the *Capsicum rhomboideum* accession were resistant. These results provide several accessions with resistance that can be used in breeding programs. Especially important are the *C. annuum* resistant accessions, as this resistance can be more quickly incorporated into commercially important *C. annuum* cultivars as compared with interspecific hybridizations.

Chile peppers (*Capsicum* sp.) are a widely cultivated crop that is a staple in the diet of many cultures worldwide and is used as a vegetable, spice, ornamental, and medicinal plant (Bosland and Votava, 2012). There are five domesticated species: *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz and Pav., with *C. annuum* providing most of the commercial production in the world. In New Mexico, the chile pepper industry has cultural and economic significance with New Mexico having the largest area of chile pepper production in the United States (USDA, 2017).

A relatively new disease affecting chile pepper production in New Mexico is powdery mildew incited by *L. taurica* (Lév.) Arn. Powdery mildew is a major foliar disease that can cause serious economic losses (Palti, 1988). It is widely distributed in chile pepper production areas with confirmed existence from dry regions of Asia and the Mediterranean to more humid regions (Palti, 1988). It was reported in the North-Central Mexico states of Chihuahua, Zacatecas, and Aguascalientes in 1999 (Velásquez-Valle and Valle-García, 1999). Since its introduction into the southwest region of the United States, losses to powdery mildew have varied year to year, with severe infection early in the season causing heavy yield losses (Murray, 2000). Application of fungicides may manage the

disease; however, effectiveness depends on early detection and thorough coverage of the plant, which can be difficult (Goldberg, 2004). The most economically and environmentally sustainable management of powdery mildew is through resistant cultivars. In this study, a wide range of germplasm was evaluated for resistance to powdery mildew.

Materials and Methods

Plant material. The Chile Pepper Institute (CPI) plants a teaching garden annually at the Fabian Garcia Science Center in Las Cruces, NM (lat. 32.3199°N, long. 106.7637°W). During the 2017 growing season, environmental conditions were highly favorable for powdery mildew development and severe infection occurred throughout the garden. In fact, the disease level was the highest seen in more than a decade. The garden has been used in the past to identify serendipitous sources of salt tolerance (Votava and Bosland, 2002) and curly top virus resistance (Bosland, 2000) within *Capsicum*. With more than 152 accessions growing in the garden, it provided a unique and exceptional opportunity to evaluate for novel sources of powdery mildew resistance (Table 1). This included six *Capsicum* species: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, *C. chacoense* Hunz., and *C. rhomboideum* (Dunal) Kuntze.

Plant culture. The CPI Teaching Garden is managed according to standard New Mexico cultivation practices (Bosland and Walker, 2014). Seeds were sown into a planting medium (Sunshine Redi-Earth Plug & Seedling Mix; Sun Gro Horticulture, Bellevue, WA) in 12-celled bedding plant containers (Hummert Intl., Earth City, MO) and placed in a greenhouse maintained at 27 °C

day/18 °C night $\pm$ 3 °C. Plants were transplanted from the greenhouse to the CPI Teaching Garden location, a Glendale loam soil. The plants were spaced 30 cm apart within row in 1-m-wide rows, and furrow irrigated and fertilized as needed.

Disease scoring. Plants were grown under natural infection conditions and were evaluated once when disease had reached its highest level throughout the field. Plants were scored based on a modified DI developed by Zheng et al. (2013). The DI is a scale of 0 to 5, where 0 = no visible sporulation, 1 = restricted chlorotic spots on the adaxial leaf surface, with weak or no sporulation on the abaxial leaf area; 2 = several isolated sporulation spots on the abaxial leaf area; 3 = numerous sporulation spots covering up to 40% to 50% of the abaxial leaf area; 4 = numerous coalescent sporulation spots covering up to 75% of the abaxial leaf area; and 5 = the whole abaxial surface of the leaf, and also parts of the adaxial leaf surface, covered with dense sporulation (Zheng et al., 2013; Fig. 1). Plants were considered to be resistant if $DI \leq 1$. Moderately resistant accessions are also noted and are defined as plants with an average score of less than 2 and with no more than five individual leaves with a score of 2 or less. Within each accession, five random plants were chosen and three individual leaves per plant were assigned a DI rating. Disease scores were analyzed using R version 3.4.2 (2017).

Results

Of the 152 accessions, evaluated for their reaction to natural field incidence and severity of powdery mildew in the CPI Teaching Garden, 53 were resistant, i.e., a DI score of ≤ 1 (Table 2). When examining across *Capsicum* species, 16 of 115 *C. annuum* accessions were resistant, as well as the 8 of 8 *C. baccatum* accessions, 21 of 21 *C. chinense* accessions, 5 of 6 *C. frutescens* accessions, the *C. chacoense* accession and the *C. rhomboideum* accession. In addition, seven accessions were moderately resistant, with low levels of sporulation and few chlorotic lesions (Table 3).

