



# Abstracts of M.Sc. and Ph.D. Dissertations on Spice Crops



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

2007-08

Technical Bulletin No. 8

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**Correct Citation :**

Indian Institute of Spices Research  
Abstracts of M.Sc. and Ph.D. Dissertations on spice crops, Calicut.

**Published by :**

Dr. V. A. Parthasarathy  
Director

**Compiled and Edited by :**

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**Front & Back Cover :**

IISR Laboratories

**Cover design :**

A. Sudhakaran

**Year of Publication :**

2005

**ISBN No. 81 - 86872 - 24 -8**

**Printed at :**

Ideal Printers  
Calicut, Ph : 2301715

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## FOREWORD

The Indian Institute of Spices Research in its existence for nearly three decades has carved a niche for itself as a centre of excellence in spices research. The institute established as a Regional Station of Central Plantation Crops Research Institute in 1975 has blossomed into a full-fledged institute of world repute involved in research on spice crops. In its mandate of developing appropriate technologies for use by farmers, the institute attempts to do a large number of basic, applied and strategic researches.

The institute is recognized for the purpose of higher studies by various universities. In its portal, about 107 masters candidates have done their research and 30 have carried out doctoral research. The dissertations are important source of information in spice crops. Considering the immense wealth of information available in these dissertations, it was decided to bring out a publication of abstracts of M.Sc. and Ph.D. theses available at IISR library.

I hope, this would be very valuable to all research workers on spice crops. I compliment the editors for having done a good job of compiling the abstracts of these theses.



[V.A. Parthasarathy]  
Director

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# M.Sc. DISSERTATION

## Abstracts

### BLACK PEPPER

1. **Studies on RAPD polymorphism in varieties and related species of black pepper (*Piper nigrum* L.) (PR 89)**

Hareesh, P. S. / Cochin University of Science and Technology / Guide -  
*K. Nirmal Babu*

A study was taken up with an objective to standardize RAPD protocols for *Piper* and related species and to use the RAPD polymorphism to estimate inter relationships between 8 cultivated types - Panniyur-1, Sreekara, Subhakara and Panchami, P 24, P 1095, HP-1, HP 780 and 4 related species - *P. longum*, *P. chaba*, *P. colubrinum* and *P. arboreum*. RAPD protocols were standardized for black pepper and their related species. Eight random primers were screened and two of them, OPA 04 and OPE 14 which amplified all the genotypes were used in the study. The studies indicated that all the cultivated types tested genetically differ from each other to a large extent. The RAPD profiles also indicated that Sreekara and Subhakara differ from each other though it is difficult to distinguish between them morphologically. The primers gave good polymorphism among the species. The study indicated that *P. longum* and *P. chaba* are distant from each other.

2. **Molecular characterization of black pepper and related species using RAPD polymorphism (PR 93)**

Sinoj, J. / Bharathidasan University / Guide - *K. Nirmal Babu*

The present investigation was an effort to use RAPD polymorphism as a tool to study the interrelationships between different species of *Piper*. Male and female lines of *P. longum*, *P. hapnium*, *P. peepuloides*, *P. chaba*, *P. mullesua*, *P. silentvalleyensis*, *P. attenuatum*, *P. argyrophyllum*, *P. hymenophyllum*, *P. galeatum*, *P. trichostachyon*, *P. sugandhi*, *P. nigrum*, *P. schmidti*, *P. wightii*, *P. cubeba*, *P. colubrinum*, *P. barberi*, *P. magnificum*,

*P. arboreum* were used in the study. Genomic DNA was isolated and RAPD profiles were developed using the methods standardized at IISR. Six polymorphic primers i.e., OPA-09, OPC-13, OPD-02, OPE-11, OPF-09 and OPF-14 were used for developing RAPD profiles. The dendrogram indicating the relationships of various species studied gave interesting observations. *P. attenuatum*, *P. argyrophyllum* and *P. hymenophyllum* formed a group and *P. schmidtii* and *P. wightii* were placed in another group. *P. galeatum* and *P. sugandhi* were also in similar group. These agree with the earlier understanding of the species relationships. But *P. longum* and *P. hapnium* formed one group though two collections of *P. longum* and *P. hapnium* male and female lines were placed in distinctly separate groups.

3. Studies on interrelationships among black pepper and related species as expressed by RAPD polymorphism (PR 98)

Kripa, J. K. / Acharya Nagarjuna University / Guide - K. Nirmal Babu

Female plants of 22 species of *Piper*, namely *P. longum*, *P. hapnium*, *P. mullesua*, *P. attenuatum*, *P. argyrophyllum*, *P. hymenophyllum*, *P. bababudani*, *P. trichostachyon*, *P. galeatum*, *P. sugandhi*, *P. pseudonigrum*, *P. nigrum*, *P. schmidtii*, *P. wightii*, *P. barberi*, *P. betel*, two collections of *P. chaba*, *P. magnificum* and two collections of *P. colubrinum*, *P. arboreum* and *P. ornatum* and one black pepper cultivar were used to study their RAPD profiles. Five Operon primers - OPA 09, OPB 07, OPD 03, OPE 11 and OPF 09 were used to develop RAPD profiles. The dendrogram grouped *P. longum*, *P. hapnium* and *P. mullesua* in one group and *P. attenuatum* and *P. argyrophyllum* in another group. *P. pseudonigrum*, *P. nigrum* and *P. galeatum* are clustered together. However, grouping of *P. magnificum* and *P. arboreum* with *P. hapnium* & *P. mullesua*, separate and unique clustering of *P. hymenophyllum* are against the earlier observations and understanding.

4. Molecular characterization of *Phytophthora* tolerant and susceptible lines of black pepper, *Piper nigrum* L. (PR 97)

Reema, K. K. / Kannur University / Guide - K. Nirmal Babu

RAPD profiles of 11 cultivars each of black pepper showing resistance (P-24,

P-334, P-339, P-1534, C-847, C-1047, C-1090, C-1095, HP-1, HP-780 and HP-1411) and susceptibility (Doddigae, Kaniakkadan, Chumalakodi, Nedumchola, Malamundi, Karuthapirimunda, TMB VI, Valiyakarimunda, Karimcotta, Panniyur-1 and Karimunda) to *Phytophthora* were analysed to study their interrelationships and to identify RAPD marker, if any, associated with *Phytophthora* resistance. The study indicated Kaniakkadan and Chumalanampan are nearest to each other followed by C-1090 and C-1095. Though in general the tolerant lines formed a cluster of their own a few susceptibles were also grouped with tolerants and vice versa. The most probable reason for this anomaly may be that the tolerant or susceptible lines may be heterozygous for their traits thus having the other template also. No marker was found to be consistently associated with *Phytophthora* resistance.

5. **Studies on genetic fidelity of micropropagated plants of black pepper using RAPD polymorphism (PR 94)**

**Karthik Prabhu, T / Bharathidasan University / Guide - K. Nirmal Babu**

Genetic fidelity of micropropagated plants of black pepper (var: Subhakara and Aimpiriyan) was studied using both morphological and RAPD characterization. Morphological characters such as vine column height, vine column width, leaf length/breadth, spike length and number of laterals were recorded from two year old tissue cultured plants and there was no significant difference between the control and tissue cultured plants. This indicated that the micropropagated plants were morphologically similar and no variation could be detected. Genomic DNA was isolated from black pepper using CTAB method. Template DNA concentration of 45 ng/ml of DNA, dNTP concentration of 125 mM, 1.5 U / ml. of Taq DNA polymerase, primer concentration 10 picomoles /ml, additional 10mM magnesium ion ( $Mg^{2+}$ ) concentration gave best results for PCR amplification. Two primers OPF 04 and OPC 13 were used for developing RAPDs. Though two genotypes showed minor band differences, morphological characters coupled with RAPD profiles have indicated genetic fidelity among the micropropagated plants of black pepper.



