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ORIGINAL ARTICLE

Identification of *Piper* species that are resistant to *Phytophthora capsici*, *Meloidogyne incognita*, and waterlogging in Vietnam

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Abstract

Black pepper (*Piper nigrum*) is a spice commonly used in kitchens throughout the world. Black pepper production is devastated by a range of pathogenic agents, including *Phytophthora capsici* and *Meloidogyne incognita*. Many efforts have been directed towards finding black pepper cultivars that are resistant to these pathogens. In this work, a 39-accession germplasm panel of species in the *Piper* family collected throughout Vietnam was described. Preliminary tests using *P. capsici* inoculation onto leaves were carried out to identify potentially resistant accessions. Next, candidate plants were inoculated with *P. capsici* mycelial suspension and survival rates were assessed 15, 30 and 45 days postinoculation. In addition, *Piper* plants were challenged with *M. incognita* by adding larvae/juveniles to growing pots. Resistance to *M. incognita* was determined by the number of root galls and the percentage of plants with yellow leaves 1, 2 and 4 months after treatment. *Piper* accessions were also subjected to a 4-day waterlogged treatment. Two accessions (HUIB_PH30 and HUIB_PD36) demonstrated high levels of resistance to all biological and water stresses. Micromorphological characterizations revealed that the amount of intercellular spaces in the root cortex correlated with the resistance to *P. capsici* and waterlogging tolerance. Hence, the abundance of intercellular spaces can serve as a guide for further selection of black pepper accessions that are resistant to common diseases and tolerant to waterlogged conditions.

KEYWORDS
black pepper, germplasm, morphology, quick death, slow death, waterlogging tolerance

1 | INTRODUCTION

Black pepper (*Piper nigrum*) is a valuable spice crop and plays an important role in the economies of countries in South-east Asia, South Asia and Brazil. Crop loss due to pathogens represents a major challenge for the black pepper industry. Two common pathogens severely affecting black pepper cultivation are *Phytophthora capsici* and *Meloidogyne incognita* (Indu et al., 2022; Mahadevan et al., 2016; Tran et al., 2019; Truong et al., 2010; Verma et al., 2023). In addition, it is well known that black pepper plants require well-drained soil (Nair, 2011; Sadanandan, 2000), which means that black pepper cultivation is hampered in parts of Asia where frequent rainfall and flooding are common. Therefore, a high-yielding cultivar that is also resistant to common pathogenic agents and tolerant to waterlogging is invaluable to the black pepper industry.

P. capsici is a soilborne pathogenic oomycete that causes foot rot in black pepper and other crops (Lamour et al., 2012). Rotting of the collar prevents the transfer of water and nutrients from the roots to aerial parts of the plant, causing rapid leaf wilting and dropping, and eventually plant death (Ravindran et al., 2000). The broad host range, combined with long-lived dormant sexual spores and extensive genetic diversity, make it challenging to eradicate *P. capsici* (Anandaraj

& Sarma, 1995; Bi et al., 2014; Lamour & Hausbeck, 2001). In addition to oomycetes, plant-parasitic nematodes represent a major class of pathogens infesting black pepper plants worldwide. Among nematodes, *M. incognita* is the most destructive parasite in black pepper cultivation (Thuy et al., 2012). Symptoms of *M. incognita* infestation include swellings on the thick primary roots, knots on secondary roots and leaves turning yellow. These parasites destroy the root vascular tissues, hence interfering with the transport of water and nutrients from the roots (Ramana & Eapen, 2000). As a result, infested plants display poor growth and a gradual decline in health and vigour.

Current management strategies against *P. capsici* and *M. incognita* rely on chemical treatments, which are not environmentally friendly (Narayana et al., 2018; Rini & Remya, 2020; Verma et al., 2023). Hence, it is critical to identify resistant genetic resources and introgress resistance against *P. capsici* and *M. incognita*, and also waterlogging tolerance, into high-yielding commercial varieties. This can be achieved via grafting, breeding or somatic fusion. Towards this goal, it is useful to develop a large germplasm panel of *Piper* spp. and an efficient screening approach for disease-resistant and waterlogging-tolerant plants.

Vietnam has an abundance of *Piper* cultivars and related species for which resistance to diseases and tolerance to waterlogged conditions have not been fully evaluated. In this study, a 39-accession germplasm of *Piper* spp. in Vietnam was characterized in terms of their resistance to *P. capsici*, *M. incognita* and waterlogging tolerance. This work provides valuable genetic materials for future studies to improve the resilience of commercial black pepper cultivars.

2 | MATERIALS AND METHODS

2.1 | Plant materials

From 2019 to 2021, we collected 39 *Piper* accessions from different black pepper production areas in eight provinces throughout Vietnam: Quang Tri, Quang Nam, Quang Ngai, Gia Lai, Binh Phuoc, Dak Lak, Dong Nai and Kien Giang. Three-internode cuttings from the lateral branches were dipped in rooting hormone to promote root formation. Five cuttings from each accession were planted in 23 × 13 cm pots containing a 3:1 mixture of sterile soil and compost. After 3 months, each plant was transferred to a 36 × 29 × 29 cm pot containing soil and compost mixture and placed in an open-net house at the Institute of Biotechnology, Hue University.

2.2 | Morphological characterization

Sixteen qualitative morphological traits were assessed using criteria as previously described (International Plant Genetic Resources Institute, 1995): plant growth habit, branching type, young orthotropic shoot tip colour, runner shoot production, holding capacity, adventitious root production, pubescence on the stem, lateral branch habit, leaf lamina shape, leaf base shape, leaf margin, type of veining, spike orientation, spike shape, type of hermaphroditism and fruit shape. The shoot colour was recorded using a Royal Horticultural Society colour chart. For the cluster analysis (R Development Core Team, 2008), all the traits of each accession were standardized, and the Euclidean distances were calculated using the unweighted pair group method with arithmetic mean (UPGMA).

