RPF-III
(PROFORMA FOR SUBMISSION OF FINAL REPORT OF RESEARCH PROJECTS)
Part-I: General Information

800 Project Code:
8001 Institute Project Code Number : HORT-V (813)
8002 ICAR Project Code Number : Not allotted

801 Name of the Institute and Division
8011 Name and address of Institute : Indian Institute of Spices Research, Kozhikode
8012 Name of Division/Section : Crop Improvement/ Horticulture Section
8013 Location of the Project : IISR Experimental Farm, Peruvannamuzhi

802 Project Title : Rootstock intervention to manage root infection of *Phytophthora* and nematodes in black pepper.

803 Priority Area : Black pepper improvement
8031Research Approach: Applied Res./ Basic Res./Process/ Transfer of Or Technolo. of Tech. Develop.

01 02 03 04

804 Specific Area : Rootstock studies for foot rot management

805 Duration of Project :
8051 Date of start : 01.04.2006
8052 Date of completion : 31.03.2013
806 Total cost/Expenditure incurred **:Rs. 23,08,100/-**
(Give reasons for variation, if any from original estimated cost)
The initial cost envisaged from 2006 to 2009 was 14.59 lakhs. The reasons for escalation are increased salaries due to adoption of new pay scales and extension of Project up to 2013 in view of the continuous nature of work.

807 Executive Summary :

a)**Grafting Sreekara variety on resistant lines of pepper and Screening**

The black pepper lines identified as resistant to nematodes i) C-820 and ii) HP-39 and *Phytophthora capsici* i) C-1090 ii) IISR-Shakthi(P-24) and iii)IISR Thevam were multiplied and grafted with the susceptible variety Sreekara. The success was 60% on C-820, 10% on H.P-39, 66.67% on P-24(IISR-Shakthi) and 78% on C-1090. The line HP-39 had poor growth, less vigour and poor graft take and IISR Thevam was found susceptible to *Phytophthora.* Hence, these two lines were discarded and grafts on C-820, P-24(IISR-Shakthi) and C-1090 were screened against pathogens

specific for the resistance. Trials indicated that C-820 is not resistant against the nematode *Radopholus similis.* Hence it cannot be considered as a nematode resistant rootstock for pepper. The resistance of P-24(IISR-Shakthi) and C-1090 against *Phytophthora capsici* was revealed from the trials but control of nematodes with phorate is necessary. The root rot was less than 10% in both rootstocks. The growth of Sreekara on these rootstocks did not show any significant variation in terms of number of nodes, height and shoot dry weight. Since long term yield evaluation is needed grafts of Sreekara on P-24(IISR-Shakthi) and C-1090 have been established in the field. The line C-1090 has been found susceptible to leaf rot (anthracnose) caused by *Colletotrichum* sp.

b) Screening wild *Piper Species* against *Phytophthora capsici* and nematodes

A total of 22 wild *Piper* accessions were screened against *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita.* Out of these, Piper *colubrinum* (Acc.392), *Piper Sp.* (Acc.5815), and *P.ornatum* (Acc.3362) were found resistant to all the above pathogens whereas *P.sylvaticum* (Acc.3177) and *P.hamiltoni* (Acc.5532) were found resistant only to *Phytophthora capsici* alone. Among these, *P.ornatum* was found susceptible to *Sclerotium rolfsii* especially under high humidity / water logged conditions.

c) Grafting Sreekara variety on wild resistant *Piper species.*

Since grafting of *Piper colubrinum* with pepper varieties is already known and practised, no trials were conducted using this species except as interstocks. All the other *Piper* species identified as resistant were used as rootstock for grafting Sreekara variety of pepper. Good survival and growth of Sreekara was seen only on *P.hamiltoni* rootstock giving 50% success. However, it was found susceptible to drought and hence it can be used only under irrigated conditions. Besides, it needs excellent nematode control measures to get good growth being susceptible to nematodes.

Interstock grafting of the other two resistant species; *P.ornatum* and *Piper* acc.5815 was done with other *Piper* species for grafting Sreekara. Good success was obtained on *P.ornatum* with *P.hamiltoni* giving (100% success) whereas good growth was seen in *P.chaba* though the percentage take was poor (30%) out of five *Piper* species tried. Sreekara was grafted on the combination *P.ornatum* + *P.hamiltoni.* Though initial survival of Sreekara was 80% all died subsequently with stunted growth. However, grafts of *P.chaba on P.ornatum* grew excellently well and survived more than one year indicating that wherever chaba is cultivated, *P.ornatum* can be used as a resistant rootstock if footrot is observed.

Out of nine *Piper sp.* tested as interstock for *Piper* acc. 5815 only *P.colubrinum* and *P.hamiltoni* were successful (70-100%) . Though union was obtained with other species all died subsequently. Sreekara was grafted on the interstock *P.colubrinum* and *P.hamiltoni* but the survival

and growth was very poor (less than 6%). So *Piper* acc. 5815 cannot be directly used as rootstock but can be used in breeding. This species is vigorous growing with profuse root formation and survives under rainfed conditions.

d) Nurse grafting of Sreekara with *P.colubrinum* and *piper* acc.5815.

Approach grafts of Sreekara with *P.colubrinum* and *Piper* acc.5815 were grown together with out detopping. Examination of the root system of Sreekara indicated that the presence of root system of the above wild species did not prevent root rot of Sreekara by pathogens. It also indicated that no antagonistic chemicals are transmitted through the graft union from resistant stocks to black pepper when grown together as approach grafts – called nurse grafting to prevent root rot.

e) Grafting of laterals of black pepper as bush pepper.

Large scale grafting of 10 varieties of black pepper was done using *Piper colubrinum* as rootstock using laterals as scion and gave a success of 46.91 %. The bush grafts were established in the field and are growing satisfactorily and started yielding.

f) Mentor grafting of Panniyur -1

Panniyur – I laterals grafted on *P.colubrinum* were grown with out leaves on the scion part to get mentor grafted seedlings. Nine seedlings were obtained that showed variations. One seedling showed extreme variation with poor growth having yellowish leaves with vein pattern similar to colubrinum. So ISSR marker studies were carried out. No evidence of transfer of genetic material from colubrinum to any of the seedlings could be detected. Probably more specific markers may be needed. When shoots of this variant was grafted on pepper as well as colubrinum, good growth has been observed. Further studies will help to use this method in improving varieties of black pepper.

Salient Outcome

1. P-24(IISR-Shakthi) and C-1090 can be used as moderately resistant root stocks for black pepper against *Phytophthora capsici.*

2.Out of 22 wild *Piper acc.* tested, *P.colubrinum, P.ornatum* , *Piper acc.5815* and *P. magnificum* are resistant to *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita*, whereas *P. sylvaticum* and *P.hamiltoni* are resistant only to *Phytophthora capsici.* Among resistant species, *P.colubrinum* and *P.hamiltoni* only are graftable with black pepper.