6. **Studies on the genetic fidelity of micropropagated black pepper using RAPD and ISSR polymorphism (PR 47)**

**Bhavitha Kumaran** / Bharathidasan University / Guide - *K. Nirmal Babu*

Morphological characters coupled with molecular profiling were used to estimate the extent of genetic variability in micropropagated plants of two popular varieties Aimpiriyan and Subhakara. Standardization of Inter Simple Sequence Repeat (ISSR) profiling was done and this along with RAPD profiling was used to estimate genetic fidelity of micropropagated plants. Morphological characters such as spike colour, leaf lamina shape, leaf margin, leaf veining, shoot tip colour, spike orientation did not show any differences indicating that micropropagated plants are morphologically similar. RAPD and ISSR profiling also did not show detectable variations. Thus morphological supplemented by molecular characterization indicated that the micropropagated plants of black pepper are similar to their parents and are genetically stable.

7. **Quality profiling and isolation of genomic DNA from dried berries of Malabar, Vietnam and Lampong black pepper (PR 54)**

**Subba Rao, M.** / Acharya Nagarjuna University / Guide - *B. Sasikumar*

Quality profiling of Malabar pepper as against Vietnam and Lampong pepper and isolation of genomic DNA from the dried black pepper berries of these three samples were done. Bulk density was found to be highest in Vietnam (573 g litre<sup>-1</sup>) followed by Lampong (569g litre<sup>-1</sup>) and Malabar (460 g litre<sup>-1</sup>) and in the case of size, Vietnam pepper recorded maximum percentage of bold berries of size 4.75 mm (70%) followed by Malabar pepper (50%). Lampong pepper showed highest content of piperine (5.9%) while the Malabar showed the least (3.3%). The volatile oil content in Malabar pepper was found to be maximum (4.0%) followed by Lampong pepper (2.6%) and Vietnam pepper (2.3%). Vietnam pepper showed the highest content of oleoresin (9.2%) followed by Lampong pepper (7.2%) and Malabar pepper (6.6%). Good quality DNA was isolated from the powdered samples of dried black pepper berries by a modified CTAB method. The purification step was slightly modified by including PEG purification after the usual phenol: chloroform: isoamylalcohol (25:24:1) purification step. This increased the yield and quality of DNA.

8. Variation in yield and growth performance of cuttings derived from top, middle and bottom nodal explants of different varieties of black pepper under nursery condition (PR 1)

Manoj, K. A. / University of Calicut / Guide - B. Sasikumar

Growth variation of black pepper cuttings derived from different nodal segments viz., top, middle and bottom of the five high yielding varieties viz., Panchami, Pournami, Panniyur-1, Panniyur-3 and Panniyur-5 and the yield of single nodal rooted cuttings through rapid multiplication technique were studied. The top nodal explants exhibited rapid growth in vine length with an increase of 0.64 cm per day. Pooled over varieties the leaves of the vines derived from the top nodal explants exhibited more growth in leaf length (1.02 cm per day), leaf breadth (0.65 cm per day), and leaf area (4.43 cm<sup>2</sup> per day) compared to middle and bottom nodal explants. In the yield of harvestable nodes from different nodal explants varieties behaved differently.

9. An investigation on intracloonal variability in a black pepper hybrid and variety using molecular techniques (PR 14)

Shahanas, C. H. / Periyar University / Guide - B. Sasikumar

Variability, if any in vegetative cuttings of black pepper was investigated using RAPD technique. Different rooted cuttings derived from top middle and bottom portion of bamboo groove vines of 'Panniyur-1' and 'Sreekara' were used in the study. Ten selected random decamer primers generated 120 numbers of products in 'Panniyur-1' and 124 fragments in 'Sreekara'. Mean number of amplified products ranged from 2 (OPC 10 & OPC 15) to 8 (OPC 03) in case of 'Panniyur 1'. In the case of 'Sreekara' the range of product was 2 (OPC 14) to 6 (OPC 03). The smallest fragment in the case of 'Panniyur-1' was of size 72.35 bp and the largest 3406.55 bp whereas the size range of fragments in 'Sreekara' was 82.30 to 2230.40 bp. The ten decamer primers did not produce any variation in size or number of the amplified products in 'Panniyur-1' cuttings. In the case of 'Sreekara' also there was no polymorphic band except in case of the primer OPC 20 where an extra band of size 900.06 bp could be observed in the bottom nodal explants.

10. Molecular and morphological characterization of black pepper lines (PR 50)

Sreedevi, M. / Bharathidasan University / Guide - B. Sasikumar

Different black pepper lines were characterized based on their molecular profiling RAPD and morphological features. A total of sixteen unique bands were produced by nine of three primers studied making an average of 1.7 bands per primer. All the seven black pepper lines exhibited common features with respect to plant growth habit (climbing), branching (polymorphic), colour of the young orthotropic shoot tip (light purple), pubescence on the stem (no pubescence), lateral branch habit (erect, except in the case of OPKm which was hanging), juvenile leaf length (intermediate), leaf texture (glabrous coriaceous), leaf hairiness (no hairs), leaf scales (no scales), flower arrangement (free), flower nature (sessile), fruit shape (round) and fruit size (intermediate). Maximum unique bands by different primers were observed in OPKm (OPA-06, OPA-08, OPA-17 and OPC-13) followed by HP 1411 (OPA-06, OPA-08 and OPE-18) and HP 105 (OPA-03, OPA-06 and OPA-17). The maximum distinct morphological features were observed in the case of OPKm, HP 1411 and HP 105.

11. *In vitro* propagation of *Piper longum* (PR 51)

Nirmal Joy /Allahabad Agricultural Institute - Deemed University / Guide - J. Rema

Nine different genotypes of *Piper longum* were grown *in vitro* in order to find out a better responding one. The *in vitro* raised plants were hardened in different conditions and an efficient protocol was identified for the hardening. The major impediment during the course of experiment was the bacterial and fungal contamination. Higher concentrations of BAP (BAP 1 mg/litre, BAP 0.5 mg/litre and BAP 1 mg/litre + NAA 0.5 mg/litre) were found to be the best growth regulator combinations along with Woody Plant Medium in micropropagation of *P. longum* as they induced maximum number of shoots. Accession number 3319 was found to be the most responding genotype under *in vitro* as it produced maximum multiple shoots as well as maximum shoot length. Sterile sand was found to be the best hardening media along with incubation conditions of 70% relative humidity combined with a temperature of 28-30°C and a shade of 50%.

12. **Tagging of disease response gene beta-1,3- glucanase in *Piper colubrinum* through RNA finger printing (PR 24)**

Jaleel Kizhakkayil / Periyar University / Guide - Johnson George K.

RT-PCR was used to identify the presence of beta 1,3-glucanase in *Piper colubrinum*. Already identified beta 1,3 - glucanase gene sequences of various crops were retrieved from NCBI site. Enough homology was found among the beta-1,3 glucanase classes to generate primers to target the beta -1,3 glucanase genes from different plants. Conserved sequence motifs were obtained using CLUSTAL W and the primers for amplification of the gene from *Piper colubrinum* were designed manually. A putative beta 1,3-glucanase cDNA fragment of approximately 350 bp in length was amplified in RNA sample from *Piper colubrinum* challenged with *Phytophthora capsici*.

13. **Random Amplified Polymorphic DNA (RAPD) analysis in *Piper nigrum* and identification of true hybrids (PR 70)**

Baby Jooju / Bharatidasan University / Guide - Johnson George K.

This study was conducted in *Piper* species for the identification of true hybrids using RAPD markers. The genomic DNA was isolated from black pepper plants (parents and hybrids) using CTAB method and good quality and high molecular weight DNA was obtained. DNA quantification was done by comparing band intensity of different concentrations of standard genomic DNA markers using agarose gel electrophoresis. For the optimization of PCR, three different components such as dNTP's, Taq DNA polymerase and MgCl<sub>2</sub> were tested in different combination. Out of twenty-three primers tested, six of them showed good amplification. OPE-01 was found to be useful in identification of hybrids in the cross HP780 x *P. nigrum* (Wild). The result reported in this study confirms, the utility of RAPD in hybrid identification.