2.3 | Identification of *P. capsici*-resistant accessions

Infected root-zone soil (5–10 cm below the surface) was collected from black pepper farms in Dak Krong commune, Dak Doa district, Gia Lai, Vietnam. The *P. capsici* strain was isolated from infected soil following a published protocol (Tran et al., 2023) with modifications. Briefly, 200 g of soil was soaked in 400 mL of distilled water. A rose petal was placed onto the water surface and incubation was carried out between 25 and 30°C for 2–3 days. Petals whose colour turned from red to brown were cut into 5-mm squared pieces and placed on a *Phytophthora*-selective medium (Tsao & Ocana, 1969). Plates were incubated in the dark at 25–30°C and microscopic morphologies were examined after 4–5 days. Based on morphology, *Phytophthora* spp. were transferred to potato dextrose agar (PDA) plates and incubation was carried out at 28°C for 7 days (Figure S1). For molecular verification, about 50 mg mycelia was ground with a mortar and pestle, and genomic DNA was extracted as previously described (Zelaya-Molina et al., 2011). Oomycete DNA was amplified using MyTaq polymerase (Bioline) with rDNA internal transcribed spacer (ITS) primers (ITS6: 5′-GAAGGTGAAGTCGTAACAAGG-3′ and ITS4: 5′-TCCTCCGCTTATTGATATGC-3′) (Cooke et al., 2000). PCR products were sequenced to confirm species identity (GenBank accession number OQ781193).

Mycelial suspension was prepared as previously described (Truong et al., 2012). Briefly, mycelial plugs of *P. capsici* isolate were cultured on PDA plates and incubated at 28°C for 7 days with constant light. The PDA plates with mycelial growth were sliced into 5-mm squares, transferred to fresh PDA plates and incubated for 8–9 days or until the plates were uniformly covered with mycelia. The mycelia were harvested and ground in a blender. A PDA plate (100 × 15 mm) fully covered with mycelia was required to prepare 50 mL of mycelial suspension.

To prescreen for *P. capsici* resistance, the leaves were placed in sterile Petri dishes. Mycelial suspension (50 μL) was added onto the lower surface of the leaves with a layer of wet cotton over it. The experiment was laid out in a randomized complete block design (RCBD) with three repeats, each involving three leaves. The inoculated leaves were incubated for 6 days at room temperature with regular wetting. The data were recorded at 2, 4 and 6 days following inoculation.

To validate the prescreening test, plants (two- to three-leaf stage) in 13 × 23 cm pots were inoculated with 50 mL of *P. capsici* mycelial suspension. The suspension was added onto the soil surface of each pot. The experiment was laid out in an RCBD and repeated three times, each involving 10 replicates per accession. The survival rate was determined at 15, 30 and 45 days following inoculation. Tap water was used for watering.

TABLE 1 Morphological characterization of the *Piper* germplasm in Vietnam.

	Morphological feature[a]															
Accession	PGH	BT	YOSTC	RSP	HC	ARP	POS	LBH	LLS	LBS	LM	TOV	SO	SS	TH	FS
HUIB_PN10	1	2	3	5	7	7	1	1	2	1	1	2	2	1	3	1
HUIB_PN20	1	2	2	7	7	7	1	1	3	2	2	2	2	1	3	1
HUIB_PN21	1	2	3	7	7	7	1	2	1	2	1	2	2	1	3	1
HUIB_PN27	1	2	3	7	7	7	1	1	4	2	1	2	2	1	3	1
HUIB_PN29	1	2	3	7	7	7	1	2	4	2	1	2	2	1	3	1
HUIB_PH30	1	2	4	7	7	7	1	3	5	3	1	1	1	2	2	99
HUIB_PN34	1	2	1	5	7	7	1	1	5	2	1	2	2	1	3	1
HUIB_PN35	1	2	5	7	7	7	1	1	4	3	2	2	2	1	3	1
HUIB_PD36	3	3	5	7	3	5	1	1	4	3	2	3	1	2	3	99
HUIB_PN38	1	2	5	7	7	7	1	2	1	2	1	2	2	1	3	1
HUIB_PR41	1	2	2	7	5	5	1	2	4	1	2	2	1	4	2	99
HUIB_PN42	1	2	3	7	7	7	1	1	4	2	1	2	2	1	3	1
HUIB_PN43	1	2	2	7	7	7	1	2	1	1	1	2	2	1	3	1
HUIB_PN45	1	2	1	7	7	7	1	2	2	3	1	2	2	1	3	1
HUIB_PH46	1	2	2	7	7	7	1	3	4	3	2	1	1	2	2	99
HUIB_PN47	1	2	2	7	5	7	1	2	1	1	1	2	2	1	3	1
HUIB_PR48	2	2	2	7	3	5	1	2	5	2	2	2	–	99	99	99
HUIB_PN50	1	2	1	7	7	7	1	2	3	3	1	2	2	1	3	1
HUIB_PN52	1	2	5	7	7	7	1	1	4	3	2	2	2	1	3	1
HUIB_PN54	1	2	3	7	7	7	1	1	4	2	1	2	2	1	3	1
HUIB_PN55	1	2	3	7	7	7	1	2	4	2	1	2	2	1	3	1
HUIB_PN56	1	2	3	7	5	7	1	2	4	2	1	2	2	1	3	1
HUIB_PN69	1	2	2	7	7	7	1	1	2	3	2	2	2	1	3	1
HUIB_PN70	1	2	2	7	7	7	1	1	5	2	2	2	2	1	3	1
HUIB_PN84	1	2	3	7	7	7	1	1	2	1	1	2	2	1	3	1
HUIB_PN87	1	2	2	7	7	7	1	1	2	1	1	2	2	1	3	1
HUIB_PN89	1	2	1	7	7	7	1	2	1	1	1	2	2	1	3	1
HUIB_PN91	1	2	3	7	7	7	1	3	3	2	1	2	2	1	3	1
HUIB_PN93	1	2	2	7	7	7	1	1	4	2	1	2	2	1	3	1
HUIB_PN95	1	2	3	7	7	7	1	1	2	1	1	2	2	1	3	1
HUIB_PN96	1	2	1	5	7	7	1	1	5	2	1	2	2	1	3	1
HUIB_PN97	1	2	1	5	7	7	1	1	5	2	1	2	2	1	3	1
HUIB_PN101	1	2	3	5	3	3	1	1	4	1	1	2	2	1	3	1
HUIB_PN102	1	2	2	5	7	7	1	1	2	1	2	2	2	1	3	1
HUIB_PN105	1	2	2	7	7	7	1	1	5	2	1	2	2	1	3	1
HUIB_PN113	1	2	1	5	7	7	1	1	5	2	1	2	2	1	3	1
HUIB_PN114	1	2	2	7	7	7	1	1	4	1	1	2	2	1	3	1
HUIB_PN115	1	2	2	7	7	7	1	1	4	3	1	2	2	1	3	1
HUIB_PN116	1	2	2	5	7	7	1	1	1	1	1	2	2	1	3	1