808 Key Words

Key words : Black pepper, Resistant/tolerant Rootstocks/*Phytophthora capsici Radopholus similis, Melodogyne incognita,* Grafting, footrot,testing

Part -II: Investigator Profile

810	**Principal investigator**	:
8101	Name	: P.A. Mathew
8102	Designation	: Principal Scientist (Hort.)
8103	Division/Section	: Crop improvement, Horticulture Section
8104	Location	: IISR Expl. Farm, Peruvannamuzhi
8105	Institute Address	: Indian Institute of Spices Research, Marikunnu (P.O.), Kozhikode -673012.
811	**Co-Investigator**	:
8111	Name	: K.Abhirami
8112	Designation	: Scientist (Horticulture)
8113	Division/Section	: Crop Improvement
8114	Location	: IISR Experimental Farm, Peruvannamuzhi (2007-08)
8115	Institute Address	: (Transferred after wards)
812	**Co-Investigator**	:
8121	Name	: R.Suseela Bhai
8122	Designation	: Principal Scientist (Plant Pathology)
8123	Division/Section	: Crop Protection
8124	Location	: IISR, Kozhikode
8125	Institute Address	: Indian Institute of Spices Research, Marikunnu (P.O.), Kozhikode -6730 (2006-07 and External support from 2007-08 onwards)
813	**Co-Investigator**	:
8131	Name	: Santhosh J.Eapen
8132	Designation	: Principal Scientist (Nematology)
8133	Division/Section	: Crop Protection
8134	Location	: IISR, Kozhikode
8135	Institute Address	: Indian Institute of Spices Research, Marikunnu (P.O.), Kozhikode -6730 (2006-07 and External support from 2007-08 onwards

Part-III: Technical Details

820 Introduction and objectives :

8201 The project envisaged to fulfil the following objectives:

- To find out the effectiveness of identified types of pepper against pathogens as a rootstock with Sreekara as scion.

- To test the wild species resistant to *Phytophthora* and nematodes as a nurse rootstock to manage this disease.
- To study the population dynamics of *Phytophthora* and nematodes in the presence of tolerant or resistant lines.
- To study the growth of Sreekara grafts.
- To monitor the existing colubrinum grafts for long term survival.

8202 Background information and importance of the project

Root rot is a very serious problem in black pepper caused by a combination of *Phytophthora capsici* and nematodes especially *Radopholus similis.* Chemical methods are becoming costly and residues are creating marketing problems. Nowadays organic pepper is becoming important and alternative to chemical control are needed.

Biocontrol is quite effective against fungus foot rot but no effective biocontrol agent is available for nematodes. Though some types are found tolerant or resistant to these pathogens, they are not very high yielding or lack certain quality attributes. Besides, the root system of some varieties like Sreekara is weak. Similar problems have been tackled in other horticultural crops through the use of rootstocks. In this project, it is envisaged to graft one variety Sreekara with moderately resistant varieties such as Thevam, C-1090, P-24(IISR-Shakthi), HP-39, C-820, or other identified materials as root stocks and see the performance. Some wild species resistant to these pathogens exist. No attempt has been made to use these materials to prevent root rot. Nurse grafting is proposed to be used for this purpose because it has been observed that these species form union with black pepper but the growth is poor as individual rootstock but may work as a nurse rootstock. It is also possible that the root exudates of these wild relatives may curb population build up of these pathogens. Besides this, long term survival of grafts of black pepper on *Piper colubrinum* that are 3 years old also need to be monitored.

821 Project Technical Profile :

8211 Technical Programme :
(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments etc.)

a) Grafting Sreekara variety on resistant lines of black pepper and screening.

Black pepper lines C-820 and HP-39 (resistant to nematode *Radopholus similis*); C-1090, P-24(IISR-Shakthi) and IISR Thevam (moderately resistant to *Phytophtora capsici*) were collected and multiplied for grafting Sreekara in sufficient numbers through serpentine method and

raised in bags of 25cm x 15cm size with sterilized potting mixture. Runners of Sreekara variety of black pepper were cleft grafted on the resistant lines to get sufficient number of grafts for screening trial. In C-820 the numbers of cuttings raised were 156 and a graft success of 60% (94/156) was obtained and these grafts were used for nematode resistance trial during 2007-08 and 2008-09. The number of rooted cuttings raised in HP-39 and C-1090 were 100 each and 120 in P-24(IISR-Shakthi). These were grafted with Sreekara that gave a success of 10%, 66.67% and 78% respectively for HP-39, P-24(IISR-Shakthi) and C-1090. All these grafts were again raised in big polybags with sterilized potting mixture for screening. The growth and graft success were poor in HP-39 due to virus problem and IISR Thevam was found susceptible to *Phytophthora.* So these two lines were discarded from trials.

For nematode screening the treatments were given as follows with C-820 as rootstock with Sreekara as scion .*Phytophthora* was inoculated as inoculum of 10mm size (10 Nos.) discs inserted to the plant base whereas nematodes (*Radopholus similis*) was inoculated as suspension of culture .

Sl. No.	Treatment	Number of grafts treated
1	Absolute control	10
2	Phytophthora alone	10
3	Nematode alone	10
4	Phytophthora +Nematode	10
5	COC treatment	10

COC (copper oxychloride) was given at 3 monthly intervals @ 2.5gm / litre as soil drench. The trial was monitored for 8 months. Survival of grafts and root rot were recorded counting infected roots. Presence of nematode was confirmed with the help of nematologist.

Screening of Sreekara grafts on C-1090 and P-24(IISR-Shakthi) as rootstocks was done initially with following treatments

Sl. No.	Treatment	Number of grafts treated
1	Absolute control	12
2	Phytophthora alone	12
3	Nematode alone	12
4	Phytophthora +Nematode	12
5	Phorate 1g per bag	12

Inoculation with *Phytophthora* was done as above. The trial was monitored for six months. Survival of grafts and root rot were recorded in percentage. Since comparison cannot be made easily between rootstocks and grafts, the IRC in 2009 suggested to repeat the trial with the following treatments. Two separate trials were carried out respectively for grafts on P-24(IISR – Shakthi) and C-1090 raising 60 grafts of Sreekara in each rootstock with non grafted rootstock. COC was given @ 2.5gm per litre of water as soil drench and phorate @ 1g per bag.

