HUE UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY
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SONEXAY RASPHONE

RESEARCH ON PEPPER VARIETIES (*Piper* spp.) RESISTANCE TO *Meloidogyne incognita* BY MOLECULAR MARKERS IN VIETNAM

Major: Biology
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SUMMARY OF DOCTORAL THESIS IN BIOLOGY

Scientific supervisors:
ASSOCIATE PROFESSOR PH.D TRUONG THI HONG HAI
PH.D NGUYEN QUANG CO

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PREAMBLE

1. The urgency of the Thesis

Pepper (*Piper* spp.) is a crop with great economic value in Vietnam. In 2022, the pepper growing area across the country will be 131.8 thousand hectares, and exports will reach 228.7 thousand tons, with total export turnover increasing by 3.5% compared to 2021. Vietnam accounts for 40% of output and 60% of the global pepper market share, while maintaining the number one position in the world in terms of production and export (Vietnambiz, 2023).

In Vietnam, pepper varieties being grown popularly in production can be classified into three groups: small-leaved pepper including Se, Se Dat Do, Vinh Linh, Tieu Son, Di Linh, Phu Quoc, and Nam Vang; Medium leaf pepper is usually imported from Madagascar, India and, Indonesia such as Lada Belangtoeng, Karimunda, Kuching and Panniyur; Large-leaf peppers include Se Mo and Trau Dat varieties, among which the three most commonly grown groups are Lada Belangtoeng (Sung, 2001). In recent years, due to climate change combined with the development of pepper trees beyond the orientation and not according to the plan, the situation of pests and diseases on pepper plants has appeared more and more, including the two most serious diseases the fast-dead and the slow-dead. According to a report by the Plant Protection Department at the beginning of 2019, the area of pepper trees that died was more than 10 thousand hectares, mainly due to harmful diseases, in which the disease died quickly due to *Phytophthora* fungus and the disease died slowly caused by the fungus *Phytophthora*. *Meloidogyne incognita* is considered the most harmful disease for pepper plants.

According to research and experiences in pepper cultivation in the world and in Vietnam, the control of harmful nematodes on pepper plants by chemical drugs is very ineffective, costly, and pollutes the environment (Youssef & El-Nagdi, 2021). In addition, the use of crop rotation techniques and the use of *Mycorrhizal arbuscular* fungi (Mandou et al., 2023) or the use of biological products to control nematodes have also been published (Lockett et al., 2000; Xuyen, 2000; Dong & Zhang, 2006; Anwar & Rashid, 2007; Caillaud et al., 2008; Claudius-Cole et al., 2010; Sowley et al., 2014; El-Nagdi & Youssef, 2015; El-Nagdi et al., 2019; Mhatre et al., 2019; Thuy et al., 2019; Youssef & El-Nagdi, 2021; Lawal et al., 2022; Burns et al., 2023; Bhat et al., 2023). The use of parasites in nematode control has also been studied (Rahanandeh, 2012; Mukhta & Pervaz, 2013; Mukhta et al., 2013; Saad et al., 2022). However, the most effective method to control nematodes is the use of resistant pepper varieties (Eapen & Pandey, 2018; Ngoc et al.,

2021). Therefore, the research and breeding of pepper resistant to nematodes is very necessary for current and future pepper production. Local varieties are used in breeding programs because of their potential to carry genes for resistance to plant diseases and pests, as well as providing a source of genetic diversity for plant breeding (Nas et al., 2023.). However, as pepper is a perennial plant, it takes a lot of time and effort to select and create new varieties according to traditional methods to select varieties with desired traits, especially tolerance traits. adapt to the changing environmental conditions.

There have been many research projects on breeding pepper plants with high quality, efficiency, and productivity. In particular, the South American wild pepper (*Piper colubrinum*) and the betel nut (*Piper betle*) are quite resistant to the fungus *Phytophthora capsici* and the nematode Meloidogyne incognita (Hien et al., 2019) and have good compatibility when grafted with Vinh Linh pepper (*Piper Nigrum*) (Ngoc et al., 2021).

Nowadays, with the development of the biotechnology industry, the work of selecting and creating new plant varieties has become more convenient and easier, especially using molecular marker techniques in breeding that can quickly and accurately select desired traits, shorten time, increase yield, select and create genetically accurate target varieties as well as save effort (compared to traditional breeding and selection). So, "Research on pepper varieties (*Piper* spp.) resistance to *Meloidogyne incognita* by molecular markers in Vietnam" is urgent to select nematode-resistant varieties and develop solutions for stable and sustainable pepper production. In this study, waterlogging-tolerant black pepper varieties were also selected to select varieties suitable for the frequently flooded conditions of Thua Thien Hue Province.

1.1. Objectives of the study

Overall objective

Research on pepper varieties (*Piper* spp.) resistance to *Meloidogyne incognita* by molecular markers in Vietnam.

Details objective

Evaluation of genetic diversity of pepper groups collected in Vietnam

Selection of some pepper lines/varieties that are resistant to root knot nematode (*M. incognita*) and tolerant to waterlogging

The development of molecular markers helps identify nematode resistance of pepper strains/varieties.

Evaluation of flowering characteristics of some pepper lines/varieties of *P. nigrum* L. and the possibility of crossbreeding with

nematode-resistant *P. divaricatum* to create new pepper lines/varieties for Vietnam

Selection of some good conjugative graft combinations that are resistant to nematodes

Evaluation of the growth and development ability of a nematode-resistant pepper graft combination under greenhouse conditions.

1.2. New points of the thesis

Successfully identified and evaluated genetic diversity using morphology and molecular markers of pepper lines/varieties collected in Vietnam

Selected a pepper line/cultivar of *Piper hancei* (HUIB_PH30) and a pepper line/cultivar of *Piper devaricatum* (HUIB_PD36) that are resistant to gall nematode *M. incognita* and have good waterlogging tolerance.

The molecular marker SCAR 30 - 360F1R2 associated with nematode resistance of pepper plants has been developed.

Flowering characteristics of *P. nigrum* L. and the possibility of weak hybridization between *P. nigrum* L and *P. divaricatum* were evaluated.

Selected two pepper graft combinations (HUIB_PH30 - Vinh Linh and HUIB_PD36 - Vinh Linh) that are well compatible, resistant to gall nematodes and grow and develop well in greenhouse conditions

CHAPTER 1
DOCUMENTARY OVERVIEW

1.1. Theoretical basis of the research

1.1.1. Synopsis of Nematodes

1.1.1.1. Introduction to nematodes

The nodule nematode, of the genus *Meloidogyne* (Trinh et al., 2019), family Meloidogynidae, order Tylenchida (Kofoid & White, 1919), is one of the main pathogens found in many different plant species (Sikandan et al., 2020; Yang et al., 2020). Root-knot nematode (*Meloidogyne* spp.) is a pathogen affecting the quality and yield of pepper varieties, and *M. incognita* is economically one of the most important plant parasitic nematodes. worldwide due to its increasing geographical distribution, wide host range, and pathogenicity (Nas et al., 2023).

1.1.1.2. Classification of nematodes

In Vietnam, *Meloidogyne* spp., *Tylenchus* sp., *Rotylenchulus reniformis*, *Ditylenchus ausafi*, and *Aphelenchus avenae* are five plant parasitic nematodes found in all studied provinces. *Meloidogyne* spp, is the common taxon found and all *Meloidogyne* is recognized as *M. incognita*.

