

## Screening of Resistance Melon Germplasm to Phytophthora Rot caused by *Phytophthora Capsici*

Min-Jeong Kim, Chang-Ki Shim<sup>†</sup>, Yong-Ki Kim, Hyeong-Jin Jee, Sung-Jun Hong, Jong-Ho Park, Min-Ho Lee, and Eun-Jung Han

Organic Agriculture Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Korea

**ABSTRACT** Melon (*Cucumis melo*) is an annual herbaceous plant of the family Cucurbitaceae. Phytophthora rot, caused by *Phytophthora capsici* is a serious threat to cucurbits crops production as it directly infects the host plant, and it is difficult to control because of variable pathogenicity. This study investigated the resistance of 450 accessions of melon germplasm against Phytophthora rot by inoculating the seedlings with sporangial suspension ( $10^5$  or  $10^6$  zoosporangia/ml) of *P. capsici*. Disease incidence of Phytophthora rot was observed on the melon germplasm at 7-day intervals for 35 days after inoculation. Susceptible melon germplasm showed either severe symptoms of stem and root rot or death of the whole plant. Twenty out of 450 tested accessions showed less than 20% disease incidence, of which five accessions showed a high level of resistance against Phytophthora rot. Five resistant accessions, namely IT119813, IT138016, IT174911, IT174927, and IT906998, scored 0% disease incidence under high inoculum density of *P. capsici* ( $10^6$  zoosporangia/mL). We recommend that these candidate melon germplasm may be used as genetic resources in the breeding of melon varieties resistant to Phytophthora rot.

**Keywords** : *Cucumis melo*, *Phytophthora capsici*, tolerance, artificial inoculum

**Melon** has come from different geographical origins and has very diverse varieties. Nonetheless, all melons are of the same species, *Cucumis melo*. Morphological diversity resulted from natural hybridization of melon crops, and as such, domestic and international classifications are confusing (Kerje and Grum, 2000; Robinson and Decker-Walters; Yi *et al.*, 2004).

Many kinds of melon are grown in various parts of Asia. Korea has a long history of oriental melon cultivation.

Oriental melon is characterized by its golden skin color that has silver lines running from end to end. The fruit is oblong in shape and has a white flesh that is very crispy and sweet (Kwak, 1998; Lee and Kim, 2003; Mo *et al.*, 1998; Yi *et al.*, 2004).

The genus *Phytophthora* inflicts significant damage to the production of cucurbits and solanaceous crops in the world, sometimes causing up to 100% yield loss (Babadoost, 2004; Hausbeck and Lamour, 2004; Zhang *et al.*, 2006). The pathogen, *Phytophthora capsici*, has a wide host range including pepper, tomato, eggplant, and most cucurbits such as cucumber, squash, pumpkins, watermelon and muskmelons (Erwin and Ribeiro, 1996; Islam and Babadoost, 2004; Jee *et al.*, 2000a; Kim and Shon, 1991).

Four species of *Phytophthora*, *P. capsici*, *P. drechsleri*, *P. melonis*, and *P. nicotianae*, were described as the causal agents of the Phytophthora rot of oriental melon and melon in Korea (Kim, 1998; Jee *et al.*, 2000a, 2000b).

*Phytophthora capsici* causes seedling damping-off, leaf spot, foliar blight, root and crown rot, stem lesion, and fruit rot (Erwin and Ribeiro, 1996; Gubler and Davis, 1996; Jee *et al.*, 2000a). Crown rot is commonly the first field symptom on cucurbits. Crown rot causes the entire plant to completely collapse and die in a short period of time. Affected vine tissue is brown, appears water-soaked and often collapse. Infected fruits develop dark, water-soaked or slightly sunken lesions, which expand and become covered with white fungus (Erwin and Ribeiro, 1996; Kim and Shon, 1991).

The pathogen is favored by high soil moisture, frequent rains or irrigation, and warm temperatures (Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004; Jee *et al.*, 2000b; Kim

<sup>†</sup>Corresponding author: (Phone) +82-31-290-0545 (E-mail) ckshim@korea.kr

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and Kim, 2002; Pavón *et al.*, 2008).