Sl. No.	Treatment	Number of grafts treated	Absolute control (No. of non grafted rootstock)
1	Sterilized potting mixture alone	10	10
2	Chemical control (COC+Phorate)	10	10
3	Phytophthora alone	10	10
4	Nematode alone	10	10
5	Phytophthora+ Nematode	10	10
6	Phorate alone	10	10

The second trial was monitored for six months and survival and percentage root rot were recorded. Since there was anthracnose infection on C-1090 and many plants died, the trial on C-1090 was repeated during 2010-11 by providing control for *Colletotrichum* infection. Bavistin was sprayed at 0.1 g per litre of water at 3 month intervals. Here also 60 grafts were prepared with 60 rooted cuttings of C-1090. **Growth studies** on Sreekara grafts 30 each on P-24(IISR-Shakthi) and C-1090 were carried out for 5 months in pot culture. Data on height, number of nodes and shoot dry weight were recorded and analysed. Similarly data on number of nodes and height were also recorded in another group of grafts aged 10 months. These grafts were field planted and established at 3mx3m spacing for yield evaluation.

b) Screening of wild *Piper* accessions against pathogens.

The black pepper germplasm of IISR at Experimental farm holds several wild *Piper* accessions. The reaction of these germplasm materials to the pathogens of pepper is not yet studied. In order to identify and characterise resistant wild accessions for use as resistant rootstocks for pepper, rooted cuttings of these wild accessions were prepared in polybags and screened against *Phytophthora capsici* through artificial inoculation and against the nematodes, *Radopholus similis* and *Meloidogyne incognita* by growing in sick plots. Ten rooted cuttings were used against each pathogen and a total of 30 cuttings were prepared and handed over to the pathologist and nematologist for screening. This is the first time that such an evaluation has been done and that has resulted in very useful and hither to unknown information. The following 22 wild *Piper* accessions and IISR Thevam variety were screened. Due to lack of cuttings *P.magnificum* could not be screened against nematodes.

I) Wild *Piper* accessions screened against pathogens

1) *Piper colubrinum acc. 392*
2) *Piper sp. acc. 5817*
3) *Piper sp.acc. 5815*
4) *Piper sp.acc. 5587*

5) *Piper arboretum acc. 3363*

6) *Piper sylvaticum acc. 3177*

7) *Piper ribusoides acc.5225*

8) *Piper chaba acc.692*

9) *Piper betel acc.3357*

10) *Piper ornatum acc.3362*

11) *Piper galeatum acc. 3030*

12) *Piper attenuatum acc.664*

13) *Piper trichostachyon acc.611*

14) *Piper hymenophyllum acc.5315*

15) *Piper argyrophyllum acc.5369*

16) *Piper magnificum acc.5816*

17) *Piper hmiltoni acc.5532*

18) *Piper peepuloides acc.5526*

19) *Piper longum acc.6052*

20) *Piper thomsoni acc.5528*

21) *Piper sp. Acc. 6046*

22) *Piper sermantosum acc.4381*

II) Variety IISR Thevam

c) Grafting of Sreekara variety on wild resistant *Piper* accessions

Grafting of the wild *Piper colubrinum* using black pepper varieties has been done earlier and grafted pepper is being grown by farmers as well as nurserymen. So no new trial with this species was done except as interstock. Grafting of black pepper on *P.magnificum* was a failure in earlier attempts. So the species tried for direct grafting of Sreekara are *P.ornatum, P.hamiltoni, P.sylvaticum, Piper sp.*acc 5815 and *Piper acc.6046.* Sreekara was grafted with *P.sylvaticum* (10 numbers each) as rootstock and monitored for six months (All died). *P.ornatum* was grafted with Sreekara (65 Nos) and monitored for two years. *Piper* acc. 6046 was grafted with Sreekara (21 Nos) and monitored for six months since the species initially did not take up infection but after two inoculations with *Phytophthora* the accession was found susceptible and discarded as rootstock (All grafts died after developing yellowing of rootstock).

Since the shoots of *P.hamiltoni* were thin, initially 10 grafts were made with Sreekara till more thicker rootstocks were made available. The successful grafts (8 nos.) were field planted under rainfed conditions and monitored for 2 years. *P.hamiltoni* was grafted with runners (25 Nos), laterals(35 Nos)

and top shoots (35 Nos) of Karimunda acc.5835 and monitored. Though initial success was good, all died subsequently and hence Sreekara was grafted with it again (50 Nos) and monitored for 15 months to see survival. These grafts were planted in the field in 2012 season with irrigation to see survival and yield.

c-i)Interstock grafting

Since *P.ornatum* was not graft compatible with Sreekara, seven other *Piper sp.* were grafted on *P.ornatum* for use as interstock. Only 5 graftings were done in each initially due to shortage of cuttings and monitored. The species grafted were *P.galeatum, P.attenuatum, P.colubrinum, P.argyrophyllum, P.longum, P.betel* and *P.chaba* .Due to lack of thickening of root stock and aerial root formation in *P.betel* and *P.colubrinum* (signs of incompatibility) these two species were discarded from further trials. To improve the success rate *P.attenuatum, P.argyrophyllum, P.lonum, P.chaba and P.betel* (acc.4380) was again grafted on *P.ornatum* along with untested species- *P.hymenophyllum, P.sermantosum, P.hamiltoni, P.hapnium, P.peepuloides, Piper* acc.6046 and *P.suganthi,* 10-20 numbers each and monitored. Though the success was poor Sreekara was grafted on to these interstock species as per availability i.e. *P.ornatum+P.sermantosum* (2nos), *P.ornatum+ P.attenuatum* (2nos) , *P.ornatum+P.argyrophyllum* (2 nos) , *P.ornatum+ P.chaba* (1no) and monitored for one year. Since casualities were observed due to incidence of disease and thinness of stock/scions further grafting was done with *P.ornatum* and *P.hamiltoni* (interstock) only both being resistant to pathogens, after raising them in bags and obtaining thicker shoots. The resistant 14 grafts out of 57 (33.3% success) were grafted with Sreekara and monitored for one year. Rotting of rootstock was seen resulting in low graft take. The scions remain green and fresh even though rootstock was rotten and gone. The sample was given to pathologist for identification.

Piper acc.5815 having vigorous growth with profuse root system and resistant to all major pathogens was multiplied as rooted cuttings (100 numbers) and grafted with nine *Piper* species for use as interstock since it is incompatible with pepper. Ten graftings (cleft graft) were done with each species namely, *P.colubrinum, P.chaba, P.hamiltoni, P.attenuatum, P.argyrophyllum, P.longum, P.hymenophyllum, P.sermantosum* and *P.hapnium* and monitored for 8 months. Since *P.colubrinum* and *P.hamiltoni* gave 70% success all other species with low success were discarded from further trials. Fifty graftings were done with *P.colubrinum* and *P.hamiltoni* each on *Piper sp.* Acc.5815 and gave 100% graft take. These were grafted with Sreekara along with the seven available in each from earlier trials and success was monitored for 5 months. The success was noted in terms of survival and growth.

d) Nurse grafting of Sreekara with resistant *Piper sp.* to induce systematic protection

In nurse grafting, the rootstock and scion are allowed to grow together to induce growth or protection or to enhance some other aspect of a plant to obtain good productivity. So Sreekara, highly susceptible to *Phytophthora* and nematodes was approach grafted with the resistant species *P.colubrinum and Piper acc.*(5815) and the union was maintained to see transmission of any antagonistic factors from stock to scion giving protection against the pathogens.

