JARQ 60 (3), 273-281 (2026) https://www.jircas.go.jp

Comparative Evaluation of Phytophthora Blight Resistance in *Helleborus* Cultivars

Lifeng LONG[1], Thiara Celine SUAREZ[1,2], Takashi NAKATSUKA[1,3,4], Kunio YAMADA[1,5]* and Masaki OCHIAI[1,5]

[1] United Graduate School of Agricultural Science, Gifu University, Gifu, Japan
[2] Mariano Marcos State University, City of Batac, Ilocos Norte, Philippines
[3] Faculty of Agriculture, Shizuoka University, Shizuoka, Japan
[4] College of Agriculture, Academic Institute, Shizuoka University, Shizuoka, Japan
[5] Faculty of Applied Biological Science, Gifu University, Gifu, Japan

Abstract

Phytophthora blight, primarily caused by *Phytophthora nicotianae*, poses a significant threat to *Helleborus* production under warm and humid conditions. However, the tolerance levels of different *Helleborus* cultivars to this disease have not yet been compared. Thus, this study aimed to assess disease tolerance in the 17 major *Helleborus* cultivars available in Japan, all propagated via tissue culture to ensure genetic uniformity. The plants were inoculated with *P. nicotianae*, and the disease progression was monitored over 20 days. Our results revealed substantial variation in disease severity among the cultivars. The 'Petit doll' cultivars, particularly 'Petit doll Shiori,' exhibited strong and consistent tolerance, whereas the others, such as 'White sugar chiffon' and 'Mayuhiki,' showed high susceptibility. Some cultivars displayed delayed symptom development followed by rapid progression. In several cases, discrepancies were observed between root and foliar symptoms, indicating that above- and below-ground responses do not always correlate. This highlights the diversity of disease responses and the need for integrated evaluation methods when screening for disease tolerance. The tolerant cultivars identified in this study are valuable resources for breeding programs to improve resistance and ensure the sustainability of *Helleborus* production.

Discipline: Horticulture
Additional key words: Christmas rose, cultivar screening, disease progression, *Phytophthora nicotianae*, root rot tolerance

Introduction

Helleborus, a small genus of the family Ranunculaceae, comprises approximately 19 species of herbaceous perennials (Sun et al. 2001). Several *Helleborus* hybrids and cultivars have been produced by interspecific hybridization in horticultural breeding to improve flower color and size. Some *Helleborus* plants are grown in gardens or pots, whereas others are grown as cut flowers (e.g., *H. niger* and *H.* ×*hybridus*) (Lowder et al. 2010, Poupet et al. 2006). *Helleborus* is a popular Christmas flower in Europe. In the Netherlands, about 500 thousand potted plants and 1.5 million cut flowers of *Helleborus* were sold in 2007-2009 (Beruto et al. 2013). According to Flora Holland auction data, *Helleborus* ranked eighth in sales value among ornamental crops in 2014, with the total sales reaching 12 million euros (Šušek 2016). In Japan, all *Helleborus* species are commonly referred to as Christmas roses, but the English name refers to the original species *H. niger* (Nonokuchi & Yokoyama 2016). This broader usage is due to the plant's popularity and blooming during the winter months, which align with the Christmas season.

In September 2013, root and stem rot symptoms resembling *Phytophthora* infection were first reported in *Helleborus* in Chiba Prefecture, Japan. Kanegae et al. (2019) subsequently isolated the causal fungus and identified it as *Phytophthora nicotianae* using species-specific primers and have reported that similar outbreaks have occurred recurrently during the summer

*Corresponding author: yamada.kunio.k0@f.gifu-u.ac.jp
Received 10 June 2025; accepted 18 September 2025; J-STAGE Advanced Epub 30 April 2026.
https://doi.org/106090/jarq.25J07

in Japan since that initial observation. Effective control remains challenging due to the lack of registered fungicides for ornamental crops in Japan and the thermophilic nature of *Phytophthora*, which further promotes disease development under warm, humid conditions. *Phytophthora* species are serious threats to global agricultural production because they cause destructive diseases in a broad spectrum of plant hosts (Cline et al. 2008, Scott et al. 2019). These fungus-like eukaryotes, which are taxonomically classified as oomycetes, produce a range of asexual and sexual spores (Judelson & Blanco 2005). The biological characteristics of these spores are central to their ability to effectively infect and spread within host populations. *Phytophthora nicotianae* is a well-known soil-borne pathogen that produces long-lived chlamydospores, allowing it to persist in the soil under unfavorable conditions. Under suitable soil moisture levels, these chlamydospores germinate and release motile zoospores that actively infect plant roots (McCarren et al. 2005). The pathogen induces root and stem rot, characterized by brown-to-black discoloration and tissue decay (Erwin & Ribeiro 1996, Bodah 2017). It can cause a broad range of above-ground symptoms depending on the host, including foliar blight and tuber rot in potatoes (Taylor et al. 2012), trunk and branch cankers in citrus (Meitz-Hopkins et al. 2014), pre-emergence and damping-off in soybeans (Kato et al. 2013), water-soaked roots and stems in kalanchoe (Watanabe et al. 2007), and bluish-green discoloration of young leaves followed by fruit browning with a leathery texture in strawberries (Madden et al. 1991, Marin et al. 2024).

