**PROFORMA FOR SUBMISSION OF FINAL REPORT OF RESEARCH PROJECTS**
**Part – I : General Information**

**800 Project Code** :
8001 Institute Project Code No. : Gen XVII (813)

8002 ICAR Project Code no. :

**801 Name of the Institute and Division**

8011 Name and address of the Institute : Indian Institute of Spices Research, Calicut – 673 012

8012 Name of Division / Section : Crop Improvement and Biotechnology

8013 Location of the project : Indian Institute of Spices Research, Calicut – 673 012

**Project Title : Breeding for Resistance to *Phytophthora* in Black pepper**

**802 Priority area:**
8031 Research approach:

**Applied res.**; **Basic res.**; Process/tech development/Transfer of technology
**01*** **02*** 03 04

**803 Specific area : Breeding, *Phytophthora* Resistance in Black pepper**

804 Duration of project : 2006- 2012

8051 Date of start : 31- 3-2006

8052 Date of completion : 31-3- 2012

**830 Total cost / Expenditure incurred** (Give reasons for variations, if any, from original estimated cost): **26.95 lakhs**

**805 Executive summary**

Black pepper (*Piper nigrum* L) is the worlds most important spice and is considered as 'king' of spices. It has originated and are cultivated in the hilly tracts of India and is an important part of coastal ecosystem of western cost of India. India has the largest variability in black pepper .

Black pepper is a perennial and this delays the conventional genetic characterization significantly. In this context preparation of molecular profiles will come a long way in characterizing the genetic resources and effectively utilizing them in crop improvement programmes. In addition absence of many classical phenotypic markers, molecular markers which are more reliable can supplement the phenotypic markers in characterizing the germplasm. The finger printing also helps in registering the varieties which is essential to avoid gene piracy. DNA markers (molecular markers) like RFLP, RAPD, AFLP, SSR, ISSR etc have very efficiently supplemented phenotypic markers in characterizing the germplasm and latter in preparation of genetic maps. This also helps in tagging many agronomically important characters. These maps will become a base by which Marker Assisted Selection (MAS) can be done in black pepper and reduce the breeding time.

It is imperative to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional as well as biotechnological approaches.

***Definition of Problem***

Black pepper (*Piper nigrum* L) is the world's most important spice and in India. The production of black pepper is stagnant irrespective of the development of many high yielding varieties. This is mainly due to the prevalence of epidemic diseases like *Phytophthora* foot rot in black pepper caused by *Phytophthora capsici.* The strategy of controlling the disease by chemical fungicides though effective leads to their indiscriminate use, chemical pollution and occurrence of pesticide residue in the produce. Developing disease resistance varieties is an important input in disease management programme. No known sources of high resistance is available in cultivated germplasm.

One variety IISR Sakthi with good resistance and one IISR Thevam field tolerant to *Phytophthora* were released for cultivation. Still no source for absolute resistance was found in cultivated black pepper. So far *Piper colubrinum* a distant species of Piper is the only source of resistance *Phytophthora capsici.* Much about inheritance patterns of *Phytophthora* resistance need to be understood. It is imperative to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional approaches.

In addition black pepper is a perennial and this delays the conventional genetic characterization significantly. Molecular tagging of genes of importance will lead to MAS and detection of *Phytophthora* resistant genotypes in seedling stage itself thus considerably reducing the breeding cycle in Black pepper. TRhis also helps in marker assisted gene isolation.

The present project was taken with an objective of achieving the above and the project proposes to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional and biotechnological approaches for identifying, understanding the inheritance patterns and tagging *Phytophthora* resistance short as well as long term perspective.

The main objectives of this project are

*Immediate objectives*

1. Evaluation of the tolerant lines identified so far for identifying the superior genotypes for immediate varietal development.
2. Use the information from the tolerant lines identified so far for studying the inheritance patterns.
3. Develop structured mapping population for preparation of molecular and genetic maps and tagging understanding the inheritance patterns through association mapping and pseudo test cross approaches.

*Long term objectives*

4. Identify good parental combination, develop hybrids and if possible differentials for *Phytophthora* resistance.
5. Attempt convergent breeding and MAS to reduce breeding time.

**The summery of achievements are**

**Development of mapping populations**

Two mapping populations of about 100 each were developed involving Panniyur 1 X Subhakara and P 24 (IISR Shakti ) X Subhakara for tagging of agronomically important charecters and Phyto[hthora resistance respectively.

These were maintained both in the field for phenotypic characterization and multiplied and maintained in the nursery for disease indexing/ screening. Panniyur and Subhakara (Karimunda selection) being most distinct in various morphological characters this population segregates for many morphological and yield attributing characters

In order to estimate the level of heterozygosity , ***Supporting populations of*** selfed progenies (about 100 lines) of the parents Sakthi, Subhakara and Panniyur 1 were developed for phenotyping and genotyping.

P 24 (IISR Sakthi - tolerant to *Phytophthora)* has its origin as open pollinated progeny of local variety Perambramunda. Screening next generation open pollinated progeny of P 24 lead to identification of P-24-O-4 which has higher level of resistance that its parent P-24. This may possibly indicate that selfing may lead to homozygosity of the alleles involved in resistance thus increasing its effectiveness. Hence another selfed population (can be called pseudo 'F3') of P-24-O-4 was developed for screening and obtaining higher degree of resistance

Accordingly a populations of 57 genotypes were selected from germplasm containing both resistant as well as susceptible genotypes. These were maintained both in the field ( Fig 3) for phenotypic characterization and multiplied and maintained in the nursery for disease indexing/ screening.

**Phenotyping of mapping population 1 (Panniyur 1 X Subhakara cross)**

P1 X Subhakara mapping population was phenotyped for floral and other segregating morphological characters like leaf shape and size, shoot tip colour, spike length, berry size etc . Most characters were seen segregating among progenies. Among the 95 progenies, 56 were of female parent type (Panniyur 1), 11 of male parent type (Subhakara) and 28 progenies were found to be recombinants.

Spike characters like spike length, fruit set, fruit size, male female flower ratio were seen segregating and most of the progenies are found to be inter mediate forms

A few promising lines were identified for yield characters also.

**Molecular characterization of mapping population**

*RAPD profiling of progenies of P1 X Subhakara cross*

In addition to **150 polymorphic markers already scored earlier** the mapping population P1 X Subhakara was screened and 10 additional marker were added. scored .

*ISSR profiling of progenies of P1 X Subhakara cross*

Twenty ISSR primers has been screened with parents-Panniyur1 and Subhakara. 10 primers that show polymorphism between parents is used to profile the progenies. ISSR profiling is being continued in PHYTOFURA Project.

*Molecular Characterization of Association Mapping Population*

DNA was isolated from 57 genotypes following modified Doyle and Doyle method. 57 genotypes of black pepper selected for Association Mapping were profiled with five universal primers of UBC series. The work will be continued in Phytofura Project.

**Screening of the progenies of mapping populations against *Phytophthora***

Twenty seven lines selected as association mapping population were screened using leaf, stem and root inoculation methods. Most of the genotypes were grouped as

either susceptible or moderately resistant. None of the genotypes were found to give highly resistant reaction. Accession number 1386 and 1389 both named *Kattanadan local* gave most tolerant reaction along with Hybrid *HP 750*. Cultivar 1324 was the best giving resistance reaction both stem and leaf screening

***New source of resistance to Phytophthora:***

An ornamental and exotic species of Piper, *Piper ornatum* was found to give resistant reaction against *Phytohthora*.

THE LEADS OBTAINED WERE BEEING UTILISED IN PHYTOFURA PROJECT .

**Key words** :
Black pepper, Breeding , Phytophthora resistance, Molecular maps, Tagging of resistance genes, Molecular characterization, *Piper nigrum*, Mapping population, Association mapping, Pseudo test cross.

**Part-II : Investigator Profile (Please identify clearly changes, if any in Project personnel)**

**810 Principal -Investigator**

| | | |
|---|---|---|
| 8101 | Name | : Dr K Nirmal Babu |
| 8102 | Designation | : Senior Scientist |
| 8103 | Division/Section | : Crop Improvement |
| 8104 | Location | : Indian Institute of Spices Research, Calicut |
| 8105 | Institute Address | : Indian Institute of Spices Research, Marikunnu P.O., Calicut – 673 012, Kerala, India |

**811 Principal -Investigator**

| | | |
|---|---|---|
| 8111 | Name | : Dr R Suseela Bhai |
| 8112 | Designation | : Senior Scientist |
| 8113 | Division/Section | : Crop Protection |
| 8114 | Location | : Indian Institute of Spices Research, Calicut |
| 8115 | Institute Address | : Indian Institute of Spices Research, Marikunnu P.O., Calicut – 673 012, Kerala, India |

**812 Co Principal -Investigator**

| | | |
|---|---|---|
| 8121 | Name | : Dr TE Sheeja |
| 8122 | Designation | : Sr. Scientist |
| 8123 | Division/Section | : Crop Improvement |
| 8124 | Location | : Cardamom Research Center (IISR) |
| 8125 | Institute Address | : Cardamom Research Center (IISR) Madikeri, Coog District, Karnataka |

**813 Co Principal -Investigator**

| | | |
|---|---|---|
| 8131 | Name | : Ms. Krishna Radhika |
| 8132 | Designation | : Scientist |
| 8133 | Division/Section | : Crop Improvement |
| 8134 | Location | : Indian Institute of Spices Research, Calicut |
| 8135 | Institute Address | : Indian Institute of Spices Research, |

## Part – III : Technical Details

### 820 Introduction and objectives

***Origin***

Black pepper (*Piper nigrum* L) is the worlds most important spice and is considered as 'king' of spices. It has originated and are cultivated in the hilly tracts of India and is an important part of coastal ecosystem of western cost of India. India has the largest variability in black pepper .