Two approach grafts were made and grown in a 12`` pot and grown for six months. A total of 60 such grafts were prepared in 30 pots for each rootstock species. Grafts with good union were selected and the following treatments were given to 10 grafts each i.e 40 plants for each species.

1)Absolute control - - 10 plants
2)*Phytophthora* inoculation alone - 10 plants
3) *Radopholus similis* inoculation – 10 plants
4) *Phytophthora* and *Radopholus similis* inoculation – 10 plants

Observations were taken after six months of growth and survival of scion and root rot were recorded.

e) Maintenance of graft plot.

A total of 214 grafts of black pepper established in the low lying area of farm was maintained by irrigation, gap filling etc. In 2009 an unprecedented flood caused death of 115 pepper scions indicating that graft union should be above flood level point in low lying areas. The plot was subsequently maintained for supply of colubrinum cuttings to farmers because the damage from elephants also increased.

f) Bush pepper production

To produce bush pepper plants using *P.colubrinum* as rootstock 650 rooted cutting were raised in polybags. Laterals of the following varieties were grafted for this. Simple cleft grafting was done and success monitored for six months.

Sl. No.	Variety	Number grafted
1	Kathirinmel kathir	10
2	Panniyur -5	30
3	Sreekara	120
4	Muzhakkunnam local	30
5	Pournami	14
6	Kottanadan	44
7	Kumbhakkal	4
8	Konnikodi	18
9	Wayandan Chettimulaku	11
10	Karimunda	350
	Total	**631**

The successful grafts were planted in the field as maintained up to 31.3.13

g) Studies on mentor graft seedlings.

Laterals of Panniyur – I variety was grafted on *P.colubrinum* and grown in nursery and allowed to grow without leaves, allowing transport of food materials to the scion only from the rootstock. After ripening of the spikes the ripe berries were buried in soil to obtain seedlings. A total of ten seedlings were obtained out of which three died. There were variations in the number of cotyledons among the seedlings. These were grown in bigger bags and leaf sample were taken for DNA analysis from 9 seedlings. One seedling No.9 was very weak with yellowish leaves showing drastic variation compared to other seedlings. The seedlings that have grown were planted in field to see spike formation and are growing satisfactorily. Due to poor growth and yellowing of seedling No.9 variant it was grafted with 2 colubrinum and 2 pepper (P-24) rooted cuttings.

g-i)Molecular studies on mentor graft seedlings Panniyur-1

Leaf samples of 9 seedlings of mentor graft of P-1 on colubrinum were collected, high quality genomic DNA was isolated and ISSR profiling was performed in a total volume of 25 µl including 2.5µ of 10 X Taq DNA polymerase Buffer, 50 ng of total cellular DNA, 0.15 mMdNTP (Fermentas), 1.5 mM $MgCl_2$, 0.4 µM primers (Sigma Aldrich, Germany) and 1 U Taq DNA Polymerase (Bangalore Genei, Bangalore). PCR amplification were performed in a LabnetMultigene gradient Thermocycler as follows: an initial step of 4 min 94° C for 5 mins, followed by 33 cycles, each one including 45s at 94° C for denaturation, 45 s at 50° C for annealing and 2 mins at 72° C for elongation. A 10 mins step at 72° C is programmed as a final extension.

Amplification products were separated by electrophoresis on 1.5% and 3%agarose gel (Lonza) and in 1X TAE buffer (pH 8.0) stained with Ethidium Bromide for ISSR profiling.

8212 Total man months involvement of component project workers

P.A.Mathew - 20mm

K Abhirami - 3mm

R Suseela Bhai – 2mm

S.J.Eapen - 2mm

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results (not exceeding 2-5 pages)

8221 Achievements in terms of targets fixed for each activity

a) Grafting Sreekara variety on resistant lines of pepper and screening

The total numbers of cuttings raised in each resistant line were 100 in HP-39,156 in C-820, 220 in C-1090 and 120 in P-24. Grafting of Sreekara gave a success of 10%, 60%, 66.67% and 78% respectively on HP-39,C-820, P-24 and C-1090. The growth and success of grafting was poor on HP-39 due to severe virus problem. IISR Thevam, though reported to be tolerant to *Phytophthora capsici* in field, it was found susceptible when inoculated and is not suitable as rootstock. C-1090 was found susceptible to anthracnose (*Colletorichum sp.*) and hence carbendazim application is necessary wherever it is used. The results of nematode resistance trial are given in table I.

Table-I.Results of screening of C-820 as resistant rootstock against *Radopholus similis* for Sreekara

Sl. No.	Treatment	No.of grafts	Death	Root rot %
1.	Absolute control	10	7	94.44
2.	*Phytophthora* alone	10	7	100.00
3.	Nematode alone	10	8	100.00
4	*Phytophthora*+Nematode	10	8	100.00
5.	COC treatment	10	7	100.00

The results indicated that C-820 is not suitable as resistant rootstock against *Radopholus similis*. There was severe infection of roots with nematodes with high death of plants. It's resistance appeared to be broken down.

The results of the first trial of Sreekara grafts on P-24(IISR –Shakthi) and C-1090 are presented in table II

Table –II. Results of screening *Phytophthora* tolerant lines P-24(IISR –Shakthi) and C-1090 as rootstocks for Sreekara

Sl. No.	Treatment	No of grafts	P-24		C-1090	
			Survival %	Root rot %	Survival %	Root rot %
1.	Absolute control	12	6 (50.00)	60.14	10 (83.33)	79.30
2.	*Phytophthora* alone	12	7 (58.33)	95.69	10(83.33)	79.58
3.	Nematode alone	12	6(50.00)	95.13	9(75.00)	95.21
4.	*Phytophthora*+Nematode	12	5(41.67)	96.36	9(75.00)	97.43
5.	Phorate (1g/bag)	12	10(83.33)	25.63	11(91.67)	14.00

The survival of grafts is very good but the root rot is severe in all cases where phorate is not given. The root rot was mainly caused by *Radopholus similis* on examination. The intensity of nematode infection was less in C-1090 as compared to P-24(IISR –Shakthi). Since C-1090 is tolerant to *Phytophthora* and the nematode *Meloidogyne incognita* , it can be successfully used as a rootstock to control foot rot if complete control of nematode is ensured.