[a]PGH, plant growth habit: 1, climbing (on support); 2, trailing (on the ground); 3, erect. BT, branching type: 1, dimorphic; 2, polymorphic; 99, other. YOSTC, young (emerging) orthotropic shoot tip colour: 1, greenish yellow; 2, light purple; 3, dark purple; 4, light red. RSP, runner shoot production: 3, few; 5, medium; 7, many. HC, holding capacity: 3, weak; 5, medium; 7, strong. ARP, adventitious root production: 3, weak; 5, medium; 7, strong. POS, pubescence on stem: 1, absence; 2, presence. LBH, lateral branch habit: 1, erect; 2, horizontal; 3, hanging. LLS, leaf lamina shape: 1, ovate; 2, ovate-elliptic; 3, ovate-lanceolate; 4, elliptic-lanceolate; 5, cordate. LBS, leaf base shape: 1, round; 2, cordate; 3, acute; 4, oblique. LM, leaf margin: 1, even; 2, wavy. TOV, type of veining: 1, acrodromous; 2, campylodromous; 3, eucamptodromous. SO, spike orientation: 1, erect; 2, prostrate; –, data not collected. SS, spike shape: 1, filiform; 2, cylindrical; 3, globular; 4, conical; 99, other. TH, type of hermaphroditism: 1, staminate flowers only; 2, pistillate flowers only; 3, bisexual flowers only; 99, other. FS, fruit shape: 1, round; 2, ovate; 3, oblong; 99, other.

2.4 | Waterlogging survival

Twelve months following transplantation, five plants from each accession were subjected to a waterlogging treatment in which the entire pots were submerged under water for 4 days. The survival rate was determined at 15 days after treatment.

2.5 | Identification of *M. incognita*-resistant accessions

Infested roots and soil samples were collected from black pepper fields in Dak Krong commune, Dak Doa district, Gia Lai, Vietnam. Active nematodes were then isolated for 24 h using modified Baermann funnels as described by Hooper (1990) and microscopic morphology was examined (Figure S2). Genomic DNA was extracted from approximately 300 nematode individuals using FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen), following the manufacturer's protocol. PCRs were carried out to amplify ITS (forward primer ITS-F: 5′-TGTAGGTGAACCTGCTGCTGGATC-3′ and reverse primer ITS-R: 5′-CCTATTTAGTTTCTTTTCCTCCGC-3′) or oesophageal gland protein SEC 1 (forward primer SEC1-F: 5′-GGGCAAGTAAGGATGCTCTG-3′ and reverse primer SEC1-R: 5′-GCACCTCTTTCATAGCCACG-3′) gene region of genomic DNA for molecular identification (Saeki et al., 2003; Tesařová et al., 2003). Briefly, 25-μL reactions contained 12.5 μL of MyTaq 2× Master mix (Bioline), 20 ng genomic DNA, 0.5 μM of each primer and water. The thermocycling programme involved an initial denaturation (95°C for 4 min), 40 cycles of amplification (95°C for 15 s, 60°C for 15 s and 72°C for 1 min), and a final extension at 72°C for 5 min. PCR products were sequenced to confirm species identity (GenBank accession numbers OQ784598 and OQ784301).

The nematodes were propagated in tomato plants and *M. incognita* inocula were obtained from infested roots (Figure S3). In particular, roots from 9-week-old tomato plants were washed under tap water, cut into 1–2 cm segments and blended for 40 s in a 5% sodium hypochlorite solution. The mixture was clarified using a 200 μm sieve and nematode eggs were retained on a 50 μm sieve. Eggs were hatched by incubation at room temperature for 1 day. Single cuttings of black pepper were planted in 13 × 23 cm pots for 3 months prior to inoculation. The experiment was conducted in an RCBD and repeated three times, each involving 10 replicates per accession. Nematodes were added twice (each time with 1000 larvae/juveniles per pot, approximately 1 kg of soil) with a 15-day gap. At 1, 2 and 4 months after the second inoculation, the percentage of plants with yellow leaves and the root gall numbers were determined to assess the resistance towards *M. incognita*. Sterile water was used for watering.

2.6 | Micromorphological characterization of waterlogging-resistant accessions

Cross sections were prepared from the radial base of the roots and stems. The cross sections were soaked in sodium hypochlorite (5%) for 20 min before being washed with distilled water. Then, the cross sections were incubated in acetic acid (1%) for 2 min and washed with distilled water before being stained with methyl blue and carmine as described by Tran et al. (2022). Image analysis was performed in CorelDRAW. The experiment was repeated three times.