14. **ISSR and RAPD markers in diversity analysis of *Piper* species (PR 112)**

Renuka, M. / University of Calicut / Guide - Johnson George K.

ISSR and RAPD markers were found to be useful in analyzing the diversity among seven *Piper* species. The present study revealed that genetic differentiation of the selected

species of *Piper* could be done with a single ISSR primer. The ISSR profiling was found to be more efficient compared to RAPD because of relatively low frequency of reproducible polymorphisms in the latter. The broader implications of the results obtained suggest that the success of molecular markers in genetic diversity studies depend on the type of marker used and ISSR has proved to be of better option in genetic diversity studies. This study can be further extended to the identification of *Piper* cultivars and hybrids and also in assessment of genetic diversity among other species as well.

**15. Genetic analysis of black pepper (*Piper nigrum* L.) hybrids and its parents based on Random Amplified Polymorphic DNA (RAPD) markers (PR 4)**

**Kishore, K. K.** / Mangalore University / Guide - Johnson George K.

The study was undertaken for identification and to examine the genetic relationship (genetic similarity) in cultivated *P. nigrum* (var. Irumaniyan, Karimunda, Panniyur-1, Aimpiriyan) and *P. attenuatum* and The usefulness of RAPD markers in identification of true hybrids was also attempted. The protocol for DNA extraction was modified to get good quality DNA. In this study, the 12 primers were selected for the RAPD reactions, yielded promising results. Among the accessions tested, the wild species, *P. attenuatum* showed a distinct banding pattern in all RAPD reactions indicating that any of the 12 primers used in this study can be utilized for distinguishing this species. Unique bands were observed for all the accessions tested using different primers. All accessions could be distinguished from each other with a minimum of 4 primers tested. A dendrogram was constructed using NTSYS programme. The RAPD analysis revealed a moderate degree of diversity among the cultivars examined in this study.

**16. Assessment of genetic diversity among released varieties of black pepper (*Piper nigrum* L.) using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat Markers (PR 121)**

**Hidayath, K. P.** / Bharathidasan University / Guide - Johnson George K.

In the present study, twelve released varieties of black pepper were characterized using two ISSR and RAPD markers. PCR products were electrophoresed in agarose gel, documented and the bands were analyzed to detect polymorphism. Both ISSR and RAPD markers were found to be useful in the characterization of released varieties of black

pepper. The use of more number of primers is suggested to reveal complete genetic diversity among the twelve black pepper varieties selected for the study. This study can be further extended for accurate identification of varieties of black pepper, cultivated and wild relatives of *Piper* in the germplasm collections.

**17. Random Amplified Polymorphic DNA (RAPD) analysis of selected *Piper* species of South India (PR 120)**

Raji, K. / Periyar University / Guide - Johnson George K.

RAPD analysis was under taken in *Piper* spp. to examine the genetic relationships between different species. In this study, 3 primers (OPE-11, OPE-08, OPE-20) were selected for the RAPD reactions. *P. attenuatum* showed a clear differentiation from others when amplified by OPE-11 and *P. hapniium* could also be differentiated from other samples studied when amplified by OPE-18. A dendrogram was also generated for 13 *Piper* accessions by cluster (UPGMA) analysis. The study demonstrated the utility of RAPD analysis to differentiate different species of *Piper*. However; more studies are required to get complete picture of genetic diversity and relationship between different species of *Piper*.

**18. DNA profiling of selected varieties of black pepper (*Piper nigrum* L.) using ISSR markers.(PR-126)**

Sarath Reddy, T. / Acharya Nagarjuna University/Guide-Johnson George K.

A study was under taken to attempt assessment of genetic diversity among four released varieties of black pepper with the objective to find the suitability of using ISSR primers for distinguishing different varieties of black pepper. Three varieties were successfully discriminated from each other by the five primers used in the study. Primer ISSR02C3 generated a maximum number of three unique polymorphic bands and discriminated two varieties i.e. Panchami and PLD-2 from varieties tested. Primer ISSR02C4 generated one polymorphic band and discriminated Subhakara variety. Primers ISSR08 and ISSR02C4 were also successful in discrimination of varieties. The ISSR marker analysis is therefore found to be useful in discriminating cultivated varieties of black pepper. Further work using ISSR markers required to identify different released varieties of black pepper.

**19. Identification of proteinase inhibitors from *Piper* species (PR 72)**

Nitha, M. P. / Bharathidasan University / Guide - Johnson George K.

Presence of trypsin and chymotrypsin inhibitors in three *Piper* species were investigated under the study. Inhibitory activities of these proteins towards casein were detected in considerable amounts in two wild *Piper* species, namely *P. colubrinum* and *P. chaba*, whereas in the cultivar *P. nigrum*, the activity was detected at low concentrations. These inhibitor proteins were tolerant to heat, acid and alkali but were denatured at extreme conditions of pH and boiling temperature. Optimum activity was detected at neutral conditions of pH. The heat treated samples retained up to 98% of their inhibitory activity towards casein. Preliminary studies by western blotting indicated the presence of a trypsin inhibitor of approximately 58 KDa.

**20. Studies on genetic fidelity of plants regenerated from somatic embryos of black pepper (*Piper nigrum* L.) using RAPD polymorphism (PR 9)**

Anila Das / Periyar University / Guide - R. Ramakrishnan Nair

The plants regenerated from somatic embryos of cv. 'Subhakara' maintained at IISR nursery (eight months after transfer to soil) were tested for genetic stability using morphological characters and RAPD polymorphism. Morphological characters were found to be similar in somatic embryo derived plants as well as the original parent. RAPD characterization indicated, with three operon primers showed 100% uniformity between regenerated plants and with the original parent. But with one primer shown a few variable bands in four of the regenerated plants. The results indicated a large extent of similarity of regenerated plants with the mother plant.

**21. Studies on inheritance of *Phytophthora* resistance in selfed progenies of black pepper (*Piper nigrum* L.) Panniyur-1, Subhakara and P-24. (PR 37)**

Iswarya, K. / University of Calicut / Guide - M. Anandraj

Panniyur-1 and Subhakara cultivars gave susceptible reaction for *Phytophthora* by leaf as well as stem inoculation. But a few of their progenies gave highly resistant reaction in leaf lesion index indicating residual heterozygosity of these varieties for this trait. But

P24 showed tolerant reaction in both leaf and stem inoculations. P-24 was earlier reported as resistant to *Phytophthora* infection. Double the number of P-24 progenies gave resistant reaction compared to those of Panniyur-1 and Subhakara. The study showed that there were differences in the progenies with regard to leaf and stem screening and these characters segregated independently indicating that disease resistance is controlled by more than one gene. The degree of resistance also showed a sort of continuous variation indicating either quantitative or multi allelic interaction controlling disease resistance. Susceptible Panniyur-1 and Subhakara throws off resistant lines in the segregating population indicating that they can be used as parents in hybridization programme to produce resistant hybrids.