Among the diverse strategies developed to manage *Phytophthora* diseases, the breeding and deployment of resistant cultivars have emerged as a core approach due to their long-term effectiveness, sustainability, and minimal environmental impact. Tolerance to *Phytophthora* has been identified in soybeans (Sahoo et al. 2017), peppers (Foster & Hausbeck 2010), potatoes (Taylor et al. 2012), and squash (Padley et al. 2009). It is currently regarded as a cornerstone of integrated disease management. Susceptibility to *Phytophthora* varies widely among plant species and cultivars (Anacker et al. 2007, Glosier et al. 2008, Oelke et al. 2003), underscoring the importance of systematic screening of germplasm to identify tolerant cultivars. Such evaluations are essential for ranking cultivars and selecting highly tolerant lines that can be directly deployed in production or incorporated into breeding programs. The use of disease-resistant cultivars remains the most cost-effective and environmentally sustainable strategy for long-term disease control.

Despite the increasing commercial importance of *Helleborus*, the tolerance of its cultivars to *Phytophthora nicotianae* has not yet been systematically assessed, and information on intraspecific variation in resistance remains limited. Therefore, this study aimed to evaluate the tolerance of major commercial Japanese *Helleborus* cultivars to Phytophthora blight through quantitative phenotypic screening. The findings of this research will facilitate the identification of promising resistant cultivars and contribute to the development of practical breeding strategies and integrated disease management programs for *Helleborus* cultivation.

Materials and methods

1. *Helleborus* plant material

Seventeen commercial cultivars of *Helleborus* were used in this study: 'Apricot gelato,' 'Cranberry chiffon,' 'Elegance flare pink,' 'Elegance purple,' 'Elicsmithii,' 'Grace prima dress,' 'Harushimo,' 'Kurosuzu,' 'Kozakura,' 'Mayuhiki,' 'Miyabi,' 'Peach prima dress,' 'Petit doll Yui,' 'Petit doll Aya,' 'Petit doll Shiori,' 'White prima dress,' and 'White sugar chiffon.' All cultivars were clonally propagated via tissue culture and were commercially available in the Japanese horticulture market. The 17 cultivars represented a diverse range of flower colors and shapes. Plants were grown in plastic pots (7.6φ × 6.4 cm) filled with sterilized soil media. The pots were placed on saucers and cultivated in a cultivation room under a 12 h photoperiod, with a photosynthetic photon flux density of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using fluorescent light. Day and night temperatures were maintained at 24°C-28°C and 22°C-26°C, respectively. The plants were irrigated daily to maintain optimal soil moisture for growth.

2. Pathogen culturing and inoculum preparation

Phytophthora nicotianae provided by Kanegae in Chiba Prefectural Agriculture and Forestry Research Center was cultured on potato dextrose agar (PDA) consisting of potato infusion (prepared from 200 g of potato), 4 g of dextrose, 20 g of agar, and 1,000 mL of distilled water, with a final pH of 5.6 ± 0.2. The cultures were maintained in 9 cm Petri dishes and incubated at 25 and 30°C in darkness for one week (Pettitt et al. 2002). The zoospore inoculum was prepared in a grass leaf blade culture. Autoclaved bent grass leaf blades were placed onto *P. nicotianae* cultures that had been incubated for 7 days on PDA. After 24 h of incubation at 25°C, once the pathogen had colonized the grass leaf blades, four to six infected leaf materials were transferred to 0.5 mL of a sterile culture solution composed of pond water collected

at Gifu University and distilled water with a 1:2 (v/v) ratio in clean Petri dishes (Kageyama et al. 2002, Ahonsi et al. 2007). The dishes were incubated for 18 h at 20°C under fluorescent light to promote sporangia development. The cultures were chilled at 4°C in darkness for 1 h and then rewarmed to room temperature (20 ± 1°C) under fluorescent light to induce zoospore release. Zoospore release typically occurred within 10-20 min after rewarming. The zoospore-containing suspension was then decanted into a sterile Erlenmeyer flask. The concentration of the suspension was immediately measured using a hemocytometer and adjusted to 1×10^4 zoospores/mL. Zoospore motility was confirmed under a light microscope prior to inoculation.

3. Inoculation and resistance evaluation

Prior to inoculation, the plants were thoroughly irrigated, and soil moisture was maintained for 24 h to optimize the conditions for zoospore infection. Each plant was inoculated by applying 30 mL of *P. nicotianae* zoospore suspension (1×10^4 zoospores/mL) directly onto the soil surface. After inoculation, the cultivation room was maintained at 26°C-30°C and 60%-80% relative humidity.

The plants were then returned to the cultivation room and monitored at 5-day intervals by photographing and observing disease progression. To assess the root part of the plant, we carefully removed each one from the pot with the soil intact, photographed the bottom of the root ball, and then evaluated the extent of discoloration visually on a 0–5 scale as follows: 0 = no symptoms; 1 = minor (< 30% discoloration); 2 = moderate (≥ 30% and < 50%); 3 = evident (≥ 50% and < 70%); 4 = severe (≥ 70% and < 90%); 5 = extreme (≥ 90% discoloration or complete plant death) (Fig. 1). To assess the above-ground part, we took overhead photographs of the entire plant to document foliar symptoms. These observations were continued until the plants died. The area under the disease progression curve (AUDPC) was calculated to quantify disease progression over time. This method is widely used in plant pathology studies to evaluate cultivar resistance. Severity scores were recorded at 5-day intervals from 5 to 20 days post-inoculation (dpi), and AUDPC values were computed using the trapezoidal integration method, following the formula described by Shaner & Finney (1977) and further detailed by Simko & Piepho (2012). This approach integrates the intensity and duration of disease symptoms, providing a comprehensive measure of cumulative disease pressure. Five individual plants were used per replicate for each treatment, and the experiment was independently repeated three times, resulting in 15 biological samples per treatment.