1.1.1.3. The harmful effects of nematodes

Meloidogyne is known to be one of the major pests of vegetable, medicinal and other crops. In pepper, this group of nematodes is a major cause of the disease of "slow death", yellowing of leaves, and reduced yield of pepper (Quyen et al., 2019). Nematodes cause a 15% annual crop loss, estimated at $100-157 billion worldwide (Abd-Elgawad & Askary, 2015). In the pepper-growing countries of Southeast Asia, *Meloidogyne* spp causes losses of up to 16% (Sasser, 1979).

1.1.1.4. Measures to treat nematodes

Various synthetic nematodes have been used to control nematodes, however, most pesticides have been removed from the market due to off-target effects and effects on human health and the environment. *Trichoderma, mycorrhizal* and *endophytic* fungi are the main filamentous fungi used to confer nematode resistance. They can reduce damage caused by parasitic nematodes on plants by producing enzymes that break down, antibiotic, paralyze, and parasitize. In addition, many species of fungi with the ability to kill nematodes have been tested such as *Dactylella oviparasitica, Arthrobotrys oligospore, Monacrosporium gepgyropagum, Verticillium chlamydosporium.*

1.1.2. Summary of black pepper

1.1.2.1. About black pepper

Pepper (*Piper nigrum* L.) is a perennial climbing plant belonging to the Piperaceace family (Bui et al., 2017), originating from India, then introduced to tropical countries in Asia and America such as Indonesia, Vietnam, Brazil, etc. Pepper is often called the king of the most used spices in the world (Khew et al., 2020; Dongare et al., 2023) and it has become familiar in people's daily dishes. Besides, pepper is also used in medicine to treat many diseases such as flu, congestion, arthritis,... (Wang et al., 2017).

1.1.2.2. The role and effects of black pepper

Pepper is a perennial crop with high economic value. Pepper is used as a spice, in the flavoring industry, in medicine, and as an insecticide (Hoa, 2001).

1.1.2.3. Morphological characteristics of black pepper

Pepper roots include 3-6 taproots and a bunch of auxiliary roots below the ground, on the stem with lizard roots (root attachments). Pepper is a flexible herbaceous plant that is divided into several segments, each with a single, heart-shaped, alternate leaf. In the leaf axils, there are dormant sprouts that can arise into twigs, eel branches, and evil branches (left branches) depending on the stage of development. Pepper plants flower in the form of spike-shaped flowers, dangling, 7-12 cm long, depending on pepper varieties and care conditions. On the

flower spike, there are an average of 20-60 flowers arranged in a spiral, the pilot bisexual or unisexual. The fruit is a nut, without a stem, bearing 1 spherical seed. From full flowering to fruit, ripening lasts 7-10 months.

1.1.2.4. Distribution of pepper

Pepper originated in India, then imported to tropical countries such as Brazil, Indonesia, Malaysia, Thailand, Sri Lanka, and Vietnam. Today, although pepper is found in almost all tropical countries, the main production areas are concentrated in a few countries of South Asia, Southeast Asia, and Brazil. Pepper is widely grown in many places: India, Brazil, Indonesia, Malaysia, Sri Lanka, Vietnam, and China (Xuyen et al., 2019).

1.1.2.5. Pepper varieties in use

In 2021, Thuy et al. collected 33 pepper samples from the Southeast, Phu Quoc, and Central Highlands regions, including Vinh Linh, Sri Lanka, Brazil, India, and Se pepper varieties. Based on morphology, pepper can be divided into two lines: small-leaved small-leaved pepper includes: Se Dak Lak, Phu Quoc and Vinh Linh pepper collected in the Southeast, and Central Highlands. Large-leaved pepper varieties include Brazilian pepper grown in Binh Duong, Indian pepper grown in Dong Nai, and Sri Lankan pepper grown in Gia Lai and Dong Nai.

1.1.2.6. Breeding methods of pepper varieties

To improve the characteristics of pepper varieties and increase resistance to pests and diseases, cross-breeding methods are essential, to create new varieties, and increase the diversity and richness of pepper genetic resources. There are two methods, natural hybridization, and artificial hybridization. Breeding through free pollination (natural hybridization) is increasingly popular and gives very good yields.

1.1.3. Grafting methods applied on black pepper

Grafting of pepper (*Piper nigrum*) on resistant root-stocks of *P. colubrinum* is a widely accepted technique for the management of *Phytophthora* diseases. To evaluate the effect of variety and season on graft recovery, a preliminary study was performed. In which the lateral shoots of eight varieties of *P. nigrum* were grafted on *P. colubrinum* root-stock. The results show that regardless of variety, February and March are the best times for grafting (Vanaja et al., 2007).

1.1.4. Molecular Marker

1.1.4.1. Definition of a molecular marker

Molecular markers, or DNA markers, are markers that are located only near or associated with genes and have little or no effect on phenotype. DNA markers are changes in DNA and are divided into several types based on different methods and techniques for identifying

polymorphisms (Thanh, 2014).

1.1.4.2. Types of Molecular Markers

Current markers include: Restriction Fragment Length Polymorphism (RFLP), Short Tandem Repeat (STR), Variable Number of Tandem Repeat (VNTR), Single-Strand Conformation Polymorphism (SSCP), Sequences-tagged sites (STS), Random Amplified Polymorphic DNA (RAPD), Single Nucleotide Polymorphism (SND), Restriction Fragment Length Polymorphism (RFLD), Sequence tagged microsatellite site (STMS), DNA amplification fingerprinting (DAF), Expressed Sequence Tags (EST) (Adams et al., 1991), Simple Sequence Length Polymorphism (SSLP), Cleaved Amplified Polymorphic Sequence (CAPS), Distribution of single-dose allele (SDA), Simple Sequence Repeat (SSR), Sequence Characterized Amplified Region (SCAR), SAM sub-satellite repeat polymorphism, Inter Simple Sequence Repeat (ISSR), Allele Specific Associated Primers (ASAP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Microsatellite Polymorphism (RAMP), Sequence-Specific Amplification Polymorphism (S-SAP), Integrated Political Crisis Response (IPCR), Short Tandem Repeat (STR),...

1.1.4.3. The role of molecular markers

DNA marker techniques play an important role in the study of genetic diversity, phylogenetics, taxonomy, marker and gene identification; selection of genetic resources, and selection of varieties by molecular markers. However, there is currently no directive that meets all of the above requirements. Depending on the research problem, choose the appropriate techniques.

1.2. The practical basis of the topic

1.2.1. Situation of pepper production and use of pepper varieties in the world and Vietnam

- ***In the world***

According to statistics from the International Pepper Association, the total area of pepper in the world from 2008 to 2017 was almost unchanged. The total area of pepper in 2008 was 459,886 ha, by 2017 the total area of pepper in the world was 458,731 ha. India, Vietnam, and Indonesia are the 3 countries with the largest pepper area, the total area of these 3 countries accounts for 78.86% of the total area of the world (Pepper Statistical Yearbook 2017, International Pepper Community). Vietnam is the largest producer and exporter of pepper (100,000 tons), followed by India (48,000 tons), Indonesia (37,000 tons), Brazil (35,000 tons), and Malaysia (25,672 tons).

- ***In Vietnam***

Vietnam is the most prominent pepper producer and exporter in

the world. In 2020, Vietnam's black pepper growing area is 132,000 hectares, and the output is 270,000 tons, of which the Central Highlands region accounts for about 70% of both area and output. Therefore, this area is considered the capital of pepper cultivation and production in Vietnam (Tran et al., 2022).