22. **Reaction of black pepper (*Piper nigrum* L.) seedlings to infection by *Phytophthora capsici*. (PR 85)**

Ganesh, T. / Acharya Nagarjuna University / Guide - M. Anandaraj

Open pollinated seedling progenies of P 24, the *Phytophthora* tolerant line and KS 27 susceptible line were screened for their reaction to inoculation by *P. capsici*. The response of the seedling progenies varied from highly susceptible to tolerant ones. The size of the lesion also varied depending upon the maturity of the tissue. In the present study 3 stages of leaf (tender, immature, mature) were tested for the response. Maximum lesion of more than 40mm in 72 hrs was recorded in tender leaves. In the seedling progenies of P24 the minimum lesion on a mature leaf was 2 mm, whereas the maximum was 24.5 mm. In a tender leaf the maximum lesion in progenies of P24 was 42.5mm whereas in progenies of KS 27 it was 47.5mm. From both the groups the seedlings showing minimum lesion and maximum lesion were short listed for studying their host pathogen interaction. The b 1,3 glucanase activity after 48 hrs of incubation with *P.capsici* was 3.03 (mg/ml) of the extract in case of P24 and in the progenies it ranged from 2.08 – 2.65 (mg/ml). Whereas in case of KS 27 the parental line was showing 0.56 (mg/ml) and in the progenies it ranged from 0.37-1.89 (mg/ml). SDS PAGE protein profile revealed that higher activity of b 1,3 glucanase in progenies of P24 compared to KS 27. In KS 27 two out of three showing tolerant reaction, b 1,3 glucanase activity was 2-3 fold higher than parent KS 27. In P 24 progenies though there is induction of b 1,3 glucanase the plant showed susceptible reaction. This indicates that the pathogen is able to overcome the defense mechanism put forth by the host plant. From the study it is observed that the mechanism of resistance in the progenies is by induction of PR proteins.



23. *In-vitro* evaluation of *Trichoderma* spp. for their antagonism to *Phytophthora capsici*, the foot rot pathogen of black pepper (*Piper nigrum* L.) (PR 83)

Deepa Gopinath, S. / Bharathiar University / Guide - M. Anandaraj

Of the 81 isolates of *Trichoderma* screened for antagonism, a good number of isolates showed significant inhibition but variation occurred in their percentage of inhibition. When the first ten highly antagonistic strains were studied for mycoparasitism only one isolate was found to show mycoparasitic activity, even though all the strains showed high degree of antagonism. The pH ranges were found to be insignificant for mycoparasitic activity. Analysis of volatile production by the same highly antagonist strains showed relatively no degree of inhibition. Thus the study of the mode of inhibition by the highly antagonistic isolates showed that different mechanisms were employed by different isolates. The mode of biocontrol is thus dependant on the isolate used and targets the organism. The physiological condition of pH plays a very insignificant role in the mode of interaction. It would also be likely that a combination of antagonistic mechanisms can be used by some isolates. *In vitro* screening can thus be an effective tool for the initial analysis of interaction of various *Trichoderma* spp. with the pathogen. The high percentage of inhibition of isolates IISR 1413, IISR 1424, IISR 1451, IISR 1450, IISR 1444, IISR 1394, IISR 1440, IISR 1428, IISR 1448, IISR 1432 displayed its potential for study as biocontrol agent against *P. capsici*.

24. Effect of Plant Growth Promoting Rhizobacteria on growth of black pepper (*Piper nigrum* L.) and suppression of *Phytophthora* foot rot (PR 81)

Minimol, T. P. / University of Madras / Guide - M. Anandaraj

The biocontrol agents used in the present study were three strains of *P. fluorescens*, two strains of *Bacillus* and a strain of Phosphobacteria. All the strains were found to inhibit the growth of *P. capsici* above 50% in the dual culture assays conducted. The experiments conducted, short listed two strains of *P. fluorescens* viz IISR-396 and IISR-51 as most efficient out of the seven bacteria tested for its phosphate solubilising efficiency. Even though, IISR-400 could efficiently solubilize the soil phosphorus making it available to the plants, its disease suppressive ability was comparatively less. In a view to understand the endophytic nature of these strains in black pepper system, root bacterized cuttings were tested after a month for its movement and occupation in different parts such as

root, shoot and leaf. Except *Bacillus* spp. IISR-398, all other strains could reside in the internal tissues of the black pepper plant. The strains were also found to solubilize phosphate, which is in the unavailable form to its available form to the plants. Phosphobacteria strains, IISR-400 was most efficient in phosphate solubilization. The endophytic nature of these strains make them efficient biocontrol agent that can be effectively used for root rot management in black pepper. Growth enhancement was observed in black pepper cuttings treated with the test bacterial strains viz. *P. fluorescens* strains, IISR-36, IISR-51 followed by IISR-396. Percentage of root rot in the bacterized black pepper cuttings after challenge inoculation with *P. capsici* was found to be significantly less than the untreated plants. The strains were also efficient in reducing pathogen population the soil, IISR- 396 was the most efficient which could reduce the DPI from 32 to 8.

25. Ecology of *Trichoderma harzianum*-an introduced biocontrol agent in black pepper soil (PR 6)

Sibi, M. C. / Bharathiar University / Guide - M. Anandaraj

*Trichoderma, harzianum* grew better on sorghum followed by neem cake. Soil applied with sorghum and neem cake caused greater increase of *T. harzianum* than other fungi and bacteria. There was no difference in the population of actinomycetes, which remained static in all treatments. In soil applied with *T. harzianum* saprophytic nematodes and mites increased greatly after 15 days and declined after 30 days. Predatory nematodes multiplication increased in all treatments and parasitic nematodes and mites declined to zero. In all the treatments, saprophytic mite population increased and recorded maximum number 15 days after treatment and declined after 30 days. The Collembola population in soil applied with neem cake and sorghum remained unchanged. However, the population declined in other treatments. The organic and inert substrates were ineffective on larvae and beetle population.

26. Screening of *Phytophthora capsici* isolates for their pathogenic variation on black pepper (*Piper nigrum* L.) (PR 22)

Bineesh Bhaskaran, C.P. / University of Madras / Guide - M. Anandaraj

The genus *Phytophthora* is known to exhibit variability for morphology and

pathogenic characters. In this study 46 *Phytophthora capsici* obtained from various area were characterized based on morphology and pathogenicity. When these 46 isolates were tested on a single susceptible variety Karimunda (Subhakara) there were pathogenic variations. Among 46 isolates tested five isolates were less pathogenic causing less than 10 mm lesion after 48 hrs of incubation and hence called as less virulent. Twenty isolates were moderately virulent and others were highly virulent causing lesion size of >20mm. There were variability in pedicel length and sporangial L/B ratio among all the 46 isolates, but there was no correlation between pathogenic variation and morphological variability in the isolates studied.

**27. Effect of plant growth promoting rhizobacteria on slow decline disease of black pepper (*Piper nigrum* L.) (PR 10)**

Sabir, R. K. / University of Calicut / Guide - M. Anandaraj

Plant growth promoting rhizobacteria (PGPR) were tested for their effect on slow decline. After 60 days of application of PGPR, it was found that all the bacterial treatments significantly reduced the foliar yellowing in black pepper. The intensity of yellowing was graded based on visual observations. There were three grades (upto 25%, upto 50% and greater than 50% yellowing). In all the PGPR treated vines the grades have come down and the vines produced new foliage. The recovery of vines was total in vines treated with PGPR isolate IISR-853 followed by IISR-51. Whereas the untreated vines deteriorated in health as the intensity of yellowing had increased. Among the strains, IISR-853 and IISR-51 were found to be most effective in reducing the disease. There was no variation in the population of indigenous bacteria, fungi and actinomycetes after introduction of a larger population of PGPR.

**28. Studies on inheritance of *Phytophthora* resistance in hybrid progenies of black pepper (*Piper nigrum* L.) (PR 38)**

Sunaina, R. / University of Calicut / Guide - K. Nirmal Babu and M. Anandaraj

A mapping population of black pepper (169 progenies) developed at IISR with Panniyur-1 and Subhakara as parents were studied for their segregation patterns of a few juvenile characters like shoot tip colour, leaf shape and size internodal distance and resistance to *Phytophthora*. Various parameters like leafing lesion length, stemming lesion

length, and stem penetration index were used to study the reaction against *Phytophthora*. Female parent Panniyur 1 has greenish white shoot tip colour and the male parent Subhakara has purple shoot tip colour. The progenies segregated as 81.66% greenish white, 5.92% green, 10.06% purple and 4 2.37% light purple shoot tips. The study showed intermediate forms between parental characters. Cytologically Pepper is considered a tetra polyploid on the basic chromosome number  $X = 13$  hence at least 4 loci must be operating in complementary or co dominant fashion with incomplete dominance giving quantitative differences in green and purple coloration. There are differences in the parents and the progenies with regard to *Phytophthora* resistance as expressed by leaf and stem screening. These characters segregated independently indicating that disease resistance is controlled by more than one gene. The degree of resistance also showed a sort of continuous variation indicating either quantitative or multi allelic interaction may be controlling disease resistance. Though susceptible themselves both Panniyur 1 and Karimunda gave resistant lines in the segregating populations indicating that they can be used as parents in hybridisation programme to produce resistant hybrids.

