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Piper Grafts Resistant to Both Pathogenic *Phytophthora capsici* and *Meloidogyne incognita*

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ABSTRACT

Background: Quick death and slow death are common diseases affecting black pepper cultivation. Quick death is caused by the pathogenic *Phytophthora capsici* whereas slow death is caused by the pathogenic nematode *Meloidogyne incognita*. *Piper hancei* and *Piper divaricatum* are two species in the *Piper* genus that are resistant to these pathogenic agents. In this work, grafts between these disease-resistant rootstocks and high-yielding scions were created and the disease resistance of these grafts was characterized.

Methods: Twelve graft combinations were created, using rootstocks of the disease-resistant HUIB_PD36 HUIB_PH30 and HUIB_PH46. The high-yielding *P. nigrum* cultivars (HUIB_PN27, HUIB_PN45, HUIB_PN69 and HUIB_PN97) were used as scions. The graft survival rates were evaluated and graft compatibility was examined using microscopy. Furthermore, pathogenic inoculation was employed to study graft susceptibility towards *Phytophthora capsici* and *Meloidogyne incognita*.

Result: High survival rates, with high degrees of graft compatibility were observed in grafts derived from HUIB_PN27 scions. Furthermore, grafts derived from HUIB_PH30 and HUIB_PD36 rootstocks demonstrated robust resistance towards both *Phytophthora capsici* and *Meloidogyne incognita*.

Key words: Black pepper, Graft, Quick death, Slow death.

INTRODUCTION

Black pepper (*Piper nigrum* L.) is an important spice used in kitchens worldwide. Disease occurrence lowers the yield and hampers black pepper production (Krishnamoorthy and Parthasarathy, 2010). Among common diseases, quick death, caused by the oomycete *Phytophthora capsici* and slow death, caused by the plant-parasitic nematode *Meloidogyne incognita* have been enduring threats, with few effective management strategies (Umadevi and Anandaraj, 2017; Saad *et al*., 2022; Thuy *et al*., 2012; Izzah and Wan Asrina, 2019; Ravindran *et al*., 2000; Suhail, 2023). The primary strategy to control these pathogenic agents relies on chemical pesticides, which is costly and poses substantial health risks to humans and the environment (Narayana *et al*., 2018; Rini and Remya, 2020; Verma *et al*., 2023). More recently, the control of parasitic nematodes and oomycetes using microorganisms and eco-friendly approaches has been investigated (Nysanth *et al*., 2022; Saad *et al*., 2022; Tran *et al*., 2019; Rani and Kayang, 2023).

A sustainable strategy to manage disease is to create disease-resistant cultivars. The long-pursuing approach is to screen for *Phytophthora*-resistant or *M. incognita*-resistant *Piper* species before the resistance genes could be introgressed into high yielding commercial varieties. Introgression can be achieved via breeding, somatic fusion, but primarily via grafting. Grafts between high-yielding scions and *Phytophthora*-resistant rootstocks have been attempted with *P. colubrinum* and *P. flaviflorum* (Albuquerque, 1969; Fan *et al*., 2022). However, a major concern is graft incompatibility between *P. colubrinum,* a woody shrub and *P. nigrum*, a vine species. This led to cracks at graft junctions, resulting in poor yield and poor survival in the field (Nguyen *et al*., 2019). Furthermore, grafts employing *M. incognita*-resistant rootstocks have not been reported.

Recently, we have collected a *Piper* germplasm containing 39 *Piper* accessions across Vietnam, three of which displayed robust resistance to *P. capsici*:

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HUIB_PH30, HUIB_PD36 and HUIB_PH46 (Truong *et al*., 2023). Among these three accessions, HUIB_PH30 and HUIB_PD36 (of *Piper hancei* and *Piper divaricatum* species respectively) displayed robust resistance towards *M. incognita* infestation. These resistant materials are valuable resources towards the development of *Piper* cultivars/grafts that are resistant to both *P. capsici* and *M. incognita*. This is especially important for a major black pepper exporter like Vietnam, where high-yielding and widely grown varieties: Vinh Linh (HUIB_PN27, VL) and Loc Ninh (HUIB_PN45, LN) are highly susceptible to both *P. capsici* and *M. incognita* (Truong *et al*., 2023). In this work, we created 12 graft combinations between disease-resistant rootstocks and common high-yielding cultivars. We identified graft combinations with high survival rates and robust resistance to *P. capsici* and *M. incognita*. This work forms the basis, from which further examination of graft growth, disease resistance and yield on the field can be carried out.

MATERIALS AND METHODS

Plant materials

The experiments were conducted at the Institute of Biotechnology, Hue University in Thua Thien Hue province and the Center for Black pepper Research and Development, Gia Lai province, Vietnam from April to November, 2022. Rootstocks from *P. hancei* (HUIB_PH30 and HUIB_PH46) and *P. divaricatum* (HUIB_PD36) and scions from *P. nigrum*: HUIB_PN27 (VL), HUIB_PN45 (LN), HUIB_PN69 (SR) and HUIB_PN97 (AD) were used to create grafts (Truong *et al*., 2023). Another local *P. nigrum* variety, HUIB_PN105, was used as a disease-sensitive control (Truong *et al*., 2023). Three-internodal cuttings of black pepper were dipped in rooting hormones to promote root formation. Cuttings were planted in 13 × 23 cm pots containing sterilized soil and compost (3:1 ratio). Three-month-old cuttings were used to create grafts.

Grafting

Cleft graft was performed as previously described (Quyen *et al*., 2020). Briefly, the stem of the rootstock was cut and split of 2 cm was made along the stem. The single-node or two-node scions were sliced to create a 2 cm wedge (V-shaped; Fig 1A). The wedge was then inserted onto the rootstock (Fig 1B) and held in place with wrapping materials (Fig 1C).