3 | RESULTS

3.1 | Morphological characterization of the *Piper* germplasm in Vietnam

Sixteen morphological traits in all *Piper* accessions were studied (Table 1). Plant growth habit is one of the most important traits in the selection of commercial cultivars of black pepper and is primarily determined by genetics. The most common growth habit was

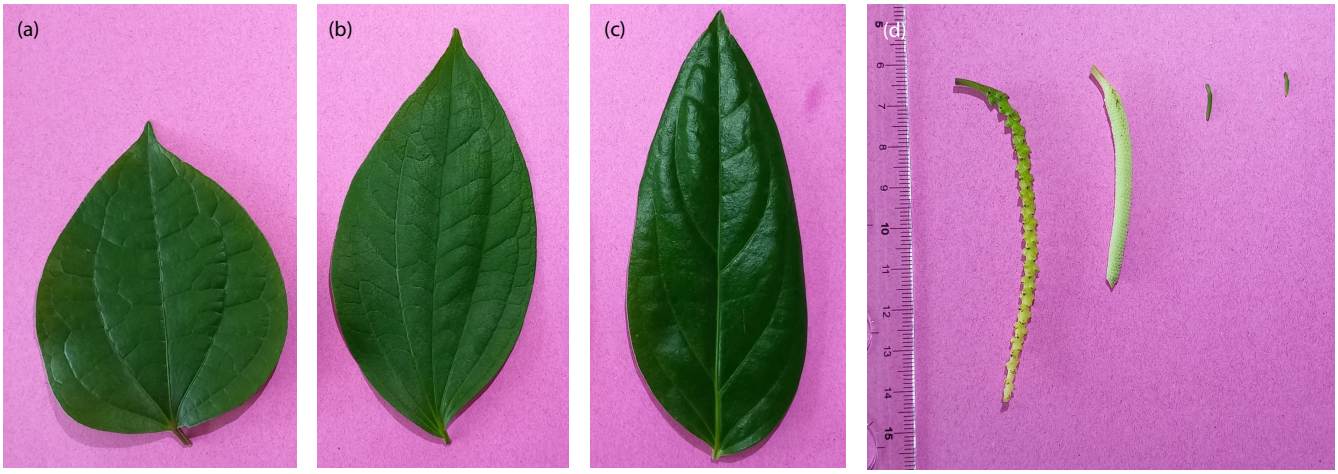

FIGURE 1 Variations in leaf and spike shapes and sizes. Leaf from accession (a) HUIB_PH30, (b) HUIB_PH46 and (c) HUIB_PD36. (d) From left to right, spike of accession HUIB_PN27, HUIB_PD36, HUIB_PH30 and HUIB_PH46.

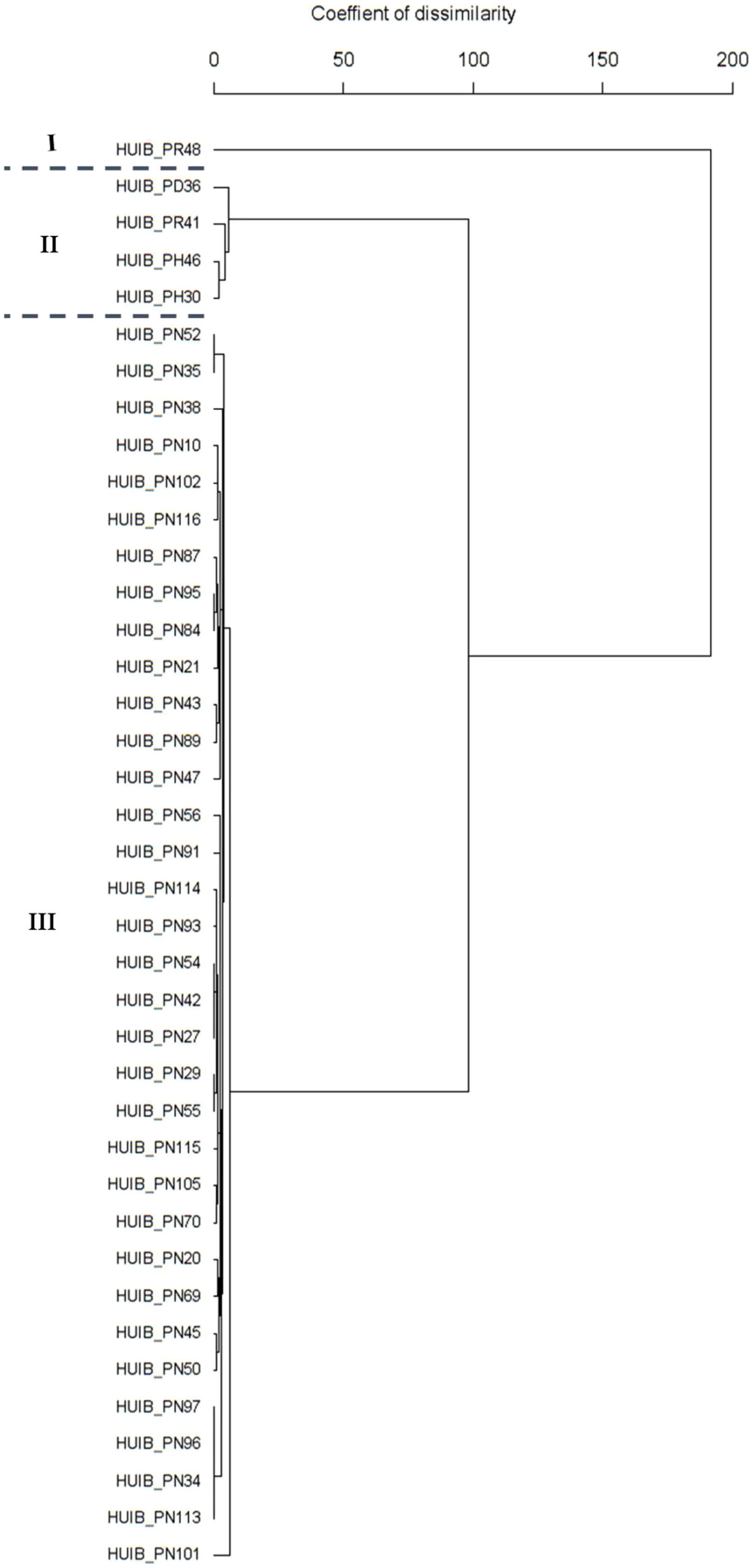

FIGURE 2 UPGMA cluster dendrogram showing the relationship of 39 *Piper* spp. accessions based on morphological data analysed with the Euclidean distance coefficient.

climbing (37 accessions) whereas trailing (HUIB_PR48) and erect (HUIB_PD36) were observed in only two accessions. All accessions had polymorphic branching type with the production of many runners except HUIB_PD36.