Results

The *Helleborus* cultivars infected with *P. nicotianae* exhibited characteristic root rot symptoms, including dark brown-to-black lesions, discoloration, and softened or decayed root tissue. No symptoms were observed in any of the non-inoculated control plants, confirming the pathogenicity of *P. nicotianae* and the suitability of the inoculation method. Symptom development in *Helleborus* cultivars was monitored over a 20-day period and quantified using AUDPC to evaluate their disease susceptibility. As shown in Table 1, the disease progression patterns varied among the 17 cultivars. The AUDPC values (Table 2) ranged from 280.0 in 'Petit doll Shiori' to 692.5 in 'White sugar chiffon,' with an overall mean of 471.3 ± 117.5. Results of one-way ANOVA followed by Tukey's HSD post-hoc test revealed significant differences ($p < 0.05$) in disease susceptibility among several cultivar pairs. The most susceptible

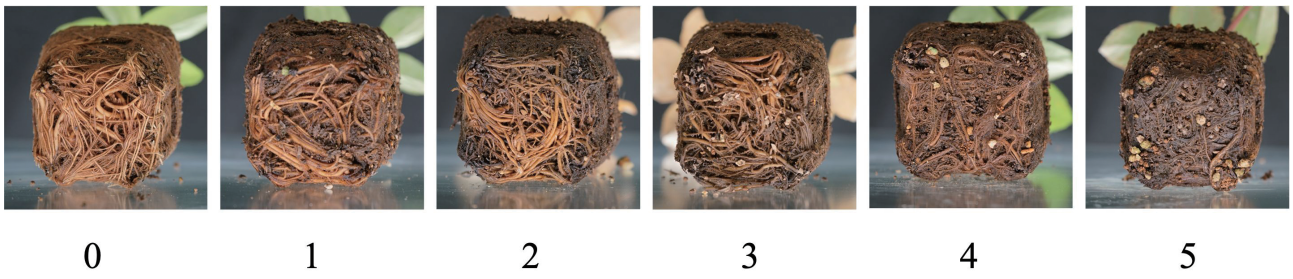

Fig. 1. Representative root images illustrating the disease severity scale used for visual assessment of root rot symptoms in *Helleborus* plants

Severity was rated on a 0-5 scale based on the extent of root discoloration observed from 5 to 20 dpi: 0 = no symptoms; 1 = minor (< 30% discoloration); 2 = moderate (≥ 30% and < 50%); 3 = evident (≥ 50% and < 70%); 4 = severe (≥ 70% and < 90%); 5 = extreme (≥ 90% discoloration or complete plant death).

cultivar ('White sugar chiffon') had an AUDPC nearly 2.5 times as high as that of the most tolerant one ('Petit doll Shiori'). Based on the AUDPC values and Tukey's HSD results, the 17 cultivars were grouped into five resistance classes. Specifically, based on the AUDPC values, 'Petit doll Shiori' and 'Petit doll Yui' were categorized as highly and moderately tolerant, respectively, while 'Mayuhiki' and 'White sugar chiffon' were categorized as highly susceptible. For the remaining 13 cultivars, Tukey's HSD results divided them into two categories: low tolerant and susceptible (Table 2).

Because this study is the first systematic evaluation of *Phytophthora* tolerance in *Helleborus* using a controlled root inoculation assay, the AUDPC method requires a 20-day follow-up period, so a simpler and more rapid evaluation method is needed. To this end, we summarize the percentages of plants with severity scores ≥ 3 at 10 and 15 dpi, and ≥ 4 at 15 and 20 dpi in Table 3 to indicate whether these thresholds offer meaningful insights for streamlined assessments. Although differences among cultivars were evident at 10 dpi, they evolved over time, with several cultivars exhibiting marked changes in symptom expression. As the infection progressed, symptom severity escalated in several cultivars. For example, 'Miyabi' exhibited a relatively low percentage (13.3%) of plants with severity ≥ 3 at 10 dpi, but this value increased sharply to 86.7% at 15 dpi, indicating a delayed onset of severe symptoms. Similarly, several cultivars, including 'White prima dress,' 'Elicsmithii,' 'Harushimo,' and 'Apricot gelato,' appeared relatively tolerant at 10 dpi but subsequently developed severe symptoms by 15 dpi. To evaluate the diagnostic value of the severity thresholds in Table 3, we performed hierarchical clustering analysis using the proportions of plants with severity scores of ≥ 3 at 10 and 15 dpi, and ≥ 4 at 15 and 20 dpi. According to the results of hierarchical clustering analysis, the grouping based on severity ≥ 4 at 20 dpi showed the highest concordance with the AUDPC-based tolerance categories in Table 2. Although the pathogen was inoculated into the roots, foliar disease symptoms were also monitored. The foliar symptoms are shown in Figure 2, and the corresponding root rot severity scores at 15 dpi for these cultivars are shown in Figure 3. In most cultivars, the severity of root and foliar symptoms was generally aligned; however, a few exceptions were observed where above- and below-ground responses did not correlate. Six representative cultivars were selected to illustrate these discrepancies: three Petit doll series cultivars spanning from highly to moderately tolerant, one low-tolerant cultivar ('Elicsmithii'), and two susceptible cultivars ('Apricot gelato' and 'Elegance flare pink'). Among the six observed plants of 'Petit doll

Table 1. Disease severity progression of 17 *Helleborus* cultivars after 5, 10, 15, and 20 days post-inoculation (dpi) response to root rot caused by *Phytophthora nicotianae*