Inoculation *piper* plants and grafts with *P. capsici* and *M. incognita*

The experiments were conducted at in March-2020. The pathogenic *P. capsici* and *M. incognita* were isolated from black pepper fields and propagated as described previously (Tsao and Ocana, 1969; Hooper, 1990; Tran *et al*., 2023; Truong *et al*., 2023). The *P. capsici* strain was cultured on potato dextrose agar (PDA) plates, incubated at 27-28°C with constant light for seven days. The formation of *P. capsici* spores was observed under the microscope. Sterile distilled water (20 mL) was added on the surface and the plates were placed at room temperature with light for two days to stimulate sporangia formation. The PDA plates were incubated at 4°C for three hours before the spore inocula were harvested. To each pot, 50 mL of *P. capsici* spore suspension (4.5×10^4 CFU/mL) were added. Plant height, the number of leaves and survival rates were measured at 15, 30 and 60 days after inoculation. The experiment was repeated three times, with 10 replicates each repeat. At 60 days after inoculation, the percentage of root rot (%) was determined, as the number of soft, brown roots divided by the total number of roots examined.

The plant-parasitic nematode *M. incognita* was propagated in tomato plants and infested roots showing galls were harvested as described (Truong *et al*., 2023). The roots were washed in tap water and the first moult, eggs and juveniles were collected by filtering. For each pot, 2000 nematode larvae/juveniles were added. Plant height, the number of leaves and the percentage of plants

(A) Scion wedge. (B) Scion insertion onto the rootstock. (C) Stabilization of the graft with nylon.

Fig 1: Grafting of black pepper scion onto disease-resistant rootstocks.

showing yellow leaves were recorded at 30, 60, 90 and 120 days after inoculation. At 120 days post inoculation, the number of larvae/juveniles per 100 g of soil, the number of larvae/juveniles per 5 g of root and infested root with galls were determined. The experiment was repeated three times, with 10 replicates each repeat.

Micro-morphological characterization of grafts

Specimens were thinly sliced for microscopic examination. Four-month-old grafts were cut at right angles to the stem axis at scions, rootstocks and graft junctions. Slices were stained with Carmine Alum and methylene blue as previously described (Truong *et al*., 2023).

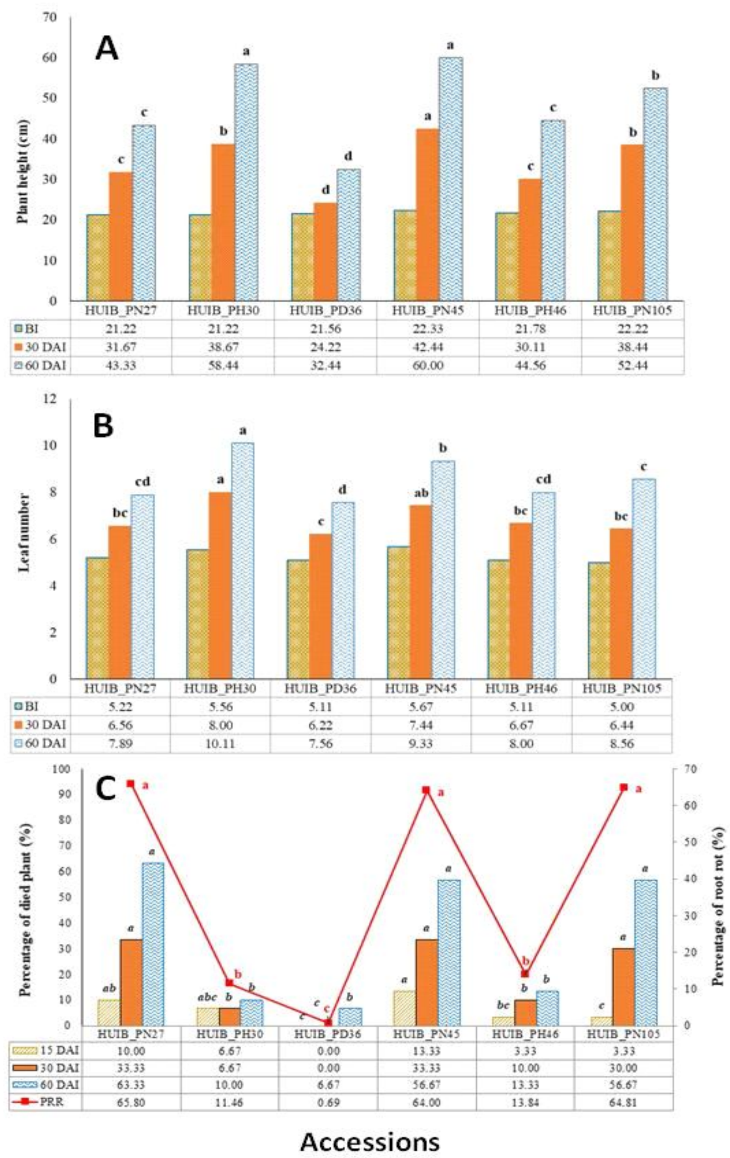
Data analysis

The data were analyzed using the Duncan test for significant character state differences (*P*<0.05) in SPSS 7.1.

RESULTS AND DISCUSSION

Resistance of *Piper spp.* towards *P. capsici* and *M. incognita*

Prior to grafting, the resistance of *Piper* spp. towards *P. capsici* and *M. incognita* was tested. In normal growth conditions, plant height and the number of leaves were comparable among *Piper* spp. (Fig 2A-B). However, at 30 and 60 days after *P. capsici* inoculation (dai), plant height



(A) Plant height, (B) the number of leaves and (C) percentage of died plants (bar charts) and root rot (line) after inoculation with *P. capsici*. BI: before inoculation, DAI: days after inoculation; PRR: percentage of root rot.