1.2.2. Situation of nematode diseases affecting pepper in the world and Vietnam

- ***In the world***

Nematodes were discovered in 1902 in the Cochin pepper region of China. In 1918, Wynad, India also reported harmful nematodes on pepper. *M. incognita* and *M. javanica* are pests of pepper in many countries such as Brazil, Sarawak, Borneo, China, Malaysia, Brunei, Cambodia, Indonesia, Philippines, Thailand, and Vietnam. *M. arenaria* species has been reported to cause damage in Sri Lanka (Koshy & Geetha, 1992).

- ***In Vietnam***

According to the inspection results of pepper production in early 2019 by the Plant Protection Department, the Central Highlands provinces alone have shown that the area of dead pepper trees has exceeded 10,000 hectares (Gia Lai is 5,547 hectares; Dak Lak is 2,774 hectares; Dak Nong is 1,827 hectares).

1.2.3. The situation of applying molecular markers in the selection and breeding of nematode-resistant varieties in pepper in the world and Vietnam

Studies on the application of molecular markers in breeding pepper resistant to nematodes in the world and in Vietnam are still limited because the nuclear genome of pepper has not been fully sequenced.

1.2.4. The situation of nematode-resistant pepper breeding in the world and Vietnam

For the first time in the history of pepper cultivation, a partially fertile hybrid pepper resistant to *Phytophthora* was developed by crossing *Piper nigrum* with the wild species *P. colubrinnum*. However, there is no research on breeding nematode-resistant pepper varieties. As molecular biology and plant breeding advance, two important approaches that become important, marker-assisted selection (MAS) and gene editing, are becoming prominent. This can be a step forward for application in the study of nematode-resistant pepper varieties.

1.2.5. Production and use of nematode-resistant pepper grafts in the world and Vietnam

So far in the world there have been studies on pepper grafting. However, no country has yet successfully developed grafted pepper, bringing grafted pepper to pepper cultivation as a popular propagation

method, and most countries are continuing the traditional propagation by stem cuttings and eel wire cuttings. In Vietnam, the cultivation of grafted pepper is mainly done by spontaneous farmers. There are planting areas that failed but there are also areas that initially developed very well and evenly. This may be due to different planting and fertilizing techniques for field-grown pepper plants among gardeners.

CHAPTER 2
RESEARCH SUBJECTS, MATERIALS, AND METHODS

2.1. Research subjects

- Evaluation of genetic diversity of pepper groups collected in Vietnam
- Selection of pepper lines/varieties that are resistant to nematode (*M. incognita*) and waterlogged
- Development of DNA markers associated with nematode resistance genes of pepper plants by BSA method
- Evaluation of flowering characteristics of some varieties of *P. nigrum* L. and the ability to cross-breed with nematode-resistant *P. divaricatum* to create new pepper varieties.
- Selection of nematode-resistant root-stocks and evaluation of successful grafting on nematode resistant root-stocks for some commercial pepper varieties
- Evaluation of the growth and development ability of nematode-resistant pepper grafts under greenhouse conditions

2.2. Research Materials

Black pepper samples were collected from pepper growing areas in 8 provinces, 39 varieties, and 100 RAPD primers were used to assess genetic diversity.

Source of nematode seed Meloidogyne: Nematodes were obtained from the roots of peppers in gardens infected with slow-killing yellow leaf disease in Gia Lai, Vietnam, and then extracted the nematodes by filtration method described by Hooper (1986).

Breeding materials: 5 varieties of pepper belonging to the species *Piper nigrum* L. with common names Vinh Linh (HUIB_PN27), Srilanka (HUIB_PN97), India (HUIB_PN69), Phu Quoc (HUIB_PN101), Malaysia (HUIB_PN96), and 01 South American wild pepper *Piper divaricatum* (HUIB_PD36).

Root-stock and top-stock materials: 6 types of root-stock and 4 types of grafted tops were used. In which, the root-stock types HUIB_PN105; HUIB_PN45; HUIB_PN27; HUIB_PH30; HUIB_PD36, HUIB_PH46 were incubated in clean potting soil that was autoclaved (1.5 kg of substrate) with the quantity of 30 pots/rootstock. The types of grafted tops are Vinh Linh -

VL, Loc Ninh - LN, Srilanka - SR, and India - AD.

2.3. Research Methods

2.3.1. Evaluation of genetic diversity of pepper corporations collected in Vietnam

2.3.1.1. Evaluation of genetic diversity of pepper lines/varieties by morphological characteristics

The detailed description of the collected materials was based on the criteria of the International Plant Genetic Resources Research Institute (IPGRI, 1995), including 16 characteristics. For cluster analysis (R Development Core Team, 2008), all features of each join were normalized, and Euclidean distances were calculated using the unweighted pair group method with arithmetic mean (UPGMA).

2.3.1.2. Identification of collected pepper lines/varieties based on ITS sequences

DNA of pepper lines/varieties was extracted from young leaves by CTAB method and purified through a silica column.

The ITSu1-4 gene region of the pepper lines/varieties was amplified in a volume of 25 µL, using OneTaq® DNA Polymerase (Biolabs Inc., New England). PCR products were checked by electrophoresis on 1% agarose gel. Samples showing a clear single band were sent for sequencing to Macrogen Co., Korea. The results will be analyzed for species identification.

2.3.1.3. Evaluation of genetic diversity of pepper lines/varieties by molecular markers

First, 3 out of 39 cultivars were randomly selected to screen 100 RAPD primers to select the one with the highest polymorphism rate. Polymorphic primers were then used to amplify 39 cultivars to assess genetic diversity. The PCR reaction was performed according to Truong et al. (2013) procedure with a volume of 15 µL.

2.3.2. Selection of pepper lines/varieties that are resistant to nematode and waterlogged

2.3.2.1. Evaluation of the resistance to nematode of the pepper corporation

- ***Prepare pepper lines/varieties for an experiment:*** Pepper is nursed with 2 cuttings/pot, each cutting has 3 eyes plugged into the potting soil, the size of the pot is 13 x 23 cm. When the plant has 3-5 leaves, the nematode infection is carried out.
- ***Experimental design method:*** The experiment was arranged in a net house in a completely randomized fashion, each recipe included 3 replicates, 10 plants each time.
- ***M. incognita collection method:*** The roots of peppers infected with yellow nodules were collected from Gia Lai. Application of TCVN

12194-1: 2019 on the identification of plant pathogenic nematodes to collect eggs and 2-year-old nematodes (J2) (Chau & Thanh, 2000). Conduct culture of J2 *M. incognita* on tomato growing medium.

- ***Method to extract nematodes from roots:*** Using filtration method (Maceration - sieving method) (Hooper, 1986).
- ***Total DNA extraction:*** About 300 nematodes were centrifuged and transferred to 1.5 mL tubes.
- ***Method of infection:*** Inoculate once when the seedling is 3 months old. The density of *M. incognita* nematodes was 100 2-year-olds (J2)/100 g of substrate. Pour 50 mL of a solution containing approximately 1,500 *M. incognita* nematodes into each seedling pot (1.5 kg of media). Monitoring indicators: Rate of infected plants with yellow leaves (%), percentage of roots with nodules (%)

2.3.2.2. Evaluation of water-logging tolerance of some pepper lines/ varieties

Evaluation of water-logging tolerance: Peppers after 12 months of being moved to pots will be treated for water-logging. The whole pot is soaked in water for four days. The survival rate was determined after 15 days and evaluated the microscopic characteristics of the waterlogged samples as described by Tran et al (2022).

2.3.3. Development of DNA markers associated with nematode resistance genes of pepper plants by BSA method

2.3.3.1. Study of electromechanical recognition of molecular markers associated with nematode resistance genes by BSA method

RAPD technique: From the results of the section on Evaluation of Genetic Diversity of Pepper Group, to find more bands specific to nematode-resistant samples, screen 100 more RAPD primers.