Commercial cultivars	Disease severity			
	5 dpi	10 dpi	15 dpi	20 dpi
Petit doll Shiori	0.53 ± 0.50 a**	0.93 ± 0.25 a**	1.53 ± 0.50 a**	2.00 ± 0.63 a**
Petit doll Yui	0.87 ± 0.50 ab	1.53 ± 0.50 ab	2.13 ± 0.34 ab	2.73 ± 0.57 ab
Petit doll Aya	1.20 ± 0.54 bcd	1.87 ± 0.50 bc	2.53 ± 0.62 bc	3.13 ± 0.50 cd
Elegance purple	1.20 ± 0.40 bcd	1.87 ± 0.50 bc	2.60 ± 0.61 bcd	3.27 ± 0.44 bcd
White prima dress	1.33 ± 0.47 bcd	2.07 ± 0.25 bcd	2.67 ± 0.47 bcd	3.33 ± 0.47 bcd
Elicsmithii	1.20 ± 0.40 bcd	2.00 ± 0.00 bcd	2.67 ± 0.47 bcd	3.40 ± 0.49 bcde
Grace prima dress	1.33 ± 0.47 bcd	2.13 ± 0.62 bcd	2.67 ± 0.70 bcd	3.40 ± 0.71 bcde
Harushimo	1.33 ± 0.47 bcd	2.07 ± 0.57 bcd	2.67 ± 0.60 bcd	3.53 ± 0.62 bcde
Apricot gelato	1.53 ± 0.62 cd	2.27 ± 0.57 cde	2.87 ± 0.81 bcde	3.53 ± 0.72 bcde
Cranberry chiffon	1.67 ± 0.47 cd	2.40 ± 0.71 cde	3.00 ± 0.52 cde	3.73 ± 0.68 cdef
Elegance flare pink	1.47 ± 0.50 bcd	2.47 ± 0.62 cde	3.07 ± 0.68 cde	3.73 ± 0.85 cdef
Peach prima dress	1.47 ± 0.50 bcd	2.47 ± 0.62 cde	3.13 ± 0.62 cde	3.73 ± 0.85 cdef
Miyabi	1.13 ± 0.34 abc	2.07 ± 0.44 bcd	2.93 ± 0.44 cde	3.80 ± 0.65 cdef
Kozakura	1.67 ± 0.47 cd	2.40 ± 0.49 cde	2.80 ± 0.54 bcd	3.93 ± 0.57 cdef
Kurosuzu	1.73 ± 0.44 cd	2.60 ± 0.49 de	3.20 ± 0.40 cde	4.00 ± 0.73 def
Mayuhiki	1.80 ± 0.40 d	2.87 ± 0.50 e	3.33 ± 0.47 de	4.20 ± 0.40 ef
White sugar chiffon	1.60 ± 0.49 cd	2.67 ± 0.47 de	3.60 ± 0.49 e	4.33 ± 0.60 f

**Significantly different according to Tukey test at $P = 0.05$ if letters differ among the same dpi (n= 15).

Table 2. Area under the disease progress curve (AUDPC) values for disease severity in 17 *Helleborus* cultivars in response to root rot caused by *Phytophthora nicotianae*

Commercial cultivars	AUDPC*		Category
Petit doll Shiori	280.0	a**	Highly Tolerant
Petit doll Yui	410.0	ab	Moderately Tolerant
Petit doll Aya	492.5	bc	Low Tolerant
Elegance purple	502.5	bc	
Elicsmithii	522.5	bcd	
White prima dress	530.0	bcd	
Grace prima dress	537.5	bcdef	
Harushimo	537.5	bcdef	
Miyabi	560.0	cdef	Susceptible
Apricot gelato	575.0	cdef	
Kozakura	600.0	cdef	
Cranberry chiffon	607.5	cdef	
Elegance flare pink	610.0	cdef	
Peach prima dress	615.0	cdef	
Kurosuzu	650.0	def	
Mayuhiki	690.0	ef	Highly Susceptible
White sugar chiffon	692.5	f	

*AUDPC values were calculated by using disease severity rating assessed at 5 d intervals from 0 to 20 d post-inoculation.
**Significantly different according to Tukey test at $P = 0.05$ if letters differ (n= 15).

Table 3. Percentages of plant disease severity scores ≥ 3 or ≥ 4 at 10, 15, and 20 days post-inoculation across 17 *Helleborus* cultivars

Commercial cultivars	Severity* ≥ 3 (%) at 10 dpi	Severity ≥ 3 (%) at 15 dpi	Severity ≥ 4 (%) at 15 dpi	Severity ≥ 4 (%) at 20 dpi
Petit doll Shiori	0.0%	0.0%	0.0%	0.0%
Petit doll Yui	0.0%	13.3%	0.0%	6.7%
Petit doll Aya	6.7%	46.7%	6.7%	20.0%
Elegance purple	6.7%	53.3%	6.7%	26.7%
Grace prima dress	26.7%	53.3%	13.3%	26.7%
White prima dress	6.7%	66.7%	0.0%	33.3%
Elicsmithii	0.0%	66.7%	0.0%	40.0%
Apricot gelato	33.3%	60.0%	26.7%	40.0%
Harushimo	20.0%	60.0%	6.7%	46.7%
Elegance flare pink	40.0%	80.0%	26.7%	46.7%
Peach prima dress	40.0%	86.7%	26.7%	46.7%
Cranberry chiffon	40.0%	86.7%	13.3%	60.0%
Miyabi	13.3%	86.7%	6.7%	66.7%
Kurosuzu	60.0%	100.0%	20.0%	73.3%
Kozakura	40.0%	73.3%	6.7%	80.0%
White sugar chiffon	66.7%	100.0%	60.0%	93.3%
Mayuhiki	80.0%	100.0%	33.3%	100.0%

*Plant disease severity was assessed based on the extent of root discoloration caused by root rot, using a 1–5 rating scale as follows: 1 = minor (≤ 30% of discoloration); 2 = moderate (≥ 30% and < 50%); 3 = evident (≥ 50% and < 70%); 4 = severe (≥ 70% and < 90%); 5 = extreme (≥ 90% discoloration or complete plant death).