Fig 2: Resistance of *Piper* accessions towards *P. capsici*.

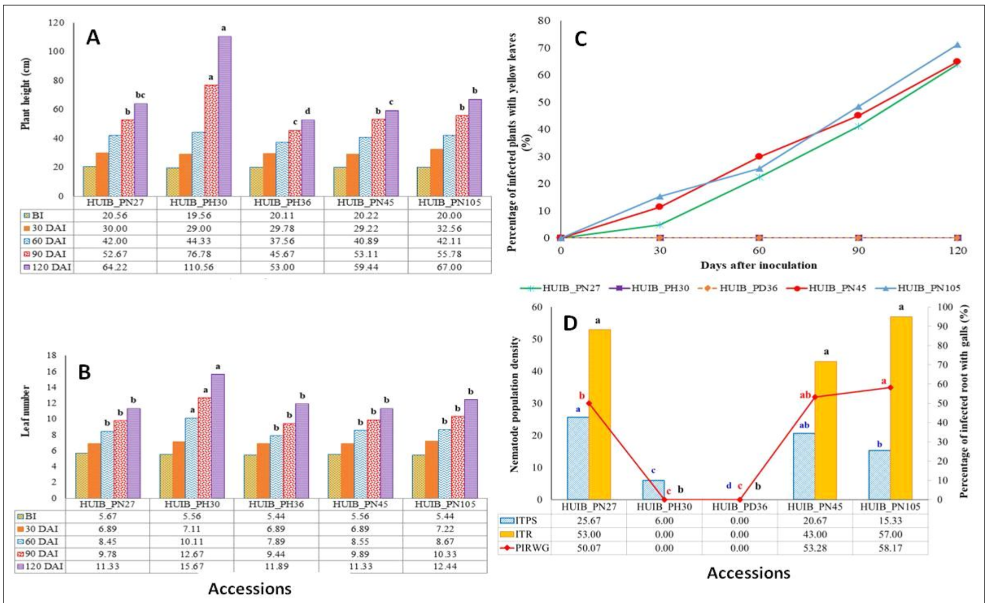
and leaf number varied significantly. Plant height ranged from 24.2 cm (HUIB_PD36) to 42.4 cm (HUIB_PN45) at 30 dai and 32.4 cm (HUIB_PD36) to 60.0 cm (HUIB_PN45) at 60 dai. On the other hand, leaf number varied between 6.2 (HUIB_PD36) and 8.0 (HUIB_PH30) at 30 dai, 7.6 (HUIB_PD36) and 10.1 (HUIB_PH30) at 60 dai. While survival rates remained high at 15 dai, the survival rates of HUIB_PN27, HUIB_PN45 and HUIB_PN105 reduced significantly at 30 dai (67-70%) and 60 dai (37-43%) (Fig 2C). In contrast, HUIB_PD36, HUIB_PH30 and HUIB_PH46 were highly resistant to *P. capsici*. Their survival rates were 93, 90 and 87% respectively at 60 dai. When roots were examined at 60 dai, susceptible lines (HUIB_PN27, HUIB_PN45 and HUIB_PN105) showed high levels of root rot while the majority of roots in HUIB_PD36, HUIB_PH30 and HUIB_PH46 were healthy (Fig 2C).

On the other hand, prior to inoculation and up to 60 days post inoculation with *M. incognita*, plant heights were comparable between *Piper* spp. (Fig 3A). Only 90 and 120 days after inoculation, significant differences in plant heights were observed, with HUIB_PH30 being the tallest (111 cm) and HUIB_PD36 being the shortest (53 cm) at 120 dai (Fig 3A). Similarly, leaf numbers differed significantly at 60, 90 and 120 dai (Fig 3B). HUIB_PH30 had the highest leaf number (10, 13 and 16 leaves at 60, 90 and 120 dai respectively), whereas HUIB_PD36 had the fewest leaves at 60 and 90 dai (8 and 9 leaves, respectively). HUIB_PN45 and HUIB_PN27 had the fewest leaves at 120 dai (11 leaves). Signs of *M. incognita* infestation include yellow leaves, root galls and high nematode density in soil (Thuy *et al*., 2012). Up to 120 days after inoculation, yellow leaves and root galls were absent in both HUIB_PH30 and HUIB_PD36 (Fig 3C-D). These results demonstrated robust resistance towards *M. incognita* by these two accessions. In contrast, at 90 and 120 dai, large percentages of plants in other accessions showed yellow leaves (more than 40%) and roots with galls (more than 50%) (Fig 3C-D). In addition, *M. incognita* was absent in potted soil with HUIB_PD36 while they were found in the potted soil with HUIB_PH30, HUIB_PN105, HUIB_PN45 and HUIB_PN27 (6, 15, 21 and 26 larvae/juveniles per 100 g soil respectively). Consistent with these results, *M. incognita* was absent in roots from HUIB_PH30 and HUIB_PD36, but was observed in roots of other accessions (43 to 57 larvae/juveniles per 5 g of root) (Fig 3D).

Graft survival and compatibility

Twelve graft combinations showed high survival rates (more than 93%) at 30 days post grafting (Table 1). However, the survival rates dropped at a function of time. At 120 dpg, the highest survival rates were observed in the combinations of VL scion grafted onto the rootstocks of HUIB_PH30 (97%), HUIB_PH46 (93%) and HUIB_PD36 (93%), whereas the lowest survival rates were found in the combinations of LN scion grafted onto the rootstocks HUIB_PH30 (67%), HUIB_PH46 (67%) and HUIB_PD36 (70%).