A hypersensitive response (HR) and early leaf senescence were observed in some accessions, where small chlorotic lesions were noted. This indicated that powdery mildew infection had clearly occurred but did not spread and had no visible sporulation (Fig. 2). In *C. frutescens* ‘Siling Labuyo’ and in *C. baccatum* ‘Suriname Color’ an HR was observed, and both had a DI rating of 0.73. Early leaf senescence was observed for *C. rhomboideum* and *C. chinense* ‘NuMex Farolito’, where leaves appeared to shed early in infection, preventing the spread of the disease on the plant.

Discussion

Results of this investigation suggest significant sources of resistance for powdery mildew exist within *Capsicum* species. This

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Table 1. List of *Capsicum* accessions evaluated for powdery mildew (*Leveillula taurica*) resistance in the 2017 Chile Pepper Institute Teaching Garden.

Species	Accession ^z
<i>Capsicum annuum</i>	Abay, Aci Sivri, Admiral, Alma Paprika, Ancho San Luis, Barker's Hot, Bianca, Blacina, Blazing Banana, Bola, Brebes, Bulgarian Carrot, Cajun Belle, Calico, Canary, Cherry Pick, Chile Negro, Chilhuacle, Chilly Chilli, Chimayo, Chocolate Beauty, Sigarreta di Bergamo, Corcel, Costeno Amarillo, Cubanella, Da Vinci, Dashen, de Arbol, Delirio, Devil, Dhing Shan, Early Summer, Edesalma, Feher Ozon, Floral Gem, Guatemalan, Fu Di Jian, Guajillo, Hontaka, Jalafuego, Jigsaw, Kalmicho, Kundu, Large Red Thick Cayenne, LB25, Marbles, Moms Pic, Mulato, New Mexico 6-4, New Mexico 9, NuMex April Fool's Day, NuMex Baily Piquin, NuMex Big Jim, NuMex Centennial, NuMex Chinese New Year, NuMex Christmas, NuMex Cinco de Mayo, NuMex Conquistador, NuMex Easter, NuMex Eclipse, NuMex Espanola Improved, NuMex Garnet, NuMex Halloween, NuMex Heritage 6-4, NuMex Heritage Big Jim, NuMex Jalundo, NuMex Joe E. Parker, NuMex Las Cruces Cayenne, NuMex Lemon Spice, NuMex Memorial Day, NuMex Mirasol, NuMex Nematador, NuMex Orange Spice, NuMex Primavera, NuMex Pumpkin Spice, NuMex R Naky, NuMex Sandia Select, NuMex St. Patrick's Day, NuMex Summer Solstice, NuMex Sunburst, NuMex Sunflare, NuMex Sunglow, NuMex Sunrise, NuMex Sunset, NuMex Sweet, NuMex Thanksgiving, NuMex Twilight, NuMex Valentine's Day, NuMex Vaquero, NuMex Veteran's Day, Pasilla Bajio, Pepperoncino, Picante Calabrese, Piment d'Espelette, Pimiento L, PX207, Purple Flash, Red Fly, Rio Grande21, Sandia, Santa Fe Grande, Serrano, Shishito, Spanish Piquillo, Sprinter, Takanotsume, Tequila, Thai Demon, Thai Fogo, Thai Hot, Urfa, Yum Yum, and Zunla
<i>C. annuum</i> var. <i>glabriusculum</i>	Chiltepín
<i>C. baccatum</i>	Aji Limon, Aji Red, Aji Verde, Bolivian Aji, Brazilian Wild, Omnicolor, Peri Peri, and Uba Tuba
<i>C. chinense</i>	Aji Rojo, Bhut Jolokia, Brown Habanero, Cajamarca, Chichen Itza, Datil, Fatalii, Habanada, Maya, NuMex Farolito, NuMex Suave Orange, NuMex Suave Red, NuMex Trick-or-Treat, Petch Mordindang, Piminet Boda Ma Jacques, Red Caribbean Habanero, Rocotillo, Senegal, Surinam Color, Trinidad Perfume, and Trinidad Moruga Scorpion
<i>C. chacoense</i>	NMCA 50009
<i>C. frutescens</i>	Birds Eye, Diente de Perro, Puta Pario, Siling Labuyo, Tabasco, and Zimbabwe Bird
<i>C. rhomboideum</i>	NMCA 50064

^zAccessions listed in alphabetical order by species.