The production of black pepper is stagnant irrespective of the development of many high yielding varieties. This is mainly due to the prevalence of epidemic diseases like *Phytophthora* foot rot in black pepper caused by *Phytophthora capsici.* Black pepper is highly susceptible to infestation by *Phytophthora capsici.* The strategy of controlling the disease by chemical fungicides though effective leads to their indiscriminate use, chemical pollution and occurrence of pesticide residue in the produce. In many areas cultivation of black pepper has become impossible due to the prevalence of this disease. Plant protection methods have only limited success. Developing disease resistant varieties is an important input in disease management programme. No known sources of complete resistance are available in cultivated germplasm. In black pepper, after two decades of continued efforts, a few *Phytophthora* tolerant lines were identified and are being evaluated for their field tolerance. The only source of resistance is from a very distant species and a different cytotype, *Piper colubrinum*, which is not crossable to cultivated black pepper.

Black pepper is a perennial and this delays the conventional genetic characterization significantly. In this context preparation of molecular profiles will come a long way in characterizing the genetic resources and effectively utilizing them in crop improvement programmes. In addition absence of many classical phenotypic markers, molecular markers which are more reliable can supplement the phenotypic markers in characterizing the germplasm. The finger printing also helps in registering the varieties which is essential to avoid gene piracy. DNA markers (molecular markers) like RFLP, RAPD, AFLP, SSR, ISSR etc have very efficiently supplemented phenotypic markers in characterizing the germplasm and latter in preparation of genetic maps. This also helps

in tagging many agronomically important characters. These maps will become a base by which Marker Assisted Selection (MAS) can be done in black pepper and reduce the breeding time.

It is imperative to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional as well as biotechnological approaches.

***Definition of Problem***

Prevalence of epidemic diseases like *Phytophthora* foot rot in black pepper caused by *Phytophthora capsici* is a major production constraint black pepper. The strategy of controlling the disease by chemical fungicides though effective leads to their indiscriminate use, chemical pollution and occurrence of pesticide residue in the produce. Developing disease resistance varieties is an important input in disease management programme. No known sources of high resistance is available in cultivated germplasm.

In the initial stages breeding of pepper has focused only on developing high yielding and high quality genotypes through both selection and hybridization. Later Black pepper breeding focused on developing *Phytophthora capsici* resistant genotypes and varieties. The germplasm and good number of OP progenies and hybrids developed were screened for identifying tolerance/ resistance to *Phytophthora capsici* and this has resulted in identifying about 100 lines with varying degrees of tolerance but no resistant line was isolated so far from cultivated black pepper. One variety IISR Sakthi with good resistance and one IISR Thevam field tolerant to *Phytophthora* were released for cultivation. Still no source for absolute resistance was found in cultivated black pepper. So far *Piper colubrinum* a distant species of Piper is the only source of resistance *Phytophthora capsici.* Much about inheritance patterns of *Phytophthora* resistance need to be understood. It is imperative to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional approaches.

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***Long term objectives***

4. Identify good parental combination, develop hybrids and if possible differentials for *Phytophthora* resistance.
5. Attempt convergent breeding and MAS to reduce breeding time.

**8211 Total man months involvement of component project workers**

| | Months |
|---|---|
| K. Nirmal Babu | 21 |
| R Suseela Bhai | 14 |
| TE Sheeja | 14 |
| Krishna Radhika | 2 |

821 **Final report on the project.** Detailed report containing all relevant data with a summary of results (not exceeding 2-5 pages)
**See Annexure 1**

## 8221 Achievements in terms of targets fixed for each activity

### Development of mapping populations

Two mapping populations of about 100 each were developed involving Panniyur 1 X Subhakara and P 24 (IISR Shakti ) X Subhakara for tagging of agronomically important charecters and Phyto[hthora resistance respectively.

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***New source of resistance to Phytophthora:***

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**8222 Questions answered**

1. Whether *Phytophthora* resistance is qualitative or quantitative character?

   Based on the present study and based on the literature available *Phytophthora* resistance in black pepper is also a QTL. Hence finding absolute resistance is difficult in black pepper germplasm.

2. Whether multiple rersistance in genotypes is available in black pepper germlasm?

   Yes. The present study indicated that this is present. The Accession.1114 which gave tolerant reaction to *Phytophthora* was also found to be tolerant to Pollu beetle and drought in earlier studies.

**8223 Process / Product / Technology / Developed**

- **Products / Processes developed**

1. One mapping population of Panniyur 1 x Subhakara developed earlier was field maintained for field evaluation and phenotyping.
2. 3 new populations (about 100) each of P 24(Shakti) X Subhakara , OP progenies of Shakti, Subhakara and Panniyur 1 were developed for mapping
3. A population of 57 genotypes from germplasm for association mapping and an 'F3' population from P-24-04 for studying inheritance patterns of *Phytophthora* resistance.
4. Technology for ISSR and SSR profiling was developed.
5. Two genotypes (Acc.1324, Acc. 1114HP) tolerant to *Phytophthora* were identified and the tolerance of HP 780 was confirmed.

- **Patents:** nil

**3. Practical Utility (not more than 150 words)**

Three mapping were developed for the first time in black pepper in addition to supporting populations .

The molecular profiling techniques standardized will help in developing varietal specific profiles for genetic purity testing and IPR protection.

The resistant genotypes identified can be used in convergent breeding programmes for developing Phytophthora resistant varieties.

The promising high yielding lines developed can be further evaluated before releasing as varieties.

The molecular profiling and tagging will help in Marker Assisted Selection (MAS) can be done in black pepper and reduce the breeding time.

**4. Constraints, if any**

Black pepper is a perennial and study of inheritance patterns, development of resistant genotypes is a long term process especially in bringing QTLs thorough convergent breeding.

**823. Publications and materials development (one copy each to be supplied with this proforma)**

8231 Research / review papers :

1. Nirmal Babu K (2011) Breeding for *Phytophthora* resistance in black pepper. Status paper, International workshop and seminar on Phytophthora Diseases of Plantation Crops and their

Management, 12-14 September, 2011, Rubber research Institute, Kottayam, India (Abstracts: p 81-84.)

2. Nirmal Babu K, Sasikumar B, Jonson K George, Saji KV, Sheeja TE, Anandaraj M and Parthasarathy VA (2007) Molecular Markers – Spices *in* Proceedings of National Seminar on Horticultural Biotechnology : Present Status and Future Action Plan, IIHR, Bangalore,PP: 187-195.
3. Parthasarathy VA and Nirmal Babu K (2008) Approaches for Molecular Breeding in Perennial Arid Horticulture Crops, National Seminar on Opportunities and Challenges of Arid Horticulture for Nutrition and Livelihood, 7-8March, 2008, CIAH, Bikaner, India.
4. Parthasarathy VA and Nirmal Babu K (2008) Genome analysis in Plants- Molecular approaches, National Symposium on "From Chromosomes to Genomes- Challenges and Prospects", 26-28 March, 2008 Kerala University, Trivandrum- 695581, India. Abstracts, pp.20-21.

Book chapters :

1. Nirmal Babu K, Asha S, Saji KV and Parthasarathy VA (2011) Black pepper *in* HP Singh, VA Parthasarathy and K Nirmal Babu (Eds), *Advances in Horticulture Biotechnology - Vol 3 - Molecular Markers and Marker Assisted Selection - Fruit Crops, Plantation Crops and Spices*, Westville Publishing House, New Delhi, p. 247-260.
2. Nirmal Babu K, RR Nair, Saji KV and Parthasarathy VA (2012) Biotechnology *in* HP Singh, VA Parthasarathy, V Srinivasan & KV Saji (Eds), *Piperaceae* Westville Publishing House, New Delhi. p. 57-81.

**822 Popular articles/ Training:**

1. Nirmal Babu K (2009) Spices Biotechnology – Recent trends, Recent Trends in Botanical Research, 10 November 2009, Mangalore University, Mangalore.
2. Nirmal Babu K, Asha S, Jayakumar Vand Minoo D (2009) Genome Mapping in Plants, Winter School on "Bioinformatics and its Applications in Agriculture", 1-21$^{st}$ December 2009, Bioinformatics Centre, College of Horticulture, Kerala Agricultural University, Vellanikkara-680 656, Thrissur, Kerala, India.
3. Nirmal Babu K (2011) Breeding of perennial Spices. Gregor Mendal Birthday Lecture, 21 July, 2011, Gregor Mendal Foundation, Department of Botany, University of Calicut, Kerala.
4. Nirmal Babu K and Cissin J (2011) Development of Molecular Maps in Plants In Utpala P and Pervez R Eds) Summer training on Biochemistry, Biotechnology and Bioinformatics. May- June, 2011, IISR, Calicut.
5. Nirmal Babu K (2011) Molecular markers in crop management, XII Refresher course in life Sciences, (Thrust area: Molecular Biology), 4$^{th}$ August 2011, Academic Staff College, University of Calicut, Kerala.

823 **Reports** : Nil

824 Seminars, conferences and workshops (relevant to the project) in which the scientists have participated). List abstracts forwarded.

1. Gregor Mendal Birthday Lecture, 21 July, 2011, Gregor Mendal Foundation, Department of Botany, University of Calicut, Kerala.
2. Summer training on Biochemistry, Biotechnology and Bioinformatics.y, May- June, 2007-2011, IISR, Calicut.
3. International workshop and seminar on Phytophthora Diseases of Plantation Crops and their Management, 12-14 September, 2011, Rubber research Institute, Kottayam, India
4. National Symposium of Spices and Aromatic Crops 2011, University of Agricultural Sciences, Dharwar. Abstract, P. 223.