At four months since grafting, the micro-morphology at graft junctions were evaluated (Fig 4). Most of the combinations showed good conjugations, except HUIB_PD36 - SR (Fig 4.2C) and HUIB_PH46-SR (Fig 4.2K). High degrees of graft compatibility were observed in HUIB_PD36 - VL (Fig 4.2A), HUIB_PD36 - LN



(A) Plant height and (B) number of leaves, (C) the percentage of plants showing yellow leaves and (D) roots with galls and nematode density in potted soil. ITPS: *M. incognita* larvae/juveniles per 100 g soil, ITR: *M. incognita* larvae/juveniles per 5 g of root, PRWG: Percentage of roots with galls.

Fig 3: Resistance of *Piper* accessions towards *M. incognita*.

(Fig 4.2B), HUIB_PD36 - AD (Fig 4.2D), HUIB_PH30 - VL (Fig 4.2E), HUIB_PH30 - SR (Fig 4.2G), HUIB_PH30 - AD (Fig 4.2H), HUIB_PH46 - VL (Fig 4.2I) and HUIB_PH46 - AD (Fig 4.2M).

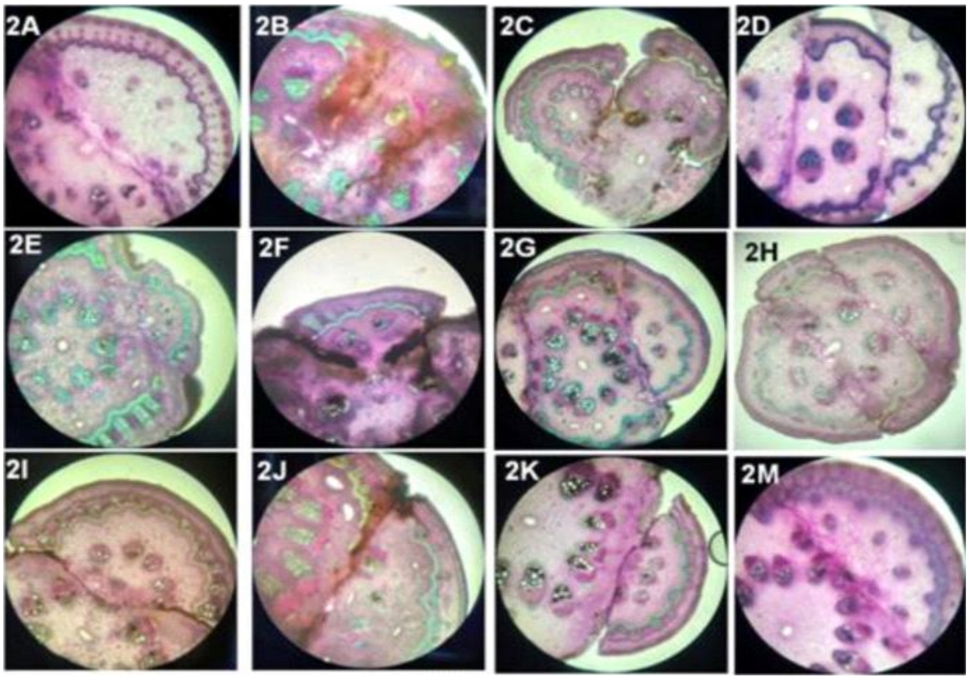
Resistance of *piper* grafts towards *P. capsici*

At two months post grafting, 12 grafting combinations were inoculated with *P. capsici* and shoot height was monitored after inoculation. While shoot heights were similar among grafts prior to inoculation, variations in shoot heights were observed at 30 and 60 days after inoculation. Shoot heights ranged from 14.7 cm (HUIB_PH30 - LN) to 20.2 cm (HUIB_PH30 - VL). Similarly, shoot heights varied from 22.4 cm (HUIB_PH46 - LN) to 26.4 cm (HUIB_PH30 - VL) at 60 dai (Fig 5A). On the other hand, the number of leaves remained relatively constant among grafts (Fig 5B). Overall, HUIB_PH30 - VL grafts displayed the fastest growth following inoculation with *P. capsici*. All grafts showed high survival rates at 15 and 30 days after inoculation (Fig 5C). However, survival rates dropped at 60 dai: 70% for HUIB_PH46 - AD, 73.3% for HUIB_PH46 - VL, HUIB_PH46 - LN and 76.7% for HUIB_PH46 - SR. The highest survival rates were observed in HUIB_PD36 - SR (96.7%), HUIB_PD36 - VL, HUIB_PD36 - LN and HUIB_PD36 - AD (93.3%), indicating high levels of resistance against *P. capsici* in grafts prepared from HUIB_PD36 rootstocks. These data were corroborated with the low percentage of root rot observed in these grafts (0-2%, Fig 5C). On the other hand, grafts from HUIB_PH30 and HUIB_PH46 appeared to be less resistant to *P. capsici* infection, with the percentage of root rot ranging from 8 to 21% (Fig 5C).

Resistance of *piper* grafts towards *M. incognita*

Based on the robust resistance of HUIB_PD36 and HUIB_PH30 against *M. incognita* (Fig 3), eight graft combinations were inoculated with *M. incognita* and shoot heights were monitored up to 120 days after inoculation (Fig 6A). In all grafts, shoot heights increased as a function of time following *M. incognita* inoculation. The longest shoots were observed with VL scions: HUIB_PH30 - VL (from 21 to 55 cm at 30 to 120 dai) and HUIB_PD36 (from 17 to 47 cm at 30 to 120 dai). On the other hand, the least growth was observed in HUIB_PH30 - LN (from 13 to 30 cm at 30 to 120 dai) (Fig 6A). The number of leaves followed the same trend as shoot heights (Fig 6A,B). At 120 dai, number of leaves varied significantly among grafts. HUIB_PH30 - VL on average yielded 9 leaves whereas HUIB_PH30 - SR had only 6 leaves at 120 dai (Fig 6B). More importantly, yellow leaves and roots with galls, common symptoms of *M. incognita* infestation, were not observed among these grafts. Consistent with these data, nematodes were not found in potted soil at 120 dai. These results suggested *Piper* grafts derived from HUIB_PD36 and HUIB_PH30 rootstocks were highly resistant to *M. incognita* infestation.