Phytophthora rot is difficult to control, particularly once established in the soil. The pathogen overwinters as oospores or mycelium in infected plant debris and soil. Oospores are resistant to desiccation, cold temperatures, and other extreme environmental conditions, and can survive in the soil, in the absence of a host plant, for many years. A combination of cultural and chemical control should be practiced to reduce Phytophthora pathogens on cucurbits (Babadoost, 2000; Hausbeck, 2004; Islam and Babadoost *et al.*, 2008).

An effective crop rotation is an excellent control management for most vegetable crop diseases. Two years has been shown to be insufficient, therefore, there is a need to select a field where susceptible crops have not been grown for at least 3 years (Jang *et al.*, 2008; Kim and Kim 2002; Noh *et al.*, 2004). Lee *et al.* (2009) suggested that soil sterilization using hot water was significantly more effective than fumigation in controlling soil-borne fungal diseases including Phytophthora rot in fields consecutively planted to muskmelon.

In Korea, farmers demand for new melon varieties that are easily cultivated and have a stable quality that is not dependent on disease and insect pest management and cultivation conditions (Munger and Robinson 1991; Kim, 1998). This study was conducted to evaluate the resistance of melon germplasm to Phytophthora rot caused by *P. capsici* using *in vitro* assay.

## MATERIALS & METHODS

### Plant materials

The list of 450 accessions of melon germplasm obtained

from the National Agrobiodiversity Center in Korea in 2010. These accessions originated from 37 different countries, of which 30 countries have fewer than ten accessions of melon germplasm represented in the national collection. The countries with the most number of accessions included in the study were Korea (137), Uzbekistan (68), Russia (30), Turkmenistan (26), Kazakhstan (20) and Tajikistan (16). There were also 29 accessions which are of unknown origin in Table 1.

Twenty seeds from each accession were disinfected with 2% sodium hypochloride (NaOCl) for two hours. The disinfected seeds were vigorously washed with distilled water three times and pre-germinated on moist filter paper in petri-dishes at 30°C for one week under light and dark (16hrs/8hrs) conditions. Fifteen pre-germinated seeds of each melon germplasm were sown into 70-hole plastic seedling trays filled with peat moss-horticultural soil mixture. The melon seedlings were grown for 45 days in the greenhouse.

