RPF III

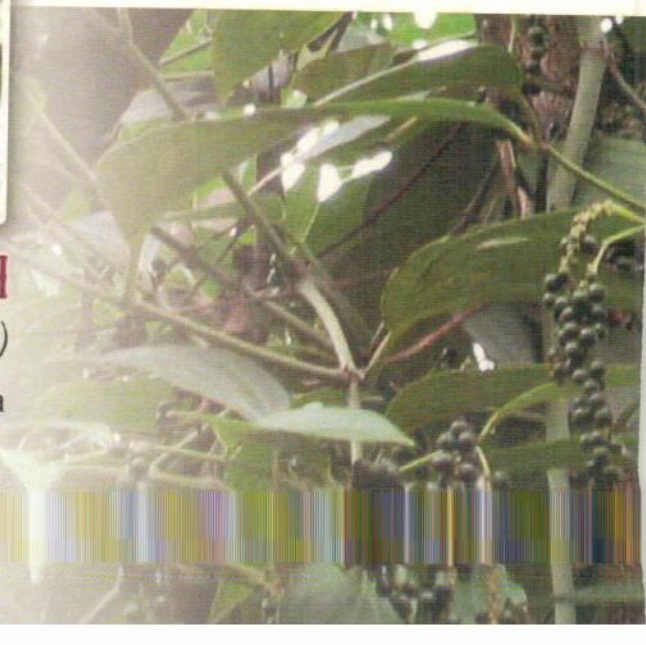
FINAL REPORT

Path XII (813)
IDENTIFICATION OF BLACK PEPPER GENOTYPES WITH MULTIPLE RESISTANCE AGAINST PHYTOPHTHORA & NEMATODES

2006- 2009

Submitted by

Dr. R. Suseela Bhai
Dr. S J Eapen &
Dr. Rashid Pervez



INDIAN INSTITUTE OF SPICES RESEARCH
(Indian Council of Agricultural Research)
Calicut - 673012, Kerala

Path XII (813) IDENTIFICATION OF BLACK PEPPER GENOTYPES WITH MULTIPLE RESISTANCE AGAINST *PHYTOPHTHORA* & NEMATODES

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PB. NO.1701, Calicut-673 012, Kerala

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CONTENTS

PART - I: GENERAL INFORMATION

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800 Project Code :

8002 ICAR Project Code No. : Path XII (813)

801 Name of the Institute and Division :

8011 Name and address of Institute : Indian Institute of Spices Research, Calicut- 673 012, Kerala

8012 Name of Division/Section : Crop Protection/ Pathology

8013 Location of the Project : Indian Institute of Spices, Calicut

802 Project Title :

IDENTIFICATION OF BLACK PEPPER GENOTYPES WITH MULTIPLE RESISTANCE AGAINST *PHYTOPHTHORA* & NEMATODES

803 Priority Area : 01, 03 & 04

8031 Research Approach :

Applied Research	Basic Research	Process/Technology development	Transfer of Technology
01	02	03	04

804 Specific area : Spices Research

805 Duration of Project : 3 Years

8051 Date of start of project : 2006

8052 Date of completion of project : 2009

8053 Period for which report submitted : 2006-2009

806 Total cost of the project/ **Rs. 10, 09, 550**

Expenditure incurred : **Rs. 6, 16, 262**

(Give reasons for variation, if any from original estimated cost)

As estimated in the proposal, glass wares were not purchased and requirement was met from the existing stock. Chemicals were purchased to the minimum. No travel expenditure was incurred. The item 'under heading 'others' were also not spent.

807 EXECUTIVE SUMMARIES

Identification and utilization of resistance to *Phytophthora capsici* in black pepper is the most cost effective method for a viable management strategy against *Phytophthora* foot rot disease, the most burning problem in black pepper. To identify source of resistance from cultivated and germplasm accessions of black pepper, different inoculation methods were compared for assessing the reaction of cultivars/accessions to *P.capsici*. Attempts were also made to develop a suitable screening strategy by integrating the reactions of leaf, stem and root to identify the resistance. There were significant differences in reaction of black pepper lines towards *P. capsici* among the various inoculation methods and the study has proved that a single method of inoculation is not sufficient to rate the cultivars. Based on the results obtained in the study, a modified protocol for screening was developed that consists of initial root inoculation of 3-month old rooted cuttings (having 3-4 leaf stage) with 5 numbers of 5 mm mycelial plugs of *P. capsici* having a zoospore spore load of 10^5/plug. The accessions/ cultivars that survived after 100day of inoculation were further evaluated for resistance by subjecting them to intact stem and leaf inoculation (aerial inoculation). 72h after inoculation, the external stem lesion length and leaf lesion diameter was indexed on a 0-4 scale. The disease severity index of the stem and leaf was calculated from the above indices and the accessions were ranked as Resistant, Moderately resistant and, Susceptible.

Based on the new protocol, 11,632 seedlings raised from 30 cultivars, 42 hybrids and OP selection were subjected for preliminary screening. Forty progenies (including 21 Karimunda progenies, 10 progenies from other cultivars, 8 progenies from hybrids and one progeny from the selection IISR Shakti) that took no infection in the preliminary screening against *P. capsici* were multiplied vegetatively and subjected to second round of screening adopting root and aerial inoculations and assessed for disease severity after 100 days of inoculation. The progenies were rated initially based on the time taken for mortality and then assayed for aerial infection and final score was based on average disease severity index. Of the 11632 progenies screened, one progeny viz. 04-P24-1 showed resistant reaction towards *P. capsici*

and one hybrid progeny viz. 04-HP -400-1 showed moderate resistant reaction. Besides, another progeny 04-HP 1533(2) showed resistance towards the root knot nematode *M. incognita*. However, in the present screening, all the shortlisted progenies (found resistant to *P. capsici)* were found susceptible to *R. similis.* The resistant progeny 04-P24-1 consistently showed resistant reaction even after repeated inoculations and is under field evaluation since 2006. This progeny is identified as a *Phytophthora* resistant source in black pepper (*Piper nigrum* L.) in addition to the existing moderately resistant IISR Shakti. The mod. resistant progeny 04-Hp-400-1 showed stunted growth and found not suitable for multiplication and further evaluation. The modified protocol can be used for screening OP progenies and rooted cuttings of cultivars and hybrids of black pepper.

Eleven wild accessions viz. Acc. 5225 (*P. sp.*), Acc.3177 (*P. sylvaticum*), Acc. 4381 (*P. sarmentosum*), Acc.3357, Acc.692, Acc. 4381, Acc. 3030, Acc. 3362, Acc. 3296, Acc.611 and Acc. 612 were evaluated for *Phytophthora* resistance using root inoculation technique. Among the eleven accessions, two accessions viz. Acc.3177 (*P. sylvaticum*), and Acc.3362 (*P. ornatum*) showed resistance towards *Phytophthora* and also towards *M. incognita* and *R. similis*.

Of the 63 hybrid black pepper lines screened, four hybrids viz. HP 449, HP 490 and HP 521 showed moderate resistance and one hybrid viz. HP 1375 showed resistance in the second round screening. But after field planting the 3 moderately resistant hybrids HP 449, HP 490, HP 521 succumbed to infection whereas **HP 1375** continued to show resistance.

In field evaluations, except **C 1090, n**one of the cultivars screened showed resistance to *Phytophthora capsici* during the period. **C 1090** was found promising in field evaluations and is under multilocation trial in AICRP centers. Among the nematode resistant lines HP 39 and C 820 are found promising and are also under multilocation trial in AICRP centers.

808 Key words: Keywords: Accessions, Black pepper, Foot rot, inoculation methods, *Meloidogyne incognita, Phytophthora capsici, Piper nigrum, Radopholus similis* Resistance, Screening,

PART II: INVESTIGATOR'S PROFILE

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810 Principal Investigator :

810	**Principal Investigator**	:	
8101	Name	:	**R. Suseela Bhai**
8102	Designation	:	Senior Scientist
8103	Division/Section	:	Crop Protection/Pathology
8104	Location	:	Calicut
8105	Institute Address	:	Indian Institute of Spices Research, Calicut 673 012

811	**Co-investigator**:		
8121	Name	:	**Santhosh J Eapen**
8122	Designation	:	Senior Scientist
8123	Division/Section	:	Crop Protection/Nematology
8124	Location	:	Calicut
8125	Institute Address	:	Indian Institute of Spices Research, Calicut, Kerala, 673 012

812	**Co-investigator**:		
8121	Name	:	**Rashid Pervez**
8122	Designation	:	Senior Scientist
8123	Division/Section	:	Crop Protection/Nematology
8124	Location	:	Calicut
8125	Institute Address	:	Indian Institute of Spices Research, Calicut, Kerala, 673 012

PART III: TECHNICAL DETAILS

820 Introduction and Objectives

8201 Project objectives

1. To develop a new screening methodology for evaluating *Phytophthora* resistance in black pepper by integrating the reaction of leaf, stem and root.
2. To identify multiple resistant genotypes of black pepper against *Phytophthora capsici, Meloidogyne incognita* and *Radopholus similis* from short listed lines.

8202 Back ground information and importance of the project

Foot rot disease of black pepper, caused by *Phytophthora capsici* Leonean, is one of the major limiting factors of black pepper production in all the pepper growing countries of the world. The disease takes a heavy toll when soil moisture and low temperature prevail and is destructive where heavy rainfall continues over a period of 2-3 months as in Kerala (Anandaraj, 2000). Several technologies are available to manage the disease through chemical, biological and integrated means (Ramachandran *et al.*, 1988, 1991; Anandaraj and Sarma, 1995). But it is imperative to search for source of resistance for an effective and long term control of the disease. Unfortunately, despite numerous screening tests conducted, no source of complete resistance to *P. capsici* has been identified in black pepper. Earlier workers identified some resistant types by adopting different methods of screening (Holiday and Mowat, 1963; Turner, 1971; 1973; Anonymous, 1977; Sarma *et al.*, 1982, Suseela Bhai *et al.* 2007). Holiday and Mowat (1963) adopted both leaf and stem inoculation technique. They used percentage of root necrosis and number of plants wilted as the criteria for measuring resistance/ tolerance of cultivars. Turner (1971 & 1973) used zoospore suspension as inoculum and adopted percentage root necrosis as the criteria for assessing degree of tolerance and reported *Piper* species such as *P. colubrinum* and *P. obliquum* as resistant. *P. guinense* was also reported as resistant to the foot rot pathogen using root inoculation method (Anonymous, 1977). Later Sarma *et al.* (1982) screened 41 cultivars and 73 wild *Piper* species against *P. palmivora* adopting root dip inoculation technique. By this method Narayakodi, Kalluvally, Uthiaramkotta and Balankotta showed low percentage of infection as compared to others and none of the wild species showed resistance in the screening. However, in all these screening tests, the degree of resistance was assessed based on a single inoculation method. Moreover there is no consistency in stem or leaf lesion size between

inoculations. Since all parts of black pepper are vulnerable to infection and the root/collar infection is more crucial as it leads to the death of the vine, a methodology of screening by integrating the reaction of leaf, stem and root is expected to provide an overall assessment of the reaction of black pepper to *P. capsici*.