In this work, grafts between disease-resistant *Piper* rootstocks and high-yielding *Piper nigrum* cultivars were created using cleft grafting method. High graft survival rates

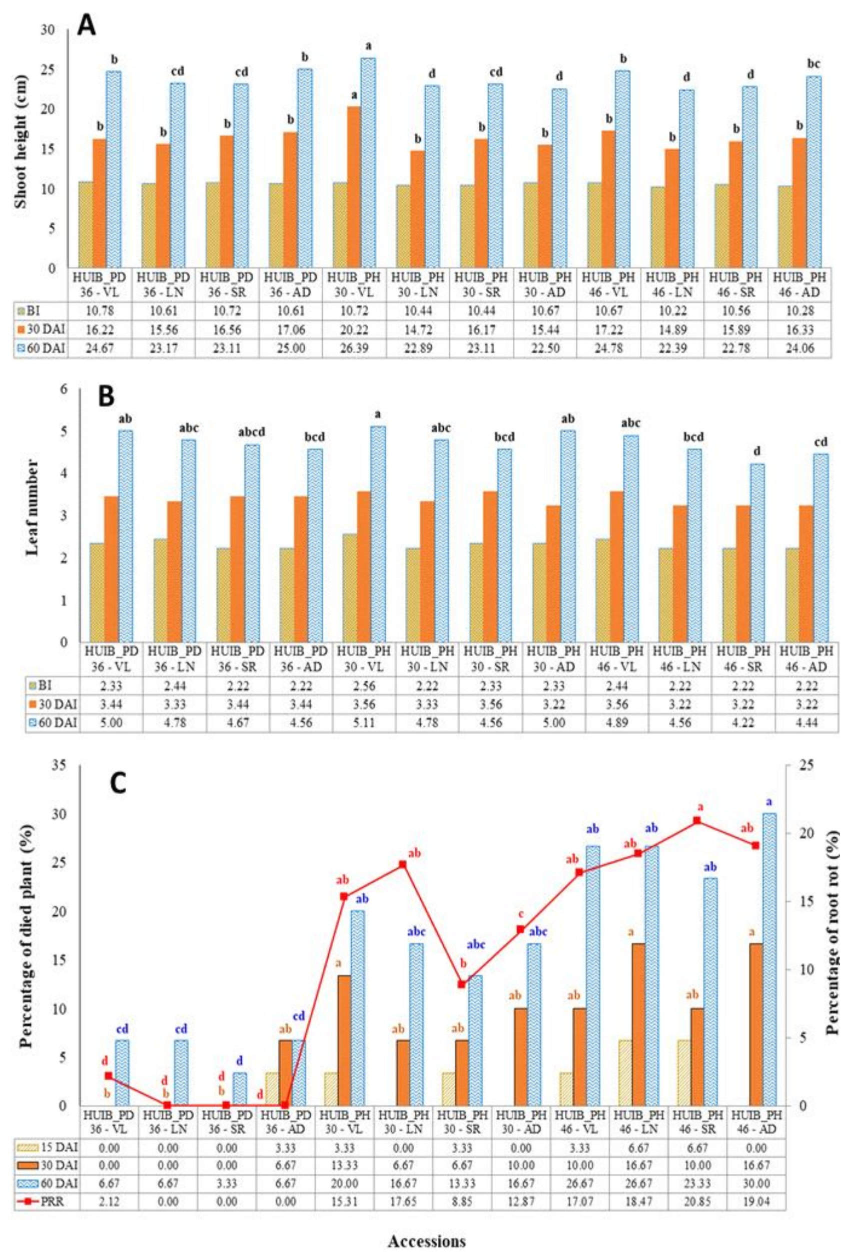


(2A) HUIB_PD36 - VL, (2B) HUIB_PD36 - LN, (2C) HUIB_PD36 - SR, (2D) HUIB_PD36 - AD, (2E) HUIB_PH30 - VL, (2F) HUIB_PH30 - LN, (2G) HUIB_PH30 - SR, (2H) HUIB_PH30 - AD, (2I) HUIB_PH46 - VL, (2J) HUIB_PH46 - LN, (2K) HUIB_PH46 - SR and (2M) HUIB_PH46 - AD.

Fig 4: Micro-morphological observation at graft junctions.

were observed among twelve graft combinations (Table 1). Furthermore, graft resistance towards *P. capsici* and *M. incognita* were evaluated, with grafts derived from HUIB_PD36 and HUIB_PH30 displaying high levels of resistance to both pathogenic agents. These results are consistent with the robust resistance of these accessions towards *P. capsici* and *M. incognita* observed in this and previous studies (Fig 2, 3; Truong *et al*., 2023).

A long-investigated strategy to create disease-resistant black pepper cultivars is to identify disease-resistant *Piper* species and to prepare grafts between disease-resistant rootstocks and high-yielding *Piper* scions. This approach has been explored for *Phytophthora*-resistant *P. colubrinum* and *P. flaviflorum* with limited success (Albuquerque, 1969; Fan *et al*., 2022). Graft deterioration is a major concern. Grafts prepared with *P. colubrinum* rootstocks were found