**29. Studies on the evaluation of antifeedant activity against pollu beetle (*Longitarsus nigripennis*) – A major pest of pepper (PR 86)**

**Shinu, P.P.** / University of Calicut / Guide - *S. Devasahayam*

Acetone extracts of leaves of *Annona squamosa*, *Chromolaena odoreta*, *Azadirachta indica*, *Pongamia pinnata* and *Strychnos nux-vomica* were evaluated for their antifeedant activity at 1%, 3% and 5% concentrations against pollu beetle (*Longitarsus nigripennis*) – a major insect pest of black pepper in laboratory bioassays. All the extracts exhibited varying degrees of antifeedant activity and when the mean antifeedant activity of all the three concentrations were taken into consideration, *S. nux-vomica* and *A. squamosa* were more effective exhibiting 90.4% and 88.7% antifeedant activity, respectively. The study indicated the potential of utilizing extracts of these plant species as a component in developing IPM schedules against the pest.

30. Purification and characterization of *Cucumber mosaic virus* infecting black pepper (*Piper nigrum* L.) (PR 19)

Faisal T Thayyullathil / Periyar University / Guide - A. Ishwara Bhat

A successful method for isolation and purification of *Cucumber mosaic virus* (CMV) from black pepper was developed. Electron microscopy of purified preparation revealed the presence of typical isometric particles measuring about 28nm in diameter. The coat protein molecular weight of the virus was 25.7 kDa. In DAC-ELISA among the three isolates of black pepper collected from Belur, Kodlepet and Walayar, Belur isolate gave a higher O.D value (0.42) indicating higher concentration of virus. The result of DAC-ELISA were further confirmed through electro blot immuno assay (EBIA) using CMV antiserum which showed a positive reaction with all the three isolates of CMV used in the present study.

31. Biochemical characterization and isoenzyme profile of few black pepper varieties (PR 62)

Sumesh Kumar, T.M. / Bharathiar University / Guide - B. Chempakam

The biochemical constituents, enzyme activities and isozyme profile of the selected seven black pepper varieties, which are characterized by high yield, quality and disease tolerance were analysed. Coll. 1041 and P 24 contained higher levels of reducing sugars, which can provide precursors for the piperine synthesis, in these varieties. Karimunda and OP karimunda had higher levels of phenols along with Coll. 1041, supporting its disease tolerance. Regarding enzyme activities, peroxidase (PRX) and polyphenol oxidase (PPO) showed higher activities in Coll. 1041, HP 813 and HP 105. These varieties exhibit tolerance to *Phytophthora* foot rot disease. Catalase activity was maximum in P 24 which is a *Phytophthora* tolerant variety. Esterase showed higher activities in coll. 1041, OPKM and P 24, reflecting the high yield, disease tolerance and better quality of the berries.

32. **Isozyme profile and enzyme changes associated with nematode tolerance in black pepper (PR 61)**

Anu Paulose / Bharathidasan University / Guide - B. Chempakam

Biochemical and enzyme profiles of black pepper accessions tolerant and susceptible to *M. incognita* exhibited wide variations. Decreased levels of carbohydrates were seen associated with tolerance in roots. Phenols and O.D Phenols showed a steep increase during the period of infection. Catalase and peroxidase isozyme showed specific bands at 24 hrs after inoculation with *M. incognita*. SOD isozyme showed an additional slow moving band 12 hrs after inoculation indicating the possibility of a hypersensitive response.

33. **Evaluation of different application methods of plant growth promoting rhizobacteria (PGPR) and *Trichoderma* (*Trichoderma harzianum*) on growth of black pepper (*Piper nigrum* L.) varieties in the nursery (PR 33)**

Sreekala, K. / University of Calicut / Guide - C. K. Thankamani

Growth parameters like height, number of leaves and leaf area were maximum with application of PGPR (*Pseudomonas fluorescense*) thrice, where as number of roots and biomass production were higher with combined application of PGPR and *Trichoderma harzianum*. Increased uptake of N, P, K, Mg, Fe and Mn was observed by the combined application of PGPR thrice and *Trichoderma*. Application of PGPR thrice in the polythene bag filled with potting mixture was helpful for the production of vigorous rooted black pepper cuttings in the nursery. Pest management strategies based on ecofriendly practices, application of PGPR thrice with *Trichoderma harzianum* or PGPR thrice in the potting mixture may be suggested for the production of healthy black pepper rooted cuttings in the nursery.

34. **Studies on foliar application of nitrogen (urea) in black pepper (*Piper nigrum* L.) under nursery condition (PR 31)**

Manju, T. M. / University of Calicut / Guide - K. Kandiannan

The spray solutions of different concentrations viz., 0.5%, 1.0%, 1.5%, 2.0% and 2.5% were prepared by diluting urea with distilled water and applied periodically and

separate check (control) with water spray was also maintained. The experiment was laid out in randomized block design with four replications using four black pepper varieties – (Panniyur-1, P-24, Pournami and Subhakara). The growth parameters such as plant height, leaf number, leaf area were recorded periodically and fresh and dry weight were also observed at the end of the experiment. The result indicated that foliar spray of urea enhanced the growth, nutrient content and nutrient uptake of black pepper cuttings compared to control under nursery conditions. The maximum growth was noticed under 2.5% spray of urea.

35. **Metabolite partitioning and isozyme profiles of peroxidase, esterase, acid phosphatase, malate dehydrogenase and poly phenol oxidase of high and low yielding black pepper accessions (PR 44)**

**Gipson Makil** / Bharathidasan University / Guide - *K. S. Krishnamurthy*

The experiment was conducted to study the metabolite partitioning and isozyme profiles of some high and low yielding black pepper accessions. The salient findings are leaves and petioles of high yielders had lower levels of carbohydrates. Leaves of low yielding accessions contained more reducing sugars when compared to that of high yielders. Leaves and roots of high yielding accessions contained higher phenol level than that of low yielding accessions. Roots of low yielding accessions showed higher protein content than that of high yielding accessions. There was no significant difference in the level of total amino acids in high and low yielding accessions. Three identical bands of peroxidase isozyme were found only in leaves of high yielding black pepper accessions. Esterase banding pattern also revealed three identical bands for high yielding accessions, which were absent in low yielders. Acid phosphatase, and MDH isozyme profiles did not vary among high and low yielding accessions. In case of polyphenol oxidase a band specific to high yielders was observed.

36. **Response of black pepper varieties to variation in temperature (PR 30)**

**Prajeena, V.** / Periyar University / Guide - *K. S. Krishnamurthy*

Response of six black pepper cultivars (Panniyur-4, Panniyur-5, Panchami, Sreekara, Acc.no 1041 and open pollinated Karimunda) to varied temperature was studied. Biochemical parameters studied included membrane leakage, total proteins, total

carbohydrates and enzymes such as catalase, super oxide dismutase (SOD) and peroxidase. Results indicated that high temperature decreased the activities of catalase and super oxide dismutase enzymes while peroxidase activity showed mixed trend. Low temperature increased the super oxide dismutase activity and decreased the catalase activity. Membrane leakage increased at high temperature and carbohydrate content decreased while the protein content increased at high temperature.