5. International symposium on Advances in Food Biotechnology and Nutrition" during 30th November- 1st December, 2007. Mar Athanasios College for Advanced Studies Tiruvalla (MACFAST).
6. Proceedings of National Seminar on Horticultural Biotechnology : Present Status and Future Action Plan, IIHR, Bangalore.
7. National Symposium on "From Chromosomes to Genomes- Challenges and Prospects", 26-28 March, 2008 Kerala University, Trivandrum.
8. Recent trends, Recent Trends in Botanical Research, 10 November 2009, Mangalore University, Mangalore.
9. Winter School on "Bioinformatics and its Applications in Agriculture", 1-21st December 2009, Bioinformatics Centre, College of Horticulture, Kerala Agricultural University, Vellanikkara-680 656, Thrissur
10. Interactive session on Biotechnology, ICAR, NASC Complex, July, 2010 New Delhi
11. National Consultative meet on Bioinformatics in Horticulture,11-12, November 2010, IISR, Calicut.
12. Consultation on Biotechnology Research in ICAR, July 26th, 2010NASC, New Delhi 110012.
13. Application of Genomicas and Bioinformatics in Phytophthora / Ralstonia Research, 8-17 February 2011, Indian Institute of Spices Research, Calicut. – Resource Person

**825 Infrastructural facilities developed (Details of field, laboratory, note books and final material and their location)**

1. One mapping population of Panniyur 1 x Subhakara developed earlier was field maintained for field evaluation and phenotyping.
2. 3 new populations (about 100) each of hybrid progenies of P 24(Sakhti) X Subhakara cross, selfed progenies of Shakti, Subhakara and Panniyur 1 were developed for mapping
3. A population of 57 genotypes from germplasm for association mapping and an 'F3' population from P-24-04 for studying inheritance patterns of *Phytophthora* resistance.

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

The genotypes identified as tolerant are being used as parental combinations along with IISR Sakthi, P24-O-4 and Panniyur-1.

The material and information generated from this project is being used to identify and isolate r genes responsible for *Phytophtohra* resistance under the national net work on Phytofura Project.

**Part – IV : Project Expenditure (Summary)**
**Year :-2009-2012**

831 Total recurring expenditure

8301 Salaries (Designation with pay scale)

| | Estimated | Actual in ;akhs |
|---|---|---|
| Scientific | | 17.70 |
| Technical | | - |
| Supporting | | 3.30 |
| Wages | | 2.10 |
| Sub total | | |

8302 Consumables

i. Chemicals -

ii. Consumables 0.90

iii. Other(over heads) 1.90

Sub Total

8303 Travel 1.05

8304 Miscellaneous (other costs)

8305 Sub total (recurring)

832 Total non recurring 1,30.000
Expenditure (Equipments and works)

**833** Total (830 and 831) **26.95 lakhs**

## Part – V : Declaration

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

Signature of the Project Investigators

| | |
|---|---|
| Principal Investigator: | K. Nirmal Babu |
| Co Investigators: | Dr R Suseela Bhai |
| | Dr TE Sheeja |
| | Ms Krishna Radhika |

**Signature and comments of the Head of the Division/Section**

**B. Krishnamoorthy**
Head, Division of Crop Improvement and Biotechnology
Indian Institute of Spices Research, Calicut – 673 012, Kerala

**Signature and comments of the Director**

3 Jan 2013

**Dr. M Anandaraj**
Director
Indian Institute of Spices Research, Calicut – 673 012, Kerala

**DETAILED REPORT OF THE PROJECT**

## Gen. XVII (813) Breeding black pepper for *Phytophthra* resistance

[2006-2011]

**(Part of this work is also reported in PhytoFuRa as both these projects go in conjunction)**

### Introduction

Black pepper ( *Piper nigrum* L) is the worlds most important spice and is considered as 'king' of spices. It has originated and are cultivated in the hilly tracts of India and is an important part of coastal ecosystem of western cost of India. India has the largest variability in black pepper .

The production of black pepper is stagnant irrespective of the development of many high yielding varieties. This is mainly due to the prevalence of epidemic diseases like *Phytophthora* foot rot in black pepper caused by *Phytophthora capsici.* Black pepper (*Piper nigrum* L.) is highly susceptible to infestation by *Phytophthora capsici.* The strategy of controlling the disease by chemical fungicides though effective leads to their indiscriminate use, chemical pollution and occurrence of pesticide residue in the produce. In many areas cultivation of black pepper has become impossible due to the prevalence of this disease. Plant protection methods have only limited success. Developing disease resistant varieties is an important input in disease management programme. No known sources of complete resistance are available in cultivated germplasm. In black pepper, after two decades of continued efforts, a few *Phytophthora* tolerant lines were identified and are being evaluated for their field tolerance. The only source of resistance is from a very distant species and a different cytotype, *Piper colubrinum*, which is not crossable to cultivated black pepper.

Black pepper is a perennial and this delays the conventional genetic characterization significantly. In this context preparation of molecular profiles will come a long way in characterizing the genetic resources and effectively utilizing them in crop improvement programmes. In addition absence of many classical phenotypic markers, molecular markers which are more reliable can supplement the phenotypic markers in

characterizing the germplasm. The finger printing also helps in registering the varieties which is essential to avoid gene piracy. DNA markers (molecular markers) like RFLP, RAPD, AFLP, SSR, ISSR etc have very efficiently supplemented phenotypic markers in characterizing the germplasm and latter in preparation of genetic maps. This also helps in tagging many agronomically important characters. These maps will become a base by which Marker Assisted Selection (MAS) can be done in black pepper and reduce the breeding time.

It is imperative to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional as well as biotechnological approaches.

***Definition of Problem***

Black pepper (*Piper nigrum* L) is the world's most important spice and in India. The production of black pepper is stagnant irrespective of the development of many high yielding varieties. This is mainly due to the prevalence of epidemic diseases like *Phytophthora* foot rot in black pepper caused by *Phytophthora capsici.* The strategy of controlling the disease by chemical fungicides though effective leads to their indiscriminate use, chemical pollution and occurrence of pesticide residue in the produce. Developing disease resistance varieties is an important input in disease management programme. No known sources of high resistance is available in cultivated germplasm.

One variety IISR Sakthi with good resistance and one IISR Thevam field tolerant to *Phytophthora* were released for cultivation. Still no source for absolute resistance was found in cultivated black pepper. So far *Piper colubrinum* a distant species of Piper is the only source of resistance *Phytophthora capsici.* Much about inheritance patterns of *Phytophthora* resistance need to be understood. It is imperative to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional approaches.

In addition black pepper is a perennial and this delays the conventional genetic characterization significantly. Molecular tagging of genes of importance will lead to MAS and detection of *Phytophthora* resistant genotypes in seedling stage itself thus considerably reducing the breeding cycle in Black pepper. TRhis also helps in marker assisted gene isolation.

**Objectives**

The present project was taken with an objective of achieving the above and the project proposes to pool up all the information available and design a breeding strategy to develop *Phytophthora capsici* resistant varieties in black pepper using conventional and biotechnological approaches for identifying, understanding the inheritance patterns and tagging *Phytophthora* resistance short as well as long term perspective.

*Main activities of the project are*

***Immediate objectives***

- Evaluation of all the tolerant lines identified so far for identifying the superior genotypes for immediate varietal development.
- Use the information from the tolerant lines identified so far for studying the inheritance patterns.
- Develop structured mapping population for preparation of molecular and genetic maps and tagging understanding the inheritance patterns through association mapping and pseudo test cross approaches.

***Immediate objectives***

- Identify good parental combination, develop hybrids and if possible differentials for *Phytophthora* resistance.
- Attempt convergent breeding and MAS to reduce breeding time.

**Achievements**

**Development of mapping populations**

***Mapping population 1*** *(Panniyur 1 X Subhakara)*

The mapping population of about 150 progenies were developed earlier using Panniyur 1 X Subhakara as parents and 144 of them were field planted earlier. These were maintained both in the field for phenotypic characterization and multiplied and maintained in the nursery for disease indexing/ screening. Panniyur and Subhakara (Karimunda selection) being most distinct in various morphological characters this population segregates for many morphological and yield attributing characters ( Fig 1).

Pepper being perennial heterozygote the mapping populations segregate mostly in F2 intercross fashion. Pseudo test cross approach would be suitable for mapping and tagging as was done in perennial Eucalyptus.