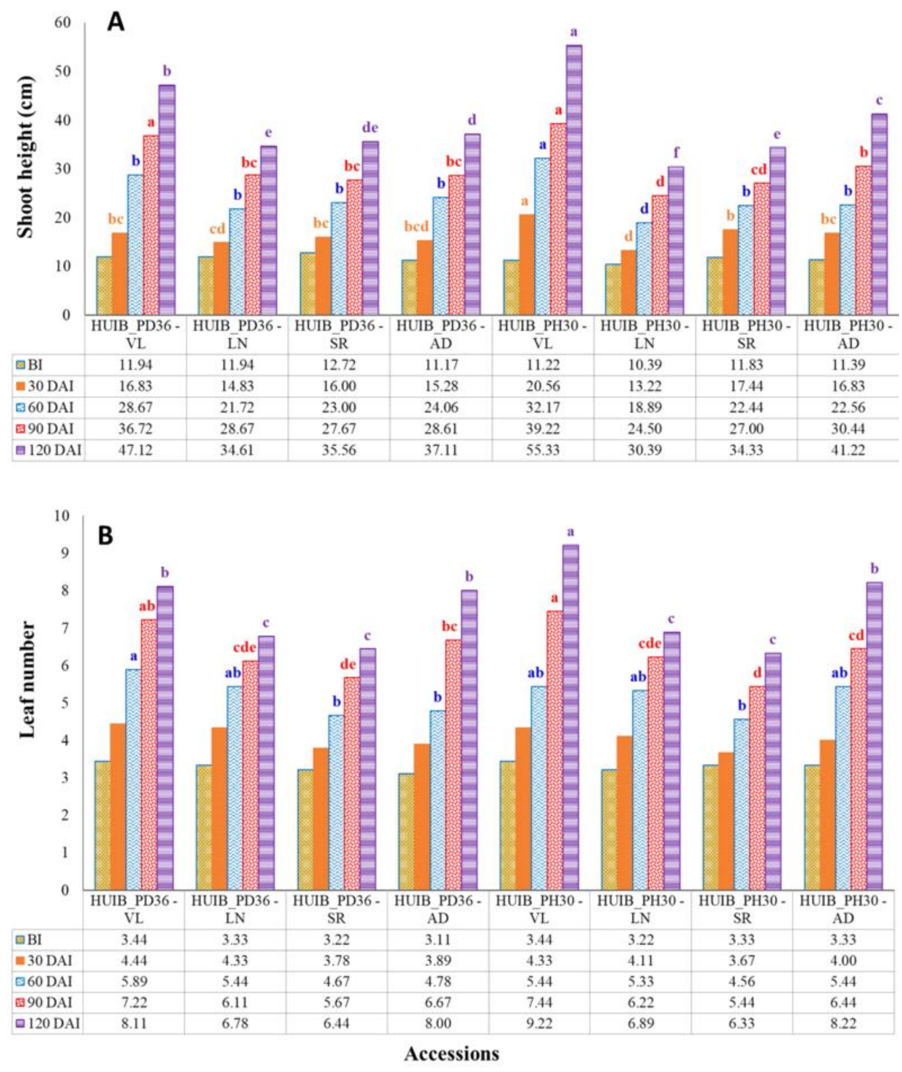


(A) Shoot height, (B) number of leaves, (C) percentages of died plants (bar charts) and root rot (red line) among grafts following inoculation with *P. capsici*. BI: Before inoculation, DAI: Days after inoculation; PRR: Percentage of root rot.

Fig 5: *Piper* graft resistance towards *P. capsici*.

to deteriorate after four years (Alconero *et al*., 1972). Furthermore, low productivity and low drought tolerance were associated with these grafts (Nguyen *et al*., 2019). Incompatibility between rootstocks and scions appeared to be a key issue. In this work, graft junctions were observed at microscopic levels, showing incompatibility in grafts between HUIB_PD36 - SR and HUIB_PH46 - SR (Fig 4). On the other hand, all grafts using VL scions appeared to have well-established junctions, suggesting high degrees of graft compatibility and long-term survival of grafts in the field. This is further supported by the highest survival rates of VL-derived grafts (Table 1).

The high levels of *P. capsici* resistance displayed by HUIB_PD36, HUIB_PH30 and HUIB_PH46 plants (Fig 2C) were consistent with survival rates observed in these accessions following *P. capsici* inoculation (Truong *et al*., 2023). The most robust resistance was observed in HUIB_PD36, followed by HUIB_PH30 and HUIB_PH46 (root rot found at less than 1%, 12% and 14% respectively). Not surprisingly, these observations were translated to *P. capsici* resistance by *Piper* grafts using these rootstocks (Fig 5C). Since HUIB_PH46 had been found to be moderately sensitive to *M. incognita* (Truong *et al*., 2023), resistance towards the plant-parasitic nematode was performed on HUIB_PH30 and HUIB_PD36, along with sensitive controls: HUIB_PN27, HUIB_PN45 and HUIB_PN105 (Fig 3). Consistent with previous work (Truong *et al*., 2023), high levels of resistance towards *M. incognita*, demonstrated by the absence of plants with yellow leaves and roots with galls, were observed in HUIB_PH30 and HUIB_PD36 but not in others (Fig 3C-D). The robust resistance was conferred to grafts prepared with HUIB_PH30 and HUIB_PD36 rootstocks (Fig 6). In general, when four parameters were evaluated: (1) graft compatibility and survival rate, (2) robust resistance to *P. capsici*, (3) robust resistance to *M. incognita* and (4) fast



	HUIB_PD36 - VL	HUIB_PD36 - LN	HUIB_PD36 - SR	HUIB_PD36 - AD	HUIB_PH30 - VL	HUIB_PH30 - LN	HUIB_PH30 - SR	HUIB_PH30 - AD
BI	11.94	11.94	12.72	11.17	11.22	10.39	11.83	11.39
30 DAI	16.83	14.83	16.00	15.28	20.56	13.22	17.44	16.83
60 DAI	28.67	21.72	23.00	24.06	32.17	18.89	22.44	22.56
90 DAI	36.72	28.67	27.67	28.61	39.22	24.50	27.00	30.44
120 DAI	47.12	34.61	35.56	37.11	55.33	30.39	34.33	41.22

	HUIB_PD36 - VL	HUIB_PD36 - LN	HUIB_PD36 - SR	HUIB_PD36 - AD	HUIB_PH30 - VL	HUIB_PH30 - LN	HUIB_PH30 - SR	HUIB_PH30 - AD
BI	3.44	3.33	3.22	3.11	3.44	3.22	3.33	3.33
30 DAI	4.44	4.33	3.78	3.89	4.33	4.11	3.67	4.00
60 DAI	5.89	5.44	4.67	4.78	5.44	5.33	4.56	5.44
90 DAI	7.22	6.11	5.67	6.67	7.44	6.22	5.44	6.44
120 DAI	8.11	6.78	6.44	8.00	9.22	6.89	6.33	8.22

(A) Shoot height and (B) Number of leaves observed in *Piper* grafts following *M. incognita* inoculation.