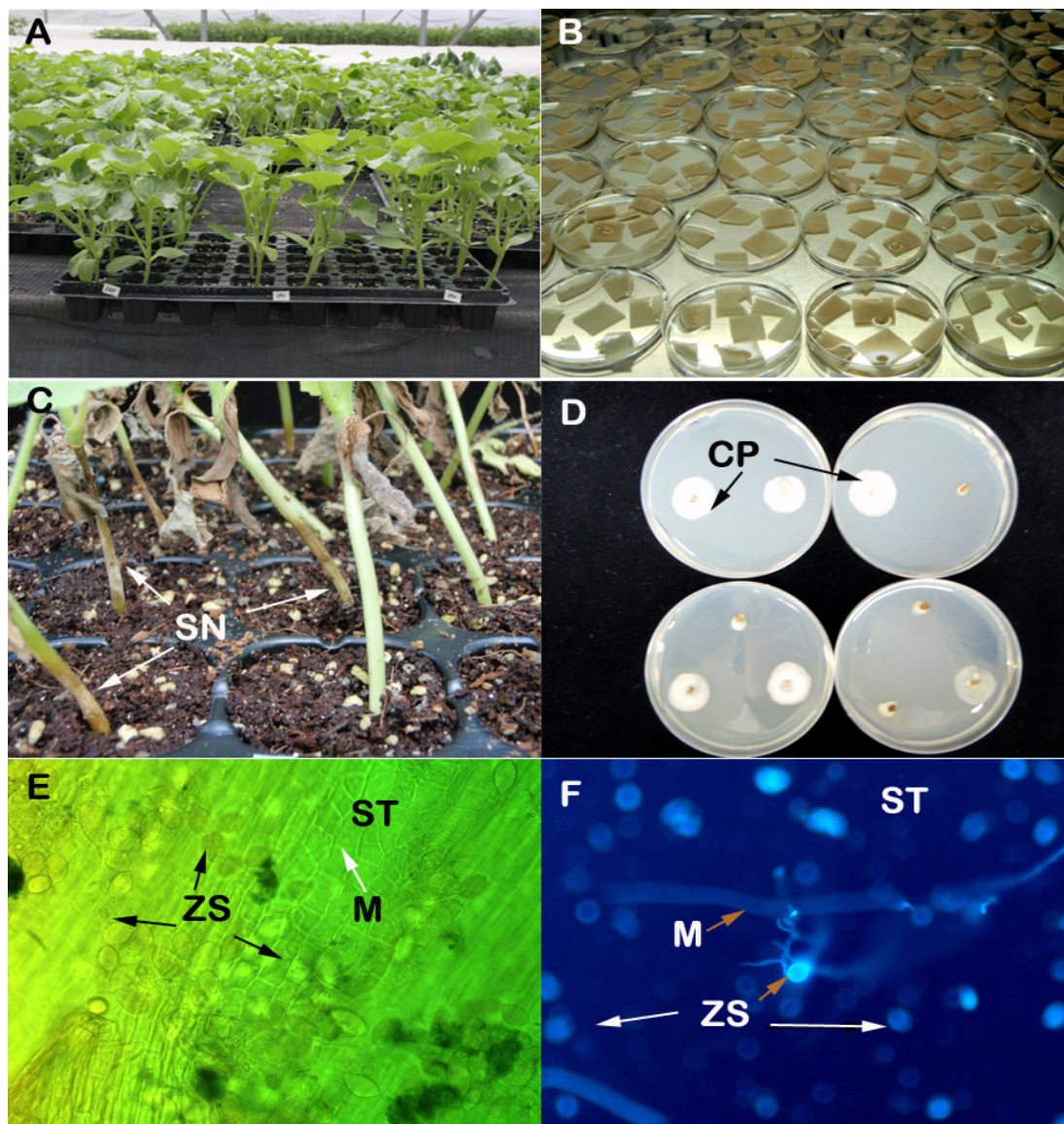
### Preparation and inoculation of pathogen

The Phytophthora rot pathogen, *P. capsici* (KACC 44716), was obtained from the Korea Agricultural Culture Collection (KACC) of the Rural Development Administration (RDA). We modified the production method of Phytophthora sporangia reported by Jee *et al.* (2000). To induce zoospore production, the pathogen was grown on 10% V-8 agar (V8 Juice Agar; V8 Juice 100 mL, CaCO<sub>3</sub> 3 g, Agar 20 g, Distilled Water 900 mL) for one week. The agar plate with fungal hyphae was placed under fluorescent light at room temperature. After one week, the sporangia and zoospores were harvested from the mycelia using an autoclaved paint brush and were cold shocked at 4-8°C for

**Table 1.** Origin and number of accessions of the 450 of melon germplasm tested in this study.

	Origin (Number of Accessions)
More than 11 accessions	Korea (137), Uzbekistan (68), China (59), Russia (30), Turkmenistan (26), Kazakhstan (20), Tajikistan (16), Unknown (29)*
Less than 10 accessions	Iran (8), Japan (7), Afghanistan (7), Kyrgyzstan (6), U.S.A. (5), Cameroon (4), Turkey (3), Taiwan (3), India (2), Thailand(2), Azerbaijan (1), Canada (1), Chile (1), Colombia (1), Dominican Republic (1), Finland (1), France (1), United Kingdom (1), Germany (1), Georgia (1), Moldova (1), Mexico (1), Myanmar (1), Mongolia (1), Netherlands (1), Nepal (1), Saudi Arabia (1), Syria (1), Ukraine (1), Yugoslavia (1)
Total	37 countries (450)

\*Unknown : These accessions have no information on origin.