BSA technique: Using BSA (Bulked Segregant Analysis) method (Michelmore et al., 1991; Truong et al., 2013) to rapidly detect DNA fragments specific for nematode-resistant individuals.

2.3.3.2. Research and development of converting the RAPD into the SCAR

SCAR primer design: SCAR primer was designed based on decoding sequences of RAPD fragments associated with nematode resistance and infection genes, using Primer3 4.0 program (Rozen & Skaletsky, 1999).

Assessment of SCAR Sensitivity: The sensitivity is defined as the lowest DNA concentration that can detect the disease through a positive PCR result. To prepare for the PCR reaction, each DNA sample was diluted to concentrations of 1, 5, 10 and 20 ng/µl.

Evaluation of SCAR specificity: The specificity is determined as

the ratio of individuals giving positive results out of the total number of individuals performing PCR with the primer pair in the Kit. The PCR-optimized SCAR primer will be used to amplify all 39 pepper lines/varieties to test the polymorphism and resistance of the SCAR molecular marker to the nematode resistance gene. Each PCR reaction was performed

2.3.4. Evaluation of flowering characteristics of some varieties of P. nigrum L. and the ability to cross-breed with nematode-resistant P. divaricatum to create new pepper varieties.

2.3.4.1. Survey on flowering characteristics of some pepper varieties

Observe the elongation period (days) (from the appearance of the bud to the time the first flower blooms), the time of flower differentiation (days) (from the appearance of the first flower to the full bloom of the flower), the interval between the stamen and the pistil (day) (from the time the flower blooms to the appearance of the stamen).

2.3.4.2. The first step in breeding pepper varieties

The experiment was arranged in a completely randomized design with 1 factor (CRD), 3 replicates, 3 plants/each replicate, 10 seeds/3 plants, and 5 flowers each. The hybrid technique is applied according to the pilot hybrid technique of the Research Institute of Spices and Medicinal Plants of Indonesia.

2.3.5. Selection of nematode-resistant root-stocks and evaluation of successful grafting on nematode-resistant root-stocks for some commercial pepper varieties

2.3.5.1. Evaluation of resistance to M. incognita nematode of pepper root-stocks

M. incognita collection method: The roots of pepper plants with many nodules were collected from pepper gardens infected with yellow leaf disease in Gia Lai. Application of TCVN 12194-1:2019 on the identification of *M. incognita* to collect eggs and 2-year-old nematodes (J2) (Chau & Thanh, 2000).

Method of infection: Inoculated once when the root-stock materials (HUIB_PN105; HUIB_PN45; HUIB_PN27; HUIB_PH30; HUIB_PD36) were 3 months old. The density of *M. incognita* nematodes was 100 2-year-olds (J2)/100 g of substrate. Pour 50mL of the solution containing about 1,500 *M. incognita* nematodes for each seedling pot (1.5 kg of media). Then monitor the infection rate.

Resistance level: divided into 5 levels based on disease index. Level 5/5 disease index < 20%, Level 4/5 disease index from 20% - 40%, Level 3/5 disease index from 40% - 60%, Level 2/5 disease index from 60% - 80%, Level 1/5 disease index > 80%.

2.3.5.2. Evaluation of successful grafting and conjugation ability of graft

combination

Grafting method: Wedge jointing.

Experiment with 2 factors: Factor A: 3 types of root-stock (HUIB_PD36 (A1), HUIB_PH30 (A2), HUIB_PH46 (A3); Factor B: 4 types of grafted tops (Vinh Linh - VL (B1), Loc Ninh - LN (B2), Sri Lanka - SR (B3), India - AD (B4)). Evaluation of successful grafting and growth in height of top-stocks after 30, 60, 90 and 120 days.

Evaluation of the ability to adapt: Through the degree of anatomical similarity between the root-stock, the top of the graft and the degree of conjugation at the graft site when the graft is 4 months old.

2.3.5.3. Evaluation of the resistance to M. incognita of the grafted pepper combinations

Infection with artificial *M. incognita*: After grafting successfully, the grafted plant is 2 months old, 10-15 cm tall with 2-3 leaves, then it will infect *M. incognita*.

2.3.6. Evaluation of growth and development ability of nematode-resistant pepper -grafted plants under greenhouse conditions

Evaluation of the growth ability of nematode-resistant varieties of pepper grafted plants based on the growth dynamics of plant height, number of leaves, leaf color, number of nodes, burning color, and number of branches.

2.4. Data processing methods

Analysis of morphological characteristics of pepper groups

Data were analyzed by the Duncan test ($P<0.05$) in the SPSS software of IBM. Cluster and principal component analysis was performed using R software (R Development Core., 2008).

Analysis of the results of the identification of pepper corporation:

The sequencing results of the ITSu1-4 gene region were collected and edited using BioEdit v7.2. The edited sequences were then aligned by ClustalW in MEGA X after which a phylogenetic tree was constructed. The barcode sequences were queried for species identification against the GeneBank database (NCBI) using the Nucleotide BLAST algorithm.

Analysis of genetic diversity assessment results of pepper corporation

Based on the electrophoresis results, bands that appear clear and undistorted will be assigned a "1", none (or too faint) will be assigned a "0". The binary matrix data will be used to calculate the genetic diversity index using POPGENE1.32 software and build a phylogenetic tree using NTSYS2.1.

Analysis of flowering characteristics of some varieties of P. nigrum L. and the ability to cross-breed with nematode-resistant P.

divaricatum to create new pepper varieties.

Methods of data processing: Data were synthesized by Excel software and processed with descriptive statistics, compared with ANOVA variance by statistical processing software SAS9.1.

Analysis of the results of nematode resistance, the possibility of successful grafting, the results of hybridization, the results of grafting, the assessment of the conjugation ability, the growth and development ability of the combination of pepper grafting:

Data were processed using Ms Excel and SAS 9.1 software.

CHAPTER 3
RESEARCH RESULTS

3.1. Evaluation of genetic diversity of pepper corporations collected in Vietnam

3.1.1. Evaluation of genetic diversity of pepper lines/varieties by morphological characteristics

Morphological characteristics of the seed material:

16 morphological characteristics in all pepper plants were studied. The most common growth is climbing (37 varieties) while HUIB_PR48 and HUIB_PH36 are trailing. All pepper varieties have polymorphic branching patterns except HUIB_PD36. Most varieties have multiple shoots arising from the stem. HUIB_PD36, HUIB_PR48, and HUIB_PN101 had weak holding capacity, while medium grip ability was observed in 3 varieties HUIB_PN56, HUIB_PR41, and HUIB_PN47. The remaining varieties show strong grip. The adventitious root production was low in HUIB_PN101, medium in HUIB_PD36, HUIB_PR41, and HUIB_PR48; the remaining varieties produced a lot of indeterminate roots. All varieties are absence of pubescence on stem. There are three lateral branch habit: erect, horizontal, hanging. Leaf lamina shape varies from ovate, ovate-elliptic, ovate-lanceolate, elliptic-lanceolate, and cordate. Leaf base shape with 4 phenotypes is round, cordate, acute, and oblique. There are two types of leaf margins: wavy and even. Most varieties have campylodromous veins, except HUIB_PH30 and HUIB_PH46 (acrodromous) and HUIB_PD36 eucamptodromous vein. The spike orientation of most varieties is prostrate, except for HUIB_PH30, HUIB_PH46, HUIB_PD36, and HUIB_PR41 (erect). Most varieties have filiform spike-shape, except HUIB_PR41 (conical), HUIB_PH30, and HUIB_PH46 (cylindrical). Most varieties have bisexual flowers. Whereas, HUIB_PH30, HUIB_PH46, and HUIB_PR41 only pistillate flowers. Fruit shape of all varieties is mostly round.