Slow decline is caused by the complex association of nematodes and *Phytophthora* sp. Both *M.incognita* and *R. similis* are involved in slow decline disease. Cropping system and microclimate appear to be the deciding factors in the incidence and spread of the disease. Since there is no spatial segregation of *Phytophthora* and nematodes in the soil under field conditions, identifying source of resistance individually against *P. capsici* or nematodes will not be sufficient to manage the disease.. Hence greater priority is to identify source with multiple resistance/ tolerance to P. *capsici, M.incognita* and *R. similis* among cultivars, accessions or wild types. The work done so far at IISR on identification of source of resistance has short listed a few numbers of accessions tolerant to *Phytophthora capsici* and a few accessions tolerant to *Meloidogyne incognita* and *Radopholus similis*. The present project aims to identify from among the short listed ones, the cultivars or accessions having multiple resistances to both *Phytophthora* and nematodes and also to identify resistant sources from open pollinated progenies by developing a modified screening protocol.

821 Project Technical Profile:

8211 **Technical Programme**

(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments etc.)

1. Development of a new screening methodology for evaluating *Phytophthora* resistance in black pepper (*Piper nigrum* L.) by integrating the reactions of leaf, stem and root.

II. Screening shortlisted cultivars / selections against *Phytophthora* and nematodes

III. Selection from Open pollinated seedlings for *Phytophthora* and nematode resistance

IV. Field Evaluation of promising *Phytophthora* and nematode resistant/moderately resistant lines

The procedure adopted for fulfilling the technical programme were

1. Development of a new screening methodology for evaluating *Phytophthora* resistance in black pepper (*Piper nigrum* L.) by integrating the reactions of leaf, stem and root.

Studying the sporulation and infectivity potential of the pathogen

Since studying the infection potential of the target pathogen *i.e. P. capsici* is a pre-requisite for any of the artificial inoculation studies including screening, the first step of the experiment consisted of estimating the sporulation and infectivity potential of *P. capsici*. This includes preparation of inoculum, studying the sporulation potential and infectivity of the isolate used in screening.

Studies on sporulation potential of Pathogen

Pure culture of a virulent isolate of *P.capsici* (IISR 02-56) was isolated from foot rot affected black pepper (*Piper nigrum* L.) collected from IISR Experimental Farm, Peruvannamuzhi using selective PVPH (Tsao and Guy, 1977) medium. This was sub cultured into freshly prepared carrot agar (CA). This culture was used as the pathogen inoculum throughout the studies.

Sporulation

P.capsici was grown in CA at 24°C for 72h in the dark. Mycelial plugs of 3mm and 5mm were cut from the edge of 72 h old colony and immersed in sterile distilled water under constant fluorescent light. Sporangial production was observed at different intervals such as 24, 48 and 72h under the microscope at 100X magnification. Sporangial count was recorded from 15 discs at random and from each disc 10 microscopic fields were counted (Charles *et al.* 1995; Zhang Songlin, 1988). Altogether 150 observations were recorded for each size of the disc and calculated the sporulation potential (Table 1).

Zoospore production and germination

Zoospore production was calculated from inoculum plugs as described above after cold shock treatment. The sporulated inoculum plugs of 3mm and 5mm were taken in a Petri dish individually, immersed in sterile distilled water and incubated in refrigerator at 4°C for 10 -30 min (cold shock treatment). This was taken out and kept at room temperature for 10 min for zoospore release. After complete release of zoospores, the zoospore suspension was strained through two layers of cheese cloth and counted the number of zoospores using a cell counting hemocytometer (Eva Petrikkou *et al.*, 2000). From the released zoospores, germination count was also recorded after 2h incubation of released zoospores (**Table 1**).

Infectivity of the pathogen

The zoospore suspension having a spore load of $5x10^9$ cfu ml^{-1} was inoculated to rooted cuttings of 3-4 leaf stage (3 month old) plants in polybags @ 1ml, ($5x10^9$zoospores ml^{-1}), 10ml and 20ml. There were 4 replications /treatment. The plants were incubated under

open conditions (temp of 27±1°C). Observations were recorded on the time taken for infection and also the percent infection.

In the experiment to study the disease potential index in the soil required to express the symptoms of *Phytophthora* infection, one kg sterile soil was mixed thoroughly with the spore suspension having a spore load of 5x 10^{11} zoospores. Serial dilutions of this soil was made up to 1/16 fold and baited with leaves of *Albizzia falcataria (*Anandaraj 1990*)* There were four replications for each dilution and the bait infection was recorded after 72h. (Denman *et al.*, 2005).

3. Inoculation methods

Three month old rooted cuttings of a single variety of black pepper was used through out for all the tests unless otherwise specified. Nine different inoculation methods were tested as follows

A) Detached leaf assay

a) Detached leaf inoculation with injury, **b**) Detached leaf inoculation without injury

B) Intact leaf assay

a) Intact leaf inoculation with injury, **d**) intact leaf inoculation without injury

C) Stem assay

a) Stem inoculation with injury, **b)** Stem inoculation without injury

D) Root assay

a) Root drenching with zoospore suspension, **b**) Root /collar inoculation with mycelial plug and c) Root dip in zoospore suspension.(hydroponic method)

A) Detached leaf assay (Inoculation with and without injury)

Detached leaves taken from rooted cuttings of 3-4 leaf stage was used for the test. The third leaf from the top was excised at the base of the petiole, washed in sterile water and blotted dry. Ten leaves each were placed upside down in a plastic box lined with moistened filter paper for creating a moist incubation chamber (Tedford *et al.*, 1990, Denman *et al.*, 2005). The isolate of *P. capsici* was grown in CA for 72h under fluorescent light and was used as the pathogen inoculum. Inoculum plugs of 3mm size were cut using a sterile cork borer from the growing margin of the colony and placed at the centre of the leaf and a moist cotton strip was placed over it to keep the inoculum plug moist. Another set of the same was inoculated with a slight pin prick. The box was closed with a lid and incubated at 24±1°C. Observation on lesion diameter was taken at 24 h interval for 72 hours. The disease severity

of the leaf was scored using a 0- 4 scale as 0 = no lesion, 1-5mm lesion diameter, 2= 6-10mm, 3= 11-15 mm and 4= >15mm.

B. Intact leaf assay

Intact plants (rooted cuttings of 3-4 leaf stage) raised in sterile potting mixture (1:1:1) in polythene bag of size 21cm x 15cm were used for the experiment. Plants were divided into two sets. One set was inoculated with injury by giving a slight pin prick on the lower surface and another set without injury. Inoculum plugs of 3 mm were placed on the lower surface of the leaves in both cases. A moist cotton strip was placed over the inoculum plug and was kept in position by pasting a cello tape over it. Twenty plants each were inoculated. One set of 10 plants each was placed in the greenhouse maintaining a temperature of (19±1°) C and the other set was kept in open shed having an ambient temperature of 27-28°C. Observation on leaf lesion diameter was recorded daily for 72h. and DSI was calculated as above

C. Stem assay

Intact plants were used for inoculation. For this method, the third internode from the top of the plant was inoculated on the stem. One set was inoculated with injury and another set without injury. In the injured set, a pinprick was made on the stem and inoculated with culture disc of 3 mm size of *P. capsici* taken from the edge of a 72h old actively growing culture and covered with wet cotton wad and later tied with polythene strip to keep it continuously wet. The other set was inoculated as above without injury. After 72h, the cotton wad and polythene strips were removed and measured the lesion length. The infected stem portion was cut open longitudinally and the depth of penetration (Index) was assessed visually (as indicated by the brown coloration inside) and scored using a 0- 4 scale as 0 = no lesion/ penetration, 1= up to 25% penetration, 2= 26-50% penetration, 3= 51-75% penetration and 4= >75% penetration (Sarma *et al.* 2000). Based on the depth of penetration, the disease severity index (DSI) of the stem was calculated using the formula described above. The disease severity of the stem was also calculated as done for leaf by scoring using a 0- 4 scale as 0 = no lesion, 1-5mm lesion diameter, 2= 6-20mm, 3= 21-30 mm and 4= >30mm and calculated the DSI.

D. Root assay

Root inoculation was done in three methods using intact plants.

a) The plants were moistened thoroughly and 5 numbers of inoculum plugs of 5mm diameter were placed just beneath the collar portion encircling it and covered with soil. The observations on collar and root infection/mortality were recorded from 7th day onwards.

b) Zoospore suspension was prepared as described elsewhere. The zoospores were counted using hemocytometer and 10ml of this zoospore suspension (containing app.10^{10} spores ml^{-1}) was inoculated to the base of the plants. The observations were recorded on mortality of the plants from 7^{th} day onwards (Tedford, 1990).

c) Hydroponics method of root inoculation

Inoculum plugs of 5mm size were kept for sporulation under continuous fluorescent light for 48h and then given cold shock treatment for 10 min. These plugs were immediately transferred to sterile bottles containing 200ml of sterile distilled water. Rooted cuttings of 3-4 leaf stage were uprooted from polythene bags without disturbing the root system and thoroughly washed in running tap water to remove the soil and then rinsed with sterile water. These plants were immersed into the above bottles and incubated at 25°C for 72-96 h. The colour change occurred in the water in 72 -96h and this water was analyzed for total phenol using Folin-Ciocalteau reagent method and total carbohydrate by anthrone method (Sadasivan and Manikam, 1991). For comparison five different varieties viz. IISR Subhakara, IISR Shakti, IISR Thevam, Panniyur 1and Panniyur- 2 were used.

4. Modifying the screening methodology

Based on the results of the nine different inoculation methods, a modified protocol was designed for ranking the varieties integrating different methods. A population of 30 plants were evaluated. Initially three month old rooted cuttings having 3-4 leaf stage were raised in sterile potting mixture in Polythene bags of size 20x 15cm. These plants were inoculated with mycelial plugs (of 5mm size @ 5 discs /plant) of *P. capsici* and incubated at 24-28^{0}C for 100 days. Plants were moistened regularly to keep the soil moist in order to keep the moist conditions favourable to the development and multiplication of the inoculum. After the stipulated time the plants remained (more than 60%) were subjected to intact stem and leaf inoculation using mycelial plugs of 3 mm cut from the margin of 72h actively growing culture. After 72h of inoculation, leaf lesion was scored on a 0-4 scale as mentioned elsewhere simultaneously the external stem lesion length was also measured and scored on a 0-4 scale. From this, the disease severity (DSI) was calculated for each part using the formula of Kim *et* al., 2000

$$DSI = \frac{\sum (\text{rating of each plant})}{\text{Maximum score x No. of plants rated.}} \times 100$$

Final ranking was based on the time taken for mortality. Those plants that took > 21 days for mortality (root infection manifested as mortality) were assessed for their reaction to aerial infection.

The average DSI of both stem and leaf was taken and the DSI of the whole plant. Based on the DSI and mortality due to root infection, the plants were rated into resistant, moderately resistant and susceptible as given above.