**Fig 1 Mapping population 1 of *Panniyur 1 X Subhakara* in the field**

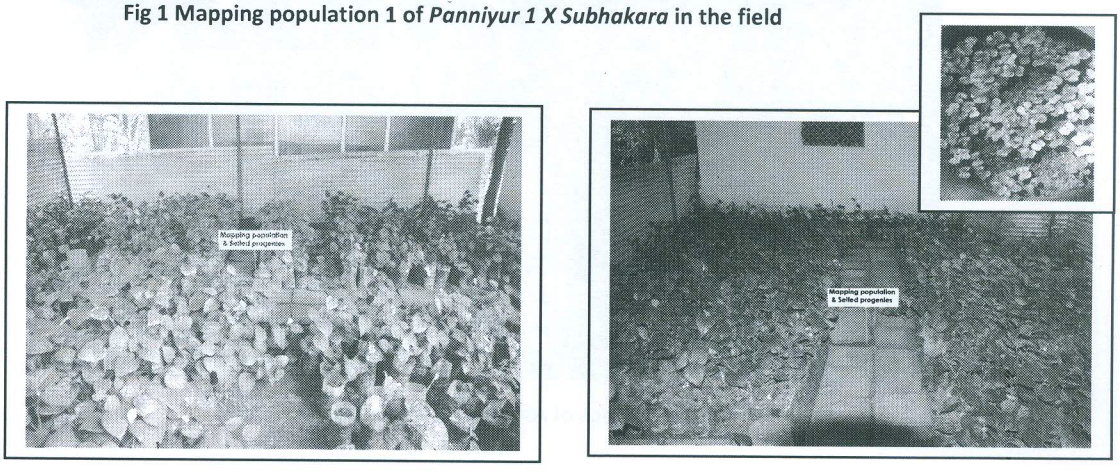


**Fig 2 Multiplication and maintenance of Mapping populations and selfed prohgenies in nursery for screening**

***Mapping population 2*** *(P 24 (IISR Shakti ) X Subhakara)*

In order to develop another mapping population segregating specially for *Phytophthora* resistance another population of about 100 progenies was developed by crossing between P 24 (IISR Sakthi - tolerant to *Phytophthora)* X Subhakara (susceptible to *Phytophthora.* These lines were multiplied and maintained in nursery( Fig 2).

***Supporting populations***

In order to estimate the level of heterozygosity , selfed progenies (about 100 lines) of the parents Sakthi, Subhakara and Panniyur 1 were developed for phenotyping and genotyping.

P 24 (IISR Sakthi - tolerant to *Phytophthora)* has its origin as open pollinated progeny of local variety Perambramunda. Screening next generation open pollinated progeny of P 24 lead to identification of P-24-O-4 which has higher level of resistance that its parent P-24. This may possibly indicate that selfing may lead to homozygosity of the alleles involved in resistance thus increasing its effectiveness. Hence another selfed population (can be called pseudo 'F3') of P-24-O-4 was developed for screening and obtaining higher degree of resistance ( Fig 2).

**Fig.3. Field maintenance of Association Mapping Population**

***Mapping population 3 for association mapping***

In a perennial heterozygote like Black pepper , sometimes it's preferable to use association mapping approach for tagging and mapping. Accordingly a populations of 57 genotypes were selected from germplasm containing both resistant as well as susceptible genotypes. These were maintained both in the field ( Fig 3) for phenotypic characterization and multiplied and maintained in the nursery for disease indexing/ screening.

**Maintenance and field characterization (phenotyping) of mapping population 1 (Panniyur 1 X Subhakara cross)**

This population was about 6 years old with varying degrees of growth. Data on floral and other segregating morphological characters which are distinct in the parents like leaf shape and size, shoot tip colour, spike length, berry size etc was recorded in P1 X Subhakara mapping population (Table 1 & 2). Most characters were seen segregating among progenies. The progenies were morphologically characterized and

classified as Panniyur type, Karimunda type and recombinant. Among the 95 progenies, 56 were of female parent type (Panniyur 1), 11 of male parent type (Subhakara) and 28 progenies were found to be recombinants.

**Table 1 Morphological characterization of Panniyur 1 x Subhakara mapping population**

| Progeny No. | Plant: Shoot Tip Colour | Leaf: Length (cm) | Leaf: Width (cm) | Leaf: Petiole Length (cm) | Leaf: Lamina Shape | Leaf: Base Shape | Leaf: Margin | Leaf: Lateral Branch Length (cm) | Number of Nodes/Lateral Branch | Lateral Branch Pattern |
|---|---|---|---|---|---|---|---|---|---|---|
| $P_1XK_1$ 1 | Light Purple | 12-Medium | 8-Medium | 2.5-Medium | Ovate-elliptic | Chordate | Even | 30-Medium | 4- Few | Semi-erect |
| $P_1XK_1$ 8 | Light Green | 12.5-Medium | 11-Medium | 4- Long | Chordate | Chordate | Even | 40-Medium | 9- Few | Hanging |
| $P_1XK_1$ 12 | Light Green | 12-Medium | 9-Medium | 3-Medium | Ovate | Round | Even | 20- Short | 7- Few | Semi-erect |
| $P_1XK_1$ 13 | Light Green | 12.5-Medium | 11.5- Broad | 5- Long | Ovate | Round | Even | 35-Medium | 4- Few | Semi-erect |
| $P_1XK_1$ 15 | Light Green | 9.5- Short | 7-Medium | 3-Medium | Ovate | Round | Even | 35-Medium | 10- Few | Horizontal |
| $P_1XK_1$ 16 | Light Green | 12.5-Medium | 7.5-Medium | 5- Long | Ovate-elliptic | Acute | Even | 70- Long | 23-Medium | Semi-erect |
| $P_1XK_1$ 20 | Light Green | 15-Medium | 12- Broad | 4.5- Long | Chordate | Chordate | Even | 40-Medium | 13- Few | Semi-erect |
| $P_1XK_121$ | Light Green | 17.5- Long | 16- Broad | 6- Long | Chordate | Chordate | Even | 60- Long | 16- Few | Hanging |
| $P_1XK_122$ | Light Green | 14-Medium | 9- Medium | 4.5- Long | Chordate | Chordate | Even | 40-Medium | 9- Few | Semi-erect |
| $P_1XK_124$ | Light Green | 16-Medium | 10-Medium | 6- Long | Ovate | Acute | Even | 30-Medium | 7- Few | Horizontal |
| $P_1XK_125$ | Light Green | 15.5-Medium | 11- Broad | 5.5- Long | Chordate | Chordate | Even | 35-Medium | 11- Few | Semi-erect |
| $P_1XK_128$ | Light Green | 15-Medium | 13- Broad | 2.5-Medium | Chordate | Chordate | Even | 25-Short | 6- Few | Semi-erect |
| $P_1XK_129$ | Light Green | 16.2- Long | 11- Broad | 3- Medium | Ovate | Round | Even | 68- Long | 16- Few | Semi-erect |
| $P_1XK_131$ | Light Green | 15.2-Medium | 13- Broad | 2.6-Medium | Chordate | Chordate | Even | 40-Medium | 6- Few | Horizontal |
| $P_1XK_137$ | Light Green | 15-Medium | 12- Broad | 2.3-Medium | Chordate | Chordate | Even | - | - | - |
| $P_1XK_138$ | Light Green | 16-Medium | 13.5- Broad | 2.5-Medium | Chordate | Chordate | Even | 32-Medium | 5- Few | Semi-erect |
| $P_1XK_143$ | Light Green | 9.5-Short | 7.5-Medium | 3- Medium | Ovate | Acute | Even | 30-Medium | 7- Few | Horizontal |
| $P_1XK_145$ | Light Green | 10.5-Medium | 8-Medium | 3- Medium | Ovate | Round | Even | 30-Medium | 9- Few | Semi-erect |
| $P_1XK_149$ | Light Green | 12.5-Medium | 9.5-Medium | 4.5- Long | Ovate | Round | Even | 35-Medium | 9- Few | Semi-erect |
| $P_1XK_151$ | Light Green | 10.5-Medium | 10-Medium | 6- Long | Chordate | Chordate | Even | | | |
| $P_1XK_153$ | Light Green | 10-Medium | 9- Medium | 2.6-Medium | Chordate | Chordate | Even | 55- Long | 22-Medium | Semi-erect |
| $P_1XK_177$ | Light Green | 10-Medium | 10.5- Broad | 6- Long | Chordate | Chordate | Even | - | - | - |
| $P_1XK_180$ | Light Green | 15-Medium | 11- Broad | 5- Long | Chordate | Chordate | Even | - | - | - |
| $P_1XK_182$ | Light Green | 12.5-Medium | 10.5- Broad | 5- Long | Chordate | Chordate | Even | 30-Medium | 5- Few | Hanging |
| $P_1XK_191$ | Light Green | 12.5-Medium | 8-Medium | 2- Medium | Ovate | Round | Even | 25- Short | 8- Few | Horizontal |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $P_1XK_1$95 | Light Green | 13- Medium | 9-Medium | 6- Long | Ovate | Round | Even | 65- Long | 21- Medium | Semi-erect |
| $P_1XK_1$97 | Light Green | 12.5- Medium | 9- Medium | 5.5- Long | Ovate | Round | Even | 35- Medium | 7-Few | Horizontal |
| $P_1XK_1$99 | Light Green | 14- Medium | 11.5- Broad | 5.5- Long | Ovate | Round | Even | 62- Long | 15- Few | Semi-erect |
| $P_1XK_1$100 | Light Green | 16.5- Long | 11- Broad | 4.5- Long | Chordate | Chordate | Even | 70- Long | 21- Medium | Semi-erect |
| $P_1XK_1$101 | Light Green | 11.5- Medium | 8- Medium | 4- Long | Ovate | Round | Even | 77- Long | 21- Medium | Semi-erect |
| $P_1XK_1$102 | Light Green | 12.1- Medium | 9.5- Broad | 4- Long | Ovate-elliptic | Round | Even | 30- Medium | 13- Few | Semi-erect |
| $P_1XK_1$104 | Light Green | 16.0- Medium | 12.0- Broad | 6- Long | Chordate | Chordate | Even | 40- Medium | 12- Few | Semi-erect |
| $P_1XK_1$107 | Light Green | 14.5- Medium | 9.5- Medium | 2.0- Medium | Ovate | Round | Even | 42.0- Long | 8- Few | Horizontal |
| $P_1XK_1$109 | Light Green | 13.0- Medium | 10.0- Medium | 4.5- Long | Ovate | Round | Even | 35- Medium | 12-Few | Semi-erect |
| $P_1XK_1$110 | Light Green | 14- Medium | 16- Broad | 6- Long | Chordate | Chordate | Even | - | - | - |
| $P_1XK_1$112 | Light Green | 8-Short | 7.5- Medium | 3.0- Medium | Ovate | Round | Even | - | - | - |
| $P_1XK_1$113 | Light Purple | 11.5- Medium | 8.0- Medium | 4-long | Ovate | Acute | Even | 30- Medium | 7- Few | Horizontal |
| $P_1XK_1$116 | Light Green | 10.5- Medium | 9.1- Medium | 4.9- Long | Chordate | Chordate | Even | - | - | - |
| $P_1XK_1$117 | Light Purple | 17- long | 12- Broad | 1.9-Short | Ovate | Acute | Even | 42- Long | 8- Few | Horizontal |
| $P_1XK_1$118 | Light Purple | 17- Long | 11- Broad | 2.0- Medium | Ovate-elliptic | Round | Even | 43- Long | 7- Few | Horizontal |
| $P_1XK_1$123 | Light Green | 15.0- Medium | 12- Broad | 3.0- Medium | Chordate | Chordate | Even | 36- Medium | 12- Few | Horizontal |
| $P_1XK_1$124 | Light Green | 17- Long | 11- Broad | 2.0- Medium | Ovate-elliptic | Acute | Even | 40- Medium | 7- Few | Hanging |
| $P_1XK_1$125 | Light Green | 14.5- Medium | 11- Broad | 2.2- Medium | Chordate | Chordate | Even | 40- Medium | 9- Few | Semi-erect |
| $P_1XK_1$135 | Light Green | 13- Medium | 9.4- Medium | 3.5-Long | Ovate | Round | Even | 40- Medium | 8- Few | Semi-erect |
| $P_1XK_1$140 | Light Green | 11- Medium | 7- Medium | 3.2- Long | Ovate-elliptic | Round | Even | - | - | - |
| $P_1XK_1$176 | Light Purple | 9-Short | 8.2- Medium | 4.0- Long | Chordate | Chordate | Even | 45- Long | 11- Few | Semi-erect |