Fig 6: Resistance of *Piper* grafts towards *M. incognita*.

Table 1: Graft survival rates at 30, 60, 90 and 120 days post graft (dpg).

	Rootstocks	Scions			
		HUIB_PN27 (VL)	HUIB_PN45 (LN)	HUIB_PN69 (SR)	HUIB_PN97 (AD)
30 dpg	HUIB_PD36	100.00	96.67	96.67	96.67
	HUIB_PH30	100.00	96.67	96.67	96.67
	HUIB_PH46	96.67	93.33	96.67	96.67
60 dpg	HUIB_PD36	93.33[a]	70.00[b]	80.00[ab]	83.33[ab]
	HUIB_PH30	96.67[a]	66.67[b]	80.00[ab]	83.33[ab]
	HUIB_PH46	93.33[a]	66.67[b]	80.00[ab]	83.33[ab]
90 dpg	HUIB_PD36	93.33[a]	70.00[b]	80.00[ab]	83.33[ab]
	HUIB_PH30	96.67[a]	66.67[b]	80.00[ab]	83.33[ab]
	HUIB_PH46	93.33[a]	66.67[b]	80.00[ab]	83.33[ab]
120 dpg	HUIB_PD36	93.33[a]	70.00[b]	80.00[ab]	83.33[ab]
	HUIB_PH30	96.67[a]	66.67[b]	80.00[ab]	83.33[ab]
	HUIB_PH46	93.33[a]	66.67[b]	80.00[ab]	83.33[ab]

growth, HUIB_PH30 - VL and HUIB_PH36 - VL, followed by other HUIB_PH30-derived grafts, stood out as promising candidates, warranting further examination on the field. In particular, their growth rate, yield and disease resistance profiles over the course of three to five years need to be determined, before these grafts are produced and adopted by farmers.

Grafting of susceptible scions on *Phytophthora*-resistant rootstock is a common strategy to create oomycete-resistant crops, including walnut (Alvarado *et al*., 2020), pepper (Shi *et al*., 2024), potato (Li and Zhao, 2021) and eggplants (Foster *et al*., 2013). Similarly, grafting scions onto nematode-resistant rootstocks is useful to manage *M. incognita* infestation in tomato, melon, pepper and watermelon (Fullana *et al*., 2023). To our knowledge, this is the first study exploring the resistance towards *M. incognita* by grafts derived from *P. hancei* and *P. divaricatum* rootstocks and high-yielding *P. nigrum* scions.

CONCLUSION

Pathogenic *P. capsici* and *M. incognita* have been causing tremendous harm to black pepper cultivation. In this work, we created 12 graft combinations between disease-resistant rootstocks and common high-yielding cultivars. High survival rates, with high degrees of graft compatibility were observed in grafts derived from HUIB_PN27 (*Piper nigrum*) scions. Furthermore, grafts derived from HUIB_PH30 (*Piper hancei*) and HUIB_PD36 (*Piper divaricatum*) rootstocks demonstrated robust resistance towards both *P. capsici* and *M. incognita*. This work forms the basis, from which further examination of graft growth, disease resistance and yield on the field can be carried out.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

Albuquerque, F. (1969). *Piper colubrinum* Link; a grafting root-stock for *Piper nigrum* L. resistant to diseases caused by *Phytophthora palmivora* (Bult) and *Fusarium solanii* f. piperi. Perqui Agropecuario. 3: 141-145.

Alconero, R., Albuquerque, F., Almeyda, N. and Santingo, A. (1972). *Phytophthora* foot rot of black pepper in Brazil and Puerto Rico. Phytopathology. 62: 144-148.

Alvarado, L., Saa, S., Cuneo, I.F., Pedreschi, R., Morales, J., Larach, A., Barros, W., Guajardo, J., Besoain, X. (2020). A comparison of immediate and short-term defensive responses to *Phytophthora* species infection in both susceptible and resistant walnut rootstocks. Plant Dis. 104(3): 921-929.

Fan, R., Tao, X.Y., Xia, Z.Q., Sim, S., Hu, L.S., Wu, B.D. *et al.* (2022). Comparative transcriptome and metabolome analysis of resistant and susceptible *Piper* species upon infection by the oomycete *Phytophthora capsici*. Frontiers in Plant Science. 13: 864927. https://doi.org/10.3389/fpls.2022.864927.

Foster, J.M., Naegele, R.P., Hausbeck, M.K. (2013). Evaluation of eggplant rootstocks and pepper varieties for potential resistance to isolates of *Phytophthora capsici* from Michigan and New York. Plant Dis. 97(8): 1037-1041.

Fullana, A.M., Expósito, A., Escudero, N., Cunquero, M., Loza-Alvarez, P., Giné, A., Sorribas, F.J. (2023). Crop rotation with *Meloidogyne*-resistant germplasm is useful to manage and revert the (a)virulent populations of *Mi1.2* gene and reduce yield losses. Front Plant Sci. 14: 1133095. https://doi.org/10.3389/fpls.2023.1133095.