The results of modified trials are given in tables III and IV. The data on death and root rot indicated that both the rootstocks had less than 10% root rot as grafts with nematode control chemically. On examination it was observed that root rot was caused by *Radopholus similis* and *Phytophthora* was not detected. So both lines show tolerance to *Phytophthora capsici* and can be used as fungus tolerant rootstock. However, the line C-1090 was severely infected with leaf rot which was later identified as anthracnose caused by *Colletotricum sp.* and resulted in death of many plants. So the trial of C-1090 was repeated with carbendazim treatment and the results are given in Table - V

Table – III. Death and root rot in P-24 **(IISR –Shakthi)** rootstock grafted with Sreekara variety

S.No	Treatments	No.of plants	Absolute control Rootstock alone		Sreekara + root stock (grafted)	
			Death (%)	Root rot (%)	Death (%)	Root rot (%)
1.	Sterilized potting mixture	10	0.0	73.2	20.0	83.33
2.	Chemical control - COC +Phorate	10	0.0	8.11	0.0	10.43
3.	*Phytophthora* alone	10	10.0	45.24	20.0	52.00
4.	Nematode alone	10	30.0	81.18	30.0	85.71
5.	*Phytophthora* +nematode	10	30.0	85.71	40.0	87.50
6.	Phorate alone	10	0.0	9.18	0.0	9.10

Table – IV. Death and root rot in C-1090 rootstock grafted with Sreekara variety

S.No	Treatments	No. of plants	Absolute control Rootstock alone		Sreekara + root stock (grafted)	
			Death (%)	Root rot (%)	Death (%)	Root rot (%)
1.	Sterilized potting mixture	10	70.0	93.55	100.00	0.0
2.	Chemical control COC +Phorate	10	40.0	25.86	0.0	0.0
3.	*Phytophthora* alone	10	90.0	100.00	100.00	0.0
4.	Nematode alone	10	30.0	100.00	100.00	0.0
5.	*Phytophthora* + nematode	10	70.0	100.00	100.00	0.0
6.	Phorate alone	10	50.0	15.91	20.0	0.1

(The death of plants was due to anthracnose infection)

Table – V. Death and root rot in C-1090 rootstock grafted with Sreekara variety *

S.No	Treatments	No. of plants	Absolute control Rootstock alone		Sreekara + root stock (grafted)	
			Death (%)	Root rot (%)	Death (%)	Root rot (%)
1.	Sterilized potting mixture	10	0.0	79.68	0.0	85.43
2.	Chemical control COC +Phorate	10	0.0	11.60	0.0	12.84
3.	*Phytophthora* alone	10	0.0	95.56	0.0	97.67
4.	Nematode alone (*Radopholus similis)*	10	10.0	99.15	20.0	98.85
5.	*Phytophthora* + nematode (*Radopholus similis*)	10	10.0	99.56	20.0	100.00
6.	Phorate alone	10	0.0	14.59	0.0	15.31

- Results of repeated trial with C-1090

The results showed that bavistin was effective in controlling leaf rot caused by *Colletotrichum sp.* as indicated by the low death rate. The root rot in non-chemical treatments is severe and it was mainly due to severe infestation of nematode (*Radopholus similis)* as identified by the nematologist. Even without COC and using phorate alone, the root rot between rootstock alone (14.59%) and graft

(15.31%) is seen not much different. So C-1090 can be successfully used as a *Phytophthora* tolerant rootstock provided effective control of nematode, *Radopholus similis* is ensured.
Finally it can be concluded that both P-24 (IISR –Shakthi) and C-1090 can be utilized as rootstocks tolerant to *Phytophthora capsici*. So grafts of Sreekara were prepared and established in the field for yield evaluation.

a-i) Growth studies:-

Since P-24 (IISR –Shakthi) and C-1090 are useful as rootstocks, growth studies on Sreekara grafted on them was carried out. The results are presented in Table VI.

Table – VI. Data on growth of Sreekara grafted on P-24(IISR –Shakthi) **and C – 1090 along with control**

S.No	Rootstock	Height (cm)	No. of nodes	Shoot dry weight(g)
1.	P-24(IISR –Shakthi)	56.36	11.11	3.27
2.	C-1090	51.96	9.74	3.07
3.	Sreekara (control)	54.14	10.68	3.19
	Mean	54.1596	10.3684	3.1742
	SED	6.8607	1.2559	0.3961
	CV %	39.04	37.33	38.46
		NS	NS	NS

When grafts were prepared for field planting, these were grown for 10 months and observations on shoot height and nodes were collected. These data are given in Table VII.

Table - VII . Data on growth of Sreekara grafted on P-24(IISR –Shakthi) and C – 1090

S.No.	Rootstock	No. of nodes	Shoot height (cm)
1.	P-24(IISR –Shakthi)	9.6	
2.	C-1090	15.0	97.33
T-value		1.69	0.50
T-value (Table)		1.72	1.72
		NS	NS

The results on growth studies indicated that there is no significant variation in scion growth between P-24(IISR –Shakthi) and C-1090 and hence both can be utilised as rootstock for Sreekara or even for other varieties. However, long term yield evaluation is necessary.

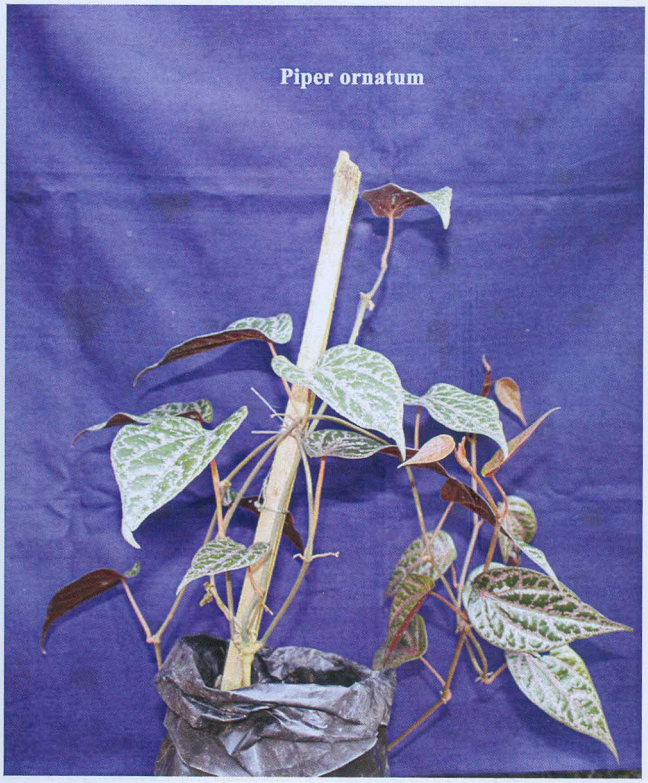
b) Screening of wild *Piper* accession against pathogens

Out of 22 wild *Piper* accessions screened against major pathogens of black pepper, *Piper colubrinum* and *Piper ornatum* were resistant to the fungus *Phytophthora capsici* and the nematodes *Radopholus similis* and *Meloidogyne incognita* whereas *P.sylvaticum, P.hamiltoni* and *P.magnificum* were found resistant only to *Phytophthora capsici.* Though *P.magnificum* could not be screened against nematodes, its rootsystem was never found infested with nematodes indicating resistance. The variety IISR Thevam also was found susceptible to all the pathogens though it is reported to be tolerant. The accessions *P.colubrinum* and *P.hamiltoni* were found highly susceptible to drought. In addition it was also seen that *P.thomsoni* was susceptible to *Pythium sp.* whereas *P.ornatum* was susceptible to *Sclerotium rolfsii* under high rainfall/ humid conditions. Considering the above resistance these species were used for grafting pepper as resistant rootstocks.