Most accessions produced multiple runner shoots, except HUIB_PN10, HUIB_PN34, HUIB_PN96, HUIB_PN97, HUIB_PN101, HUIB_PN102, HUIB_PN113 and HUIB_PN116. Three accessions (HUIB_PD36, HUIB_PR48 and HUIB_PN101) possessed weak holding capacity whereas medium holding capacity was observed in three accessions (HUIB_PR41, HUIB_PN47 and HUIB_PN56). The remaining accessions displayed strong holding capacity. Adventitious root production was minimal in accession HUIB_PN101 and moderate in HUIB_PD36, HUIB_PR41 and HUIB_PR48. The remaining accessions produced lots of adventitious roots. Pubescence on the stem was absent in all accessions. Furthermore, leaf lamina shapes varied from ovate, ovate-elliptic, elliptic-lanceolate and lastly cordate (Figure 1a–c). All accessions had campylodromous venation, except HUIB_PH30 and HUIB_PH46 (acrodromous venation).

The flower spike was prostrate in most accessions, except HUIB_PH30, HUIB_PH46, HUIB_PD36 and HUIB_PR41 (erect type, Figure 1d). The data were not collected in accession HUIB_PR48. Spike shape was filiform in most accessions, except HUIB_PR41 (conical), HUIB_PD36, HUIB_PH30 and HUIB_PH46 (cylindrical). Most accessions produced bisexual flowers. On the other hand, HUIB_PH30, HUIB_PR41 and HUIB_PH46 produced only pistillate flowers. The fruit shape was round in most accessions, except for HUIB_PH30, HUIB_PD36, HUIB_PR41, HUIB_PH46 and HUIB_PR48.

Based on Euclidean distances, which were calculated using the UPGMA, 39 *Piper* accessions were grouped into three groups (Figure 2). Group I included only one accession: HUIB_PR48. Group II included four accessions HUIB_PH30, HUIB_PD36, HUIB_PR41 and HUIB_PH46. The remaining 34 accessions of *P. nigrum* species were classified into Group III.

3.2 | *P. capsici* resistance and waterlogging survival of the black pepper germplasm

In this study, a prescreening test for *P. capsici* resistance was conducted. Leaves from 39 accessions were inoculated with *P. capsici* mycelial suspension in Petri dishes. Four to six days postinoculation, most of the leaves were seriously infected and rotted (Figure 3). Moderate infection was observed in accessions HUIB_PN35, HUIB_PN69, HUIB_PN102, HUIB_PN114 and HUIB_PN115 (Table 2). Only three accessions showed mild infection: HUIB_PH30, HUIB_PD36 and HUIB_PH46. Coincidentally, these were also the only three accessions that survived a 4-day waterlogging treatment (Table 2).

Because it could not be ruled out completely that other microbes might contribute to leaf rotting in the prescreening assay, further tests in which whole plants were inoculated with *P. capsici* were carried out. Fifteen days following inoculation, high levels of survival were observed in all the selected accessions (Figure 4). However, 30–45 days after inoculation, the number of surviving plants reduced dramatically in most accessions, except for HUIB_PH30, HUIB_PD36 and HUIB_PH46. The survival rates for HUIB_PH30, HUIB_PD36 and HUIB_PH46 plants were 86.7%, 90% and 76.7%, respectively, 45 days postinoculation. These results demonstrated a high level of resistance to *P. capsici* by these accessions. On the other hand, only 30.6% of the susceptible control, HUIB_PN27, survived the treatments after 45 days. Furthermore, all of the accessions that displayed moderate infection levels in the prescreening tests were susceptible to *P. capsici* at the plant level.

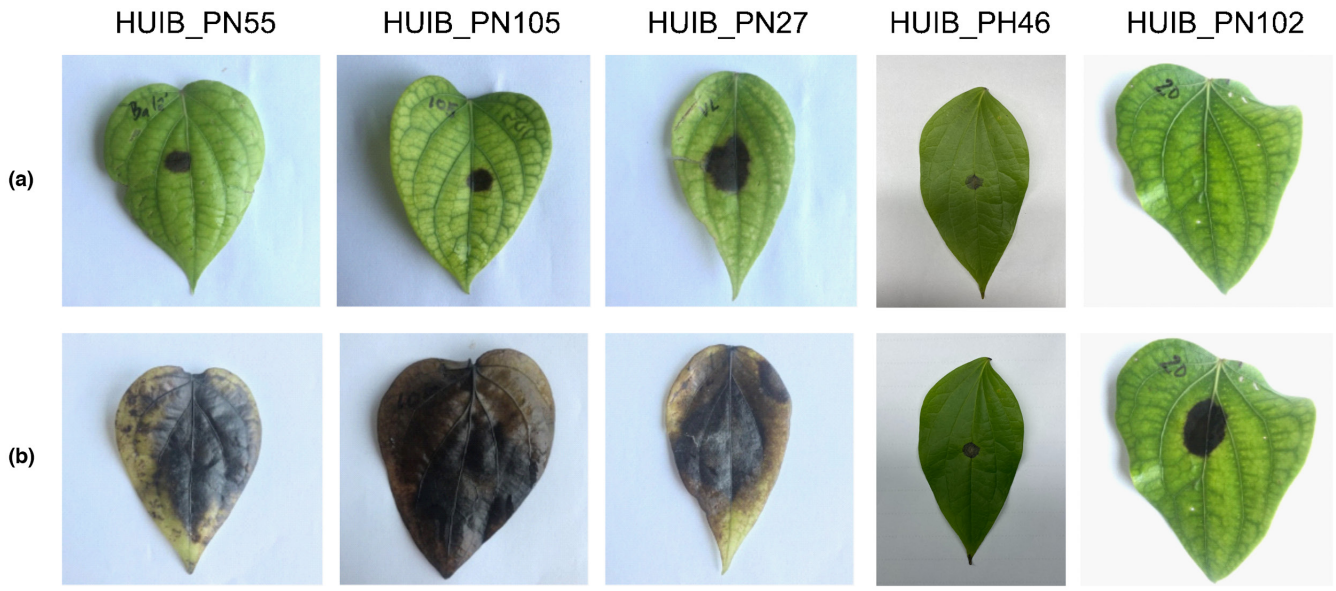

FIGURE 3 *Piper* leaves from various accessions following inoculation with *Phytophthora capsici* for 4 (row a) and 6 (row b) days.