Hooper, D. (1990). Extraction and Processing of Plant and Soil Nematodes. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. [Luc, M., Sikora, R.A. and Bridge, J. (Eds.)], CABI International: Wallingford, pp. 45-68.

Izzah, A.H., Wan Asrina,W.Y. (2019). Black pepper in Malaysia: An overview and future prospects. Agricultural Reviews. 40(4): 296-302. doi: 10.18805/ag.R-129.

Krishnamoorthy, B., Parthasarathy, V. (2010). Improvement of Black Pepper. CABI Reviews. pp 1-12.

Li, Y., Zhao, D. (2021). Transcriptome analysis of scions grafted to potato rootstock for improving late blight resistance. BMC Plant Biol. 21: 272. https://doi.org/10.1186/s12870-021-03039-w.

Narayana, R., Thomas, S., Sheela, M. (2018). Management of root-knot nematode *Meloidogyne incognita* infecting black pepper. Indian Journal of Nematology. 48: 51-55.

Nguyen, V.A., Nguyen, C.T., Nguyen, T.H., Nguyen, T.H., Phan, T.P.T., Doan, T.H.C. *et al*. (2019). Evaluate the effectiveness of grafted pepper (*Piper colubrinum* L.) production in the southeast and central highlands of Vietnam. SSRG International Journal of Agriculture and Environmental Science. 6: 78-90.

Nysanth, N.S., Divya, S., Nair, C., Anju, A.B., Ravindran, P., Anith, K. (2022). Biological control of foot rot (*Phytophthora capsici* Leonian) disease in black pepper (*Piper nigrum* L.) with rhizospheric microorganisms. Rhizosphere. 23: 100578. doi: 10.1016/j.rhisph.2022.100578.

Quyen, T., Tran, T., Duong, O., Ngoc, N., Dang, B. (2020). Determination grafting techniques and compatible grafts between piper species-a case study in Vietnam. International Journal of Chemical Studies. 8: 1817-1820.

Rani, M.B. and Kayang, H. (2023). Plant growth promoting Rhizospheric *Bacillus* Spp. isolated from black pepper (*Piper nigrum* L.). Indian Journal of Agricultural Research. 57(3): 297-303. doi: 10.18805/IJARe.A-6052.

Ravindran, P.N., Babu, K.N., Sasikumar, B. and Krishnamurthy, K.S. (2000). Botany and Crop Improvement of Black Pepper. In: Black Pepper (*Piper nigrum*). [Ravindran, P.N. (Ed.)], Reading: Harwood Academic Publishers. pp. 23-142.

Rini, C., Remya, J. (2020). Management of *Phytophthora capsici* infection in black pepper (*Piper nigrum* L.) using new generation fungicides and biopesticide. International Journal of Agriculture, Environment and Biotechnology. 13: 71-74.

Saad, A.M., Salem, H.M., El-Tahan, A.M., El-Saadony, M.T., Alotaibi, S.S., El-Shehawi, A.M., Abd El-Mageed, T.A., Taha, A.E., Alkahtani, M.A., Ezzat Ahmed, A., Swelum, A.A. (2022). Biological control: An effective approach against nematodes using black pepper plants (*Piper nigrum* L.). Saudi Journal of Biological Sciences. 29: 2047-2055.

Shi, F., Zhang, X., Wang, Z., Wang, X., Zou, C. (2024). Unveiling molecular mechanisms of pepper resistance to *Phytophthora capsici* through grafting using iTRAQ-based proteomic analysis. Sci. Rep. 14(1): 4789. https://doi.org/10.1038/s41598-024-55596-3.

Suhail, A. (2023). Control of root-knot nematode, *Meloidogyne incognita* and reniform nematode, *Rotylenchulus reniformis* on chickpea. Agricultural Science Digest. 43(2): 232-237. doi: 10.18805/ag.D-5269.

Thuy, T.T.T., Yen, N.T., Tuyet, N.T.A., Te, L.L. and De Waele, D. (2012). Plantparasitic nematodes and yellowing of leaves associated with black pepper plants in Vietnam. Archives of Phytopathology and Plant Protection. 45: 1183-1200.

Tran, T.P.H., Wang, S.L., Nguyen, V.B., Tran, D.M., Nguyen, D.S. and Nguyen, A.D. (2019). Study of novel endophytic bacteria for biocontrol of black pepper root-knot nematodes in the Central Highlands of Vietnam. Agronomy. 9(11): 714. https://doi.org/10.3390/agronomy9110714.

Tran, Q.V., Ha, C.V., Vvedensky, V.V., Le, L.T.T. and Han, V.C. (2023). Pathogenicity and fungicide sensitivity of *Phytophthora parvispora*, a new pathogen causing gummosis and root rot disease on citrus trees. Microbial Pathogenesis. 175: 105986. doi: 10.1016/j.micpath.2023.105986.

Truong, H.T.H., Rasphone, S., Nguyen, B.L.Q., Ho, H.N., Nguyen, C.Q., Tran, T.T., Hoang, T.X. and Duong, T.T. (2023). Identification of *Piper* species that are resistant to *Phytophthora capsici*, *Meloidogyne incognita* and waterlogging in Vietnam. Plant Pathology. Pp 1-11.

Tsao, P.H. and Ocana, G. (1969) Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature. 223: 636-638.

Umadevi, P. and Anandaraj, M. (2017). Genotype specific host resistance for *Phytophthora* in black pepper (*Piper nigrum* L.). Physiological and Molecular Plant Pathology. 100: 237-241.

Verma, R., Gupta, P.K. and Kaur, M. (2023). Black Pepper: Diseases and Pests. In: Pests and Disease Management of Horticultural Crops. [Hasan, W., Verma, B. and Minnatullah, M. (Eds.)], New Delhi: Biotech Books, pp. 199-208.