Av DSI (Stem & Leaf)	Reaction
<30%	Resistant
31-40%	Moderately resistant
>40%	susceptible

5. Evaluation of the new methodology

The modified screening protocol was tested among 17 promising lines of black pepper viz, Sreekara, Subhakara, Panchami, Pournami, IISR Thevam, IISR Shakti, C 1090, C 820, HP 780, HP 39, HP 813, HP 105 and Panniyur varieties such as Panniyur1, Panniyur 2, Panniyur 3, Panniyur 4 and Panniyur 5. These varieties were raised in sterilized potting mixture as above and root inoculated. The variety in which more than 60% of the plants survived after 100 day of inoculation were subjected to aerial inoculation as mentioned above and DSI of the whole plant was calculated. The varieties were then ranked based on the score card.

II. Screening short listed cultivars / selections against *Phytophthora* and nematodes

Cultivar accessions were collected from IISR farm Peruvannamuzhi, multiplied in sufficient numbers and screened using stem, leaf and root inoculation method. The accessions falling in the resistant or moderately resistant rating were multiplied and evaluated further.

B. Screening hybrids for *Phytophthora* resistance

63 hybrids short listed as moderately tolerant in the preliminary screening for *Phytophthora* resistance were subjected to root inoculation under artificially created sick plot condition along with a susceptible check Sreekara. The plants were watered regularly to keep the soil moist through out the period. The plants started dying from 4th day onwards. The plants were retained for more than 100 days. There were ten plants for each accession. The mortality of the plants due to root infection was recorded.

C. Screening wild *Piper* species for *Phytophthora* resistance

Eleven wild piper accessions viz. Acc. 5225 (*P. ribusoids*), Acc.3177 (*P.sylvaticum*), Acc. 4381 (P. sermentosa), Acc.3357, Acc.692, Acc. 4381, Acc. 3030, Acc. 3362, Acc. 3296, Acc.611 and ACC. 612 were evaluated for *Phytophthora* resistance using root inoculation technique. The plants were inoculated by root inoculation method and retained for 100days and mortality rate was recorded.

III. Selection from Open pollinated seedlings for *Phytophthora* and nematode resistance

Screening material

Ripened berries were collected from 30 germplasm accessions, 42 hybrids and one *Phytophthora* moderately resistant line viz. IISR Shakti maintained at IISR Experimental Farm, Peruvannamuzhi. Mature seeds were extracted from these and washed in running tap water and sown in sterilized nursery mixture (soil, sand and farmyard manure 1:1:1) in polythene basins of 30cm diameter.

Preliminary screening of OP seedlings

The preliminary screening was carried out during 2004-2005 season. Three month old seedlings (3-4 leaf stage) were inoculated around the roots by drenching with suspension of P. *capsici* zoospores (Sarma *et al.*, 1994). The suspension was added @ 100 ml per basin (having a spore load of 1x 10^6/ml and a germination percentage of more than 75%). The seedlings which did not succumb to infection till four months of inoculation were selected for the second round screening after multiplication.

Second round screening

Multiplication

The selected seedlings were multiplied by serpentine method in polythene bags containing sterilized nursery mixture (Thankamani *et al.*, 2007). Single node cuttings were thus produced in sufficient numbers and grown for three months. These rooted cuttings were used in the second round screening during June–August.

The three month old rooted cuttings raised in polythene bags as above were inoculated with 72h old culture of *P. capsici* in the form of mycelial discs. Five mycelial discs of 10 mm diameter were incorporated into the root zone of plants and observed for infection /mortality. There were ten replications /line. Root /collar infection is manifested as decay of the collar portion or root which extends upwards resulting in the total collapse of the plant. Here the mortality of the plant was taken as the measure of disease severity. The presence of the inoculum in the soil was determined by baiting method (Anandaraj and Sarma

1991). The plants were rated based on the number of days taken for a mortality (Eikemo *et al.*, 2001, 2003, Gevens *et al.* 2006 modified,). Those plants that showed ≤ 40% mortality in > 21 days were tested for aerial infection

Root inoculation

Forty progenies shortlisted in the first round of screening were multiplied and screened by root inoculation with mycelial discs.

Aerial inoculation (Stem and leaf inoculation)

For stem inoculation, a slight pinprick injury was made on the third internode on the stem from the top of the plants and a mycelial plug (5 mm size) of *P. capsici* cut from the edge of a 72h old actively growing colony was placed over it (Sarma *et al.* 1994). The inoculated portion was covered with wet cotton wad to keep the inoculum continuously wet and tied with polythene strip. Simultaneous with the stem inoculation, the third or fourth leaf from the top was also inoculated but without making any injury. The inoculated plants were incubated for 72h at a temperature of 24-25°C with RH 80%-90%. There were ten plants /progeny. After 72 h, leaf lesion was scored as described elsewhere.

***Ranking of progenies* for *Phytophthora* infection**

The initial selection was made based on the root infection assessed by the mortality of plants due to *Phytophthora* infection. The time taken for mortality was taken as criteria for short listing the progenies. Those plants that took > 21 days for mortality was assessed for their reaction to aerial infection and rated as follows.

Av DSI (Stem & Leaf)	Reaction
<30%	Resistant
31-40%	Moderately resistant
>40%	susceptible

Screening against nematode

To study the reaction of short listed plants against plant parasitic nematodes, the shortlisted plants for *P. capsici* were multiplied vegetatively and 10 plants each were planted in sick beds infested with either root knot nematode *M. incognita* or *R.similis*. The root galling and root decay were recoded after 4 months of planting (Ramana and Mohandas 1989)

IV. Field Evaluation of promising lines

Four promising lines viz. 04-P24-1, 04-HP 1533-2 and 04-1533 -3 short listed from open pollinated progeny after three rounds of screening were planted in the sick plot at

Peruvannamuzhi during August 2006. Three replications were maintained with 9 plants/ replication and are under evaluation.

Total man months involvement of component project workers

Suseela Bhai	R.	(2006-2009)	9 man months
Santhosh J Eapen		(2006-2008 October)	9 man months
Rashid **Pervez**		(2008 Oct- -2009)	3 man months
		TOTAL	21 man months

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results

Please See **Annexure 1**

8221 Achievements in terms of targets fixed for each activity:

Target: To identify source of multiple resistance to *Phytophthora* and nematodes

1. Identified one *Phytophthora* resistant OP progeny (04-P24-1)

2. Identified one OP progeny of HP 1533 (04-HP1533 (2) as moderately resistant to *P. capsici* and resistant to *Meloidogyne incognita,* the root knot nematode of black pepper.

3. Two wild accessions viz. Acc.3177 (*P.sylvaticum*), and Acc.3362 (*Piper ornatum*) showed resistance towards *Phytophthora* and also towards both nematodes. However no cultivar line showed multiple resistances.

8222 Questions Answered

1. Whether there are any resistant sources for *Phytophthora* and nematodes among cultivated, hybrid or wild accessions of black pepper?

   Yes, there are resistant source for *Phytophthora* and nematodes among cultivated, hybrid and wild accessions of black pepper.

1. OP progeny (04-P24-1) is resistant to *P. capsici*
2. OP progeny of Hp 1533 (04-Hp1533(2)) is resistant to *Meloidogyne incognita*
3. HP 1375 and C 1530 are found as moderately resistant to *P. capsici*
4. Wild accessions namely *Piper sylvaticum* and *P.ornatum* are found as resistant to *P. capsici*

2. Whether there are any multiple resistant sources for *Phytophthora* and nematodes among wild accessions of black pepper?

Yes, two wild accessions viz. Acc.3177 (*P.sylvaticum*), and Acc.3362 (*Piper ornatum*) showed resistance towards *Phytophthora* and also towards both nematodes.

8223 Process/Product/Technology/Developed

1. Identified one OP progeny, 04-P24-1 (a progeny of IISR Shakti) as resistant to *Phytophthora capsici*

2. Developed a modified protocol for screening black pepper accessions/OP progenies against *Phytophthora.*

8224 Practical Utility (Not more than 150 words)

The new protocol developed can be used for evaluating black pepper accessions /OP progenies for their reaction to *P. capsici* in screening programmes. The presently identified OP progeny and Hybrids can be used as resistant sources against *Phytophthora* foot rot in addition to IISR Shakti, the moderately resistant line released from the Institute. This can also be used in breeding programmes. Mapping population (F2) is also being generated from this progeny. Yield evaluation and multilocation effect, of the identified hybrid and OP progeny can be studied for making use of them in crop improvement programmes.

8225 Constraints, if any : Nil

823 PUBLICATIONS AND MATERIAL DEVELOPMENT

8232 **Popular articles**- Nil

Research article –

"Evaluation of open pollinated progenies of black pepper for resistance to *Phytophthora capsici* and plant parasitic nematodes using a modified protocol" authored by R Suseela Bhai, S J Eapen, M Anandaraj and K V Saji

(Communicated to Indian Journal of Agricultural Sciences)

8233 **Reports**

Annual report 2006 -2007, 2007-2008, 2008-2009 (Attached)

8234 Seminars, Conferences and workshops (relevant to the project) in which the scientists have participated (List of abstracts forwarded)

Nil

824 **Infrastructural facilities developed**

(Details of field, laboratory, note books and final material and their location)

8241. **Infrastructure developed**

Sick bed with brick walls was made for screening *Phytophthora* and nematodes individually in addition to the green house facilities

8242 **Details of field, laboratory books-**

Field books - 3 nos. Work book -1 Registers -3 Nos. Available in pathology section

8243 **Resistant lines /moderately resistant lines:** Resistant line (04-P-24-1) and moderately resistant lines viz. HP 1533(2), Hp 449, Hp 490, HP520 and HP 1375 were planted in the sick plot at Experimental Farm Peruvannamuzhi and at IISR Farm at Chelavoor

825 Comments/suggestions of project leader regarding possible future line of work that may be taken up arising out of this project

So far screening for *Phytophthora* tolerance was aimed to identify resistant sources for *Phytophthora* alone. As the disease is of complex nature where both foot rot and slow wilt are of serious problems, and in most cases, occurring simultaneously, it becomes essential to search sources for multiple resistances to P. *capsici*, *M. incognita* and *R. similis*, among cultivars, accessions or wild types of black pepper genotypes. Accordingly, short listed

hybrids and cultivars were screened and identified few lines which are resistant or moderately resistant to *Phytophthora* and nematodes. Open pollinated seedlings were also screened and identified few progenies which are resistant or moderately resistant to *Phytophthora* and nematodes. However no one variety is found showing multiple resistances to both nematodes and *Phytophthora* except two wild accessions. These identified lines were planted in the field for evaluation. Moreover, a new protocol is developed by integrating the reaction of stem, leaf and root to *Phytophthora* infection which gives a more precise data for resistant reaction. This protocol can be adopted for screening black pepper accessions for *Phytophthora* resistance. The identified promising lines and progenies are to be evaluated in a multilocation trial for their performance.