**37. Biochemical and physiological parameters influencing productivity in black pepper varieties (PR 80)**

**Shujari, V. P / Bharathidasan University / Guide - K.S. Krishnamurthy**

Biochemical constituents, enzyme activities and isozyme profiles of five high yielding and five low yielding juvenile black pepper vines were analysed to investigate the influence of these factors on productivity. Total chlorophyll content did not influence productivity. Starch and reducing sugar content in juvenile stage were found to have influence on productivity. Similarly, catalase activity also showed some influence on productivity while isozymes of peroxidase and polyphenol oxidase did not influence productivity. It was concluded that reducing sugar, starch, total carbohydrate and protein content present in leaves and stem of juvenile pepper vines may influence productivity.

**38. Studies on the stability of *Azospirillum* and Phosphate solubilizing bacteria in black pepper rhizosphere (PR 26)**

**Nirupama, S. / Bharathiar University / Guide - V. Srinivasan**

The population of biofertilizers namely *Azospirillum* and phosphate solubilizing bacteria PSB in the black pepper rhizosphere steadily increased from an initial '0' day population of  $14.75 \times 10^9$  and  $5.25 \times 10^9$  CFU/g up to 30 days with an average of 48 and  $63 \times 10^9$  CFU in PSB and *Azospirillum* respectively. These increased population also helped in increasing the availability of major nutrients (N & P) to a higher level over uninoculated control. The P solubilizing ability of *Azospirillum* strain (P1AR6-2) was also confirmed as it recorded a higher available P value in the rhizosphere. The study confirmed better establishment of applied biofertilizers in the black pepper rhizosphere.



39. The role of phosphate solubilizing bacteria (PSB) in phosphorus utilization efficiency of black pepper (PR 28)

Sumija Rani, P. C. / Bharathidasan University / Guide - V. Srinivasan

The culture characteristics of Phosphate Solubilizing Bacteria (PSB) isolate PB-21 was studied. The phosphobacteria on Pikovskay agar medium exhibited well defined clearing zones. The inoculation of Phosphate Solubilizing Bacterial (PSB) isolate PB-21 significantly increased the shoot length, shoot and root dry matter production and shoot and root P uptake of black pepper as compared to un inoculated check. The rhizosphere population buildup of applied isolate was highest in root dipping. The inoculation of PSB significantly improved the native P solubilization and its uptake by the roots and thereby growth promotion in black pepper. Among the methods of application root dipping of black pepper cuttings with PSB was found to be most efficient followed by soil drenching and foliar application.

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## CARDAMOM

1. Estimation of genetic fidelity of cardamom (*Elettaria cardamomum* Maton.) varieties conserved in *in vitro* repositories using RAPD polymorphism (PR 90)

Jayakumar, V.N. / Bharathidasan University / Guide - K Nirmal Babu

This study aimed to standardize isolation of genomic DNA and use RAPD profiling for estimating the genetic stability of *in vitro* conserved plants of small cardamom. *In vitro* plants of cardamom varieties NKE 9, NKE 27 and APG 50, conserved for at least three years with yearly sub culturing on minimal growth medium were used in this study. Genomic DNA was isolated from young fresh leaves using CTAB method. RAPD profiling was optimized. All the 19 primers tested showed no differences between the 6 replicates of the three genotypes studied. Only NKE 27 - 1 and NKE 27 - 3 seem to slightly differ from this pattern. Otherwise the study indicated that there is considerable uniformity between the 6 replicates of *in vitro* conserved genotypes of cardamom.

2. Studies on detection of RAPD markers linked to 'katte' disease resistance in cardamom (*Elettaria cardamomum* Maton) (PR 99)

Babitha Bai, A. P. / Kannur University / Guide - K. Nirmal Babu

An attempt was made in the present study to identify RAPD markers associated with katte resistant genotypes by comparing their RAPD profiles with those of susceptible cultivars of cardamom. Tagging of katte resistance with molecular markers could help in marker assisted breeding and isolation of resistance gene. Twenty two cardamom genotypes were used in the study. 11 of them are resistant to cardamom mosaic virus (katte) and the other 11 are susceptible. Eight random OPERON primers OPA-08, OPA-12, OPB-13, OPC-06, OPD-01, OPD-03, OPE-10 and OPE-15 were used to develop RAPD profiles. The dendrogram though gave clear grouping of NKE lines from non NKE lines except for a odd one like NKE 31. No marker which was consistently associated with katte resistance could be identified. However a few RAPD loci which could form a potential diagnostic markers were identified using OPA-08 and OPC 06.

3. **Isolation and amplification of genomic DNA from dried capsules of cardamom (*Elettaria cardamomum* M.)** (PR 49)

Mrudula Jose / Bharathiar University / Guide - B. Sasikumar

Good quality DNA was isolated from the powdered samples of cardamom capsules by a modified CTAB method. The concentration of CTAB and NaCl were increased to 3% and 1.5M, respectively in the extraction buffer. The purification step was slightly modified by including PEG purification after the usual phenol:chloroform:isoamylalcohol (25:24:1) purification step. This increased the yield and quality of DNA. The isolated DNA was amplified using standard PCR conditions.

4. **Antibiotic sensitivity assay of cultured tissues of ginger and cardamom** (PR 7)

Sabija, S. / Bangalore University / Guide - B. Sasikumar

Sensitivity of calli and /or leaf discs of ginger as well as calli of cardamom to antibiotics ampicillin, hygromycin and kanamycin was assayed in replicated experiments. The antibiotic ampicillin had a growth promoting effect as in the lower concentrations it resulted in callus proliferation and organogenesis in both ginger and cardamom calli. Similarly the lower concentration of hygromycin required for arresting growth of ginger and cardamom calli was found to be 25 mg $l^{-1}$  and 50 mg $l^{-1}$  respectively. In general it appeared that ginger calli is more sensitive to hygromycin. Both ginger and cardamom calli were found to be more tolerant to kanamycin as even at 75 mg $l^{-1}$  there was no appreciable cell death.

5. **Characterisation of small cardamom (*Elettaria cardamomum*) germplasm for its biochemical constituents** (PR 63)

Sooraj, P. K / Bharathiar University / Guide - T. John Zachariah

Thirty two different cardamom germplasm accessions maintained at Indian Institute of Spices Research (IISR), Calicut, Kerala and Cardamom Research Centre (CRC), Appangala, Karnataka was taken for the study. Biochemical parameters like moisture, oil content, protein, aminoacid, carbohydrates, starch, reducing sugar, phenolics were analysed

in the samples. Gas Liquid Chromatography (GLC) was performed to separate the different volatile oils and assess its percentage of distribution in the samples. Purity of the oil was analysed by Thin Layer Chromatography (TLC). The proteins were separated by Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) and analysed for the molecular weight. Common molecular weight of proteins ranging from 67,000 - 140,000 KDA was observed in all the cardamom samples. Cardamom accessions APG-174, APG-81, APG-173 were considered superior, because of their high volatile oil distribution and other remarkable increase in biochemical constituents. These varieties can be recommended for cultivation for good yield, best variety and other specifications.

**6. Biochemical characterization of cardamom (*Elettaria cardamomum*) accessions and database of chemical compounds and metabolic pathways in cardamom volatile oil (PR 105)**

**Helna, K.** / Bharathiar University / Guide - *T. John Zachariah*

Cardamom capsules are valued commercially for its volatile oil. The major constituents of oil are 1, 8-cineole and alpha - terpinyl acetate. Based on high oil recovery and the desirable trait of oil  $\alpha$  terpinyl acetate, APG 273, 277 and 257 were identified as the best accessions. Total carbohydrate and starch content indicated that high quality cardamom accessions contain relatively high starch, carbohydrate and GLC separated components. Bioinformatics on the biosynthesis of major products were elucidated.

**7. Characterisation of cardamom based on volatile oil profile (PR 64)**

**Dhanya, K** / Bharathiar University / Guide - *T. John Zachariah*

Thirty five cardamom (*Elletaria cardamom maton*) accessions were evaluated for volatile oil, husk-seed ratio and aroma profile. Based on the study, APG - 63, 60 and 75 were high with regard to oil content and APG - 244 and 10 were found to be good in the flavour.