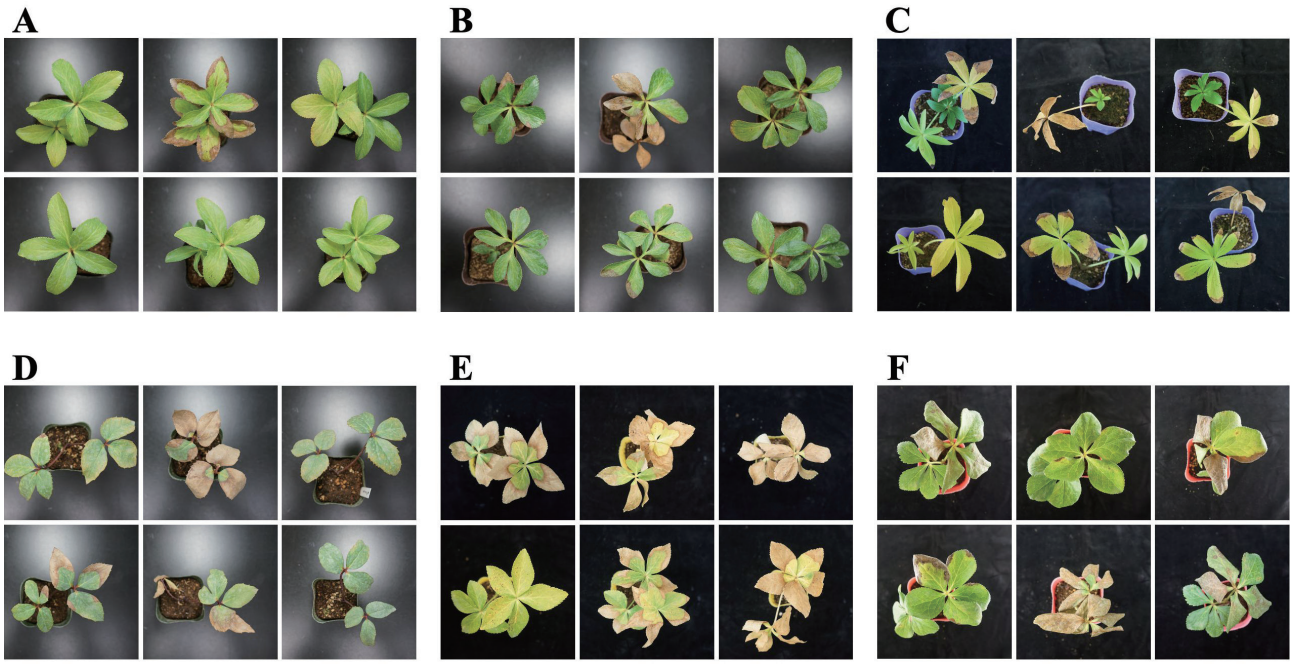

Fig. 2. Disease symptoms observed on above-ground organs of six *Helleborus* cultivars at 15 days post-inoculation with *Phytophthora nicotianae*

To illustrate the range of symptom expression, each panel (A-F) presents one of six representative samples from 15 individuals per cultivar, chosen to capture varying degrees of symptom severity: A, 'Petit doll Shiori'; B, 'Petit doll Yui'; C, 'Petit doll Aya'; D, 'Elicsmithii'; E, 'Apricot gelato'; and F, 'Elegance flare pink.'

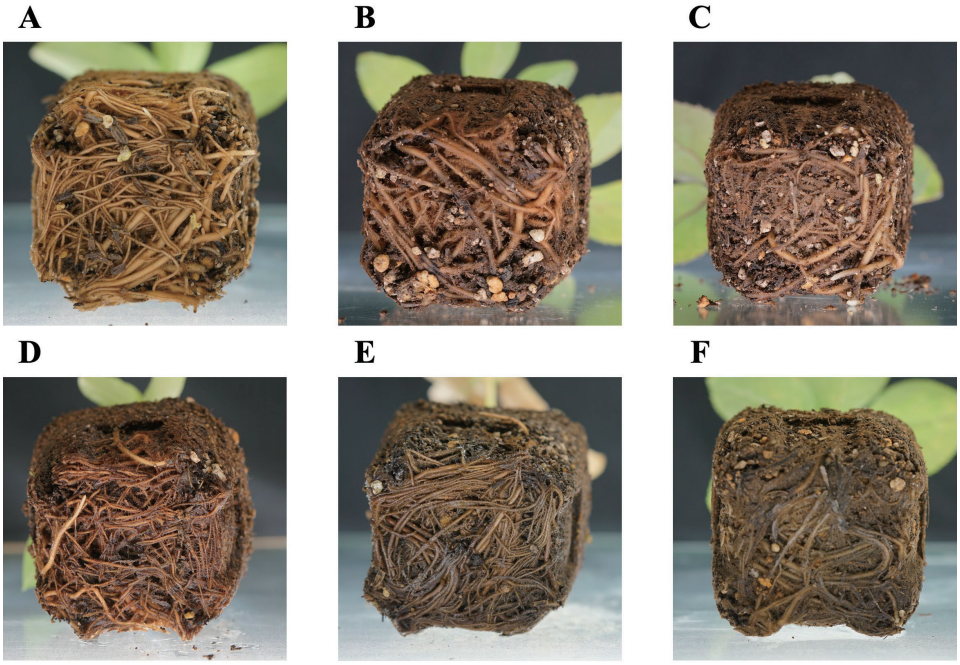

Fig. 3. Disease symptoms observed on root organs of six *Helleborus* cultivars at 15 dpi with *Phytophthora nicotianae*

Each panel (A-F) shows a representative sample from 15 individuals per cultivar, selected to reflect the average symptom severity in each group: A, 'Petit doll Shiori'; B, 'Petit doll Yui'; C, 'Petit doll Aya'; D, 'Elicsmithii'; E, 'Apricot gelato'; and F, 'Elegance flare pink.'