PART IV: PROJECT EXPENDITURE (Summary)

Year 2006-2009

830 Total Recurring Expenditure

8301 Salaries: (Designation with pay scale)

I) Scientific

Sr. Scientist - 12000-420-18300	: Rs.	3, 60,000
ii) Technical (T3) 4500-125-7000	: Rs.	1, 39. 626
iii) Supporting	: Rs.	52, 476
iv) Wages	: Rs.	40,000
Sub Total	: Rs.	6, 00,262

8302 Consumables

i) Chemicals	: Rs.	8, 000
ii) Glass wares	: Rs.	-
iii) Others	: Rs.	-
Sub Total	: Rs	8,000

8303 Travel	: Rs	-
830 Miscellaneous (Other costs)	: Rs	8, 000
8305 Sub total (Recurring)	: Rs	6, 16,262

831 Total Non-recurring Expenditure
(Equipments & works) : Nil
ii)
iii)

832 Total
(830 and 831) : Rs. **6, 16,262**

PART-V: DECLARATION

This is to certify that the final report of the project has been submitted in full consultation with the project workers as per the approved objectives and technical programme and the relevant records, note books; materials are available for the same.

Signature of the Project Investigator:

R. Suseela Bhai

Co Investigator:

Santhosh J Eapen

Rashid Pervez

Signature and comments of the Head of the Division/Section

All the technical programmes envisaged in the project have been undertaken. All the registers and field books are available in the Division.

S Devasahayam

27.03.2010

Signature and Comments of the Joint Director (Research)

Signature and Comments of the Director

6/4/10

ANNEXURE 1

ACHIEVEMENTS

I. DEVELOPMENT A NEW SCREENING METHODOLOGY FOR EVALUATING *PHYTOPHTHORA* RESISTANCE IN BLACK PEPPER (*PIPER NIGRUM* L.) BY INTEGRATING THE REACTION OF LEAF, STEM AND ROOT

There are significant differences in reaction of black pepper lines towards *P. capsici,* among the various inoculation methods and the study has proved that a single method of inoculation is not sufficient to rate the cultivars. Based on the results obtained in the study, a modified protocol for screening is developed that consists of initial root inoculation of 3-month old rooted cuttings (having 3-4 leaf stage) with 5mm mycelial plugs of *P. capsici.* The accessions/ cultivars that took > 21 days for initial infection and with ≤ 40% mortality after 100day of inoculation were further evaluated for resistance by subjecting them to intact stem and leaf inoculation (aerial inoculation). The disease severity index of the stem and leaf was calculated and the accessions were ranked as Resistant, Moderately resistant and Susceptible as specified under techniques. Unlike in the earlier screening methods, the depth of penetration was not taken into consideration since it gives only an approximate visual scoring which depends on the individual who is scoring. Moreover, it is very difficult to accurately score the actual extent of infection since the total area to be scored is too small. .

1. Studies on sporulation potential of the pathogen

When *P. capsici* culture disc was incubated in sterile distilled water for different intervals from 24-72h, maximum no. of non-dehisced sporangia was obtained at 48 h. Sporulation was very sparse at 24h whereas most of the sporangia dehisced by 72h. So 48h incubation was found to be the optimum period for getting maximum number of non-dehisced sporangia. A single 3mm mycelial plug produced approximately $8x10^2$ sporangia at 48h incubation under continuous fluorescent lighting while a 5mm disc produced around $2x10^4$ sporangia at 48h.

2) Zoospore production and germination

When the sporangia formed at 48h were subjected to cold shock treatment for 10 min at 4°C, zoospores were released in 30 min. A single 3mm mycelial plug produced approximately $2x10^5$ zoospores at 48h incubation under continuous fluorescent lighting from which 75.68% ($1x10^4$) were found germinating. Similarly a 5mm mycelial plug produced around $6x\ 10^5$ zoospores. The Sporulation potential of the pathogen or the quantity of inoculum used for inoculation was calculated based on the size of the sporulated inoculum plug (**Table 1**).

Table-1 Sporulation potential of the inoculum

Size of the inoculum plug	No. of sporangia/mf (100x)	No. of sporangia produced/ inoculum plug (48 h)	*Approx.no. of zoospores produced from the mycelial plug	**No. germinable zoospores
3mm	8.047	$8x10^2$	$2x\ 10^4$	$1x\ 10^4$
5mm	83.973	$2x10^4$	$6x10^5$	$8x\ 10^5$
P=0.05	29.685	$7x\ 10^3$	-	-

* 20-38 zoospores/sporangia; mf- microscopic field

** Percentage germination = 75.68

2. Infectivity of the pathogen

When the plants were inoculated by soil drenching with varying quantities of zoospore suspension *viz.* 1ml, (cfu $5x10^9$/ml), 10ml ($5x10^{10}$) and 20ml ($5x10^{11}$), the symptoms appeared on the third day at higher concentrations as blackening of the collar portion. This is followed by wilting and on the 12th day the whole plant collapsed (**Fig 1**). At lower concentration the symptoms appeared on the 7th day and the plants collapsed in 14-17 days. The difference in disease expression varied with varieties and concentration of the zoospores. Complete mortality was observed in Subhakara even with the lowest concentration whereas it was 50% in Panniyur 2 and zero in IISR Shakti with the lower concentrations. Only 25% of IISR Shakti took up infection at the highest concentration tested (**Fig 1**). So it is clear from the experiment that infection of the cultivar/accession is directly related to the amount of inoculum as well as the susceptibility of the plant. The disease

potential index of the soil inoculated with $5x10^{11}$ zoospores was found to be 8 in the baiting tests. In this DPI, 100% mortality was shown by highly susceptible lines. Hence it is found that a DPI of 8 obtained from a zoospore suspension of spore load $5x\ 10^{11}$ is sufficient to get 100% mortality in a susceptible variety at the 3-4 leaf stage in 12 days.

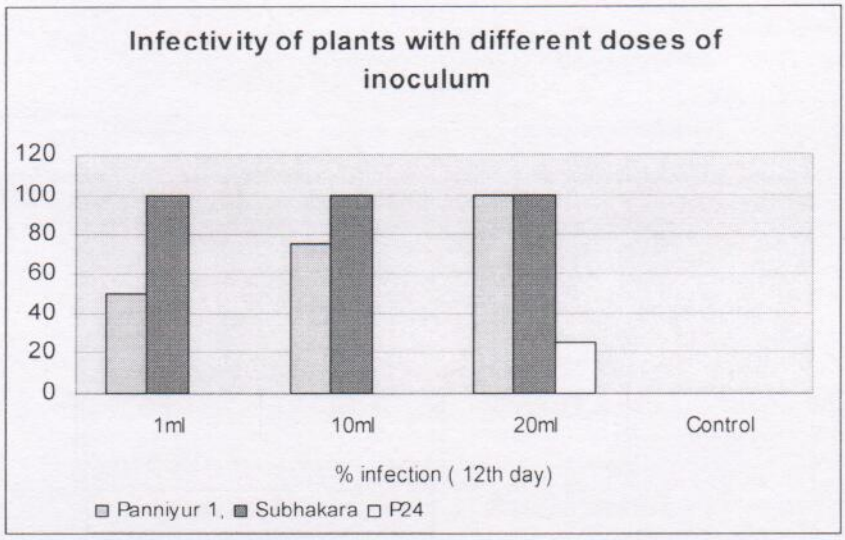

Fig.1 Infectivity of *P.capsici* in different varieties of black pepper vs. quantity of inoculum

3. Inoculation methods

A) Detached leaf assay (Inoculation with and without injury)

When the detached leaves were inoculated with or without injury, lesion development was initiated at 24h. There was a clear cut difference in lesion formation between injured and non-injured leaves and the difference also varied with varieties. Size of the lesion was comparatively larger in injured leaves when compared to non-injured leaves. Similar difference was noticed very clearly at 72h incubation. Nine different varieties were compared to confirm the results (**Table 2, Fig 2**).

In all the varieties distinct difference was noticed between lesions formed with injury and without injury. An average of 5mm lesion size difference was noticed between two types of inoculations. However much difference was not observed in Sreekara and IISR Shakti. Hence it is advisable to do inoculations without injury because injury using pinpricks vary with hands. The difference between two inoculations in different varieties is shown in Fig 2

Table-2 Reaction of detached leaves of different varieties of black pepper to *P. capsici*

Variety/Method	24h (Lesion size mm)			48h (Lesion size mm)			72 (Lesion size mm)		
	With injury	Without injury	mean	With injury	Without injury	Mean	With injury Leasion dia(mm)/score	Without injury	Mean
Panchami	0.5	0.3	0.40	2.1	1.7	1.9	7.8	4.	6.3
Pournami	0.7	0.01	0.35	2.7	0.0	1.35	5.5	2.5	4.0
Panniyur 2	2.2	0.5	1.35	5.0	2.1	3.55	11.0	5.3	8.15
IISR Thevam	1.9	0.6	1.25	7.0	3.6	5.3	15.1	9.3	12.2
IISR Shakti	1.6	2.5	2.05	4.1	5.8	4.95	7.7	8.9	8.3
Malabar excel	2.6	0.9	1.75	6.0	4.9	5.45	14.	10.3	12.55
Hp 105	2.5	0.1	1.275	7.6	3.9	5.75	17.1	9.0	13.05
Sreekara	1.4	1.2	1.30	4.7	4.5	4.6	10.25	9.3	9.775
Subhakara	1.1	0.0	0.550	6.3	1.4	3.85	13.	4.2	8.65
CD at alpha 0.05%	0.2166		0.6850	0.6007		1.900	1.097		3.470

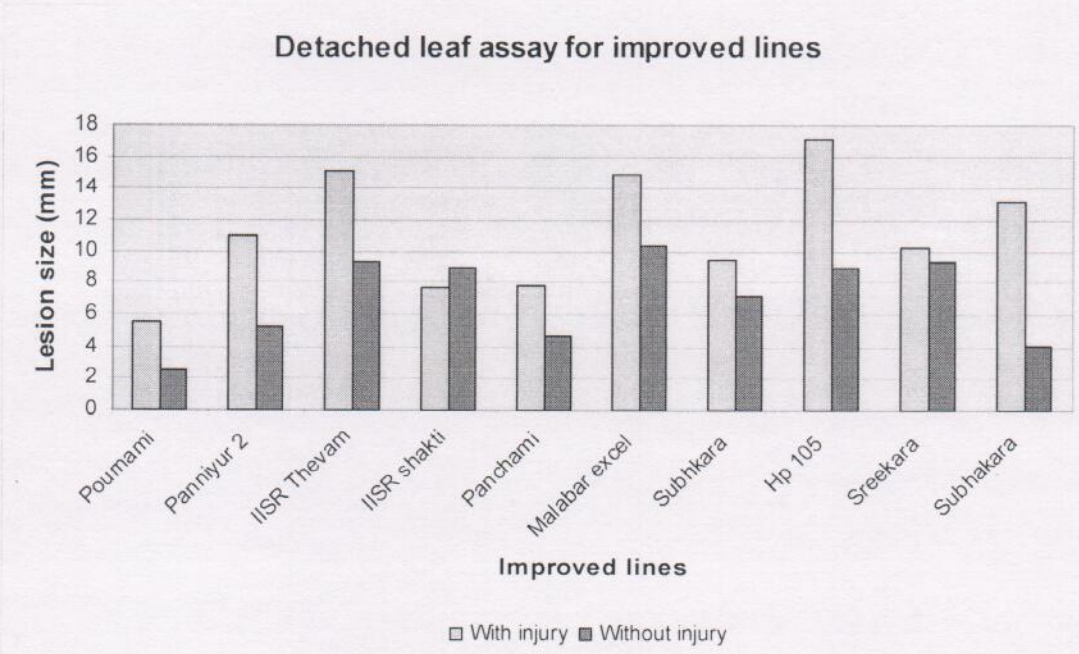

Fig 2 Detached leaf assay for improved accessions

B. Intact leaf assay

When intact plants were leaf inoculated with and without injury and incubated under open conditions, the symptom developed in 24h in injured plants when compared to uninjured plants, which developed symptoms in 48h only. But under greenhouse conditions, lesion formation occurred in injured set in 24h whereas the non-injured set developed lesions only in 72h. Significant difference could be noticed between the two methods of inoculation under two different incubation conditions. The data showed that infection is quicker with injury and is more realistic under natural conditions without injury than under artificial or greenhouse conditions (Table 3).