**Table 2: Morphological Characterization ( phenotyping ) of Mapping Population 1 (Panniyur 1 x Subhakara) showing segregation of parent specific characters**

| Sl. No | Progeny No | Shoot tip colour | Leaf base and lamina shape | Leaf Length and Width | Spike Length |
|---|---|---|---|---|---|
| 1 | $P_1XK_1$1 (PKr) | Light purple(K) | Ovate,Round(K) | Medium (K) | Long (P) |
| 2 | $P_1XK_1$ 8 (P) | Light green (P) | Cordate (P) | Medium,Broad (P) | |
| 3 | $P_1XK_1$12(PKr) | Light green (P) | Ovate, Round(K) | Medium(K) | |
| 4 | $P_1XK_1$ 13 (P) | Light green (P) | Cordate (P) | Long, Broad(P) | |
| 5 | $P_1XK_1$ 14 (P) | Light green (P) | Cordate (P) | Medium(K) | |
| 6 | $P_1XK_1$ 15 (P) | Light green (P) | Cordate (P) | Long, Broad(P) | |

| | | | | | |
|---|---|---|---|---|---|
| 7 | $P_1XK_1$ 16 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 8 | $P_1XK_1$ 20 (P) | Light green (P) | Cordate (P) | Long,Broad(P) | |
| 9 | $P_1XK_1$ 21 (P) | Light green (P) | Cordate (P) | Long,Broad(P) | |
| 10 | $P_1XK_1$ 22 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 11 | $P_1XK_1$24 (PKr) | Light green (P) | Ovate, Round(K) | Medium(K) | |
| 12 | $P_1XK_1$ 25 (P) | Light green (P) | Cordate (P) | Long,Broad(P) | |
| 13 | $P_1XK_1$ 26 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 14 | $P_1XK_1$ 28 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 15 | $P_1XK_1$29 (PKr) | Light green (P) | Ovate,Round(K) | Long, Broad(P) | |
| 16 | $P_1XK_1$31 (PKr) | Light green (P) | Cordate (P) | Medium, Broad(P) | Medium (K) |
| 17 | $P_1XK_1$ 37 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 18 | $P_1XK_1$ 38 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 19 | $P_1XK_1$43 (PKr) | Light green (P) | Cordate (P) | Medium(K) | |
| 20 | $P_1XK_1$45 (PKr) | Light green (P) | Cordate (P) | Medium (K) | |
| 21 | $P_1XK_1$ 47 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 22 | $P_1XK_1$49 (PKr) | Light green (P) | Ovate, Round(K) | Medium (K) | |
| 23 | $P_1XK_1$ 51 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 24 | $P_1XK_1$ 53 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 25 | $P_1XK_1$ 56 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 26 | $P_1XK_1$57 (PKr) | Light green (P) | Cordate (P) | Medium (K) | |
| 27 | $P_1XK_1$58 (PKr) | Light green (P) | Ovate,Round(K) | Medium (K) | |
| 28 | $P_1XK_1$59 (PKr) | Light green (P) | Ovate,Round(K) | Medium, Broad(P) | |
| 29 | $P_1XK_1$ 62 (K) | Light purple(K) | Ovate,Round(K) | Medium (K) | |
| 30 | $P_1XK_1$ 63 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 31 | $P_1XK_1$ 70 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 32 | $P_1XK_1$ 71 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 33 | $P_1XK_1$ 73 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 34 | $P_1XK_1$ 74 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 35 | $P_1XK_1$ 77 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 36 | $P_1XK_1$ 79 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 37 | $P_1XK_1$ 80 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 38 | $P_1XK_1$ 81 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 39 | $P_1XK_1$ 82 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 40 | $P_1XK_1$ 84 (P) | Light green (P) | Cordate | Medium, Broad(P) | |
| 41 | $P_1XK_1$ 91 (K) | Light purple(K) | Ovate,Round(K) | Medium (K) | |
| 42 | $P_1XK_1$ 95(PKr) | Light green (P) | Ovate,Round(K) | Medium (K) | |
| 43 | $P_1XK_1$ 97(PKr) | Light green (P) | Cordate (P) | Medium(K) | |
| 44 | $P_1XK_1$ 99 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |

| | | | | | |
|---|---|---|---|---|---|
| 45 | $P_1XK_1$ 100 (P) | Light green (P) | Cordate (P) | Long,Broad (P) | |
| 46 | $P_1XK_1$ 10(PKr) | Light green (P) | Ovate,Round(K) | Medium (K) | |
| 47 | $P_1XK_1$ 10(PKr) | Light green (P) | Cordate (P) | Long,Broad (P) | Medium (K) |
| 48 | $P_1XK_1$ 103 (P) | Light green (P) | Cordate (P) | Medium,Broad (P) | |
| 49 | $P_1XK_1$ 104 (P) | Light green (P) | Cordate (P) | Long, Broad(P) | |
| 50 | $P_1XK_1$107(PKr) | Light green (P) | Ovate,Round(K) | Medium (K) | Medium (K) |
| 51 | $P_1XK_1$ 109(PKr) | Light green (P) | Ovate,Round(K) | Medium, Broad(P) | |
| 52 | $P_1XK_1$ 110 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 53 | $P_1XK_1$ 112 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 54 | $P_1XK_1$ 113 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 55 | $P_1XK_1$ 116 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 56 | $P_1XK_1$ 117(PKr) | Light purple(K) | Ovate, Round(K) | Long,Broad (P) | Medium (K) |
| 57 | $P_1XK_1$ 118(PKr) | Light purple(K) | Ovate, Round(K) | Long,Broad (P) | Long (P) |
| 58 | $P_1XK_1$ 119 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 59 | $P_1XK_1$ 123(PKr) | Light purple(K) | Cordate (P) | Medium, Broad(P) | Long (P) |
| 60 | $P_1XK_1$ 124(PKr) | Light purple(K) | Ovate, Round(K) | Long,Broad (P) | Medium (K) |
| 61 | $P_1XK_1$ 125 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 62 | $P_1XK_1$ 128(PKr) | Light green (P) | Cordate (P) | Medium (K) | |
| 63 | $P_1XK_1$ 130 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 64 | $P_1XK_1$ 135 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 65 | $P_1XK_1$ 136 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 66 | $P_1XK_1$ 140(PKr) | Light green (P) | Ovate, Round(K) | Medium (K) | |
| 67 | $P_1XK_1$ 141 (P) | Light green (P) | Cordate (P) | Long,Broad (P) | |
| 68 | $P_1XK_1$ 143 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 69 | $P_1XK_1$ 176(PKr) | Light purple(K) | Cordate (P) | Medium (K) | |
| 70 | $P_1XK_1$ 307 (P) | Light green (P) | Cordate (P) | | |
| 71 | $P_1XK_1$ 310 (P) | Light green (P) | Cordate (P) | | |
| 72 | $P_1XK_1$ 311 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 73 | $P_1XK_1$ 312 (P) | Light green (P) | Cordate (P) | | |
| 74 | $P_1XK_1$ 314 (P) | Light green (P) | Cordate (P) | | |
| 75 | $P_1XK_1$ 315 (P) | Light green (P) | Cordate (P) | | |
| 76 | $P_1XK_1$ 317 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 77 | $P_1XK_1$ 318 (P) | Light green (P) | Cordate (P) | | |
| 78 | $P_1XK_1$ 325 (P) | Light green (P) | Cordate (P) | | |
| 79 | $P_1XK_1$ 326(PKr) | Light purple(K) | Cordate (P) | Medium (K) | |
| 80 | $P_1XK_1$ 328 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 81 | $P_1XK_1$ 330(PKr) | Light purple(K) | Ovate, Round(K) | Medium, Broad(P) | |
| 82 | $P_1XK_1$ 334 (P) | Light green (P) | Cordate (P) | Long, Broad (P) | |