Shiori,' only one exhibited minor browning along the leaf margins, while the remaining five showed no visible above-ground symptoms. By contrast, 'Petit doll Aya,' ranked third in root rot tolerance, showed clear foliar symptoms, such as yellowing and browning, across all six representative individuals, despite only mild root rot. Similar mismatches were found in the susceptible group. 'Apricot gelato' exhibited severe foliar symptoms, including extensive chlorosis and wilting. Conversely, 'Elegance flare pink', which had the highest root rot severity score among the six cultivars, displayed comparatively mild foliar symptoms, with three of six plants showing no visible above-ground symptoms.

Discussion

This study is the first evaluation of Phytophthora blight tolerance in commercially available *Helleborus* cultivars under controlled inoculation conditions. Root inoculation trials revealed substantial variation in tolerance to *Phytophthora* among cultivars. According to the AUDPC analysis (Table 2), the 17 cultivars exhibited statistically significant differences in disease severity. Based on these differences, the cultivars were classified into five categories ranging from highly tolerant to highly susceptible. 'Petit doll Shiori' exhibited high tolerance, whereas 'Petit doll Yui' demonstrated moderate levels of tolerance. In contrast, 'Petit doll Aya,' along with 'Elegance purple,' 'Elicsmithii,' 'White prima dress,' 'Grace prima dress,' and 'Harushimo,' were categorized as having low tolerance. 'White sugar chiffon,' which displayed the lowest level of tolerance, along with 'Mayuhiki,' was categorized as highly susceptible. These findings suggest that exploitable genetic diversity exists within current commercial germplasms. The tolerant cultivars identified in this study may serve as promising parental lines and genetic material for further investigation into the genetic basis of disease tolerance. Given the limited research on *Phytophthora* tolerance in *Helleborus*, it would be helpful to examine related efforts in other horticultural crops, particularly bell pepper (*Capsicum annuum*), in which extensive studies have been conducted. Developing resistant cultivars of bell peppers has been a long-standing goal, but progress has often been complicated by inconsistent resistance and the need to address different disease forms, such as root rot, stem blight, and leaf blight (Foster & Hausbeck 2010, Sy et al. 2005). To overcome these challenges, researchers have established standardized screening methods and identified reliable resistant lines such as 'Criollo de Morelos-334'(CM334) (Foster & Hausbeck 2010, Quirin et al. 2005), 'PI 201234' from Mexico (Wang et al. 2015, 2016), and 'AC2258' from Central America (Li et al. 2021, Sugita et al. 2006). These tools have played a key role in supporting breeding programs and improving disease management in peppers. In contrast, for *Helleborus*, no such resistant reference cultivars or screening protocols have been established, making it challenging to evaluate tolerance consistently. The current study provides a first step toward filling this gap by identifying tolerant *Helleborus* cultivars under controlled conditions and laying the groundwork for future breeding and management efforts. While extensive frameworks have been established for bell peppers, *Helleborus* has received limited attention with respect to *Phytophthora* tolerance breeding and currently lacks designated resistant reference lines and standardized, harmonized evaluation protocols. Despite growing concerns about disease susceptibility, systematic screening efforts remain scarce in this genus. The development of standardized resistant reference lines, as established in bell peppers, may not be feasible for *Helleborus* in the near future. Establishing a structured screening system for *Phytophthora* tolerance in *Helleborus* is urgently needed for tolerance breeding and disease management. The present study addresses this critical gap by introducing a clearly defined root inoculation protocol that simulates natural infection pathways and enables standardized evaluation.

As Table 3 shows, disease severity progressed markedly over time, depending on the cultivar. Five cultivars ('Miyabi,' 'Elicsmithii,' 'White prima dress,' 'Harushimo,' and 'Apricot gelato') exhibited relatively mild symptoms at early post-inoculation stages but later experienced a rapid escalation in disease severity, suggesting delayed susceptibility responses. In addition to calculating AUDPC, we examined alternative indicators of disease progression, including the proportion of plants with severity scores ≥ 3 or ≥ 4 at specific time points after inoculation. Among these indicators, hierarchical clustering based on the percentage of plants with severity scores ≥ 4 at 20 dpi showed the highest concordance with the AUDPC-based categorization in Table 2. The resulting grouping was consistent with the tolerance categories defined by AUDPC, confirming that AUDPC remains a robust standard method for evaluating disease progression. However, because AUDPC requires repeated assessments over a 20-day period, its application can be labor-intensive and less practical for large-scale studies. In contrast, the proportion of plants with severity ≥ 4 at 20 dpi provided a simpler yet reliable alternative, effectively distinguishing tolerant cultivars (e.g., 'Petit doll Shiori,' 'Petit doll Yui') from highly susceptible ones (e.g., 'White sugar chiffon,' 'Mayuhiki'). Future studies

should validate the consistency of this threshold under diverse environmental conditions and across broader germplasm collections to determine its reliability and utility in practical breeding and disease management programs.

Historically, breeding of *Helleborus* has primarily emphasized aesthetic traits, such as flower color and morphological variation (Rice & Strangman 2005, Long et al. 2023, Mathew 1989). This focus on ornamental value may partly explain the low levels of disease tolerance observed in the current commercial cultivars. These findings underscore the urgent need for strategic breeding approaches that incorporate disease tolerance traits to ensure greater resilience and long-term sustainability of *Helleborus* production.