Table 3 Intact leaf assay under different temperature (Green house/Open conditions)

Incubation conditions/ Vs. method	Lesion size in mm								
	24h			48h			72h		
	With injury	Without injury	Mean	With injury	Without injury	Mean	With injury	Without injury	Mean
24-28°C	2.1	0.0	1.05	5.5	3.1	4.3	12.7	8.6	10.65
17-20 °C	1.9	0.0	0.95	4.8	0.20	2.5	7.2	2.0	4.6
Mean	2.0	0.0		5.15	1.65		9.95	5.3	
CD	0.54			1.35			2.16		

C. Stem assay

When stem inoculation was given to intact plants with and without injury, either under open or greenhouse conditions, it was found that stems of non- injured plants showed no lesion formation whereas the injured ones showed well developed lesions within 72h. The depth of penetration into the cortical area and the length of lesion were also taken into consideration. Here penetration was found easier with injury under both incubation conditions (Table 4). But assessment of depth of penetration by visual scoring will not be accurate always. Hence it is advisable to measure the lesion length rather than taking the depth of penetration which always varies with observer.

Table 4 -Stem inoculation with intact plants

Incubation condition	Stem Lesion (mm)		Index	
	With injury	Without injury	With injury	Without injury
24-28°C	12.0	0.0	2.5	0.0
17-20 °C	7.0	0.0	2.4	0.0
Mean	9.5	0.0	2.45	0.0
CD for mode of inoculation for index = 0.34 (P=0.05)				
CD for type of incubation = 2.36 (P=0.05)				

D. Root assay

Root inoculation with mycelial plug or zoospore suspension resulted in collar and root infection finally leading to the death of the plants. With zoospore suspension the variety Subhakara showed collar infection on the 5th day of inoculation and by 14th day 71% of the plants had perished or collapsed. While with mycelial plug inoculation, the symptom appeared on the 10th day. But in both the cases, the plants showed collar and root infection in susceptible variety irrespective of the nature of inoculum. IISR Shakti showed no symptom of collar or root infection till 28th day of inoculation. This was tested in 9 improved lines and found that > 80% of plants in Subhakara got infection in 7-14 days followed by Sreekara and Pournami, whereas at the same time Panchami showed only <10% infection . Panniyur 2, Thevam, IISR Shakti and Girimunda showed almost same reaction (30% infection) (Fig 3 & 4).

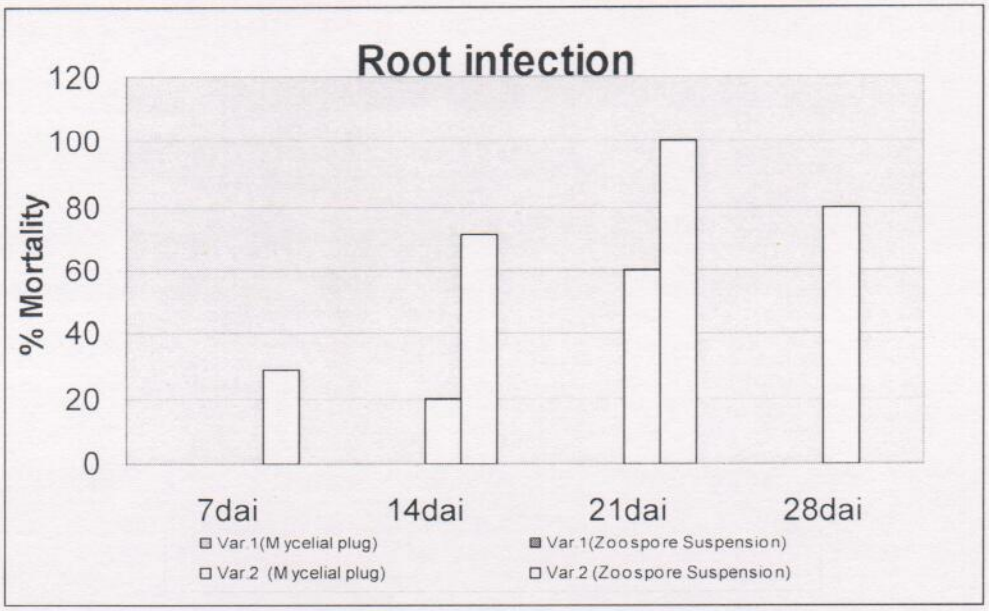

Fig 3. Mortality (%) of plants by inoculation with mycelial plug and zoospore suspension at different intervals

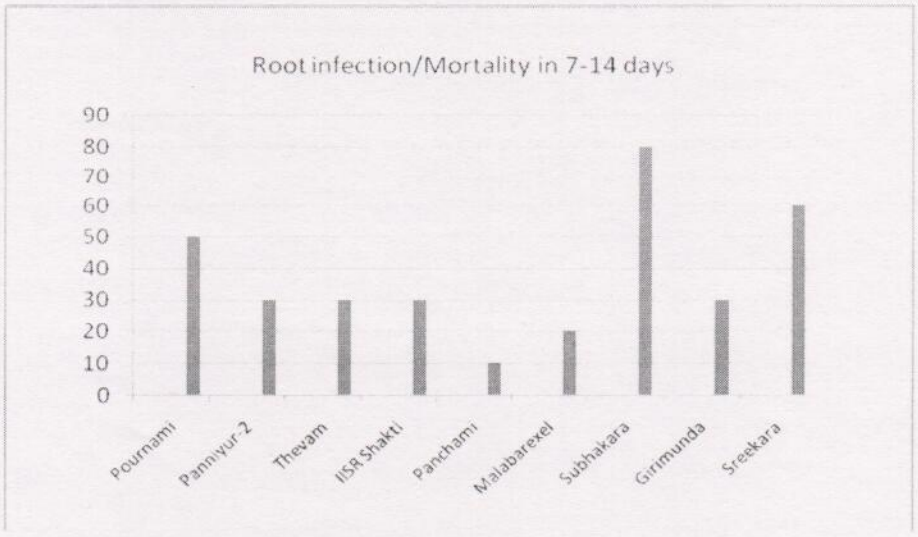

Fig-4 Root infection/mortality of improved varieties after root inoculation

Though zoospore suspension is faster in getting the infection, extraction of zoospores and its application needs more expertise. This is because the zoospores will get settled very fast and the required number will not get into the suspension while drenching the zoospore suspension to plants. This will lead to erratic results. Hence inoculation with mycelial plug of required size is recommended for inoculation in screening experiments.

c. Hydroponics method of root inoculation

When root inoculation was done by immersing the root system of plants in zoospore suspension, a bluish black coloration was noticed in the liquid suspension in 24-92h. This colour change was not observed in plants immersed in sterile distilled water without zoospores. The intensity of the colour also varied with varieties. Subhakara showed the highest intensity (which developed the color first) while IISR Shakti showed the lowest intensity of discoloration. On analyzing the colored solution for phenol content, it was observed that there was an increase in phenol content in all liquid suspensions which showed high colour intensity when compared to respective controls. The result clearly indicated the rupture of cell walls due to the host pathogen interaction (Table 5). This cell damage was less in IISR Shakti when compared to other varieties. Hence it can also be taken as a criterion for differentiating the varieties for resistance. Root and leaf carbohydrate content in different varieties showed not much variation but comparatively lowest content was observed in the moderately resistant line. Since this is only a preliminary attempt, more detailed experimentation is required for confirming the role of phenols in imparting resistance to *P. capsici*.

Table 5 Phenol release and carbohydrate content of leaf and roots of inoculated plants by Hydroponic method

Improved varieties	Phenol (%) in solution		% increase over control	Carbohydrate (%)			
				Root		Leaf	
	Treated	Control		Treated	Control	Treated	Control
IISR Subhakara	21.681	11.088	48.86	26.455	29.8	33.110	30.05
IISR Shakti	11.695	8.589	26.56	23.550	24.595	32.325	24.535
IISR Thevam	10.338	3.684	64.36	28.530	28.145	29.600	30.60
Panniyur 1	13.583	5.804	57.27	39.505	37.835	35.252	26.398
Panniyur 2	10.527	4.671	55.63	40.990	42.276	38.362	37.058
Lsd (P= 0. 05)	8.189	4.307		10.661	9.816	3.606	17.843

4. Modifying the screening methodology

By the improved method, the variety that took > 21 days with ≤ 40% mortality /root infection in 100 days was further subjected to aerial inoculation by intact stem and leaf inoculation method after 100days of incubation. The lesion size of leaf and stem was recorded and given a scoring and the scores were converted to percentage disease severity index (DSI) using the formula of Kim 2000 as given below

$$DSI = \frac{\sum \text{(rating of each plant)}}{\text{Maximum score x No. of plants rated.}} \times 100$$

The average DSI of both stem and leaf was taken and the DSI of the whole plant was calculated. Based on the DSI and mortality due to root infection, the plants were rated into resistant, moderately resistant and susceptible as below.

Av DSI (Stem & Leaf)	Reaction
<30%	Resistant
31-40%	Moderately resistant
>40%	susceptible

5. Evaluation of the improved methodology

The improved screening protocol was tested among 17 improved lines of black pepper viz, Sreekara, Subhakara, Panchami, Pournami, IISR Thevam, C 1090, C 820, HP 780, HP 39, HP 813, HP 105 and Panniyur varieties such as Panniyur1, Panniyur 2, Panniyur 3, Panniyur 4 and Panniyur 5. These varieties were raised in sterilized potting mixture as above and root inoculated. The varieties that took > 21 days for infection and in which more than 60% of the plants survived after 100 day of inoculation were subjected to aerial inoculation as mentioned above and DSI of the whole plant was calculated. The varieties were then ranked based on the score card.

Among the varieties, Panniyur1, Panniyur 3, Panniyur 4, Pournami, Sreekara , Subhakara, C 1041, HP 780, HP 39, and HP 105 > 30% infection in <21 days. Other varieties viz. Panniyur2, Panniyur 5, Panchami, C 1090, C 820, and HP 813 took > 21 days for infection and more than 40% of the plants survived even after 100 days of inoculation. These plants when subjected to aerial inoculation, all were found susceptible showing >40% aerial infection showing its susceptible nature (Table 6).