TABLE 2 Prescreening for *Phytophthora capsici* resistance and waterlogging survival among *Piper* spp. collected in Vietnam.

Accession	Place of collection	Days after inoculation			Survival rate following waterlogging treatment for 4 days (%)
		2	4	6	
HUIB_PN10	Gia Lai	++	+++	Rotted	0
HUIB_PN20	Gia Lai	++	+++	Rotted	0
HUIB_PN21	Quang Nam	+	++	Rotted	0
HUIB_PN27	Quang Tri	++	+++	Rotted	0
HUIB_PN29	Quang Tri	++	+++	Rotted	0
HUIB_PH30	Quang Tri	–	+	+	100
HUIB_PN34	Dak Lak	–	+	+++	0
HUIB_PN35	Gia Lai	–	+	++	0
HUIB_PD36	Gia Lai	–	+	+	100
HUIB_PN38	Quang Tri	++	+++	Rotted	0
HUIB_PR41	Quang Tri	–	+	Rotted	0
HUIB_PN42	Gia Lai	++	+++	Rotted	0
HUIB_PN43	Gia Lai	–	+	Rotted	0
HUIB_PN45	Gia Lai	+	++	+++	0
HUIB_PH46	Quang Tri	–	+	+	100
HUIB_PN47	Quang Nam	+	++	Rotted	0
HUIB_PR48	Quang Tri	–	+	+++	0
HUIB_PN50	Gia Lai	+	++	+++	0
HUIB_PN52	Binh Phuoc	–	+	+++	0
HUIB_PN54	Dong Nai	+	++	Rotted	0
HUIB_PN55	Kien Giang	++	+++	Rotted	0
HUIB_PN56	Quang Ngai	++	+++	Rotted	0
HUIB_PN69	Gia Lai	–	+	++	0
HUIB_PN70	Gia Lai	–	+	+++	0
HUIB_PN84	Gia Lai	++	+++	Rotted	0
HUIB_PN87	Gia Lai	+	++	+++	0
HUIB_PN89	Gia Lai	+	++	+++	0
HUIB_PN91	Gia Lai	+	++	Rotted	0
HUIB_PN93	Gia Lai	++	+++	Rotted	0
HUIB_PN95	Gia Lai	+	++	Rotted	0
HUIB_PN96	Gia Lai	+	++	Rotted	0
HUIB_PN97	Gia Lai	+	++	+++	0
HUIB_PN101	Gia Lai	++	+++	+++	0
HUIB_PN102	Gia Lai	++	+++	++	0
HUIB_PN105	Gia Lai	+	++	Rotted	0
HUIB_PN113	Gia Lai	+	++	+++	0
HUIB_PN114	Gia Lai	–	+	++	0
HUIB_PN115	Gia Lai	–	+	++	0
HUIB_PN116	Gia Lai	++	+++	Rotted	0

Note: –, not infected; +, mild infection; ++, moderate infection; +++, severe infection.

3.3 | *M. incognita* resistance in selected *Piper* spp. accessions

To identify *Piper* accessions that are resistant to both *P. capsici* and *M. incognita*, 19 accessions showing moderate to strong resistance to *P. capsici* (Table 2) were assayed for *M. incognita* resistance. Yellow leaves and root galls are typical symptoms of *M. incognita* infestation. Consistent with this, we found a strong correlation between the percentage of plants with root galls and those showing yellow leaves (Figure S4). At 4 months postinoculation, yellow leaves were

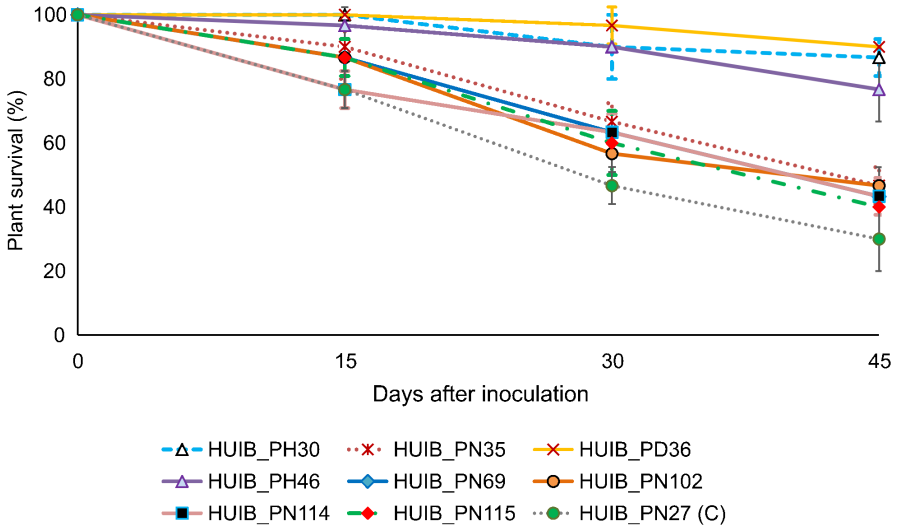

FIGURE 4 *Piper* plant survival following inoculation with *Phytophthora capsici*.