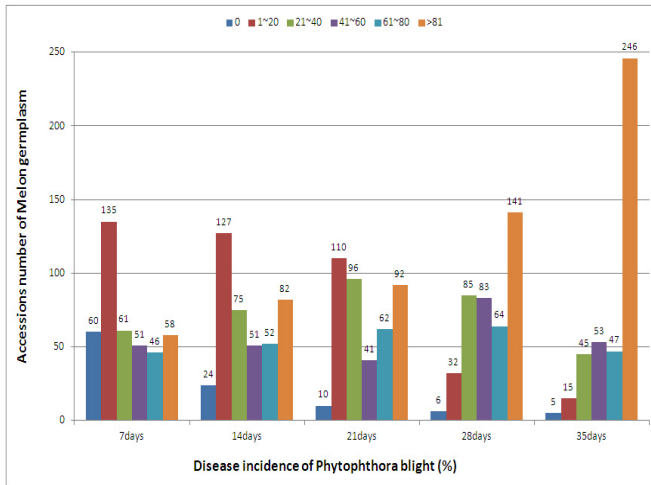
**Fig. 1.** The preparation of healthy melon seedlings (A) and artificial inoculums of *Phytophthora capsici* (B). The occurrences of Phytophthora stem rot caused by *P. capsici* at 7 days after inoculation (C). Microscopic observation of zoosporangial formation of *P. capsici* on infected stem tissues of melon seedlings (D). ST: surface of melon stem, ZS: zoosporangia.

one hour followed by one hour equilibration at room temperature (Fig. 1). The concentration of zoospores was adjusted to  $10^5$  or  $10^6$  sporangia/mL using a hemocytometer. Melon seedlings, about 45 days old, were inoculated with 5 mL of the sporangia suspension and incubated for 2 days under saturated moist condition in the greenhouse.

#### Disease evaluation on biological assay

We designed three-step experiments for redundancy check and time-saving: the mass germplasm accession broad

screening step; the early retest of entries with positive screening results, and a final retest step. Plant seedling screening methods are the most commonly used among breeders to test new cultivars and lines for resistance to plant pathogens (Zhang *et al.*, 1997; Wehner and Shetty, 2000). The disease severity was determined for 35 days, with seven days interval, after the inoculation of sporangia suspension. The severity of Phytophthora rot symptoms was assessed by assigning symptom grades based on visual inspection, as well as by measuring the healthy and diseased



**Fig. 2.** Response of 450 accessions of *Cucumis melo* germplasm to crown inoculation with a suspension of *Phytophthora capsici* for 35 days with seven days intervals after inoculation.

root tissues (Fig. 1). We determined the disease incidence of each accession of melon germplasm using the following formula:

$$\text{DiseaseIncidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of examined plants}} \times 100$$

The resistance level to *Phytophthora* rot of melon germplasm was graded based on disease incidence at 7-day intervals for 35 days after inoculation. The plants were visually rated based on a scale ranging from 0 to 5 in which 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion has progressed up to the cotyledons causing constriction at the base, 3 = plant has partially collapsed with apparent wilting of leaves, 4 = plant has completely collapsed with severe wilting present, and 5 = plant death. In all studies, plants scored as 0 were classified as resistant, 1 were classified as moderate resistant, whereas those scored 2 to 5 were classified as susceptible (Fig. 1 and Fig. 2).

### Microscopy observation

Zoospores of *P. capsici* were detected from infected tissues of inoculated seedlings with microscopy observation. Observation of epidemiological and *Phytophthora* rot symptoms was conducted by mounting the specimens in glass slides with water, without fixation, under the light microscope.

Light microscopy observation was made using a Nikon microscope (Eclipse 80i, Tokyo, Japan) fitted with a 40/0.7 objective and a 2x magnification changer. Data were recorded on a CCD camera (Nikon, Tokyo, Japan) using a video generator (Nikon Digital Sight DS-U1, Tokyo, Japan). Fluorescent microscopic observations were made with an Nikon microscope (Eclipse 80i, Tokyo, Japan) using either the wide-field epifluorescence optics. For epifluorescence, the following Nikon filter combinations were used. Excitation filter (BP360-370), dichroic mirror (DM400), and emission filter (BA420-460) were used for Calcofluor White M2R staining method. Calcofluor White M2R, prepared as a 0.1% (w/v) stock solution in distilled water, was purchased as Fluorescent brightener 28 (F-6258, Sigma, St Louis, Missouri). The fluorescent dyes (0.001-0.1% of the stock solutions) were added to the infected tissue surrounding nonfixed fungal wells for 1-5 min, after which the dye was rinsed away with either distilled water, 50 mM carbonate-bicarbonate buffer (pH 9.4). Cover glasses bearing the stained cells were mounted, cell-side down, on the top of a stainless steel microscope specimen holder with a permanently mounted bottom cover glass (Kuo and Hoch, 1996). To assess fungal growth in the dyes as well as staining of cell walls under these conditions, the dyes were added to the growth medium at the onset of growth. Later the dye was rinsed away with dye-free medium and the specimens similarly mounted as above for observation.