Table 6. Screening of improved varieties of black pepper based on the new protocol

Variety/hybrid/Cultivars	Mortality in <21 days	Mortality in >21 days	DSI (Stem)	DSI (leaf)	Av. DSI
Panniyur 1	30				
Panniyur 2		30	88.88	77.78	83.33
Panniyur 3	30				
Panniyur 4	30				
Panniyur 5		10	93.75	81.25	87.50
Panchami		20	84.38	75	79.69
Pournami	60		-	-	-
Sreekara	60		-	-	-
Subhakara	80		-	-	-
C 1041	30		100	100	100
C 1090		30	75	50	62.50
C 820		40	70.83	50	60.42
HP 780	40				
Hp 39	40				
Hp 813		10	77.77	69.44	73.61
Hp 105	40				

Conclusion

When intact black pepper plants were lightly wounded in the stem with a pin and inoculated with the mycelial disc, symptom expression occurred within 72h and differences in disease development between cultivars could be assessed by indexing the depth of cortical penetration with a visual scoring. Unwounded, inoculated plants did not develop symptoms in most of the cases. At the time of final assessment, disease was much more severe in wounded plants, but the relative susceptibility of cultivars was not affected by the wounding. Root inoculation on intact plants with mycelial discs or zoospore suspension resulted in root and collar infection within 14-21 days of inoculation in highly susceptible cultivars where as resistant cultivars remained healthy even after 100 days of inoculation (Bhai *et al.* Unpublished). This is supported by another method of root inoculation by immersing the roots of plants in zoospore suspension (hydroponics method). The uprooted plants when immersed in water containing pathogen inoculum (sporulated culture disc or zoospore suspension) developed a brown coloration in the medium within 72-96h in the case of susceptible varieties/cultivars whereas such colour development was very slow or negligible in moderately resistant or resistant lines (Bhai, unpublished). Analysis of the water suspension showed the presence of higher phenolics released by the susceptible line on

contact with the pathogen. The possibility of using this method as a complementary method along with root inoculation, for finding the source of resistance is to be explored further. Or else it is worth attempting to develop this method as a preliminary tool for screening black pepper germplasm.

Hence from a series of tests conducted, it was observed that the reaction for resistance to each plant part of black pepper is independent of each other and any one method of inoculation is not sufficient to rate black pepper cultivars for their resistance/susceptibility. Since there is differential reaction between different parts, a methodology integrating all the plant parts (stem, leaf and root) are required to rank black pepper accessions /cultivars for resistance. Since *Phytophthora* foot rot reflects both collar and root infection, root reaction should be given preference while screening for resistance.

So among the nine methods tested for screening against *P. capsici* in black pepper, preliminary screening by root inoculation and secondary screening by intact plant inoculation (leaf inoculation without injury and stem inoculation with injury) and incubation at 24-28^{0}C was found suitable for the assessment of the cultivars/accessions. Even without wounding the plants took infection due to chemotaxis. But wounding did enhance infection. Hence based on the results of the present study, a modified protocol for screening is proposed which consists of 1) inoculating the roots (basal inoculation) of 3month old rooted cuttings having 3-4 leaf stage with mycelial plugs (of 5mm size @ 5 discs /plant). If more than 60% of the plants survived after the 100th day of inoculation, they would be subjected to intact stem and leaf inoculation. From this, disease severity (DSI) would be calculated for each part and average DSI would be taken as the DSI of the whole plant. Finally accessions could be ranked integrating mortality and disease severity of the aerial portion. Hence this method is found as a suitable approach for assessing the black pepper germplasm for *Phytophthora* resistance.

I1. SCREENING SHORTLISTED CULTIVARS / SELECTIONS AGAINST *PHYTOPHTHORA* AND NEMATODES

A. Screening short listed lines for *Phytophthora* resistance

Thirty five cultivars short listed from previous years screening were subjected to root inoculation by mycelial disc inoculation method. Based on the new protocol it was found that all them took infection within 21 days except C 820 and C 1530 (Table 7). But C 820 succumbed to infection in the later stage by incubating for 100days and C 1530 survived even after 100 days.

Table 7 Reaction of short listed cultivars of black pepper screened against *P. capsici*

Sl. No	Acc. No	% Mortality in <21days	% Mortality in >21days
1	1239	√	
2	1535	√	
3	1212	√	
4	1230	√	
5	4236	√	
6	4253	√	
7	4269	√	
8	1150	√	
9	1217	√	
10	1225	√	
11	1263	√	
12	1369	√	
13	1428	√	
14	1529	√	
15	1578	√	
16	1619	√	
17	1637	√	
18	886	√	
19	1204	√	
20	1099	√	
21	809	√	
22	1095	√	
23	1090	√	
24	1112	√	
25	1099	√	
26	1204	√	
27	1485	√	
28	1386	√	
29	478	√	
30	1299	√	
31	728	√	
32	1324	√	
33	1530		√
34	27	√	
35	820		√

Likewise, already identified 15 *Phytophthora* tolerant lines (Table 8) were subjected to re-screening using stem, leaf and root inoculation methods with virulent isolate of *P. capsici*. All of them showed susceptible reaction (>30%) towards leaf and stem infection (Table 8). On root screening Acc. 1098, 847, 813 and 803 and 04-HP 1533(3) showed no root infection till 45 days after inoculation and P 339, 1093, 1052, 1038, 894, and 816 showed 20-40% infection. When these plants were subjected to aerial inoculation as per the new protocol, it was found that the average DSI of these plants are very high ranging from 76-100%.

Table-8 Screening of short listed promising lines

Sl. No	Acc. No	Leaf Infection (av. lesion length mm)	stem infection (av. lesion length mm)	Root infection
1	P 339	22.9	15.4	20.0
2	HP 1533(3)	25.0	22.0	0.0
3	HP 1	25.3	34.2	80.0
4	1099	15.3	22.5	60.0
5	1098	18.1	27.0	0.0
6	1093	26.6	44.2	20.0
7	1052	28.1	44.0	40.0
8	1047	29.7	33.4	60.0
9	1038	29.3	35.5	20.0
10	894	19.7	37.0	20.0
11	884	19.4	39.6	60.0
12	847	22.6	32.2	0.0
13	813	34.0	24.6	0.0
14	803	33.4	31.0	0.0
15	816	21.5	16.2	20.0

B. Screening Hybrids for *Phytophthora* resistance

During 2006-2008, 63 hybrids short listed as moderately tolerant in the preliminary screening for *Phytophthora* resistance were subjected to root inoculation under artificially created sick plot condition along with a susceptible check Sreekara. The plants started dying from 4th day onwards. The plants were retained for more than 100 days. The mortality of the plants due to root infection was recorded. The mortality ranged from 0-100% and the no. of days taken for death ranged from 4- 45 days. The reaction of hybrids to *Phytophthora* is presented (Table-9).

Out of 63 short listed hybrids, the mortality of the plants ranged from 0-100%. Hybrids Viz. Hp 1238, Hp 1706, 04-K17, Hp 1383 , Hp 449 (Cholamundi X Panniyur-1) , Hp 1375 (Narayakkodi X Karimunda) showed only 10-30 % infection even after 100 days, while two hybrids namely HP 490 and HP 521 showed no *Phytophthora* infection till the end of the incubation period. The hybrids found as promising were multiplied and evaluated for nematode tolerance. But all are found susceptible to both the nematodes. The hybrids that showed < 40% root infection viz. HP 449, HP 490 and HP 521 when planted in the sick plot were succumbed to infection while Hp 1375 remained uninfected and remained healthy. It is under field evaluation.

Table-9 Reaction of Black pepper accessions to *Phytophthora*

Sl.No	Reaction	Hybrid No
1	No infection / mortality up to 250 days	Hp 490, Hp 521
2	10-30% mortality in 100 days	Hp 1238, Hp 1706, 04-K17, Hp 1383 , Hp 449 (Cholamundi X Panniyur-1) , Hp 1375 (Narayakkodi X Karimunda)
3	50% mortality in 100 days	Hp 13, Hp 820, Hp920
4	100% Mortality < 21 days	Hp 987 , HP 1389 , HP 1357, Sreekara, HP 206, HP 437, Hp 900, Hp 34, HP 23, HP 96, HP 117, HP 134, HP 346, HP 348, HP 391, HP 400, HP 421, HP 427, HP 437, HP 470, HP 477, HP 478, HP 528, HP 561, HP 569, HP 599, HP 750, HP 752, HP 755, HP 780, HP 831, HP 840, HP 884, HP 984, HP 1052, HP 1382, HP 1389, HP 1599, HP 1654, HP 1660, HP 1751, HP 1789, HP 2466, HP 2433

C. Screening wild *Piper* species for *Phytophthora* resistance

Screening wild and related genera of Piper

Among the 11 wild piper accessions screened, 2 accessions viz. Acc.3177 (*P.sylvaticum*), and Acc.3362 (*P. ornatum*) (Fig 6) showed resistance towards *Phytophthora* and also towards both nematodes. Only 10% mortality was observed in Acc.3177 in six months of inoculation.

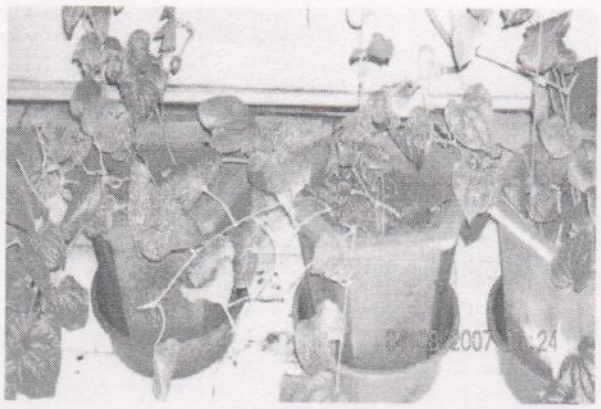

Fig-5 Acc. 3177(*P.sylvaticum*)

Fig-6 Acc.3362 *Piper ornatum*

III. Screening Open pollinated seedlings

In this study open pollinated seedling progenies of black pepper were screened to identify multiple source of resistance against *P. capsici* and nematodes.

Preliminary screening of OP seedlings

Out of 11,632 seedlings inoculated with *P. capsici*, 40 seedlings escaped infection in first round of screening. This included 21 Karimunda seedlings, 5 seedlings of the hybrid HP1533, 4 seedlings each of the cultivars 5097 and 4095, 2 seedlings of the hybrid HP1263 and 1 seedling each of IISR Shakti, cultivars C 1422, C 4098 and hybrid HP400. The percentage survival of plants after inoculation varied from 0-13.8%. Among the survived progenies, maximum survival was observed in HP 1533 (13.8%) and the minimum survival in IISR Shakti (0.12%) (Table 10 & 11).