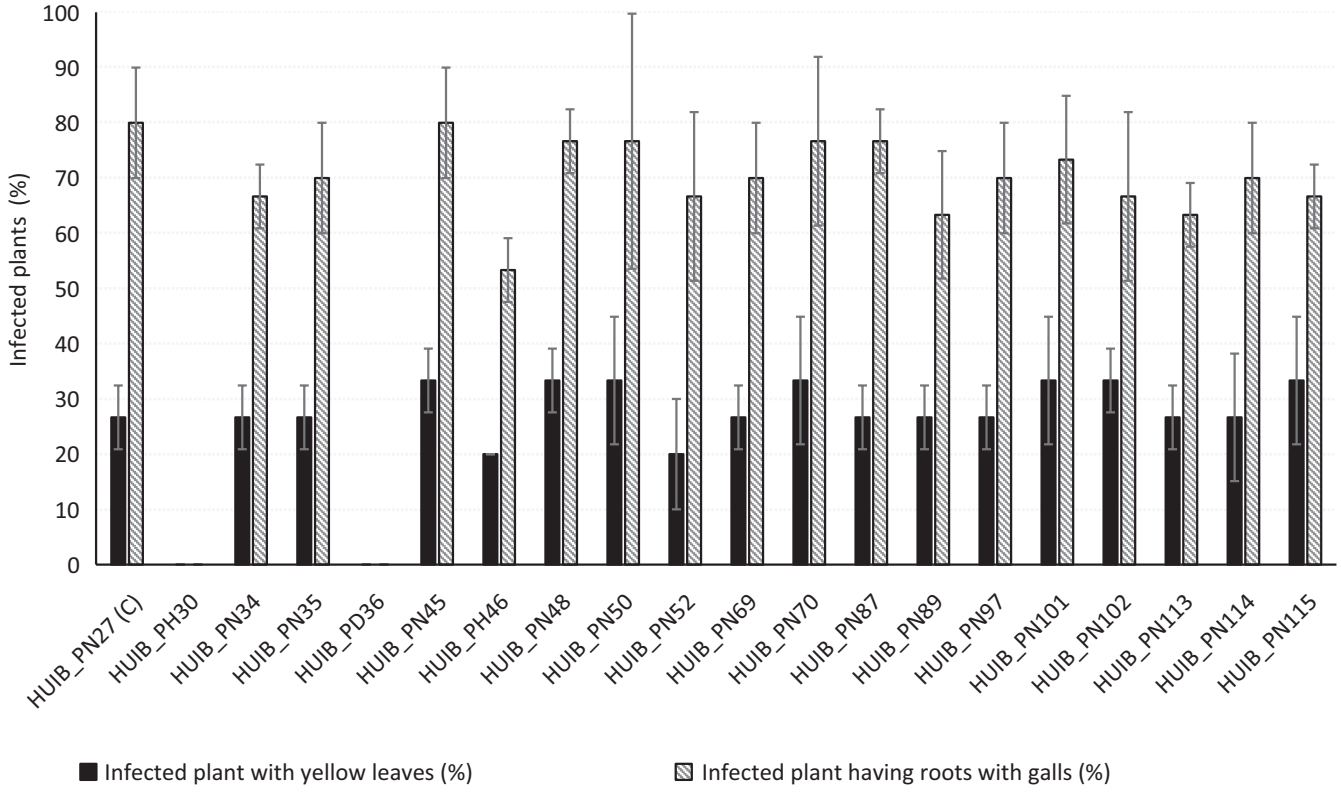

FIGURE 5 Comparison of percentage of plants with yellow leaf symptoms and percentage of plants having roots with galls after 4 months of inoculation with *Meloidogyne incognita*. The susceptible accession (HUIB_PN27) was included as the control treatment (C).

observed in most selected accessions (20% to 33% of plants in each accession), except HUIB_PH30 and HUIB_PD36 (0%, Figure 5). Similarly, when roots were investigated, galls were found in most accessions (up to 80%), except in HUIB_PH30 and HUIB_PD36 (Figure 5). These two accessions displayed no sign of *M. incognita* infestation. Another accession, HUIB_PH46, while being resistant to *P. capsici*, was moderately susceptible to *M. incognita* invasion (Figure 5).

3.4 | Micromorphological characterization of waterlogging tolerant accessions

To gain further insight into the mechanism of waterlogging tolerance by the three accessions (HUIB_PH30, HUIB_PD36 and HUIB_PH46), we investigated the micromorphological features of their stems and roots under the microscope (Figure 6). The susceptible accession (HUIB_PN27) was also examined for comparison. The stems of