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## GINGER

1. **Studies on RAPD polymorphism in ginger (*Zingiber officinale* Rosce.)**  
(PR 102)

**Rashmy, K.R.** / Cochin University of Science and Technology / Guide -  
*K. Nirmal Babu*

Standardization of protocol for RAPD profiling in ginger was attempted. DNA at 30 ng, primer concentration of ~30 pic moles, 200  $\mu$ m dNTPs and 2 units of Taq DNA Polymerase at 35 PCR cycles gave better result for developing RAPDs in ginger. Only 5 out of 21 random primers tested gave amplification.

2. **Somaclonal variation in micro propagated and callus regenerated plants of ginger (*Zingiber officinale* rosc.) as expressed by RAPD polymorphism** (PR 92)

**Suja, G** / University of Madras / Guide - *K Nirmal Babu*

Somaclonal variation forms an important source of variation especially in vegetatively propagated plant like in ginger. Selected micro propagated and callus regenerated plants of ginger, which showed clear morphological variations, were analyzed for RAPD polymorphism as an index for genetic variability. Five polymorphic primers (OPB 10, OPB 17, OPD 10, OPE 1, OPF 1) were used to develop RAPD profiles. The studies indicated that micro propagation even without callus phase induced variations as 9 out of the selected 13 plants showed profile differences. 12 out of the 15 callus regenerated plants also showed profile differences. This coupled with morphological variation indicate that somaclonal variation is an important source of variability in ginger which could be used in crop improvement programmes.

3. **Estimation of genetic variability in micropropagated plants of ginger as expressed by RAPD polymorphism** (PR 91)

**Srinivasan, V** / Bharathidasan University / Guide - *K Nirmal Babu*

Six micro propagated plants each of three varieties of ginger (Varada, Australia and Jamaica) were tested for their genetic uniformity using RAPD polymorphism. Four

primers OPB 2, OPC 5, OPE 2, OPF 1 were used. These primers could clearly detect differences between the three genotypes tested. In addition, a few of the micro propagated plants also showed profile differences indicating that they may be of possible variants. This can be expected in ginger which may be genetic due to accumulated mutations maintained by vegetative propagation. This also accounts for many varieties in ginger even though there is no sexual reproduction.

4. **Studies on cryopreservation of ginger (*Zingiber officinale* Rose) shoot buds**  
(PR 100)

Swapna, R Nair / Bangalore University / Guide - K. Nirmal Babu

An attempt was made to cryopreserve ginger shoot buds using Encapsulation-dehydration, Vitrification and Encapsulation-vitrification methods. In encapsulation-dehydration method maximum recovery was obtained in shoot buds encapsulated in 4% sodium alginate solution containing MS medium with 2M glycerol, 0.4 M sucrose and subjected to dehydration in progressive increase of sucrose concentration, together with 6 hours of desiccation. 40% of the shoot buds could be recovered and showed growth. In Encapsulation-Vitrification method encapsulated ginger shoot buds pre treated with a mixture of 2 M glycerol + 0.4 M sucrose for 3 days. About 40-50% of the shoots were recovered after cryopreservation.

5. **Callus induction and callus regeneration in *Zingiber officinale* Roscoe.**  
(PR 75)

Lincy, A. K. / University of Calicut / Guide - B. Sasikumar

In the present study, callus induction from leaf sheath and psuedostem explants and callus regeneration from psuedostem and leaf sheath derived callus were attempted in two ginger varieties. Callus was successfully induced from psuedostem and leaf sheath taken from *in vitro* grown, 2-3 months old ginger plantlets in MS full and half strength MS basal medium supplemented with 2, 4-D 1 mg<sup>l</sup><sup>-1</sup> and 2 mg<sup>l</sup><sup>-1</sup>. High amount of callus was observed with 2, 4-D 1 mg<sup>l</sup><sup>-1</sup> in Jamaica and Australia variety both in MS and MS half strength medium. Jamaica variety produced profuse callusing in MS full strength medium supplemented with 2, 4-D 1 mg<sup>l</sup><sup>-1</sup>. Australia variety produced only moderate callusing in

MS full strength medium with 2, 4-D 1 mg<sup>l</sup><sup>-1</sup>. Pseudostem have high potential to produce callus as compared to leaf sheath. Ginger calli were cultured on MS full and half strength medium supplemented with different concentrations of BAP and NAA. Plantlet regeneration was observed in callus of Australia variety cultured on MS full strength medium with BAP 2 mg<sup>l</sup><sup>-1</sup> + NAA mg<sup>l</sup><sup>-1</sup>. Shoot primordia were produced in MS half strength with all hormone concentrations in this variety. Jamaica variety showed only rhizogenesis. Direct regeneration was observed in hormone free MS medium and medium supplemented with BAP and NAA in low concentrations, in Jamaica and Australia respectively. The percentage of direct regeneration was comparatively low.

6. *In vitro* evaluation of *Pseudomonas fluorescens* against rot pathogens of ginger (PR 18)

Smitha, E. P. / Bharathidasan University / Guide - R. Suseela Bhai

*Pseudomonas fluorescens* is a plant growth promoting rhizobacteria (PGPR) and also used as biocontrol agents, which reduces the disease incidences in crop plants. The study concluded that most of the strains isolated were effective in reducing disease incidence in ginger. In the study *Pseudomonas fluorescens* strains were used against the disease caused by *Pythium*, *Fusarium* and *Ralstonia*, which cause rot and bacterial wilt in ginger. Among the ten isolates tested, the results showed that most of them were effective in inhibiting the growth of the target pathogens under *in vitro* conditions.

7. Pathogenicity and characterization of *Pythium* species causing soft rot of ginger (PR.110)

Silymol Philip / Bharathidasan University/ Guide - R. Suseela Bhai

Fungal species isolated from rhizome rot affected ginger (*Zingiber officinale*) collected from different localities in Kerala during 1996-2003 were used for the studies. Based on the cultural and morphological characteristics the fungal isolates were identified as *Pythium* species. Among this, 50 isolates were characterized and the species were identified as *P. myriolytium*, *P. graminicola*, *P. aphanidermatum*, *P. ultimum*, *P. vexans*, *P. spinosum* and *P. pleroticum*. Pathogenicity of the isolates were tested by artificial inoculation on healthy ginger plants. The studies showed that *P. myriolytium*, *P. vexans* and *P. aphanidermatum*

were pathogenic to ginger. *P. monospernum*, *P. torulosum* did not show any symptoms in ginger plants. On artificial inoculation the plants showed symptoms of yellowing and rhizome rot 7-10 days after inoculation. Several factors influenced growth and sporulation of the *Pythium*. P<sub>10</sub>VP medium was used as the selective medium for the isolation of fungi. The highest growth was observed in PDA. Most of the species produced sporangia in single culture. Calcium was the most influential cation in the substrate on development of oospores and sporangia. The studies were also conducted on the effect of different factors on the growth of *Pythium* species. Though *Pythium* species could grow on a wide range of pH, optimum pH for growth was about pH 7 ± 1. The optimum temperature for growth was about 28°C.