## RESULTS

### Microscopy observation of seedlings inoculated by *P. capsici*

Light microscopy observations showed that the zoosporangia of *P. capsici* successfully established on the outside surface of the stems of inoculated melon germplasm at 14 days after inoculation. The visible symptoms that appeared sequentially were crown rot, stem lesions, plant wilt, leaf defoliation, and damping-off. Highly susceptible melon germplasm exhibited symptoms like damping off or *Phytophthora* rot with white fungal hyphae growth and zoosporangia developed on hyphae growing out of necrotic stem surface (Fig. 1). For wide field fluorescence observations, zoosporangia developed on hyphae growing out of necrotic

stem surface were imaged with filter combinations generally used for DAPI (Calcofluor) (Fig. 1, D).

### Estimation of melon germplasm resistance to *Phytophthora rot*

About 79% of the 450 accessions of melon germplasm tested were obtained from major melon cultivation areas such as Korea, Uzbekistan, China, Russia, Turkmenistan, Kazakhstan, and Tajikistan (Table 1).

At 14 days after inoculation, disease symptoms began to appear on highly susceptible melon germplasm. From the 450 accessions of melon germplasm, 134 were found susceptible as they showed more than 61% disease incidence, exhibiting watery stem rot and seedling wilt symptoms. Twenty four accessions did not show any symptoms of the powdery mildew disease on the foliar leaves of cucumber plant (Fig. 2).

At 21 days after inoculation, 10 accessions showed no symptoms, while 110 accessions had slight symptoms and were recorded with less than 20% disease incidence. Rated as susceptible were 199 melon accessions that showed from 21% to 80% disease incidence. There were 92 accessions which showed highly susceptible symptoms by having more than 80.1% disease incidence characterized by wilting, brownish lesions and leaf defoliation (Fig. 2).

Six accessions were rated as resistant 28 days after inoculation, while 32 accessions showed moderate resistance to *Phytophthora rot*. Susceptible ratings were given to 232 accessions of melon germplasm which showed from 21% to 80% disease incidence, while 141 accessions were highly susceptible with more than 81% disease incidence accompanied by stem and root rot and seedling death (Fig. 2).

At 35 days after inoculation, five accessions of melon germplasm, namely IT119813, IT138016, IT174911, IT174927, and IT906998 showed a high level of resistance against *Phytophthora rot*. They scored 0% disease incidence even with high inoculum density of *P. capsici* ( $10^6$  zoosporangia  $\text{mL}^{-1}$ ) under green house conditions (Fig. 2 and Fig 3). There were 145 accessions of melon which were found susceptible (20% to 80% disease incidence), while 246 accessions were highly susceptible (>90.1% disease incidence), which were characterized by symptoms such as leaf defoliation, damping-off and seedling death (Fig. 2 and Fig. 3).

## DISCUSSION

*Phytophthora rot* has become a serious threat to the production of cucurbit crops in the continuous cultivation fields (Babadoost, 2004; Hausbeck and Lamour, 2004). The causal fungus occurs throughout the world with two species, *P. drechsleri* and *P. capsici*, expressing differential virulence in melon (Kim, 1995; Kim and Shon, 1991; Jee *et al.*, 2000b). Oriental melon and melon are economically important crops in Korea where production in 2011 was recorded at 207,747 tons and 41,796 tons, respectively (KOSTAT, 2012)

Cucurbit fruit exhibited variability for overall susceptibility to *P. capsici* as evidenced by the number of fruit infected, time to sporulation, and extent of infection (water soaking vs. sporulation). Of the four *C. pepo* crops, summer squash and zucchini were highly susceptible, whereas acorn squash and pumpkin were less susceptible. Also, thickness of rind



**Fig. 3.** Comparison of susceptible and resistant accessions of melon germplasm against *Phytophthora rot* at 35 days after inoculation.

did not correlate with the susceptibility, as muskmelon was one of the most susceptible, and cucumber one of the least.