In this experiment, the root systems of *Helleborus* cultivars were inoculated to evaluate their tolerance to Phytophthora blight. Disease symptoms appearing in above-ground organs, including those observed in stems and leaves, were also recorded to capture broader patterns of plant responses. Despite these observations, most cultivars, with a few exceptions, did not exhibit a clear correlation between resistance in the roots and that in the above-ground parts. As shown in Figures 2 and 3, all three Petit doll cultivars exhibited strong tolerance to root rot, but 'Petit doll Aya' displayed comparatively greater foliar symptom severity. By contrast, although 'Apricot gelato' and 'Elegance flare pink' showed similar root rot tolerance ratings, they differed in the extent of foliar symptom development. These findings highlight the complexity of plant-pathogen interactions, particularly in distinguishing between root- and foliar-based tolerance responses. These discrepancies suggest that tolerance to *Phytophthora* infection in *Helleborus* is organ-specific and governed by distinct defense pathways in roots and aerial tissues. This reinforces the need for integrated evaluation strategies that consider both root and foliar symptoms to assess cultivar performance under disease pressure more accurately.

Phytophthora-induced root rot, stem blight, and foliar blight constitute distinct disease syndromes, each governed by potentially independent genetic and physiological tolerance mechanisms (McGregor et al. 2011, Sy et al. 2005). Furthermore, these studies have consistently reported low correlations between tolerance responses in different plant organs (Candole et al. 2012). Although the pathogen primarily infects the root system, its effects often extend to the above-ground parts of the plant, causing secondary symptoms such as wilting, stem collapse, and leaf necrosis (Hausbeck & Lamour 2004).

Although the goal of tolerance breeding is to develop comprehensive cultivars, breeding programs should prioritize root rot resistance, since roots serve as the initial point of entry and are key determinants of systemic disease development. Once root-tolerant genotypes are secured, subsequent screening should be extended to stem and leaf responses to develop cultivars with multi-organ tolerance (Abebe et al. 2016, Krasnow et al. 2017).

Accordingly, the present study not only provides a practical framework for root-based resistance screening in *Helleborus* but also delivers foundational data to inform the design of multi-stage, genetically informed breeding strategies aimed at improving resistance to *Phytophthora* diseases.

Acknowledgements

We sincerely thank Mr. Kanegae of the Chiba Prefectural Agriculture and Forestry Research Center for providing the *Phytophthora nicotianae* isolate in recognition of his pioneering work on *Helleborus* pathology in Japan. We are also deeply grateful to Professor Emeritus Kageyama of Gifu University for his invaluable guidance and continued support, which greatly contributed to the direction and development of this study.

References

Abebe, A. M. et al. (2016) Evaluation of Phytophthora root rot- and bacterial wilt-resistant inbred lines and their crosses for use as rootstocks in pepper (*Capsicum annuum* L.). *Hortic. Environ. Biotechnol*., **57**, 598-605.

Ahonsi, M. O. et al. (2007) A simple *in-vitro* 'wet-plate' method for mass production of *Phytophthora nicotianae* zoospores and factors influencing zoospore production. *J. Microbiol. Methods*, **70**, 557-560.

Anacker, B. L. et al. (2007) Susceptibility to *Phytophthora ramorum* in a key infectious host: Landscape variation in host genotype, host phenotype, and environmental factors. *New Phytol*., **177**, 756-766.

Beruto, M. et al. (2013) Micropropagation of *Helleborus* through axillary budding. In Lambardi, M., Rugini, E. and Spethmann, W. (eds.), *Methods Mol. Biol*., **11013**. Humana Press, Totowa, NJ, pp. 259-267.

Bodah, E. T. (2017) Root rot diseases in plants: A review of common causal agents and management strategies. *Agri. Res. & Tech.: Open Access J*., **5**, 555661.

Candole, B. L. et al. (2012) Evaluation of phytophthora root rot-resistant *Capsicum annuum* accessions for resistance to phytophthora foliar blight and phytophthora stem blight. *Agric. Sci*., **3**, 732-737.

Cline, E. T. et al. (2008) A synopsis of *Phytophthora* with accurate scientific names, host range, and geographic distribution. *Plant Health Prog.* https://doi.org/10.1094/PHP-2008-0318-01-RS

Erwin, D. C. & Ribeiro, O. K. (1996) Phytophthora diseases worldwide. APS Press, St. Paul, MN, USA, 562 pp.

Foster, J. M. & Hausbeck, M. K. (2010) Resistance of pepper to Phytophthora crown, root, and fruit rot is affected by isolate virulence. *Plant Dis.*, **94**, 24-30.

Glosier, B. R. et al. (2008) A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora* capsici. *Euphytica*, **162**, 23-30.

Hausbeck, M. K. & Lamour, K. H. (2004) *Phytophthora capsici* on vegetable crops: research progress and management challenges. *Plant Dis.*, **88**, 1292-1303.

Judelson, H. S. & Blanco, F. A. (2005) The spores of *Phytophthora*: Weapons of the plant destroyer. *Nat. Rev. Microbiol.*, **3**, 47-58.

Kageyama, K. et al. (2002) Root rot of miniature roses caused by *Pythium helicoides*. *J. Gen. Plant Pathol.*, **68**, 15-20.

Kanegae, Y. et al. (2019) Phytophthora blight of Christmas rose (*Helleborus* sp.) caused by *Phytophthora nicotianae* in Japan. *Kanto-tozan byogaichu kenkyukaiho* (*Ann. Rept. Kanto Pl. Prot. Soc*), **66**, 36-38 [In Japanese].