3.1.2. Identification of pepper lines/varieties collected by molecular

biology technique

The sequencing results of the ITSu1-4 gene region are 667 bp in HUIB_PR41 and HUIB_PR48; 670 bp in HUIB_PN36 and HUIB_PN91; 672 bp in HUIB_PN29 and HUIB_PN38; 685 bp in HUIB_PN46 and HUIB_PN30; 671 bp for the remaining individuals. BLAST results on NCBI used to verify and compare with the sequence of *Piper* show that the obtained nucleotide sequences are 96-100% similar to *P. retrofractum* (MH493562) (HUIB_PR41 and HUIB_PR48), *P. hancei* (EF450274) (HUIB_PH30 and HUIB_PH36_PH36), and to *P. divaricatum* (DQ868714) (HUIB_PH36), and *P. nigrum* (MH493477-MH493487, KF924121, KF92411) (remaining varieties).

3.1.3. Evaluation of genetic diversity of pepper strains/varieties by molecular markers

Total DNA extraction results: Total DNA extracted from leaves gives a single, clean, unbroken, clear band. Quality DNA is guaranteed to be used as a raw material for further experiments.

RAPD primer screening results: only 12 RAPD primers with the highest number of polymorphic bands were selected to study and evaluate genetic diversity by RAPD technique for 39 pepper individuals.

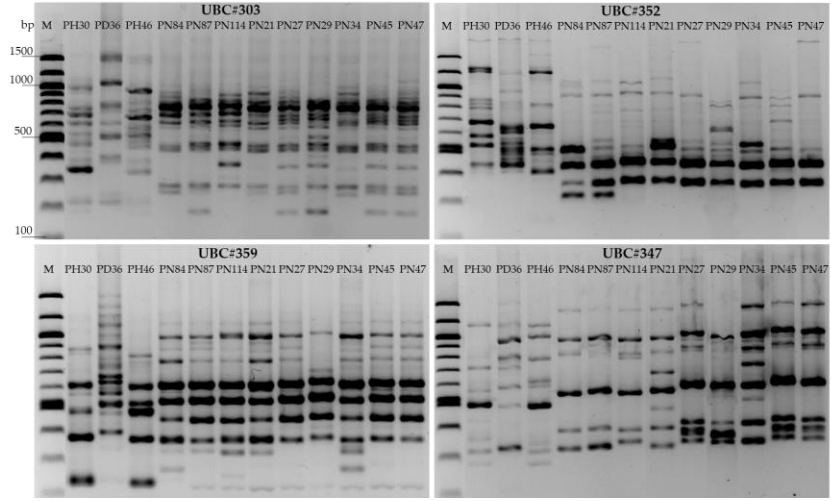

Figure 1. PCR products of primers UBC#303, UBC#352, UBC#359, UBC#347 amplifying varieties HUIB_PH30, HUIB_PD36, HUIB_PH46, HUIB_PN84, HUIB_PN87, HUIB_PN114, HUIB_PN21, HUIB_PN27, HUIB_PN45, HUIB_PN29, M: 100 bp Ladder

Results of RAPD analysis of pepper population

The number of bands amplified in different pepper individuals for 12 research primers gave a high rate (the lowest was 14 equal to 2.597% (in HUIB_PN10, HUIB_PN70, and HUIB_PN93) and the highest was 22

bands, accounting for 4.082% in HUIB_PN29. There were 40 polymorphic bands amplified from 12 random primers; band sizes ranging from 200-1400 bp. UBC#303, UBC#352, UBC#359, UBC#347, and UBC#392 were the primers with the most amplified samples (100%) followed by UBC#377 (96.774 %) with 5 DNA bands formed (Figure 1).

Analysis of the diversity of individuals in the pepper population showed that there was a great diversity in the studied samples. UBC#329 exhibited the highest diversity with an average *Ho* value of 0.533224, followed by primer UBC#317. The lowest diversity was in primer UBC#322 (Table 1).

Table 1. Genetic diversity indices of the population according to each RAPD primer

Primers	***na****	***ne****	***h****	***Ho****
UBC#303	2	1.5058	0.2926	0.4476
UBC#317	2	1.5249	0.3073	0.4676
UBC#329	2	1.6443	0.3614	0.5332
UBC#322	2	1.3268	0.2035	0.3311
UBC#333	2	1.3180	0.2114	0.3468
UBC#352	2	1.4115	0.2636	0.4195
UBC#359	2	1.5444	0.3029	0.4531
UBC#363	2	1.3851	0.2341	0.3697
UBC#377	2	1.4649	0.2754	0.4209
UBC#347	2	1.4246	0.2594	0.4072
UBC#382	2	1.5435	0.3075	0.4625
UBC#392	2	1.4751	0.2850	0.4397
Average (SE)	**2.0000 (0.172)**	**1.44 (0.343)**	**0.266 (0.171)**	**0.410 (0.226)**

See Nei (1987) Molecular Evolutionary Genetics (p. 176-187)

**na = Observed number of allele*

**ne = Effective number of alleles (Kimura and Crow, 1964)*

**h = Nei's gene diversity (1973)*

**Ho = Shannon's information Index (Lewontin, 1972)*

With 12 research primers, the genetic similarity coefficient between individuals varied from 0.418-1,000. Based on the genetic similarity coefficient, the pepper population is divided into 2 main groups.

3.2. Selection of pepper lines/varieties that are resistant to *Meloidogyne incognita* and waterlogged

3.2.1. Evaluation of the resistance to Meloidogyne incognita) of the pepper corporation

A strong correlation was found in the percentage of root nodules and leaf yellowing (Figure 2). 4 months after nematode infection, yellow leaves appeared in most varieties (20.00-33.33%), except HUIB_PH30 and HUIB_PD36 (0%). Similarly, upon root investigation, nodules were

found in most varieties (80%), except in HUIB_PH30 and HUIB_PD36. Two varieties HUIB_PH30 and HUIB_PD36 showed no signs of infection with *M. incognita*. While, HUIB_PH46 was relatively sensitive to *M. incognita* (Figure 3).

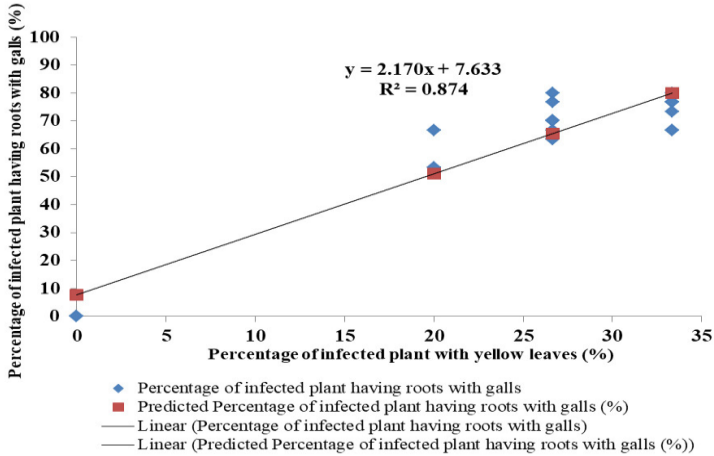

Figure 2. Correlation between the percentage of plants showing yellow leaves and nodules on the roots