Recently, Phytophthora rot disease has been increasing in severity in the world, and outbreaks have threatened the yield of many types of vegetable crops. The pathogen is difficult to control because of variable genetic diversity and viability in the soil. Different pathogenic strains have the ability to infect different crops, and there are also differences in virulence or the ability to cause the disease in host plants (Babadoost, 2004; Erwin and Ribeiro, 1996; Yi *et al.*, 2004). In this study, 450 accessions of melon germplasm were tested for resistance to Phytophthora rot. They have varied morphological characteristics and origins, having been introduced from 37 countries including Korea, Uzbekistan, China, Russia, Turkmenistan, Kazakhstan, and Tajikistan. We tested a high percentage of melon germplasm from Korea because there are a lot of Korean landraces and hybrid lines of Oriental melon.

Twenty accessions among the 450 accessions of melon germplasm tested showed resistance to Phytophthora rot caused by *P. capsici* 35 days after inoculation. However, only five accessions, IT119813, IT138016, IT174911, IT174927, and IT906998 showed a high level of resistance against *P. capsici*, with 0% disease incidence under high inoculum density of  $10^6$  zoospores per milliliter. Susceptible and highly susceptible accessions showed brownish lesions on melon roots and stems which extended rapidly into the upper parts of the plants. These were also accompanied by wilting, leaf defoliation, and damping-off. Host-plant resistance is probably the easiest, most economical and environment friendly method for managing plant disease, and is readily acceptable to growers. The identification of host plant resistance to existing, new, and emerging pathogens in vegetable crops is critical for the development of efficient and effective disease management (Kousik *et al.*, 2008).

Three of five resistant cultivar accessions, IT174911 (Gaegurichamyoi), IT174927(Chosunchamyoi), and IT906998 (Geumnodajieuncheonchamyoi) were a breed or landrace resources collected in Korea. Other two resistant accessions, IT119813(PI403994) and IT138016(青蛙香瓜) were introduced from USA and China, respectively.

IT119813 melon germplasm accessions in namely PI403994 introduced from USDA's National Plant Germplasm System.

Hassan *et al* (1991) reported that PI 403994 were the best performing accession in estimating resistance to the Yellow-Stunting Disorder caused by *Cucurbit yellow stunting disorder virus*, was given MDSs of 1.68 and 2.95 (or was classified as slightly and moderately susceptible) at 8 and 10 weeks after seed sowing, respectively; while susceptible cultivar, 'Ananas' was rated 3.77 and 4.00 at the 2 dates, respectively.

Developmentally regulated expression of single-gene-mediated resistance also has been observed for potyviruses infection of cucumber seedlings (Ullah and Grumet, 2002; Wai and Grumet, 1995). The reduced susceptibility was suggested to be related to physiological changes in root and stem tissues. Similar to the response of cucumber fruit to *P. capsici*, developing grape berries (*Vitis vinifera*) also showed decreased susceptibility to infection by *Ucinula necator*, causal agent of powdery mildew, and to *Plasmopara viticola*, causal agent of downy mildew (Ficke *et al.*, 2002; Gadoury *et al.*, 2003; Kennelly *et al.*, 2005).

Disease resistance is an important objective in most breeding programs. Screening for resistance to several important diseases through greenhouse seedling tests is useful, and provides several advantages (Munger *et al.*, 1984; Norton *et al.*, 1986; Song *et al.*, 2004). The most desirable solution would be to find resistant cultivars, but this has met with mixed success. Efforts are underway to identify sources of resistance in *Cucurbita pepo* (Padley *et al.*, 2008) and to introgress resistance from the wild *Cucurbita* species, *C. lundelliana*, into *C. mocshata* (Padley *et al.*, 2009).

To our knowledge, there have not been studies to identify sources of resistance to *P. capsici* in melon. In our results, five accessions of melon germplasm were found resistant to phytophthora rot. They survived after the flowering stage with no disease symptoms on the whole plant. We recommend the use of these genetic resources to backcross resistance into adapted cultivars, and as rootstocks for phytophthora rot resistance.

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