| | | | | | |
|---|---|---|---|---|---|
| 83 | $P_1XK_2$ 2 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 84 | $P_1XK_2$ 4 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 85 | $P_1XK_2$ 18 (K) | Light purple(K) | Ovate, Round(K) | | |
| 86 | $P_1XK_2$ 32 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 87 | $P_1XK_2$ 84 (P) | Light green (P) | Cordate (P) | | |
| 88 | $P_1XK_2$ 110 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 89 | $P_1XK_2$ 135 (K) | Light purple(K) | Ovate, Round(K) | Medium (K) | |
| 90 | MP 11 (PKr) | Light green (P) | Ovate, Round(K) | Medium, Broad(P) | Long (P) |
| 91 | MP 74 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 92 | MP 88 (P) | Light green (P) | Cordate (P) | Long, Broad (P) | |
| 93 | MP 108 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 94 | MP 126 (P) | Light green (P) | Cordate (P) | Medium, Broad(P) | |
| 95 | MP 128 (PKr) | Light green (P) | Ovate, Round(K) | Medium (K) | |

Note: P: Panniyur 1type, K: Karimunda type, PKr: Panniyur1 Karimunda recombinant

Spike characters like spike length, fruit set, fruit size, male female flower ratio were seen segregating and most of the progenies are found to be inter mediate forms (Fig 4 ).

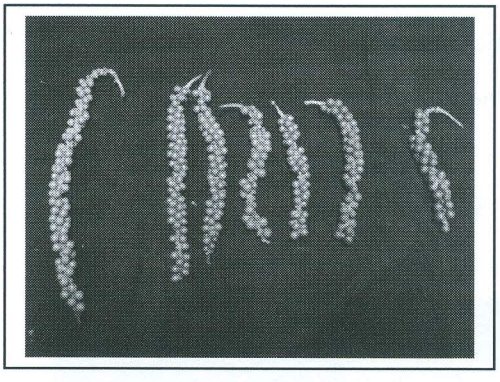

**Fig 4 Segregation of spike characters among progenies of Subhakara (Right) and Panniyur 1 (left)**

### Promising lines identified

A few promising lines (Fig 5) were identified for yield characters also.

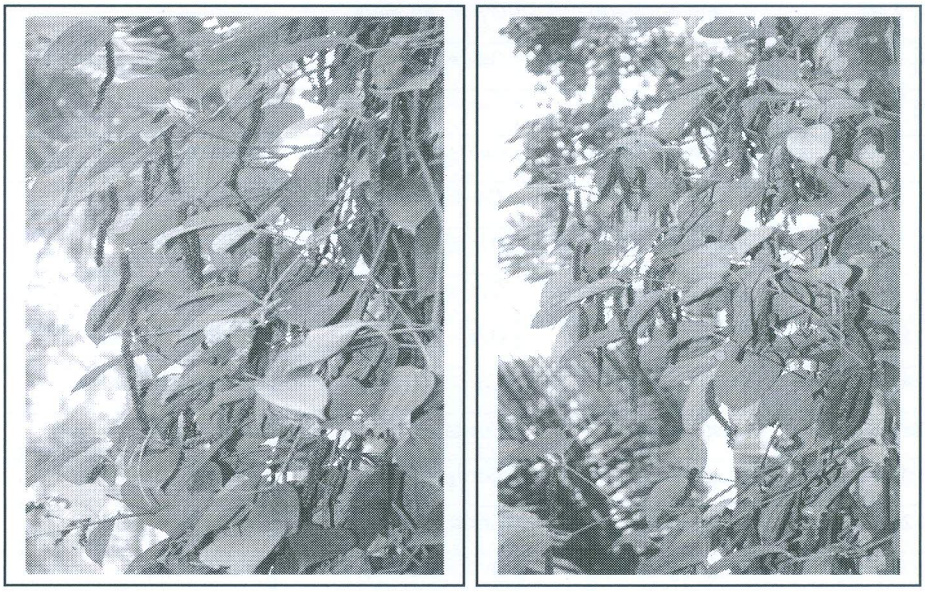

**Fig 5 Promising lines were identified FROM panniyur 1 x Subhakars cross**

## Molecular characterization of mapping population

### *RAPD profiling of progenies of P1 X Subhakara cross*

DNA was isolated from 100 progenies of Panniyur1 X Subhakara cross. RAPD profiling of 96 progenies of first mapping population Panniyur 1 X Subhakara was done along with their parents and 150 polymorphic markers segregating in the population were scored.

In addition the mapping population P1 X Subhakara was screened with 5 RAPD primers and 10 polymorphic marker scored (Fig 6).

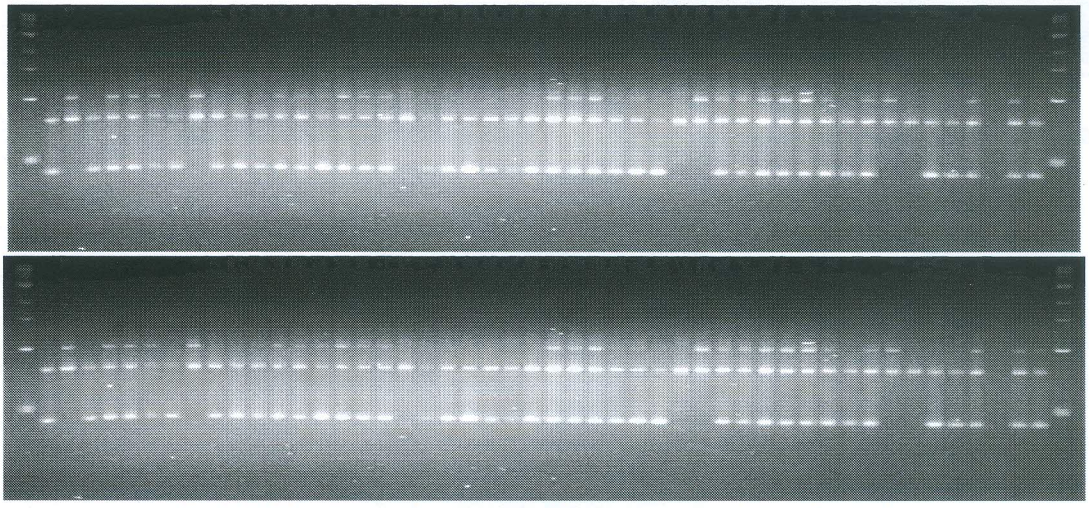

**Fig. 6 RAPD polymorphism expressed in mapping population of P1 X K 1**

***ISSR profiling of progenies of P1 X Subhakara cross***

Twenty ISSR primers has been screened with parents-Panniyur1 and Subhakara. 10 primers that show polymorphism between parents (Fig 7) is used to profile 94 selected progenies of Panniyur1 X Subhakara (Fig 8) ISSR profiling is being continued in PHYTOFURA Project.

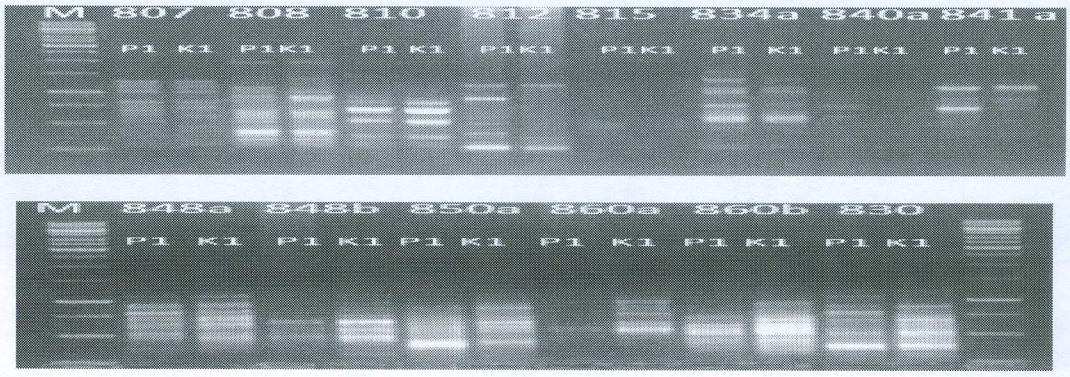


**Fig . 7 Screening of ISSR primers fort detecting polymorphisam between parents -P1 and Subhakara using1.5% agarose gel. M-1Kb ladder**