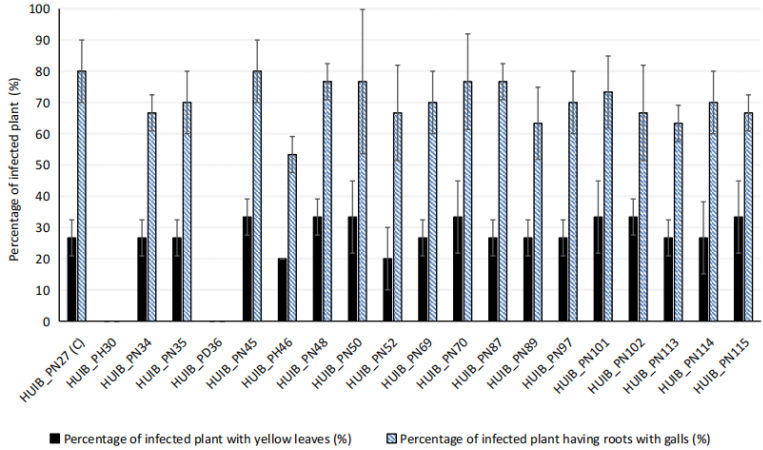

Figure 3. Comparison of percentage of plants with yellow leaf symptom and percentage of plants having roots with galls after four months of inoculation with *M. incognita*. The susceptible accession (HUIB_PN27) was included as the control treatment (C).

3.2.2. Evaluation of waterlogging tolerance of some pepper lines/ varieties

Only 3 samples HUIB_PH30, HUIB_PD36, and HUIB_PH46 had 100% survival rate after flooding treatment. The microscopic

characteristics of stems and roots showed that all 3 samples with good waterlogging tolerance, HUIB_PH30, HUIB_PD36, and HUIB_PH46 had a larger intercellular space than HUIB_PN27, helping to facilitate oxygen in the stem and roots (Figure 4).

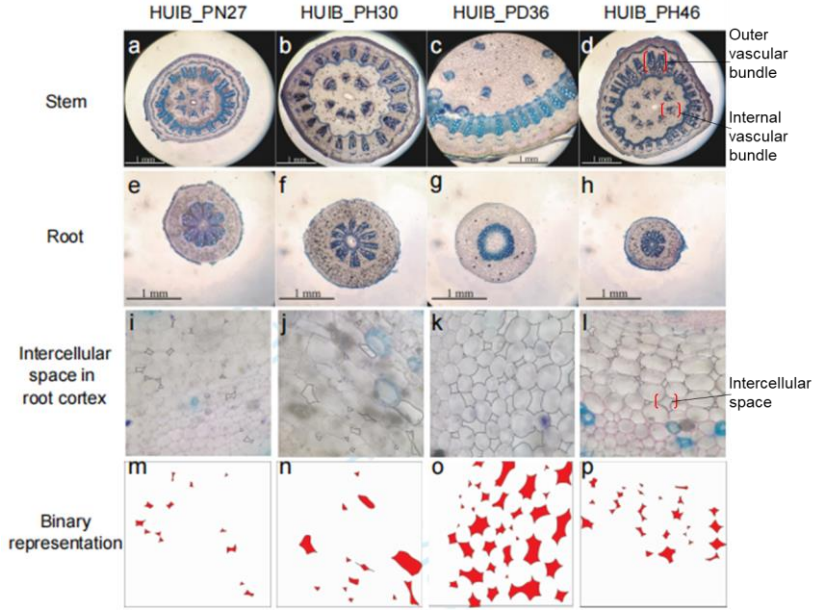

Figure 4. Micro-morphological characterization of (a-d) stems and (e-h) roots obtained from the waterlogged tolerant accessions and control. (i-l) Root cortex. The overlaid (drawn in CorelDRAW) indicated intercellular spaces in root cortex. (m-p) Binary images obtained from the corresponding root cortex (i-l), in which intercellular spaces were coloured red and the white spaces represented root cells.

3.3. Development of DNA markers associated with nematode resistance genes of pepper plants by BSA method

3.3.1. Study of electromechanical recognition of molecular markers associated with nematode resistance genes by BSA method

From the amplification results for 200 RAPD primers, 3 primers UBC#401, UBC#408, and UBC#360 were found, showing the presence of typical bands of HUIB_PH30, and HUIB_PD36 compared to the other 2 lines/varieties.

Three primers UBC#401, UBC#408, and UBC#360 were used to amplify the resistance pool, infection pool, and pepper lines/varieties to create two pools. The results showed that only 2 primers UBC#408 and UBC#360 produced two stable characteristic bands for two pepper varieties HUIB_PH30 and HUIB_PD36 (Figure 5). Which, the specific band or DNA

segment associated with the nematode resistance gene is the 1450 bp band at primer UBC#408 and the 300 bp band at primer UBC#360.

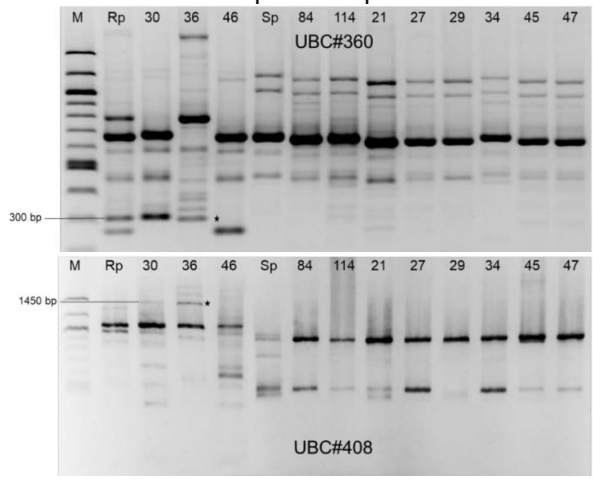

Figure 5. Amplification results of two primers UBC#360 and UBC#408 for a resistant pool (Rp), infected pool (Sp), and pooled pepper lines/varieties. *In which: HUIB_PH30 (30), HUIB_PD36 (36), HUIB_PH46 (46), HUIB_PN84 (84), HUIB_PN114 (114), HUIB_PN21 (21), HUIB_PN27 (27), HUIB_PN29 (29), HUIB_PN34 (34), HUIB_PN45 (45), HUIB); stars are markers of DNA bands associated with nematode resistance genes.*

3.3.2. Research and development of convert RAPD marker to SCAR marker

Cloning and sequencing of RAPD fragments

Of the two clones-only cassettes, sequencing was successful for one band specific for the nematode-resistant sample (300bp of primer UBC#360). Based on this sequence, 2 pairs of SCAR primers were designed (Table 2). From these 2 pairs of primers can be paired to create 4 pairs of primers to conduct SCAR analysis. The results of the amplification reaction with 4 pairs of SCAR primers showed that: only primer pairs 30- 360F1R2 showed that HUIB_PH30 and HUIB_PD36 had 1 DNA band of equal size, while samples HUIB_PN46 and HUIB_P70 had larger bands. This pair of primers was chosen to distinguish nematode-resistant/infected individuals.

Table 2. Primers designed for SCAR analysis

Primers	Sequence (5'-3')	Annealing temperature (^oC)
30-360F1	CTCTCCAGGCCTTCCCCATC	64.6
30-360R1	CTCTCCAGGCAAAACCAGTT	58.4
30-360F2	GCCCTCCTCATCTTGCCAAT	60.5
30-360R2	TCGGTCTACAGCTTCTTTCCA	59.4

The SCAR had good sensitive in low DNA concentrations (5-10

ng/ul). The results of the specificity analysis showed that the primer pairs had high specificity (100%) for the line/variety that was resistant to or infected with nematodes (Figure 6).