P. sylvaticum


Piper acc. 5815


Piper colubrinum


Piper ornatum


Piper hamiltoni

c) Grafting Sreekara variety on wild resistant *Piper* accessions

Piper colubrinum resistant to the three known pathogens of pepper is already used a rootstock by farmers based on earlier experiments, under irrigated conditions. Earlier exploratory attempts of grafting pepper on *P.magnificum* was a failure. So in this project these were not directly used as rootstock for pepper.

Piper sylvaticum and *Piper sp. acc.5815* were grafted with Sreekara but death of scion was observed after six months. So, these are unsuitable as rootstock for pepper. It was observed that the virus infecting pepper is easily transmited to *P. sylvaticum* and can be used as an indicator plant. *Piper acc.*6046 was grafted with Sreekara since it was found resistant initially but all grafts died after developing yellowing of rootstock and the accession was found susceptible to all pathogens.

In *Piper ornatum 62* graftings were done with Sreekara though initially 10% success was observed, only one graft survived and remained alive for two years and died showing that the species is incompatible with black pepper and cannot be directly used.

Initial grafting of *P.hamiltoni* with Sreekara gave 80% success. The grafts grew successfully in the field and grown to a height of 1.5 meters in nine months. However all grafts died during summer due to drought. Hence it cannot be grown as rainfed crop. Studies also indicated that it is highly susceptible to water deficit. Grafting of Acc.5835 (Karimunda) with *P.hamiltoni* using top shoots (35Nos), laterals (35 Nos) and runners (25Nos) were not successful. Probably the rootstock might have some specificity towards varieties of pepper. However, grafting of Sreekara on *P.hamiltoni* gave 50% success when monitored for 15 months and the growth was normal. The grafts obtained from this (17 Nos) were established in field in 2012 and maintained with irrigation to assess yields.

c-i) Interstock grafting:-

The results of grafting seven *Piper sp.* as scion on *P.ornatum* indicated the best success was obtained with *P.colubrinum* followed by *P.betel* and *P.longum.* However, it was observed that there was lack of thickening of *P.ornatum* rootstock and profuse root formation in scions of *P.colubrinum* and *P.betel.* Since these are signs of incompatibility these species were discarded from further trials as rootstock.

The results of grafting of *P.ornatum* with 12 *Piper* species are given below. The success rate obtained is low probably because of the thin nature of the scion.

S.No.	Species grafted on *P.ornatum*	Success
1.	*Piper acc.*4380	0 out of 10 *P.ornatum stock*
2.	*P.chaba*	1 out of 20 *P.ornatum stock*
3.	*P.longum*	0 out of 10 *P.ornatum stock*
4.	*P.argyrophyllum*	1 out of 30 *P.ornatum stock*
5.	*P.hymenophyllum*	8 out of 30 *P.ornatum stock*
6.	*P.sermantosum*	2 out of 10 *P.ornatum stock*
7.	*P.attenuatum*	4 out of *20 P.ornatum stock*
8.	*P.hamiltoni*	1 out of 10 *P.ornatum stock*
9	*P.hapnium*	0 out of 10 *P.ornatum stock*
10.	*P.peepuloides*	0 out of 10 *P.ornatum stock*
11.	*Piper acc.6046*	0 out of 10 *P.ornatum stock*
12.	*P.suganthi*	0 out of 10 *P.ornatum stock*

Grafting of Sreekara on these interstock with *P.ornatum* as base did not succeed since most of them were susceptible to *Phytophthora* and died. It may be pointed out that though success of *P.chaba* on *P.ornatum* was poor, the graft survived for two years with good growth and started giving spikes. So with better scion selection, success may be improved and further studies are warranted. There was uniform growth of *P.ornatum* and *P.chaba* indicating close affinity. Nevertheless, *P.chaba* being grown as a medicinal plant and equally susceptible to foot rot, *P.ornatum* may be recommended as a resistant rootstock. Since *P.hamiltoni* and *P.ornatum* only were disease resistant, grafting of these species after obtaining thick shoots was done but the success was generally poor. Only 14 grafts were obtained out of 57 (33.3%) done and these were grafted with Sreekara. Though initial sprouting and growth were good, all died subsequently indicating incompatibility. The low success of grafting in *P.ornatum* with *P.hamiltoni* was because of the death of *P.ornatum* due to infection of *Scleortium rolfsii* in the rainy season when grafting is generally done. Scions remained green even after stock has rotten completely.

The results of cleft grafting *Piper acc.*5815 with nine species are given below observed after eight months.

S.No.	Species grafted as interstock on *Piper acc.*5815	Success
1.	*P.colubrinum*	7 out of 10
2.	*P.hamiltoni*	7 out of 10
3.	*P.longum*	3 out of 10
4.	*P.chaba*	0 out of 10
5.	*P.hymenophyllum*	0 out of 10
6.	*P.sermantosum*	0 out of 10
7.	*P.attenuatum*	0 out of *10*
8.	*P.hapnium*	0 out of 10
9	*P.argyrophyllum*	0 out of10

It is seen that *P.colubrinum* and *P.hamiltoni* gave 70% survival and good growth. So regrafting of these species on *Piper acc.5815* was done with 50 numbers each and the success obtained was 100%.

Sreekara was grafted again on these interstocks 57 each and the success was only two with *P.colubrinum* and three with *P.hamiltoni* interstock and the growth was very poor. So interstock grafting is not successful.

In conclusion, it can be assumed that interstock grafting of *P.ornatum* and *Piper acc.*5815 cannot be done with the existing species tested and more explorations may have to be done to use resistant species directly.

b) Nurse grafting of Sreekara with resistant *Piper sp.* to induce systemic resistance.

Nurse grafting is a method in which the rootstock and scion are allowed to grow together to induce growth to obtain good productivity. So Sreekara, being highly susceptible to *Phytophthora* and nematodes was approach grafted with the resistant species. *P.colubrinum* and *Piper acc.(5815)* and were grown for six months. The results showed that there was severe root rot in Sreekara roots caused by *Radopholus similis* and *Phytophthora* in all the four treatments including casualties. In absolute control 20% causality was observed whereas in combined inoculated treatment 70% mortality was seen. However no mortality or root rot were seen in the rootstocks. Severe root rot in Sreekara roots indicated that no antagonistic compounds from the grafted rootstock passed on to the scion 'Sreekara' to give any systemic protection to the roots of Sreekara. So this method is not feasible for protection of pepper against pathogens.