Table 10 Survival rate of seedlings of cultivars/hybrids

Sl. No	Acc No	% survival	Sl. No	Acc No	% survival
1	C 1209	0	41	HP840	0
2	C1353	0	42	HP900	0
3	C 1390	0	43	HP920	0
4	C1393	0	44	HP1211	0
5	C1422	3.85	45	HP1231	0
6	C1445	0	46	HP1246	0
7	C1461	0	47	HP1261	0
8	C1462	0	48	HP 1263	11.11
9	C1496	0	49	HP1264	0
10	C1630	0	50	HP1288	0
11	C1510	0	51	HP1289	0
12	C4021	0	52	HP1290	0
13	C4023	0	53	HP1291	0
14	C4036	0	54	HP 1300	0
15	C4043	0	55	HP1319	0
16	C4076	0	56	HP 1335	0
17	C4077	0	57	HP 1336	0
18	C4090	0	58	HP 1337	0
19	C4092	0	59	HP1354	0
20	C4094	0	60	HP1508	0
21	C4095	3.73	61	HP1519	0
22	C4098	5.71	62	HP1523	0
23	C4117	0	63	HP1533	13.80
24	C4185	0	64	HP1539	0
25	C4189	0	65	HP1582	0
26	C4257	0	66	HP1597	0
27	C4263	0	67	HP1604	0
28	C5097	7.80	68	HP1637	0
29	HP400	5.00	69	HP4022	0
30	Kottanadan	0	70	HP4033	0
31	Subhakara	0.40	71	HP4070	0
32	IISR Shakti	0.12	72	HP4076	0
33	HP451	0	73	HP4041	0
34	HP466	0			
35	HP569	0			
36	HP750	0			
37	HP799	0			
38	HP811	0			
39	HP820	0			
40	HP837	0			

Table 11 Seedlings survived in initial screening for *Phytophthora* resistance

Sl. No.	Cultivar/hybrid	No. of seedlings without infection
1	Subhakara	21 (04-K 1-21)
2	IISR Shakti	1 (04-P 24-1)
3	HP 1533	5 (04-Hp 1533- 1-5)
4	HP 400	1 (04-Hp400- 1)
5	HP 1263	2 (04-Hp1263- 2)
6	C 5097	4 (04-C5097- 1-4)
7	C4095	4 (04-C4095- 1-4)
8	C4098	1 (04-C4098- 1)
9	C1422	1 (04-C1422- 1)
	Total	40

Second round screening

Root inoculation

Forty progenies shortlisted in the preliminary screening were multiplied and screened by root inoculation with mycelial discs. Certain progenies showed mortality in 7 days whereas certain other progenies took 65 days for the same. Of the 21 progenies obtained from cultivar Karimunda, 16 progenies showed 100 % mortality in 7-28 days. Among the 19 progenies obtained from hybrids / cultivars/selections, six progenies (04-P24 -1, 04-C 4095 -3, 04-C 1422 -1, 04-C 4098 -1 and 04-HP-1533 -5,) took >21 days for infection of which progeny 04-P24-1 showed no mortality even after 100 days (Table 12). Progenies of 04-HP-400-1, 04-K17, 04-HP 1533-2 and 04 -HP 1533-3 showed only 10- 30% mortality in 38-41 days. Some progenies took 15 -30 days whereas some others took only <15days for 100% mortality. The susceptible check, Subhakara showed 100% mortality in 21 days (Table 13). Hence based on the time taken for mortality, the progenies were classified into two groups. The group one took only < 21 days whereas the group two took > 21 days for mortality (Table 13).

Table.12 Mortality of black pepper progenies due to *Phytophthora* root infection

Sl. No.	Progenies	No. of days taken for mortality	Root infection in 100dai (%)
1	04-P24 -1	**0**	**0**
2	04-C 5097 -1	12	100
3	04-C 5097 -2	11-13	100
4	04-C 5097 -3	11-13	50
5	04-C 5097 -4	11	70
6	04-C 4095 -1	7-24	50
7	04-C 4095 -2	24-65	60
8	04-C 4095 -3	24-65	100
9	04-C 4095 -4	24-31	50
10	04-C 1422 -1	9	40
11	04-C 4098 -1	11	40
12	04-HP1533 -1	90	60
13	04-HP1533 -2	39-41	**20**
14	04-HP1533 -3	39-40	**30**
15	04-HP1533 -4	9-14	70
16	04-HP1533 -5	7	40
17	04-HP-400 -1	**38**	**10**
18	04- Hp 1263-1	9	80
19	04- Hp 1263-2	3-9	80
20	04-K-1	5-16	100
21	04-K-2	5-12	100
22	04-K-3	5-13	100
23	04-K-4	5-13	100
24	04-K-6	11-21	100
25	04-K-7	11	100
26	04-K-8	20	100
27	04-K-9	18-20	100
28	04-K-10	18-31	60
29	04-K-12	9-12	100
30	04-K-13	28	100
31	04-K-14	18	80
32	04-K-15	28	40
33	04-K-16	18	100
34	04-K-17	**39**	**10**
35	04-K-18	7	100
36	04-K-19	9-39	60
37	04-K- 20	7	100
38	04-K -21	7	100
39	04-K-28	11	100
40	04-K-29	11	100
41	Subhakara (Check)	16- 21	100

Aerial inoculation (Stem and leaf inoculation)

The rooted cuttings when subjected to infection by stem and leaf inoculation method, showed varying levels of reaction towards *P.capsici* (Table 13). The leaf lesion size varied from 2.8 mm-36.3 mm and DSI due to leaf infection varied from 7.5% -100% (Table 14). Among the progenies, 04-P24-1 showed the smallest leaf lesion size (2.8 mm) and DSI (7.5%) followed by 04-HP-400 -1 (leaf lesion size 11.2 mm and DSI 37.50%).

Table 13 Classification of black pepper progenies based on time taken for mortality

Sl. No.	Progenies	% Mortality within 21 days	% Mortality in > 21 days
1	04-P24 -1	0	0
2	04-C 5097 -1	100	-
3	04-C 5097 -2	100	-
4	04-C 5097 -3	50	-
5	04-C 5097 -4	70	-
6	04-C 4095 -1	-	50
7	04-C 4095 -2	-	60
8	04-C 4095 -3	-	100
9	04-C 4095 -4	-	50
10	04-C 1422 -1	40	-
11	04-C 4098 -1	40	-
12	04-HP1533 -1	-	60
13	04-HP1533 -2	-	20
14	04-HP1533 -3	-	30
15	04-HP1533 -4	70	-
16	04-HP1533 -5	40	-
17	04-HP-400 -1	-	10
18	04- Hp 1263-1	80	-
19	04- Hp 1263-2	80	-
20	04-K-1	100	-
21	04-K-2	100	-
22	04-K-3	100	-
23	04-K-4	100	-
24	04-K-6	100	-
25	04-K-7	100	-
26	04-K-8	100	-
27	04-K-9	100	-
28	04-K-10	60	-
29	04-K-12	100	-
30	04-K-13	-	100
31	04-K-14	80	-
32	04-K-15	-	40
33	04-K-16	100	-
34	04-K-17	-	10
35	04-K-18	100	-
36	04-K-19	-	60
37	04-K- 20	100	-
38	04-K -21	100	-
39	04-K-28	100	-
40	04-K-29	100	-
41	Subhakara (Check)	100	-

Similarly the stem lesion size varied from 5.2 mm-51.22 mm and DSI due to stem infection varied from 25% -100% (Table 5). Here also among the progenies, 04-P24-1 showed the smallest stem lesion size (5.2 mm) and DSI (27.5%). This is followed by 04-HP-400 -1 (stem lesion size 4.9 mm and DSI 37.5%) (Table 13& 14)

Table 14 Leaf and stem reaction of black pepper progenies

Sl. No.	Progenies	Leaf lesion (mm)	Stem lesion (mm)
1	04-P24 -1	2.80 a	5.20 a
2	04-C 5097 -1	11.00 bc	17.00 cdef
3	04-C 5097 -2	10.92 bc	17.00 abcd
4	04-C 5097 -3	10.00 bc	8.67 ab
5	04-C 5097 -4	27.60 g	24.88 def
6	04-C 4095 -1	10.88 bc	12.60 abc
7	04-C 4095 -2	14.25 bcd	17.80 bcde
8	04-C 4095 -3	16.45 bcd	16.33 bcde
9	04-C 4095 -4	25.40 fg	10.45 abcd
10	04-C 1422 -1	24.80 fg	37.91 fg
11	04-C 4098 -1	18.00 de	6.27 ab
12	04-HP1533 -1	13.55 d	12.45 abcd
13	04-HP1533 -2	36.33 h	32.80 efg
14	04-HP1533 -3	29.60 g	37.72 fg
15	04-HP1533 -4	35.60 h	33.09 ef
16	04-HP1533 -5	26.80 g	31.70 ef
17	04-HP-400 -1	11.20 bc	4.90 ab
18	04- Hp 1263-1	24.20 fg	48.20 g
19	04- Hp 1263-2	20.70 ef	20.10 bcde
20	04-K-1	4.2 ab	8.7 ab
21	04-K-2	3.6 a	6.3 a
22	04-K-3	10.3 bcd	20.8 abcde
23	04-K-4	19.16 fg	22.5 bcdef
24	04-K-6	16.80 def	23.7 bcdef
25	04-K-7	9.20 bcd	31.3 efg
26	04-K-8	12.50 cde	18.9 defg
27	04-K-9	15.91 def	32.4 bcdef
28	04-K-10	17.38 ef	19.9 defg
29	04-K-12	13.56 cdef	41.0 abcde
30	04-K-13	27.7 hi	11.1 efg
31	04-K-14	27.9 hi	37.3 gh
32	04-K-15	8.70 abc	51.22 abc
33	04-K-16	30.78 ij	32.5 fgh
34	04-K-17	31.33 ij	31.8 h
35	04-K-18	35.70 j	26.2 defg
36	04-K-19	25.90 hi	33.4 defg
37	04-K- 20	29.0 hi	26.6 cdefg
38	04-K -21	23.75 gh	15.60 efg
39	04-K-28	27.90 hi	31.6 cdefg
40	04-K-29	23.50 gh	34.2 abcd
41	Subbharaja (Check)	10.80 bc	16.6 abc

The overall DSI of the progenies due to aerial infection varied from 17.5% -100%. Among the progenies screened 04-P24-1 showed the lowest DSI (17.5%) followed by 04-HP-400 -1 (37.5%). Rest of the progenies showed more than 75% DSI (Table 15). Finally the assessment was made in such a way that the progenies that took > 21 days for root infection with < 30% DSI as resistant, 31-40% as moderately resistant and > 40% as susceptible(Table 16).