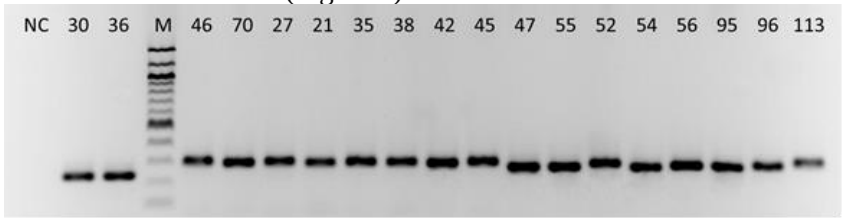

Figure 6. Results of amplification of primer pair 39-360F1R2 with some pepper lines/varieties using optimal conditions for PCR reaction. M: 100 bp Ladder; *HUIB_PH30 (30), HUIB_PD36 (36), HUIB_PH46 (46), HUIB_PN70 (70), HUIB_PN27 (27), HUIB_PN21 (21), HUIB_ PN35 (35), HUIB_PN38 (38), HUIB_ PN42 (42), HUIB_PN45 (45), HUIB_PN47 (47), HUIB_PN55 (55), HUIB_PN52 (52), HUIB_PN54 (54), HUIB_PN56 (56), HUIB_PN95 (95), HUIB_PN96 (96) and HUIB_PN113 (113).*

3.4. Evaluation of flowering characteristics of some varieties of *P. nigrum* L. and the ability to cross-breed with nematode-resistant *P. divaricatum* to create new pepper varieties.

3.4.1. Flowering characteristics of pepper varieties

The development of pepper shoots can be divided into 5 stages: (1) The stage of sprouting; (2) The elongation period; (3) The period of pollination - fertilization; (4) The post-pollination stage – spiked fruit and (5) The ripening stage. Prolongation period is the period that determines the number of flowers per stem lasting from 14.0 to 22.1 days. The pepper variety HUIB_PN101 had the longest growing period of 22.1 days and was significantly longer than the other varieties. The pepper varieties HUIB_PN27, HUIB_PN96, and HUIB_PN69 had no significant difference in lengthening time at $P < 0.01$.

Pepper seeds tend to bloom from top to bottom, from stalk to tip. There is a difference in the flowering time of the pepper varieties and this difference is statistically significant ($P < 0.01$). Flowering time ranges from 8.1 to 17.3 days, the longest is the variety HUIB_PN27 (17.3 days). The interbreeding interval of the studied varieties is from 1.7 to 7.6 days. The variety HUIB_PN101 has the shortest distance between pistils and stamens (1.7 days).

3.4.2. Results of hybridization of hybrid combinations with P. divaricatum (HUIB_PD36)

The rate of shedding and fruiting of the hybrids

The rate of shedding after pollination of hybrids of different species ranged from 30.00 to 56.67%. Specifically, the hybrid combination ♂HUIB_PD36 x ♀HUIB_PN97 has the highest abortion

rate (56.67%), and the hybrid combination ♂HUIB_PN97 x ♀HUIB_PN27 has the lowest abortion rate (30.00%). When performing out-crossing between planted pepper and South American forest pepper (HUIB_PD36), the fruiting rate of hybrid combinations is very low, only 4.67-7.33%.

Figure 7. Hybrid tree of ♂HUIB_PD36 x ♀ HUIB_PN97 (left), Hybrid tree of ♂HUIB_PD36 x ♀ HUIB_PN27 (right)

In 2021, hybrid seeds were obtained and nursed on dicotyledonous plants of the hybrid combination ♂HUIB_PD36 x ♀HUIB_PN97. However, this hybrid plant did not develop further, but stopped at the cotyledon stage, then weakened and died (Figure 7). Therefore, it is not possible to evaluate the morphology of the seedlings compared to the parent plants. In 2022, 37 hybrid seeds of 4 hybrid combinations were harvested and seeded. However, only 2 seeds of the hybrid ♂HUIB_PD36 x ♀HUIB_PN27 germinated after 21 days of incubation and developed into a dicotyledon (Figure 7). The remaining hybrid seeds did not germinate.

3.5. Selection of nematode-resistant root-stocks and evaluation of successful grafting on nematode-resistant root-stocks for some commercial pepper varieties

3.5.1. Evaluation of nematode-resistant of pepper root-stocks

Growth performance of root-stock materials under M. incognita infection conditions

In the pre-experiment period to 60 days after nematode inoculation, the plant height growth of the materials was not statistically different. The closer to the post-growth stages of the materials, the greater the difference. After 90 days of the experiment until the end of the experiment, HUIB_PH30 had superior plant height growth and was significantly different from the other 4 materials. The remaining materials grew the tree height at the same rate.

Resistance to the nematode M. incognita of root-stock materials

No nematodes appeared in the media, in the roots of HUIB_PD36, showing very high resistance to *M. incognita* nematodes. The material HUIB_PH30 showed only nematodes in the substrate with a very low

density of 6.00 pcs/100 g of soil, no nematodes in the roots and no nodules in the roots. This material proves to be highly resistant to nematodes. The remaining three materials have very poor resistance to nematodes, making them unsuitable as root-stock for pepper (Table 3).

Table 3. Nematode density and percentage of root nodules of root-stock materials

Name of materials	The local name of the materials	Nematode density		The proportion of swollen roots (%)
		In soil (lavae/100 g soil)	In root (lavae/5 g root)	
HUIB_PN105	Dak Nong local pepper	15.33^{b}	57.00^{a}	58.7^{a}
HUIB_PH30	Pepper with round leaves	6.00^{c}	0.00^{b}	0.00^{c}
HUIB_PN45	Loc Ninh	20.67^{ab}	43.00^{a}	53.28^{ab}
HUIB_PD36	Forest pepper Nam My	0.00^{d}	0.00^{b}	0.00^{c}
HUIB_PN27	Vinh Linh	25.67^{a}	53.00^{a}	50.07^{b}
CV%		**10.39**	**11.47**	**6.84**
F		**	**	**

3.5.2. Evaluation of successful grafting and conjugation ability of graft combinations

Evaluation of growth ability and survival rate of grafted plants

Loc Ninh grafted tops proved to be incompatible with all 3 rootstocks, so the survival rate after grafting was much lower. Vinh Linh grafted tops have the best compatibility with a very high survival rate and the best growth rate in height and number of leaves of grafted shoots. Rootstock HUIB_PH30 is well compatible with all 3 types of grafted tops Vinh Linh, Sri Lanka, and India with a good survival rate and height growth, a good number of leaves.

Evaluation of the ability to conjugate by microsurgery

The anatomical results show that the size dissimilarity of the root-stock and the graft has a great influence on the survival and growth rate of the graft complex. Vinh Linh grafted tops have the best combination, followed by Indian grafts. Srilanka grafted tops are quite large, so the ability to adapt is limited. Meanwhile, Loc Ninh grafts have incomplete fusion, forming many dead cells, this may be because the grafts are stem wires, so they will have a large diameter and age (Figure 8).