Interstock grafting
Piper acc.1815 + Colubrinum + Sreekara


Interstock grafting
Piper acc.5815 + P.hamiltoni+ Sreekara


P.ornatum + P.chaba


Mentor graft seedling no.9

c) Maintenance of graft plot.

A plot with 214 grafts of black pepper in a low-lying area was maintained. Due to an unprecedented flood in 2009 and subsequent inundation 115 scions died above graft union. Since wild animals also created problems in maintaining the plot, it was kept as a source of colubrinum cuttings demanded from farmers. The flooding indicated that colubrinum can survive flooding but not pepper. So wherever grafted pepper is grown, the graft union must be above the general flood level point.

d) Bush pepper production

The success obtained in bush pepper grafting on *P.colubrinum* is given below.

S. No.	Variety	No.of successful grafts
1.	Sreekara	70
2	Panniyur -5	15
3.	Muzhakunnam local	1
4.	Pournami	1
5.	Kottanadan	5
6.	Kumbhakkal	1
7.	Konnikodi	10
8.	Wayanadn chetti mulaku	1
9.	Karimunda	185
	Total	281

The average success is 46.91%. These grafts are established in the field (Block No.A)and are growing satisfactorily and started yielding spikes.

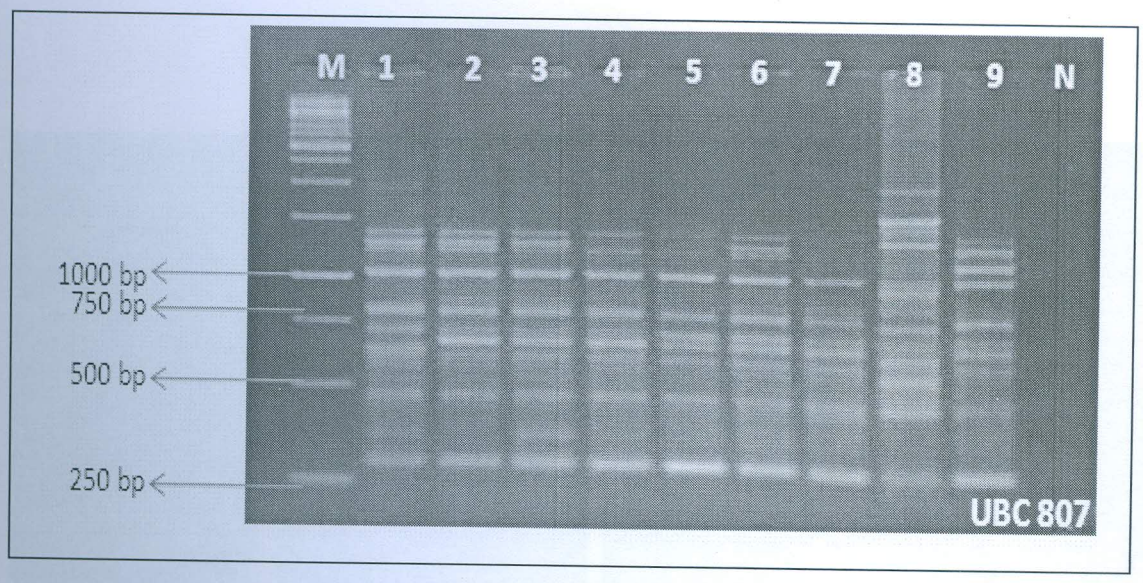
e) Studies on mentor graft seedling:-

The mentor graft technique was used to develop disease resistant pepper by grafting Panniyur – I on *P.colubrinum.* One ripe spike was obtained and 10 seedlings were raised. Out of this three weak seedlings died at different stages. Variation in the vigour of the seedlings in the number of cotyledons were observed. Five seedlings had only one cotyledon whereas 4 had two cotyledons. One seedling designated No.9 had high deviation from other seedlings with leaves resembling colubrinum having extra venation, poor growth and yellowing of leaves. This seedling was grafted on pepper (P-24) and colubrinum and it induced good growth. The seedlings exhibited root formation similar to colubrinum. The seedlings were planted in field and growing satisfactorily. Severe infection of leaves by *Colletotrichum sp.* seen in all seedlings. However, the grafts of seedling No.9 variant is growing better with more green leaves and show an extra venation similar to colubrinum as compared to pepper that need further studies.

Molecular studies

ISSR studies with two markers (UBC 807 and UBC 841a) did not show any sign of transfer of genetic material from colubrinum to the seedlings. The gel pictures are shown below.

Result



ISSR profile of Pepper samples using UBC *807 (5'- AGAGAGAGAGAGAGT -3').*

Lane M : 1 kb ladder ;Lane 1 : MGS 1, Lane 2 : MGS 2, Lane 3 : MGS 3, Lane 4 : MGS 4, Lane 5 : MGS 6, Lane 6 : MGS 7 , Lane 7 : MGS 9, Lane 8 : Colubrinum, Lane 9 : Panniyur 1

ISSR profile of Pepper samples using UBC *841a (5'- 5'- GAGAGAGAGAGAGAGACC-3').*

ane M : 1 kb ladder ;Lane 1 : MGS 1, Lane 2 : MGS 2, Lane 3 : MGS 3, Lane 4 : MGS 4, Lane 5 : MGS 6, Lane 6 : MGS 7 , Lane 7 : MGS 9, Lane 8 : Colubrinum, Lane 9 : Panniyur 1


Seedling number 9 grafted on Colubrinum


Seedling no.9 grafted on pepper


Vein pattern on seedling no.9

Summary of results

Screening of Sreekara variety of black pepper grafted on C-820 (nematode tolerant line) and C – 1090 and P-24(IISR –Shakthi) (*Phytophthora* moderately resistant lines) as rootstocks against pathogens indicated that C-820 is not resistant to the nematode *Radopholus similis* whereas C-1090 and P-24(IISR –Shakthi) are tolerant to *Phytophthora capsici.* However, *C-1090* and P-24(IISR – Shakthi) are susceptible to nematodes. Grafting of Sreekara on the above tolerant lines gave 78% success on C-1090 and 66.67% success on P-24(IISR –Shakthi). Growth studies indicated that Sreekara can grow successfully on both lines and the growth variations are insignificant. So C-1090 and P-24(IISR –Shakthi) can be considered as *Phytophthora* tolerant lines as rootstock for pepper with complete control of nematode. However, long term yield evaluation is needed. C-1090 was found susceptible to anthracnose disease. IISR Thevam was found susceptible to all the pathogens.