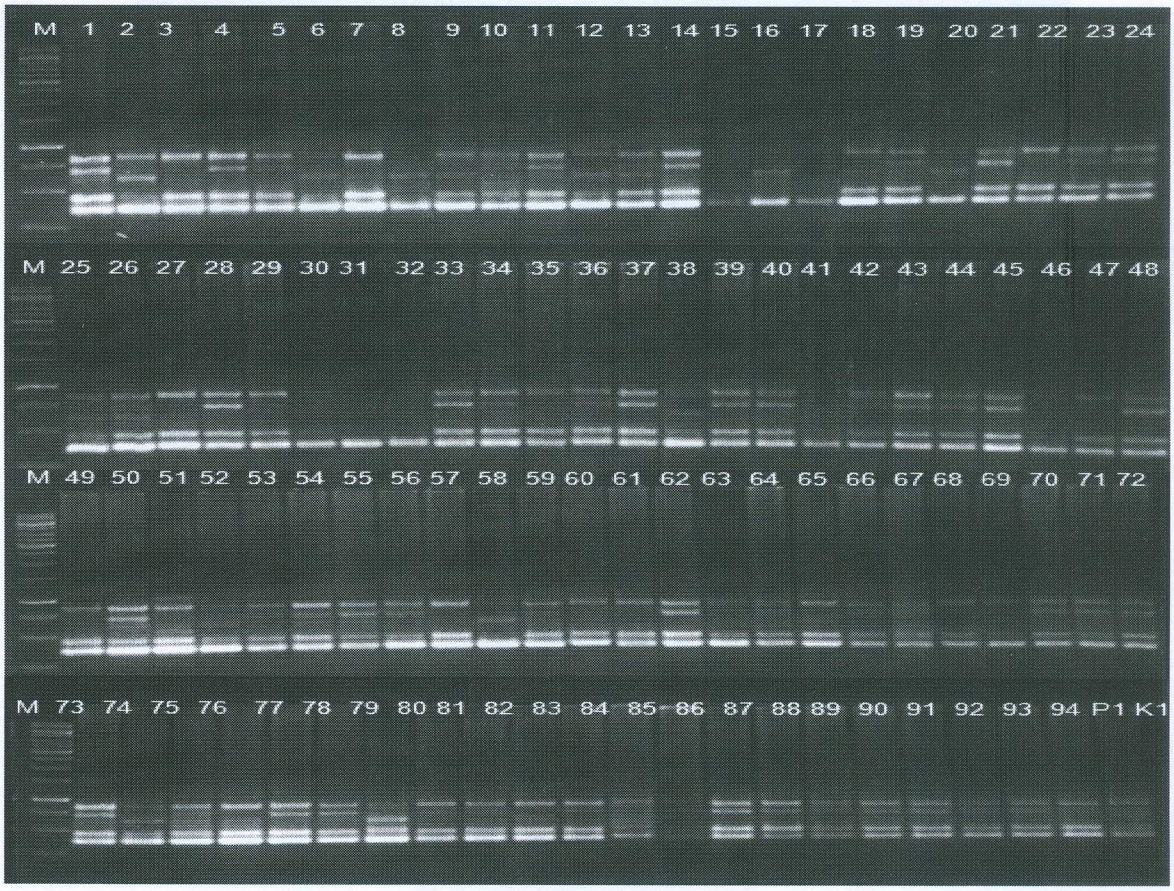


**Fig. 8 ISSR Profiling of mapping population 1.5% agarose gel using UBC 818-$(CA)_8G$ primer.**

### *Molecular Characterization of Association Mapping Population*

DNA was isolated from 57 genotypes following modified Doyle and Doyle method. 57 genotypes of black pepper selected for Association Mapping were profiled with five universal primers of UBC series (Fig . 9 & 10). The work will be continued in Phytofura Project.

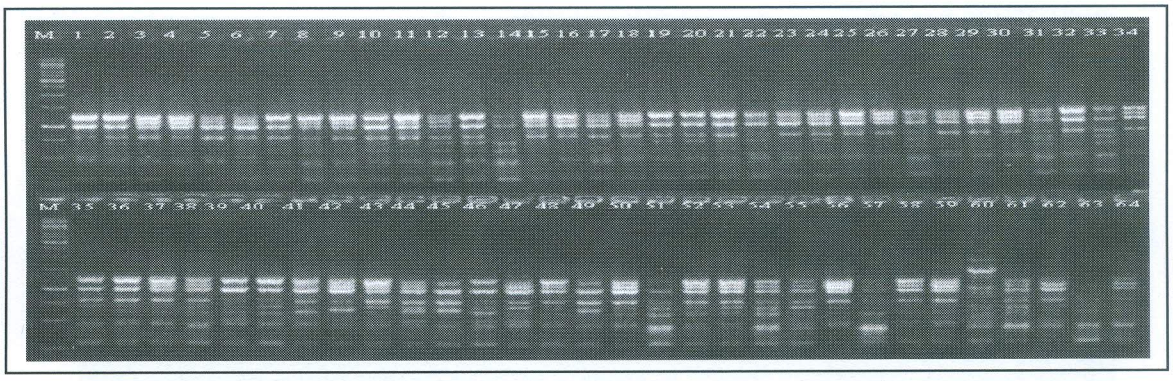

**Fig. 9:-ISSR Profiling of Association Mapping Population for tagging *Phytophthora* resistance genes using primer UBC 807**

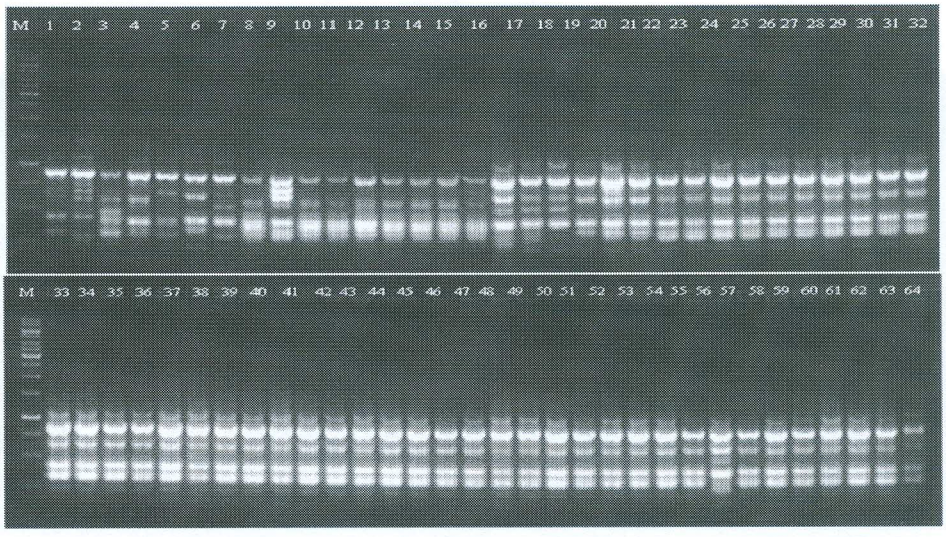


**Fig: 10 ISSR Profiling of Association Mapping Population with primer UBC 811**

### Screening of the progenies of mapping populations against *Phytophthora*

#### *Association mapping population*

Twenty seven lines selected as association mapping population were screened using leaf, stem and root inoculation methods. In Leaf and stem inoculations the size of lesion was scored as an index for disease resistance on a 0-

4 lesion scale. Disease severity index index ( DSI ) % was calculated using the formula suggested by Kim *et al.*, (2000).

$$DSI\ \% = \frac{\Sigma\ (\text{Ratings of each plant}) \times 100}{4 \times \text{No. of plants rated}}$$

The plants were rated as resistant, moderately resistant and susceptible in both leaf and stem inoculation methods separately and the average rating was taken as Disease susceptibility index and those with DSI < 30% was as Resistant, 31 - 40% as moderately Resistant and > 40% as Susceptible (Table. 3 and 4). Most of the genotypes were grouped as either susceptible or moderately resistant. None of the genotypes were found to give highly resistant reaction. Accession number 1386 and 1389 both named *Kattanadan local* (Fig.9) gave most tolerant reaction along with Hybrid *HP 750*.

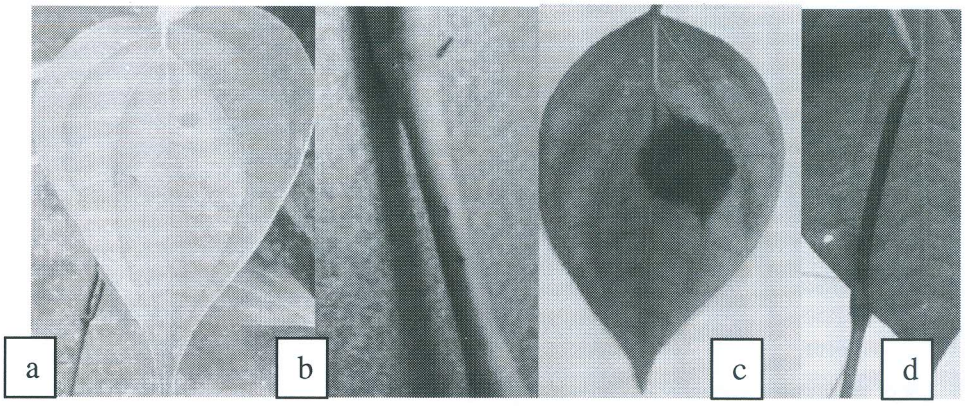


**Fig . 9 Leaf and Stem Screening of Acc. No1386 a and b with moderately resistant reaction c) and d) Control Subhakara**

**Table. 3 The list of moderately resistant genotypes and hybrids selected after leaf and stem screening**

| Sl.No | Acc.No | Genotype | Avg.Leaf Lesion (mm) | DSI (%) | Avg.Stem Lesion (mm) | DSI (%) | Avg.DSI (%) |
|---|---|---|---|---|---|---|---|
| 1 | **1386** | **Kattanadu Local** | 5.0 | 25.0 | 4.0 | 37.5 | 31.2 |
| 2 | **1389** | **Kattanadu Local** | 7.0 | 37.5 | 3.0 | 25.0 | 31.2 |
| 3 | 1605 | Mullenkolly | 8.0 | 35.0 | 6.0 | 35.0 | 35.0 |
| 4 | HP 130 | Neelamundi X Karimunda | 4 .0 | 25.0 | 10.0 | 43.75 | 34.4 |
| 5 | HP 442 | PerambramundaX Panniyur 1 | 3.3 | 25.0 | 11.3 | 50.0 | 37.5 |
| 6 | HP 581 | Panniyur 1X Balankotta | 7.3\3 | 41.6 | 7.33 | 25.0 | 33.3 |
| 7 | **HP 750** | **PerambramunbaX Karimunda** | 5.0 | 37.5 | 3.0 | 25.0 | 31.2 |
| 8 | HP 780 | Panniyur1X Karimunda | 7.6 | 33.3 | 6.2 | 31.25 | 32.3 |