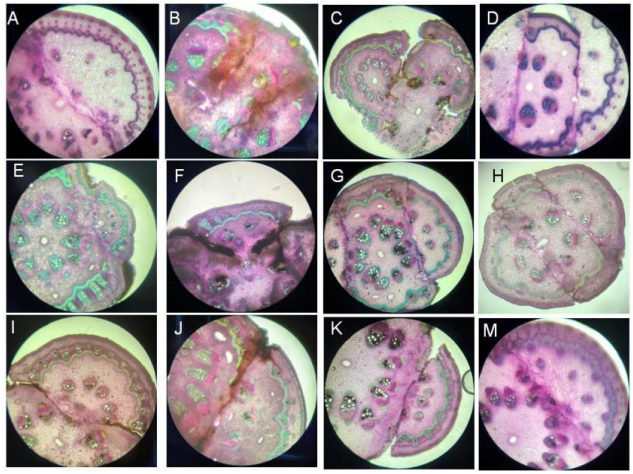

Figure 8. Compatibility of root-stocks HUIB_PH30, HUIB_PD36, HUIB_PH46 with root-stocks Vinh Linh (A), Loc Ninh (B), Siri Lanka (C), India (D)

In which: A: HUIB_PH30 - Vĩnh Linh, B: HUIB_PH30 - Lộc Ninh, C: HUIB_PH30 - Sri Lanka, D: HUIB_PH30 - Ấn Độ, E: HUIB_PD6 - Vĩnh Linh, F: HUIB_PD36 - Lộc Ninh, G: HUIB_PD36 - Sri Lanka, H: HUIB_PD36 - Ấn Độ, I: HUIB_PH46 - Vĩnh Linh, J: HUIB_PH46 - Lộc Ninh, K: HUIB_PH46 - Sri Lanka, M: HUIB_PH46 - Ấn Độ

3.5.3. Evaluation of the resistance to the nematode of the grafted pepper combinations

Growth of graft combinations under nematode infection

The shoot height of the grafted combinations before infection was similar, ranging from 10.39 to 12.72 cm. The growth rate of leaves of the grafted combination HUIB_PH30 - Vinh Linh was the best at 1.44 leaves/month, followed by the grafted combination HUIB_PH30 - India (1.22 leaves/30 days), HUIB_PH30 - Vinh Linh (1.17 leaves/30 days). The rest of the grafts have a rather slow leaf growth rate.

Resistance to nematode of graft combinations

The results of monitoring the density of nematodes in the soil, roots, and the percentage of swollen roots showed that there were no harmful nematodes in the soil and in the roots, so there were no swollen roots on all grafts. This proves that the grafted combinations have very good resistance to nematodes.

3.6. Evaluation of growth and development ability of nematode-resistant pepper grafted plants under greenhouse conditions

Height growth moves

After 30 days of growing in the greenhouse, the plant height growth of the varieties was different, the highest was M1 36-VL (21.10 cm), the lowest was M4 36-AD (0.00 cm). In the period after 60-90 days

of planting, the height growth rate in 2 varieties M1 36-VL and M5 30-VL was the greatest. While at 120 days, this remarkable growth was in 3 varieties reaching M5 30-VL, M3 36-SR, and M1 36-VL.

Leaf growth moves

After 30 days of growing in the greenhouse, the varieties increased by 1.00 - 4.40 leaves and developed rapidly in the next stages. In the period after 60-120 days, the highest leaf growth was M1 36-VL with 18.53 leaves and the least leaf growth was M4 36-AD with 4.00 leaves. In addition, leaf color is also different between varieties, most varieties have purple new leaves, except for M7 30-SR and M3 36-SR varieties which are green.

The move to increase the number of burnings

The number of burnings in the 30 days after release of the greenhouses of different varieties was statistically significant, the highest was M1 36-VL (4.60 nodes), and the lowest was M7 30-SR, M6 30-LN. and M4 36-AD (1.00 burn). The variety with the fastest growth rate at 60 days to 120 days after planting was M1 36-VL with 18.53 nodes, and M4 36-AD with the lowest number of nodes at 6.60. In addition, two varieties M5 30-VL and M3 36-SR also showed great growth in the number of nodes after 120 days.

Growth in the number of branches

Most varieties only grow 1 branch after 30 days of growing outside the carrier, except for M5 30-VL (increase of 2.4 branches). The variety with the fastest growth rate at 60-120 days after planting outside the greenhouse was M1 36-VL with 5.40 branches and M5 30-VL with 4.8 branches. Meanwhile, M6 30-LN has the lowest number of branches.

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusions

- The phylogenetic tree divided the population of 39 pepper lines/varieties into two groups. Group I consisted of 5 individuals HUIB_PH30, HUIB_PH46, HUIB_PD36, HUIB_PR41, and HUIB_PR48. Group II includes the remaining 34 individuals.

- Two varieties of pepper with resistance to nematodes (HUIB_PD36 and HUIB_PC30) have been selected. These two varieties have good water-logging tolerance.

- BSA technique has identified 2 DNA segments (1450bp and 300 bp) associated with nematode resistance from 2 RAPD markers, UBC#360 and UBC#408. Based on the 300 bp sequence amplified by primer UBC#360, a SCAR marker (30 - 360F1R2) was developed that was tightly linked to nematode resistance.

- The results of the evaluation of flowering characteristics of *P. nigrum* showed that the lengthening period ranged from 14.0 to 22.1 days; the time of flower differentiation lasted from 8.1 to 17.3 days; there was a difference between the materials and the distance between different varieties of pepper was from 1.7 to 7.6 days. When crossing between two species *P. nigrum* and *P. divaricatum*, the fruit set rate and the germination ability of the hybrid seeds were very low.

- Anatomy results show that Vinh Linh grafts have the best combination with 2 types of root−stocks. Root−stock HUIB_PH30 is well compatible with all 3 types of grafted tops Vinh Linh, Sri Lanka, and India with good survival rate and height growth, good number of leaves. In addition, the graft combinations showed good resistance to nematodes.

- Evaluation of the growth and development ability of nematode-resistant pepper grafted plants in greenhouse conditions showed that two grafting combinations, HUIB_PD36 - Vinh Linh and HUIB_PH30- Vinh Linh, grew the best.

2. Recommendations

- Develop more molecular markers (AFLD, SSR,...) and sequence gene regions in pepper lines/varieties to find nematode resistance genes in pepper.

- Continue to monitor the growth and development of grafted combinations in greenhouse conditions. At the same time, two graft combinations HUIB_PD36 - Vinh Linh and HUIB_PH30 - Vinh Linh were planted in the field to monitor and evaluate disease resistance, waterlogging resistance and growth and development.

LIST OF PUBLISHED ARTICLES

1. **Sonexay Rasphone**, Long Thanh Dang, Nhi Thi Hoang Ho, Co Quang Nguyen, Hai Thi Hong Truong. Phylogenetic analysis of black peper (*Piper* spp.) population collected in different locations of vietnam based on the ITSu1-4 gene region. *Research Journal of Biotechnology*, 2022, Vol. 17(7). 1-9.
2. **Sonexay Rasphone**, Nhi Hoang Thi Ho, Long Dang Thanh, Bao Le Quy Nguyen, Hai Thi Hong Truong. Genetic diversity analysis of black pepper (*Piper nigrum* L.) by RAPD marker. *Hue University Journal Science: Nature science*, 2022, Vol. 131, No. 1D, 49-59.
3. Hai Thi Hong Truong, **Sonexay Rasphone**, Bao Le Quy Nguyen, Han Ngoc Ho, Co Quang Nguyen, Tu Thi Tran, Thao Xuan Hoang, Thuy Thanh Duong. Identification of *Piper* species that are resistant to *Phytophthora capsici*, *Meloidogyne incognita* and waterlogging in Vietnam. *Plant Pathology*, 2023.
4. Truong Thi Hong Hai, Nguyen Quang Ngoc, Duong Thi Oanh, **Sonexay Rasphone**. Khao sat dac diem ra hoa va buoc dau lai tao mot so giong ho tieu (*Piper* spp.) o Viet Nam. *Vietnam journal of Agriculture and Rural development, 07/2023*.