A total of 22 wild *Piper* accessions were screened. *P.ornatum*, *P.colubrinum*, *P.magnificum* and *Piper*.acc.– 5815 were found resistant to *Phytophthora capsici* and nematodes affecting pepper roots. *P.hamiltoni and P.sylvaticum* were resistant only to *P. capsici*. Out of the above, *P.colubrinum*, *P.hamiltoni* were found susceptible to drought. *P.thomsoni* was found susceptible to *Pythium* species also in addition to the other three pathogens. *P.ornatum* was found highly susceptible to *Sclerotium rolfsii* during high rainfall or under high humidity

Grafting of Sreekara on the resistant species indicated that success is obtained only on *P.colubrinum* and *P.hamiltoni* and so other species cannot be directly used as rootstocks. More studies were done with *P.hamiltoni* as rootstock and it gave a success of 50% and the growth was normal. However the grafts died in field under rainfed conditions; grafting of pepper on *colubrinum* is already standardised and is an established practice by farmers.

Interstock grafting of *P.ornatum* and *Piper*.acc. 5815 was done with other *Piper* species (12 nos.) compatible with pepper but the success was poor. So this method is not feasible to control foot rot. But the species may be used in breeding. However it was observed that Java pepper (*P.chaba*) grafted on P.*ornatum* grows very well and this resistant species may be used wherever foot rot is a problem for *P.chaba.*

Nurse grafting of Sreekara on *P.colubrinum* and *Piper*.acc.5815 did not induce any systemic resistance against pathogens indicating that no specific compounds are transmitted between stock and scions to give protection.

Grafting of laterals of nine varieties on colubrinum gave 46.91% success. The bush peppers obtained this way are growing satisfactorily in the field.

Nine seedlings were obtained from Panniyur I grafted on colubrinum and maintained as mentor graft. DNA marker studies were done on these seedlings to find out transfer of genes from colubrinum. But no such evidence was obtained. However one seedling showed extreme variation with additional venation on leaves similar to colubrinum and more marker studies are needed to evolve this method as a breeding tool.

i) Can C-820 black pepper line be used as rootstock for pepper (Sreekara) to get protection from nematodes?

No. C-820 did not give any resistance as rootstock against the nematode, *Radopholus similis,* causing root rot of black pepper.

ii) What is the reaction of C-1090 and P-24(IISR –Shakthi) lines of pepper tolerant to *Phytophthora capsici* as rootstock for pepper Sreekara?

C-1090 and P-24(IISR –Shakthi) showed tolerant reaction to *Phytophthora* when grown as graft with Sreekara as scion. The root rot was below 15% with control of nematodes whereas it was 100% without nematode control indicating that both these lines are susceptible to nematodes infecting pepper.

iii) What is the success of grafting Sreekara on C-1090 and P-24(IISR –Shakthi)? How is the growth on both the stocks?

C-1090 gave 78% success whereas P-24(IISR –Shakthi) gave 66.67% success. The growth of Sreekara on both the stocks has been very good and no significant differences were observed in terms of number of nodes, height and dry weight of scion shoot.

iv) Is IISR Thevam resistant to *Phytophthora capsici?*

No. Though the variety shows field tolerance, on artificial inoculation it succumbed to the disease.

v) How many wild *Piper* accessions were tested against the pathogens of pepper? What was the reaction?

22 *Piper* species accessions were tested. *P.colubrinum, P.ornatum, P.magnificum* and *Piper acc.*5815 were found resistant to *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita. P.sylvaticum* and *P.hamiltoni* were found resistant only to *Phytophthora cpsici. P.ornatum* was found susceptible to *sclerotium rolfsii* and *P.thomsoni* to *Pythium sp.*also.

vi) Are the identified *Piper sp.* graft compatible with black pepper (Sreekara)?

Sreeekara was graftable only on *P.colubrinum* and *P.hamiltoni*

vii) Are *P.colubrinum* and *P.hamiltoni* useful as rootstocks under rainfed conditions?

No. Both species require artificial irrigation during dry period to prevent death of grafts.

viii) Have any interstock graftings been done with the resistant incompatible species *P.ornatum,* and *Piper acc.5815?* If so what are the results?

Interstock grafting has been done. *Piper ornatum* was grafted with12 *Piper* species and 9 *Piper sp.* with *Piper acc.5815. P.hamiltoni* gave the best result on *P.ornatum* whereas *P.colubrinum* and *P.hamiltoni* were successful on *Piper acc.5815.* When these successful interstocks were grafted with Sreekara pepper variety the success obtained was poor in terms of survival and growth. So this method is not feasible to utilize the

resistant species directly in the field. However, grafting of *P.chaba (*Java pepper) on *P.ornatum* is highly successful and can be used wherever chaba is cultivated, if footrot is a serious problem.

ix) Does nurse grafting with the resistant species give any protection to pepper against the pathogens?

No protection was obtained. It appears that no antagonistic compounds are transmitted from rootstock to scion for systemic action and protection of roots.

x) Is grafting laterals on *P.colubrinum* successful as bush pepper? What is the result?

Nine varieties were grafted on *P.colubrinum* stock with laterals as scion and a success of 46.91% was obtained. These were field planted and are growing satisfactorily as bush pepper. Any pepper variety can be grafted on this species.

xi) Has any variation observed between mentor graft seedlings of Panniyur-I on colubrinum? Any molecular studies have been done?

Variation was observed between seedlings (9nos) obtained from mentor graft of Panniyur-I on colubrinum. One seedling was highly variant with slow growth and yellowish leaves which showed an extra venation similar to colubrinum leaves. ISSR marker studies were done but no evidence of genetic transfer obtained. Probably more specific markers may be needed.

xii) What is the result of grafting this variant on P-24 (pepper) and colubrinum stocks?

The growth is good with reduced yellowing and broadened leaves. These grafts may be monitored for further observations to explore the possibility of this method to obtain disease resistant lines of pepper.

8223 Process/Product/ Technology/ Developed

1. C-1090 and P-24(IISR –Shakthi) are identified as *Phytophthora* tolerant lines as rootstock for black pepper.

2. *P.colubrinum, P.ornatum, Piper acc. 5815* and *P.magnificum* are resistant to *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita* wheras *P.sylvaticum* and *P.hamiltoni* are resistant only to *Phytophthora capsici* among the wild *Piper* germplasm accessions studied. *P.ornatum* is susceptible to *Scleroticum rolfsii.* These are new and useful information for breeders/Pathologists.

3. *P.colubrinum* and *P.hamiltoni* only are compatible with pepper as resistant stock. Both species being susceptible to drought these can be grown only under irrigated conditions.
4. Interstock grafting of identified species resistant to pathogens is not feasible with pepper.
5. Nurse grafting technique with resistant wild *Piper* species is not useful as a protective means to prevent root rot.
6. Bush pepper can be prepared on *P.colubrinum* stock, using laterals of pepper and provides protection from pathogens.
7. Mentor grafting of pepper on colubrinum induces variation in seedlings but needs more studies to evolve it as a technique of crop improvement.