Fig. 1. Disease index (DI) for powdery mildew based on Zheng et al. (2013). (A) DI = 1, (B) DI = 2, (C) DI = 3, (D) DI = 4, (E) DI = 5, abaxial, and (F) DI = 5, adaxial, where 0 = no visible sporulation, 1 = restricted chlorotic spots on the adaxial leaf surface, with weak or no sporulation on the abaxial leaf area; 2 = several isolated sporulation spots on the abaxial leaf area; 3 = numerous sporulation spots covering up to 40% to 50% of the abaxial leaf area; 4 = numerous coalescent sporulation spots covering up to 75% of the abaxial leaf area; and 5 = the whole abaxial surface of the leaf, and also parts of the adaxial leaf surface, covered with dense sporulation.

is the first evaluation of plant materials in New Mexico for powdery mildew resistance. In Brazil, Souza and Café-Filho (2003) evaluated 162 *Capsicum* accessions and found that most *C. annuum* material was susceptible; however, *C. baccatum*, *C. chinense*, and *C. frutescens* are mostly resistant. The findings of this study support the observations of Souza and Café-Filho (2003) that *C. baccatum*, *C. chinense*, and *C. frutescens* are

mostly resistant. However, this study identified new sources of resistance within the germplasm evaluated.

Preliminary work on the *mildew resistance locus O (Mlo)* in these chile pepper accessions was carried out. The *CaMlo2* allele, identified by Kim and Hwang (2012), was sequenced and early screening suggests it could play a role in resistance for some of the accessions in this study (data not shown).

Early reports of *mlo*-mediated resistance in barley indicate a phenotype with a tendency toward pronounced necrotic or chlorotic leaf spotting and occasional mildew colonies (Jørgensen, 1992). Necrotic and chlorotic leaf spotting reported by Jørgensen (1992) is similar to the HR characteristics observed in this study. In an investigation into accumulation of H₂O₂ because of loss-of-function of *mlo* gene, Piffanelli et al. (2002) reported a

Table 2. Resistant accessions from the 2017 Chile Pepper Institute Teaching Garden in Las Cruces, NM.

Species	Accession ^z	Mean Score ^y
<i>Capsicum annuum</i>	NuMex Veteran's Day	0.20 ± 0.41
	Costeno Amarillo	0.27 ± 0.46
	Purple Flash	0.33 ± 0.49
	NuMex St. Patrick's Day	0.53 ± 0.52
	Chilly Chili	0.60 ± 1.06
	NuMex Halloween	0.60 ± 0.51
	NuMex Thanksgiving	0.60 ± 0.51
	NuMex Chinese New Year	0.67 ± 0.49
	Jigsaw	0.73 ± 0.46
	NuMex Christmas	0.73 ± 0.46
	NuMex Bailey Piquin	0.80 ± 0.41
	Guatemalan	0.87 ± 0.35
	NuMex April Fool's Day	0.87 ± 0.35
	NuMex Las Cruces Cayenne	0.93 ± 0.26
	NuMex Summer Solstice	0.93 ± 0.26
	Corcel	1.00 ± 0.00
<i>C. annuum</i> var. <i>glabriusculum</i>	Chiltepín	0.47 ± 0.52
<i>C. baccatum</i>	Aji Verde	0.00 ± 0.00
	Uba Tuba	0.20 ± 0.41
	Bolivian Aji	0.27 ± 0.46
	Brazilian Wild	0.40 ± 0.51
	Aji Red	0.40 ± 0.51
	Omnicolor	0.47 ± 0.52
	Petch Mordindang	0.47 ± 0.52
	Aji Limon	0.53 ± 0.52
	Peri Peri	0.53 ± 0.52
	Rocotillo	0.67 ± 0.49
	Suriname Color	0.73 ± 0.46
	NuMex Trick-or-Treat	0.13 ± 0.35
	Brown Habanero	0.20 ± 0.41
	Maya	0.27 ± 0.46
	NuMex Farolito	0.27 ± 0.46
	Red Caribbean Habanero	0.27 ± 0.46
	Habanada	0.33 ± 0.49
	NuMex Suave Red	0.33 ± 0.49
	Senegal	0.33 ± 0.49
	Fatalii	0.40 ± 0.51
	Chichen Itza	0.47 ± 0.52
<i>C. chinense</i>	Bhut Jolokia	0.53 ± 0.52
	NuMex Suave Orange	0.53 ± 0.52
	Trinidad Perfume	0.53 ± 0.52
	Trinidad Moruga Scorpion	0.60 ± 0.51
	Cajamarca	0.67 ± 0.49
	Datil	0.67 ± 0.49
	Piminet Bonda ma Jacques	0.80 ± 0.41
	Aji Rojo	0.93 ± 0.23
	NMCA 50009	0.47 ± 0.52
	Putra Pario	0.07 ± 0.26
	Zimbabwe Bird	0.13 ± 0.35
	Diente de Perro	0.20 ± 0.41
	Tabasco	0.53 ± 0.52
	Birds Eye	0.67 ± 0.49
	50,064	0.20 ± 0.41
<i>C. chacoense</i>		
<i>C. frutescens</i>		

^zAccessions listed by species from lowest to highest disease rating.^yMean disease index score with corresponding SD.Table 3. Moderately resistant^z accessions from the 2017 Chile Pepper Institute Teaching Garden in Las Cruces, NM.