Table 15 Disease Severity Index (DSI) due to stem and leaf infection

Sl.No.	Progenies	DSI due to Leaf infection	DSI due to Stem lesion	Average DSI
1	04-P24 -1	**7.50**	**27.50**	**17.5**
2	04-C 5097 -1	22.50	50.00	36.25
3	04-C 5097 -2	52.50	66.67	59.6
4	04-C 5097 -3	63.90	41.67	52.79
5	04-C 5097 -4	100.00	75.00	87.5
6	04-C 4095 -1	12.50	42.50	27.5
7	04-C 4095 -2	70.00	80.00	75
8	04-C 4095 -3	83.33	75.00	79.17
9	04-C 4095 -4	97.50	61.36	79.43
10	04-C 1422 -1	72.50	90.90	81.7
11	04-C 4098 -1	92.50	29.54	61.02
12	04-HP1533 -1	60.00	65.90	62.95
13	04-HP1533 -2	100.00	100.00	100
14	04-HP1533 -3	100.00	100.00	100
15	04-HP1533 -4	100.00	100.00	100
16	04-HP1533 -5	100.00	100.00	100
17	04-HP-400 -1	37.50	37.50	37.50
18	04- Hp 1263-1	100.00	100.00	100
19	04- Hp 1263-2	97.50	77.50	87.5
20	04-K-1	7.50	52.50	30.0
21	04-K-2	2.50	27.50	15.0
22	04-K-3	25.00	65.00	45.0
23	04-K-4	70.00	67.50	68.75
24	04-K-6	82.50	77.50	80.0
25	04-K-7	47.50	77.50	62.5
26	04-K-8	72.50	77.50	75.0
27	04-K-9	75.00	92.50	83.75
28	04-K-10	62.50	72.50	67.5
29	04-K-12	100.00	100.00	100
30	04-K-13	47.50	57.50	52.5
31	04-K-14	100.00	92.50	96.25
32	04-K-15	100.00	100.00	100
33	04-K-16	100.00	100.00	100
34	04-K-17	97.50	80.00	88.75
35	04-K-18	100.00	100.0	100
36	04-K-19	95.00	100.00	97.5
37	04-K- 20	100.00	87.50	93.75
38	04-K -21	97.50	80.00	88.75
39	04-K-28	100.00	80.00	90.0
40	04-K-29	100.00	100.00	100
41	Subhakara (Check)	62.50	100.00	81.25

Av DSI (Stem & Leaf)	Reaction
<30%	Resistant
31-40%	Moderately resistant
>40%	susceptible

Based on the average disease severity index of aerial infections, the OP progeny 04-P24-1 is found as resistant showing <30 %, and progeny 04-HP-400 as moderately resistant with DSI between 31-40%. All other progenies are found susceptible with more than 75% DSI (Table 16).

Table 16. Short listing of black pepper progenies based on aerial infection and mortality

Sl. No.	Progenies	Av DSI (%) due to aerial infection	Mortality (%)	Time taken for mortality	Reaction
1	04-P24 -1	17.50	0	-	R
2	04-HP1533 -2	100.00	20	41	S
3	04-HP1533 -3	100.00	30	40	S
4	04-HP-400 -1	37.50	10	38	MR
6	04-K-17	88.75	10	39	S
7	Subhakara (Check)	81.25	100	21	S

Screening against nematodes

To study the multiple resistances, the OP progenies short listed for *P. capsici* resistance in the initial level of screening were subjected to nematode screening tests in individual beds infested with *R. similis* and *M. incognita* for three months. All the seedlings were found susceptible to *R. similis* as indicated by the lesions on the roots. Evaluation against *M.incognita* showed that all the progenies except 04-HP1533 (2) are susceptible. No root galling or knots were observed in these plants even after four months of incubation (Table 17).

Besides the above, during 2006-2007 seedlings were raised from 39 accessions and preliminary selection was made by inoculating with virulent *Phytophthora* zoospore suspension under natural conditions. 32 plants were survived. These are being multiplied for

secondary screening. Similarly from a bulk of Karimunda seeds raised, 50 plants survived after inoculation was multiplied for further round screening (Table-19).

Conclusion

Evaluating open pollinated progenies by artificial inoculation is the conventional practice followed for locating source of resistance against *P.capsici*. In the present study, seedlings raised from 11632 seeds were screened, of which one progeny from IISR Shakti was found as resistant to *Phytophthora* infection based on several methods and rounds of screening. The seedlings were retained in the inoculum till five months and the one which did not succumb to infection were transplanted, multiplied in sufficient numbers and were further subjected to several rounds of root screening as well as stem and leaf inoculation. In aerial screening the DSI was calculated and disease reactions were rated for assessing the progenies. This method of assessment was not followed in the earlier screening experiments. Though the aim of the experiment was to identify source of multiple resistance, no single line showed multiple resistance. The line which is found resistant to *Phytophthora* was found susceptible to both the nematodes.

So in the present study, only one OP progeny 04-P24-1 showed resistance, while hybrid progeny 04-HP 400-1 showed moderate resistance towards *P. capsici*. The short listed progenies when screened for nematode resistance (against R. *similis* and *M .incognita)*, one progeny 04-HP 1533(2) showed resistance to *M. incognita* whereas all others are found susceptible.

Table 17 Reaction of shortlisted black pepper progenies to plant parasitic nematodes

Sl. No	Progeny	*R. similis*	*M. incognita*
1	04-P 24-1	+	+
2	04-Hp 1533- 2	+	Resistant
3	04-Hp 1533- 3	+	+
4	04-HP-400 -1	+	+
5	04-K-17	+	+
6	Subhakara (Check)	+	+

Thirty two seedlings obtained during 2006-2007 from 39 accessions were subjected to second round screening after multiplication. Out of 32 seedlings subjected for second round screening, none of the seedlings showed resistance.

Open pollinated seedlings from 28 varieties were also raised for preliminary selection during 2006-2007 (Table -20) Seedlings were raised from 28 accessions and preliminary selection was made for *Phytophthora* resistance during 2007-2008. About 1754 seedlings were inoculated by zoospore drenching method. None of the seedlings survived infection.

Evaluation of "disease escapes" from farmers plot

Besides collecting seeds from the germplasm of IISR Calicut, seeds were also collected from promising 'disease escapes' found in farmers fields. Farmers' fields were visited and collected seeds as well as runner shoots from 30 year old cultivars appearing to be healthy good yielder and remained without any infection. The names of black pepper cultivars are listed below. They were multiplied and were tested for *Phytophthora* infection under artificially inoculated conditions. But neither seedlings nor cuttings showed resistance.

1. Chumala 1(CV3)
2. Chumala 2(CV4)
3. Kuriyilamundi 1(CV1)
4. Kuriyilamundi 2(CV7)
5. Muttuchiramunda 1(CV2)
6. Muttuchiramunda-2(CV5)
7. Jeerakamundi (CV6)
8. Vadakkan (Chettan) (CV8)

Table-18 Identification of disease resistant plants from open pollinated seedlings of black pepper

Sl No	Acc.No	Total	No. survived
1	147	368	2
2	156	460	0
3	610	644	0
4	815	1012	4
5	816	322	0
6	827	644	0
7	836	690	1
8	965	644	0
9	1036	506	0
10	1057	1518	0
11	1059	460	0
12	1080	552	0
13	1124	460	0
14	1297	598	7
15	1324	552	0
16	1525	690	2
17	1580	506	0
18	1597	460	0
19	1605	460	0
20	1616	368	0
21	1623	1702	0
22	1632	322	1
23	1636	276	0
24	4021	230	0
25	4032	690	1
26	4033	736	0
27	4047	598	0
28	4070	690	0
29	4076	736	2
30	4121	828	0
31	4225	230	1
32	4223	690	4
33	4229	736	0
34	4244	552	0
35	4257	1196	2
36	4265	276	2
37	4276	736	4
	Total	23138	32
38	Karimunda	9200	50

Table-19 Details of seedlings raised during 2006-2007

Sl No	Acc.No	Total berries
1	Sreekara	920
2	Karimunda	4600
3	CV2	848
4	CV8	575
5	OPKm-2	1288
6	HP 34	1380
7	HP780	1380
8	HP813	2760
9	HP105	1840
10	HP1411	2300
11	4199	322
12	1296	276
13	4230	1288
14	1627	920
15	4211	644
16	1041	1288
17	4234	736
18	4223	690
19	4275	736
20	1297	736
21	1467	368
22	1525	690
23	4247	736
24	Km 159	828
25	4248	276
26	1530	184
27	4291	1288
28	1260	736
Total	**28 Nos.**	**30817**

IV. Field Evaluation of promising black pepper lines

a) Field testing of promising lines

Three promising lines viz. P24-04-1, 1533-2 and 1533 -3 short listed from open pollinated progeny showed a survival rate of 77.78% for 04-P24 -1, 66.67 % for 04-HP 1533 -2 and 88% for 04-HP 1533 during the first year. No *Phytophthora* infection was observed in these plants till the reporting period. The *Phytophthora* resistant line 04-P24-1 reached the bearing stage.

Besides 04-P24-1, Hp 04-1533(2), 04-1533(3), other lines short listed during 2005-2008 viz. HP 490, HP521, HP 449, HP 1375, C1530 HP 39, were also planted in the sick plot and are under evaluation. Spike formation started in the *Phytophthora* resistant line 04-P24-1 during 2007-08. Mature berries were collected and sown for raising F2 generation. HP 04-1533(2) also reached the bearing stage as on 2009. The experiment is being continued.

b) Field screening of Nematode Resistance in black pepper

Cultivars such as C.812, C.1090 (moderately resistant to *Phytophthora)* & C.4103 and HP 60, HP 290 (resistant to M.incognita) and C.820, C.1047 & C.1204 and HP39 (resistant to R. *similis*) were planted in the field during July 2003 in CRD were monitored for nematode resistance. The yield data as well as the nematode incidence 2006-07 is given (Table 20). The field performance of two *R. similis* resistant black pepper lines HP 39 and C 820 was superior to other lines even four years after planting. C 1090 showed no yellowing or foot rot symptoms till 2009 showing its potential as a promising line. The promising lines viz. HP 39 and C 1090 are under multilocation trials at AICRP centers from 2007-08 onwards.

Table-20 Field evaluation of nematode resistant lines

Accession	Yellowing Index	Yield - dry (g)	Nematode level / g root			
			Rs	Incidence	RKN	Incidence
HP 39	0.00	285.0	0.0	0/4	0.0	0/4
HP 60	0.50	67.5	12.0	2/4	340.0	1/4
HP 290	1.00	0.0	16.7	2/4	0.0	0/4
C 812	0.75	0.0	0.0	0/4	0.0	0/4
C 820	0.75	315.0	7.7	2/4	250.2	2/4
C 1047	2.75	0.0	0.0	0/4	0.0	0/4
C 1090	0.00	32.5	27.0	2/4	0.0	0/4
C 1204	1.25	0.0	0.0	0/4	566.5	2/4
C 4103	1.00	0.0	20.3	1/4	20.2	1/4
Panniyur I	0.75	27.5	25.6	2/4	40.6	1/4

Fig-8 04-P24-1-bearing stage

ANNEXURE II

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ANNEXURE III

Research Publications

1 .Suseela Bhai R, Eapen S J, Anandaraj M, Sasikumar, B and Saji K V . Evaluation of open pollinated progenies of black pepper for resistance to *Phytophthora capsici* and plant parasitic nematodes using a modified protocol (Communicated to Indian Journal of Agricultural Sciences.)