Kato, M. et al. (2013) Association of *Pythium* and *Phytophthora* with pre-emergence seedling damping-off of soybean grown in a field converted from a paddy field in Japan. *Plant Prod. Sci.*, **16**, 95-104.

Krasnow, C. S. et al. (2017) Evaluation of pepper root rot resistance in an integrated Phytophthora blight management program. *HortTechnology*, **27**, 408-415.

Li, Y. F. et al. (2021) Generation of a high-density genetic map of pepper (*Capsicum annuum* L.) by SLAF-seq and QTL analysis of *Phytophthora capsici* resistance. *Horticulturae*, **7**, 92.

Long, L. et al. (2023) A comprehensive review of the morphological and molecular taxonomy of the genus *Helleborus* (Ranunculaceae). *Rev. Agric. Sci.*, **11**, 106-120.

Lowder, A. W. et al. (2010) Day/Night temperatures influence growth and photosynthesis during containerized production of selected species of *Helleborus* (hellebores). *J. Environ. Hort.*, **28**, 179-186.

Madden, L. V. et al. (1991) Epidemiology and control of leather rot of strawberries. *Plant Dis.*, **75**, 439-446.

Marin, M. V. et al. (2024) New genotypes of *Phytophthora nicotianae* found on strawberry in Florida. *Phytopathology* **114**, 743-751.

Mathew, B. (1989) *Hellebores*. Publications Ltd (UK) Alpine Garden Society.

McCarren, K. L. et al. (2005) The role of chlamydospores of *Phytophthora cinnamomic* — a review. *Australas. Plant Pathol.*, **34**, 333-338.

McGregor, C. et al. (2011) Genotypic and phenotypic variation among pepper accessions resistant to *Phytophthora capsici*. *HortScience*, **46**, 1235-1240.

Meitz-Hopkins, J. C. et al. (2014) *Phytophthora* species distribution in South African citrus production regions. *Eur. J. Plant Pathol.*, **138**, 733-749.

Nonokuchi, M. & Yokoyama, N. (2016) *Motto kurisumasu rozu* (*More Christmas roses*). NHK Publication, Tokyo, Japan, 128 pp. [In Japanese].

Oelke, L. M. et al. (2003) Differentiation of race specific resistance to Phytophthora root rot and foliar blight in *Capsicum annuum*. *J. Am. Soc. Hortic. Sci.*, **128**, 213-218.

Padley, L. D. et al. (2009) Inheritance of resistance to crown rot caused by *Phytophthora capsici* in Cucurbita. *HortScience*, **44**, 211-213.

Pettitt, T. R. et al. (2002) Comparison of serological, culture, and bait methods for detection of *Pythium* and *Phytophthora* zoospores in water. *Plant Pathol.*, **51**, 720-727.

Poupet, R. et al. (2006). Healthy in vitro propagation by meristem tip culture of *Helleborus niger*'s selected clone for cut flower. *Acta Hortic.*, **725**, 281-286.

Quirin, E. A. et al. (2005) Development of sequence characterized amplified region (SCAR) primers for the detection of Phyto.5.2, a major QTL for resistance to *Phytophthora capsici* Leon. in pepper. *Theor. Appl. Genet.*, **110**, 605-612.

Rice, G. & Strangman, E. (2005) The Gardener's Guide to Growing Hellebores. David & Charles, Newton Abbot, UK, 160 pp.

Sahoo, D. K. et al. (2017) A novel *Phytophthora sojae* resistance *Rps12* gene mapped to a genomic region that contains several Rps genes. *PLOS ONE*, **12**, e0169950.

Scott, P. et al. (2019) Global biogeography and invasion risk of the plant pathogen genus *Phytophthora*. *Environ. Sci. Policy.*, **101**, 175-182.

Shaner, G. & Finney, R. E. (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology*, **67**, 1051-1056.

Simko, I. & Piepho, H.-P. (2012) The area under the disease progress stairs: Calculation, advantage, and application. *Phytopathology*, **102**, 381-389.

Sugita, T. et al. (2006) QTL analysis for resistance to Phytophthora blight (*Phytophthora capsici* Leon.) using an intraspecific doubled-haploid population of *Capsicum annuum*. *Breed. Sci.*, **56**, 137-145.

Sun, H. et al. (2001) Molecular phylogeny of *Helleborus* (*Ranunculaceae*), with an emphasis on the East Asian–Mediterranean disjunction. *Taxon*, **50**, 1001-1018.

Šušek, A. (2016) Perspectives of Christmas rose (*Helleborus niger* L.) genetic improvement. *Agricultura*, **13**, 11-19.

Sy, O. et al. (2005) Inheritance of *Phytophthora* stem blight resistance as compared to *Phytophthora* root rot and *Phytophthora* foliar blight resistance in *Capsicum annuum* L. *J. Am. Soc. Hortic. Sci.*, **130**, 75-78.

Taylor, R. J. et al. (2012) Tuber rot of potato caused by *Phytophthora nicotianae*: Isolate aggressiveness and cultivar susceptibility. *Plant Dis.*, **96**, 693-704.

Wang, P. et al. (2015) Identification and expression analysis of candidate genes associated with defense responses to *Phytophthora capsici* in pepper line "PI 201234". *Int. J. Mol. Sci.*, **16**, 11417-11438.

Wang, P. et al. (2016) Molecular mapping of a gene conferring resistance to *Phytophthora capsici* Leonian race 2 in pepper line PI201234 (*Capsicum annuum* L.). *Mol. Breed.*, **36**, 66.

Watanabe, H. et al. (2007) *Pythium* and *Phytophthora* species associated with root and stem rots of kalanchoe. *J. Gen. Plant Pathol.*, **73**, 81-88.