Species	Accession ^y	Mean score ^x
<i>Capsicum annuum</i>	Calico	0.47 ± 0.74
	NuMex Twilight	0.93 ± 0.46
	Brebes	0.93 ± 0.59
	NuMex Valentine's Day	1.07 ± 0.26
	Mulato	1.07 ± 0.46
	Shishito	1.20 ± 0.56
<i>C. frutescens</i>	Siling Labuyo	0.73 ± 0.59

^zModerately resistance is defined as plants with an average score of less than 2, and no more than five individual leaves with a score of 2.^yAccessions listed by species from lowest to highest disease rating.^xMean disease index score with corresponding SD.

premature cell-death phenotype that mimics leaf senescence. The HR and early leaf senescence phenotype observed in some of the populations suggest that recessive

Camlo2-mediated resistance may explain resistance within some accessions in this study. *CaMlo2* could explain the variability within the population, but there may be other

genetic mechanisms controlling powdery mildew resistance. Recently, a novel gene, *PMR1*, was identified as conferring resistance in *C. annuum* (Jo et al., 2017). The

Fig. 2. Hypersensitive response (HR) to plant infection by powdery mildew on individual leaf (A) and entire plant (B). HR was given a disease index score of 1.

single dominant gene, *PMRI*, was identified through two mapping populations (Jo et al., 2017). It would be worthwhile to screen the evaluated material in this study with the *PMRI* marker.

Several accessions were identified in this study to have significant levels of resistance that can be used in a *Capsicum* breeding program. Especially important are the *C. annuum* resistant accessions, as their resistance can be more quickly incorporated into commercial cultivars, which are mainly *C. annuum*. A follow-up experiment using artificial inoculation of resistant germplasm identified in this study, as well as additional

breeding lines, would be valuable to confirm results. This is the first study evaluating resistance in germplasm exposed to the New Mexico powdery mildew pathotype and provides a basis for continued breeding efforts toward powdery mildew resistance in New Mexico, and globally.

Literature Cited

- Bosland, P.W. 2000. Sources of curly top virus resistance in *Capsicum*. HortScience 35:1321–1322.
- Bosland, P.W. and E.J. Votava. 2012. Peppers vegetable and spice capsicums. 2nd ed. CABI, Cambridge, MA.

- Bosland, P.W. and S. Walker. 2014. Growing chiles in New Mexico. NMSU Coop. Ext. Serv. Guide H-230.
- Goldberg, N. 2004. Powdery mildew on chile peppers. NMSU Coop. Ext. Serv. Guide H-248.
- Jo, J., J. Venkatesh, K. Han, H.Y. Lee, G.J. Choi, H.J. Lee, D. Choi, and B.C. Kang. 2017. Molecular mapping of *PMRI*, a novel locus conferring resistance to powdery mildew in pepper (*Capsicum annuum*). Front. Plant Sci. 8:1–11.
- Jørgensen, J.H. 1992. Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. Euphytica 63:141–152.
- Kim, D.S. and B.K. Hwang. 2012. The pepper *MLO* gene, *CaMLO2*, is involved in the susceptibility cell-death response and bacterial and oomycete proliferation. Plant J. 72:843–855.
- Murray, M.W. 2000. Crop profile for peppers (chile) in New Mexico. New Mexico Coop. Ext. Serv. Plant Sci. Dept.
- Palti, J. 1988. The *Leveillula* mildews. Bot. Rev. 54:423–535.
- Piffanelli, P., F. Zhou, C. Casais, J. Orme, B. Jarosch, U. Schaffrath, N.C. Collins, R. Panstruga, and P. Schulze-Lefert. 2002. The barley *MLO* modulator of defense and cell death is responsive to biotic and abiotic stress stimuli. Plant Physiol. 129:1076–1085.
- Souza, V.L. and A.C. Café-Filho. 2003. Resistance to *Leveillula taurica* in the genus *Capsicum*. Plant Pathol. 52:613–619.
- U.S. Department of Agriculture. 2017. Vegetables 2016 summary. U.S. Dept. Agr., Washington, DC.
- Velásquez-Valle, R. and P. Valle-Garcia. 1999. First report of powdery mildew of pepper in north-central Mexico. Plant Dis. 84:302.
- Votava, E.J. and P.W. Bosland. 2002. Sources of salt tolerance in *Capsicum*. Capsicum Eggplant Nwsl. 21:64–65.
- Zheng, Z., T. Nonomura, K. Bóka, Y. Matsuda, R.G.F. Visser, H. Toyoda, L. Kiss, and Y. Bai. 2013. Detection and quantification of *Leveillula taurica* growth in pepper leaves. Phytopathology 103:623–632.