**Table 4 Reaction of black pepper cultivars and hybrids for resistance to *Phytophthora***

| Sl.No | Acc.No | Name | Leaf | Stem | |
|---|---|---|---|---|---|
| | | | LL (mm) | LL (mm) | DP |
| 1 | 984 | Kalluvally | 5.2 | 3 | 1.2 |
| 2 | 1050 | - | 13.4 | 13.3 | 2.5 |
| 3 | 1109 | Vellanamban | 10.4 | 9 | 3.1 |
| 4 | 1324 | **Aimpiriyan** | No Lesion | 4 | 1.4 |
| 5 | HP 117 | Narayakodi X Neelamundi | 12 | 10.7 | 3.0 |
| 6 | HP 127 | Neelamundi X Karimunda | 13.6 | 10.6 | 2.33 |
| 7 | HP 344 | Narayakodi X Karimunda | 13.2 | 11.11 | 2.6 |
| 8 | HP 365 | Perumkodi X Karimunda | 12.8 | 18.15 | 2.35 |
| 9 | HP 427 | Panniyur 1X Balankotta | 13.8 | 10.3 | 3.18 |
| 10 | HP 441 | Perambramunda X Panniyur1 | 12.5 | 8.43 | 2.92 |

In another experiment Ten lines selected for association mapping from germplasm were screened using leaf, stem and root inoculation techniques (Table. 4). Cultivar 1324 was the best giving resistance reaction (Fig. 4 ) both stem and leaf screening (Fig .10 )

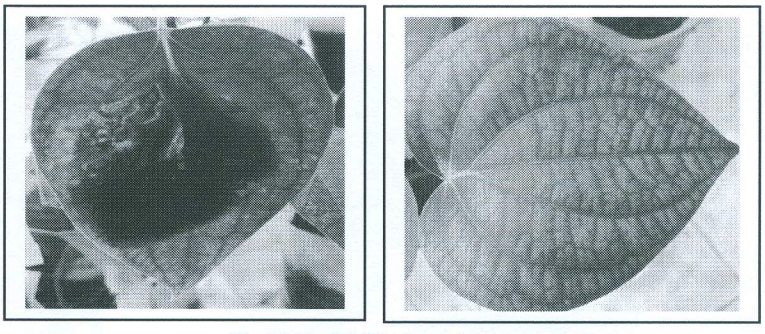

**Fig 10 Variability in resistance reaction**

**Table. 5 The list of susceptible genotypes after leaf and stem screening**

| Sl.No | Acc.No | Genotype | Avg.leaf Lesion (mm) | DSI (%) | Avg.Stem Lesion (mm) | DSI (%) | Avg. DSI(%) |
|---|---|---|---|---|---|---|---|
| 1 | HP3 | Panniyur1 X Karimunda | 13.7 | 93.0 | 17.0 | 50.0 | 71.5 |
| 2 | HP 17 | Perumkodi X Karimunda | 16.0 | 93.8 | 20.0 | 66.6 | 79.5 |
| 3 | HP23 | Unidentified X Karimunda | 13.0 | 63.3 | 19.3 | 50.0 | 56.5 |
| 4 | HP28 | Panniyur1 X Karimunda | 7.7 | 62.5 | 16.5 | 50.0 | 56.3 |
| 5 | HP37 | KalluvallyX Karimunda | 16.7 | 91.6 | 19.9 | 50.0 | 70.5 |
| 6 | HP43 | Cholamundi X Karimunda | 9.0 | 50.0 | 10.5 | 50.0 | 50.0 |
| 7 | HP45 | Narayakodi X Karimunda | 11.0 | 62.5 | 11.0 | 50.0 | 56.3 |
| 8 | HP102 | Narayakodi X Karimunda | 15.0 | 62.5 | 10.0 | 50.0 | 62.5 |
| 9 | HP117 | Narayakodi X Karimunda | 13.2 | 75.0 | 5.8 | 35.0 | 55.0 |
| 10 | HP127 | Neelamundi X Karimunda | 9.0 | 55.0 | 11.2 | 40.0 | 47.5 |
| 11 | HP203 | CheriakaniakadanX Karimunda | 9.0 | 55.0 | 11.2 | 40.0 | 42.5 |
| 12 | HP206 | CheriakaniakadanXKarimunda | 7.0 | 43.8 | 6.0 | 37.5 | 41.0 |
| 13 | HP344 | NarayakodiXKarimunda | 10.4 | 65.0 | 10.4 | 45.0 | 55.0 |
| 14 | HP365 | PerumkodiX Karimunda | 12.6 | 70.0 | 17.0 | 50.0 | 60.0 |
| 15 | HP427 | Panniyur 1 XBalankotta | 11.2 | 65.0 | 11.4 | 45.0 | 55.0 |
| 16 | HP441 | Perambramunda X P1 | 16.4 | 85.0 | 8.6 | 30.0 | 57.5 |
| 17 | HP631 | NeelamundiXKarimunda | 12.5 | 62.5 | 5.33 | 33.3 | 52.9 |
| 18 | HP726 | PerambramundaX Karimunda | 6.7 | 33.3 | 13.0 | 50.0 | 41.7 |
| 19 | HP1 | Panniyur1 X Karimunda | 25.3 | 100 | 34.0 | 85.0 | 92.5 |

***Mapping population 1 of P1 X Subhakara cross***

The material was multiplied for screening. The work will be continued in PhytoFuRa project.

***New source of resistance to Phytophthora:***

An ornamental and exotic species of Piper, *Piper ornatum* was found to give resistant reaction against *Phytohthora*.

**Summary of Achievements**

**Development of mapping populations**

Two mapping populations of about 100 each were developed involving Panniyur 1 X Subhakara and P 24 (IISR Shakti ) X Subhakara for tagging of agronomically important charecters and Phyto[hthora resistance respectively.

These were maintained both in the field for phenotypic characterization and multiplied and maintained in the nursery for disease indexing/ screening. Panniyur and Subhakara (Karimunda selection) being most distinct in various morphological characters this population segregates for many morphological and yield attributing characters

In order to estimate the level of heterozygosity , ***Supporting populations of*** selfed progenies (about 100 lines) of the parents Sakthi, Subhakara and Panniyur 1 were developed for phenotyping and genotyping.

P 24 (IISR Sakthi - tolerant to *Phytophthora)* has its origin as open pollinated progeny of local variety Perambramunda. Screening next generation open pollinated progeny of P 24 lead to identification of P-24-O-4 which has higher level of resistance that its parent P-24. This may possibly indicate that selfing may lead to homozygosity of the alleles involved in resistance thus increasing its effectiveness. Hence another selfed population (can be called pseudo 'F3') of P-24-O-4 was developed for screening and obtaining higher degree of resistance

Accordingly a populations of 57 genotypes were selected from germplasm containing both resistant as well as susceptible genotypes. These were maintained both in the field ( Fig 3) for phenotypic characterization and multiplied and maintained in the nursery for disease indexing/ screening.

**Phenotyping of mapping population 1 (Panniyur 1 X Subhakara cross)**

P1 X Subhakara mapping population was phenotyped for floral and other segregating morphological characters like leaf shape and size, shoot tip colour, spike length, berry size etc . Most characters were seen segregating among progenies. Among the 95 progenies, 56 were of female parent type (Panniyur 1), 11 of male parent type (Subhakara) and 28 progenies were found to be recombinants.

Spike characters like spike length, fruit set, fruit size, male female flower ratio were seen segregating and most of the progenies are found to be inter mediate forms

A few promising lines were identified for yield characters also.

**Molecular characterization of mapping population**

*RAPD profiling of progenies of P1 X Subhakara cross*

In addition to **150 polymorphic markers already scored earlier** the mapping population P1 X Subhakara was screened and 10 additional marker were added.

scored .

*ISSR profiling of progenies of P1 X Subhakara cross*

Twenty ISSR primers has been screened with parents-Panniyur1 and Subhakara. 10 primers that show polymorphism between parents is used to profile the progenies. ISSR profiling is being continued in PHYTOFURA Project.

*Molecular Characterization of Association Mapping Population*

DNA was isolated from 57 genotypes following modified Doyle and Doyle method. 57 genotypes of black pepper selected for Association Mapping were profiled with five universal primers of UBC series. The work will be continued in Phytofura Project.

**Screening of the progenies of mapping populations against *Phytophthora***

Twenty seven lines selected as association mapping population were screened using leaf, stem and root inoculation methods. Most of the genotypes were grouped as either susceptible or moderately resistant. None of the genotypes were found to give highly resistant reaction. Accession number 1386 and 1389 both named *Kattanadan local* gave most tolerant reaction along with Hybrid *HP 750*. Cultivar 1324 was the best giving resistance reaction both stem and leaf screening

***New source of resistance to Phytophthora:***

An ornamental and exotic species of Piper, *Piper ornatum* was found to give resistant reaction against *Phytohthora*.

THE LEADS OBTAINED WERE BEEING UTILISED IN PHYTOFURA PROJECT .