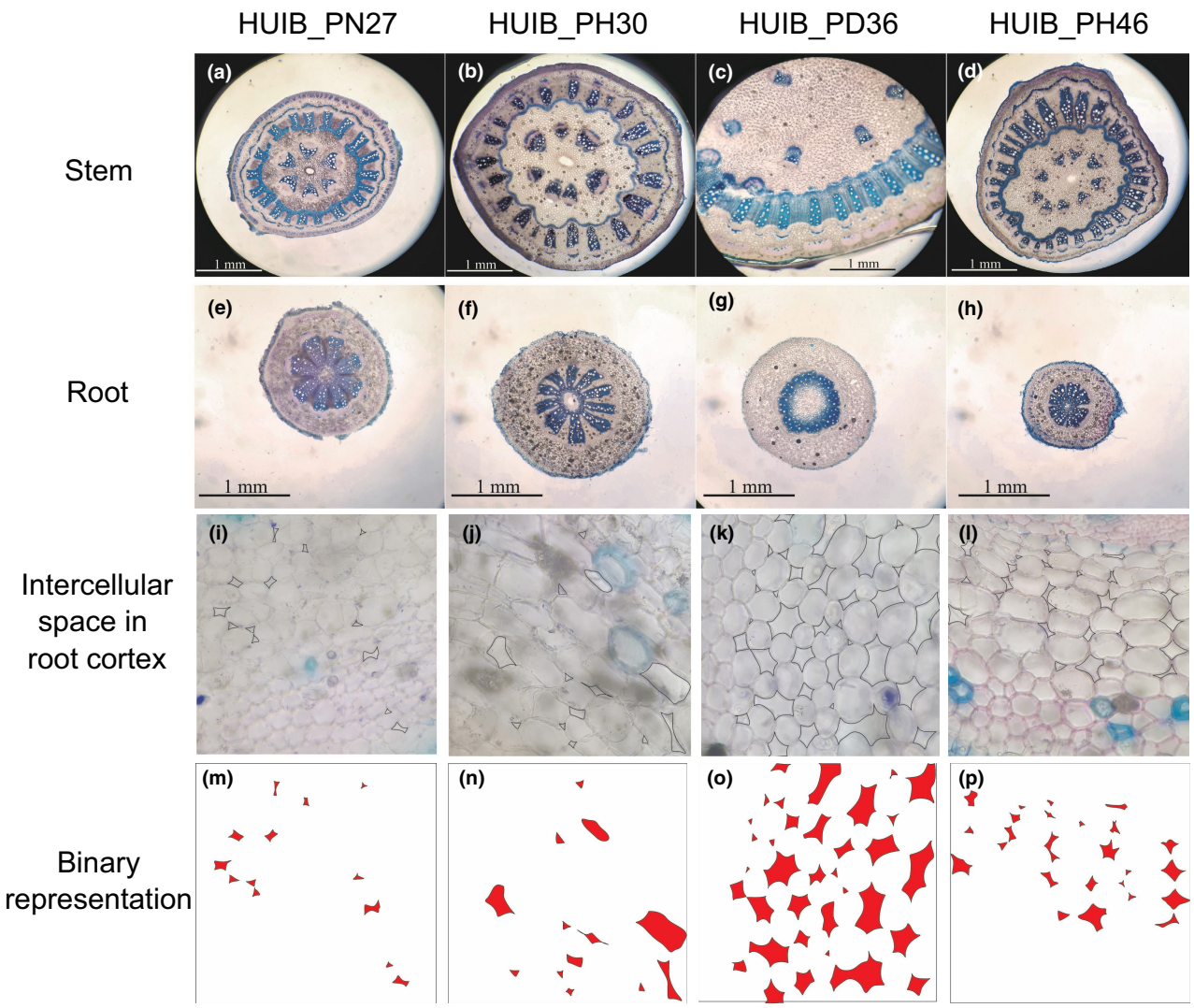

FIGURE 6 Micromorphological characterization of (a–d) stems and (e–h) roots obtained from the waterlogging-tolerant *Piper* accessions and control. (i–l) Root cortex. The overlay (drawn in CorelDRAW) indicates intercellular spaces in the root cortex. (m–p) Binary images are obtained from the corresponding root cortex images (i–l), in which intercellular spaces are coloured red and the white spaces represent root cells.

HUIB_PD36 stood out as their diameters were three to four times larger than those of the other three accessions. The roots of HUIB_PD36 were also different from the other three accessions in terms of the shape of the vascular bundles. Upon closer examination, the root cortex of HUIB_PN27 displayed little intercellular space (1.1 ± 0.1% of cortex cross-sectional area). On the other hand, all three waterlogging-tolerant accessions possessed significantly larger intercellular spaces (HUIB_PH30: 5 ± 2%, HUIB_PD36: 16 ± 6% and HUIB_PH46: 5 ± 2%). The intercellular space facilitates oxygen exchange between roots and shoots; hence, the larger intercellular spaces help to explain how these three accessions coped better in waterlogged conditions.

4 | DISCUSSION

In this work, a 39-accession germplasm panel of *Piper* species collected throughout Vietnam was characterized. Morphological and micromorphological features were examined and resistance to *P. capsici* and *M. incognita*, and tolerance to waterlogged conditions, were evaluated. Three accessions, HUIB_PH30 (*P. hancei*), HUIB_PD36 (*P. divaricatum*) and HUIB_PH46 (*P. hancei*), stood out as they displayed robust resistance to *P. capsici* and tolerance to waterlogged conditions. Among these, HUIB_PD36 was the most resistant to *P. capsici* (90% survival at 45 days postinfection), followed by HUIB_PH30 (87%) and HUIB_PH46 (77%). In contrast, only about 31% of the susceptible control (HUIB_PN27) survived the oomycete treatment. Additionally, HUIB_PD36 and HUIB_PH30 also displayed robust resistance against *M. incognita*. It is also worth noting that HUIB_PH30, HUIB_PD36 and HUIB_PH46, together with HUIB_PR41, belonged to a separate cluster (Group II) in terms of morphological traits. Apart from HUIB_PR48 (Group I), all other accessions belonged to Group III (*P. nigrum* species).

Screening a wide range of genetically diverse *Piper* spp. remains critical to discover novel resistant stocks for future breeding and

grafting works to create elite cultivars that are resistant to *P. capsici* and *M. incognita*. Our data suggest that future efforts to screen disease-resistant materials should prioritize *Piper* species other than *P. nigrum* and that morphological and micromorphological characterization can serve as useful measures in this process. Examples of such species include *P. kadsura* (Japanese pepper), *P. longum* (long pepper) and *P. laetispicum*, all of which are closely related to *P. hancei* (Zhang et al., 2021).

The mechanism of resistance towards *P. capsici* and *M. incognita* displayed by HUIB_PH30 and HUIB_PD36 remains unclear. The ability to tolerate waterlogged conditions is potentially an important factor, as root physical integrity presents a strong barrier that denies microbial entry and subsequent pathogenicity (Vandana et al., 2019). Another potential mechanism of resistance is the inhibition of microbial growth by secondary metabolites. It has been shown that essential oils in *P. divaricatum* containing methyleugenol and eugenol display robust antifungal activities (da Silva et al., 2014). Similarly, extracts from *P. hancei* possess compounds with insecticidal and cytotoxic activities (Wu et al., 2021; Yang et al., 2022). Future works will explore the ability of extracts from HUIB_PH30 and HUIB_PD36 to inhibit the growth of *P. capsici* and *M. incognita*. This has implications beyond black pepper cultivation because these pathogenic agents cause destruction to a wide range of economically important crops, and effective treatment is in high demand.

Another promising avenue to exploit the resistance of HUIB_PH30 and HUIB_PD36 towards *P. capsici* and *M. incognita* is to graft high-yielding cultivars onto the rootstocks of these accessions. Previously, *P. colubrinum*, a *Phytophthora*-resistant species originating from Brazil, had been used as rootstock for grafting *P. nigrum* to create *P. capsici*-resistant black pepper (Albuquerque, 1969) although grafts appeared to deteriorate after 4 years (Alconero et al., 1972). Furthermore, grafts suffered from lower productivity and low drought tolerance (Nguyen et al., 2019). More recently, *P. flaviflorum* has been tested as a rootstock to create oomycete-resistant black pepper grafts (Fan et al., 2022). Graft compatibility between resistant rootstocks and high-yielding scions will probably determine the success of these programmes. Along these lines, the application of hydroponic grafting techniques can be considered to increase graft success while reducing the time taken to create ready-to-plant grafts (Ajith & Rini, 2023).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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