8. Influence of Plant Growth Promoting Rhizobacteria (PGPR) on Vesicular Arbuscular Mycorrhizal (VAM) colonization in ginger and their combined effect on soft rot disease (PR 109)

Kishor, V. K. / Bharathidasan University / Guide - R. Suseela Bhai

Out of the 20 different Plant Growth Promoting Rhizobacteria (PGPR) studied, IISR 51, IISR 914 and IISR 915 were found to be better followed by IISR 912, IISR 913 and IISR 152 in enhancing the intensity of Mycorrhizal colonization and the spore load in the soil. Colonization of roots by VAM fungi reduced damage due to different plant diseases and enhanced the plant growth. The results of this study indicated that PGPR isolates could be used as biofertilizers to enhance VAM colonization which in other way is effective in increasing the nutrient uptake from the soil especially phosphorus and also in combating soil borne diseases. The biocontrol of soft rot disease by application of VAM and PGPR significantly resulted in reduction in the incidence of soft rot disease caused by *Pythium* species. The PGPR isolates IISR 51, IISR 151, IISR 859, IISR 906, IISR 913 and IISR 915 were found to be more promising in controlling the disease. The applications of these isolates along with VAM were suggested for the production of healthy ginger plants. *Glomus* species was found to be the predominant VAM fungi associated with ginger. Since ginger is an annual crop, the grower's can easily follow inoculation of VAM fungi in ginger by multiplying them in grass roots. This simple low cost agricultural technology would play an important role in getting healthy and vigorous ginger plants.



9. **Investigations on antibacterial metabolite production by *Trichoderma viride* against *Ralstonia solanacearum* causing bacterial wilt of ginger (PR 123)**

Asmaja, A. K. / University of Calicut / Guide - A. Kumar

Antibacterial activity of Cell Free Culture Filtrate (CFCF) of *Trichoderma viride* against *R. solanacearum* was established *in vitro*. *T. viride* wild type had high antibacterial activity against on *R. solanacearum* when compared to the *T. viride* (albino). Age of the culture got direct role in the production of antibacterial metabolite against *R. solanacearum* as maximum activity was found in 6-12 days old culture whereas for mutant the activity was observed only up to 8 days. *Trichoderma* could utilize *Ralstonia* as its substrate for growth as indicated by their profuse growth when live or dead cells of *R. solanacearum* served as carbon source. The heat labile nature of metabolites from *T. viride* was established as temperature above 70°C affected the biological activity of the metabolite.

10. **Effect of rhizome solarization on microbial population of ginger with special reference to the survival of *Ralstonia solanacearum* (PR 23)**

Praseena, E / Bharathiar University / Guide - A. Kumar

Rhizome temperature could be increased by exposing the rhizome to sunlight for a period of 2 hours. The larger sized rhizome pieces (100g) recorded higher temperature (1-3°C) when compared to smaller one (10g). Mean rhizome temperature after one hour of exposure was 40°C and the temperature increased to 50°C after 2 hours. The culturable bacterial count was reduced to a great extent (84.2% reduction) on the surface and also in vascular tissue of ginger (92% reduction) due to rhizome solarization. The survival of *R. solanacearum* on ginger rhizome was severely affected due to rhizome solarization as the  $A_{405}$  data showed negative result for *R. solanacearum* after two hours of solarization in ELISA.

11. Phenotypic characterization of *Ralstonia solanacearum* for biovar and its detection in soil and rhizomes using Double Antibody Sandwich (DAS)-ELISA (PR 13)

Anila George / Bharathiar University / Guide - A. Kumar

Phenotypic characterization of biovar confirmed that the bacterial wilt pathogen of ginger in Kerala is predominantly biovar 3 whereas the incidence of biovar 4 is less frequent. CIP's DAS-ELISA Kit was found suitable for the detection of *R. solanacearum* in bacterial wilt affected soil and ginger samples. Bacterial wilt pathogen was detected in apparently healthy rhizomes, which confirms the rhizome borne nature of *R. solanacearum* in ginger. The apparently healthy rhizomes are the protected carrier of bacterial inoculums at low levels. *R. solanacearum* can survive both in vascular tissue and external surface of naturally infected ginger rhizomes. Rhizome solarization is capable of disinfecting the rhizomes infected by *R. solanacearum* either artificially or naturally, as found using DAS-ELISA.

12. Identification of ginger germplasm accessions with low and high fibre, high oil, *Zingiberene* and oleoresin (PR 105)

Ashish, G.R. / Bharathidasan University / Guide - T. John Zachariah

The present study indicated that there is a good variability among 50 germplasm accessions of ginger selected for the study. Eight accessions each could be identified with low and high fibre content. Acc-555 is superior among the accessions with high oil, oleoresin, fibre, limonene, geraniol and pinene content in the oil. Many of the high oleoresin accessions showed high crude fibre content. Carbohydrate, reducing sugars and amino acid content did not show any correlation with the level of oil and oleoresin.

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## TURMERIC

1. **Studies on genetic stability of micropropagated and micro-rhizome propagated turmeric plants (*Curcuma longa* L) through RAPD profiles (PR 101)**

Sirajuddeen, A. / Bangalore University / Guide - K. Nirmal Babu

Protocols for micropropagation of turmeric through plantlets as well as micro rhizomes were developed at IISR were used. In the present study genetic stability of micropropagated and microrhizome derived turmeric plants were analyzed through RAPD profiles. Four operon primers (OPD-5, OPE-14, OPC-12 and OPD-8) were used to detect polymorphism among the micro propagated and micro rhizome derived plants. Variation in RAPD profiles were observed in some of the micro propagated plants indicating that micro propagation induces certain amount of variation. The RAPD profiles of micro rhizome-derived plants did not show any noticeable polymorphism except for 1 or 2 bands. The similarity in banding pattern indicates that micro rhizome derived plants are more stable when compared to micro propagated plants.

2. **Callus induction and *in vitro* regeneration from rhizome buds of *Curcuma amada* and *Curcuma aromatica* (PR 69)**

Jyothi Thomas / Mahatma Gandhi University/ Guide - B. Sasikumar

*In vitro* response of *Curcuma amada* and *Curcuma aromatica* to callus induction and direct regeneration on MS medium supplemented with various growth regulators was investigated. Callusing was successfully induced from vegetative bud of these two *Curcuma* species in MS full strength medium supplemented with 2,4-D in various concentrations (0.5, 1 & 2 mg l<sup>-1</sup>). But high rate of callusing could be observed at concentrations of 2 mg l<sup>-1</sup> 2, 4-D. In the direct regeneration study MS medium with different hormone concentration of BAP + NAA were tried. In both *C.amada* and *C.aromatica* maximum number of shoots were observed in the treatment BAP + NAA (2:1) followed by BAP + NAA (3:1). Highest number of roots were observed in the treatment BAP + NAA (1:1) implying that varying hormone requirement for shoot and root regeneration.

3. **Comparative molecular profiling and quality studies of market samples of turmeric and pure turmeric sample (PR 25)**

Remya, R. / Bharathiar University / Guide - B. Sasikumar

The study was undertaken to determine the purity / adulteration of three popular brands of turmeric powders (market samples) in comparison with genuine powder of two *Curcuma* species. Good quality of DNA was isolated from the three market samples and the two *Curcuma* species samples using a modified CTAB method. Out of 40 random decamer primers screened, 8 yielded consistent and clear banding patterns. The brands differed in the representation of the species specific markers. The curcumin content of market samples was comparable to *C.longa*, though adulterated turmeric powders are reported to have low curcumin content. It was observed that the popular brands of turmeric powder are not pure *C.longa* L. powder though they have acceptable curcumin levels.

4. **Molecular characterization of *Curcuma* species using RAPD markers (PR 5)**

Sreeja, S. G. / Periyar University / Guide - B. Sasikumar

The study was undertaken to identify different *Curcuma* species using RAPD markers. The original Gawl and Jarret protocol for DNA extraction was modified to get good quality of DNA from rhizome and PCR conditions for good amplification was standardized with DNA from rhizome. Three primers generated 11 polymorphic bands among 5 *Curcuma* spp. Primer OPA-01, OPA-04 and OPE-11 generated 3-4 polymorphic bands across 5 *Curcuma* spp. Since the amplification products were from rhizome this will further enable to easily identify market samples of the *Curcuma* species. The utility of the specific RAPD marker can be further increased by sequencing their termini and designing longer primers (SCARS) for specific amplification.

5. **Callus induction and regeneration in turmeric (*Curcuma longa* L.) (PR 108)**

Noushad Kalluvalappil / Bangalore University / Guide - B. Sasikumar

*In vitro* response of three turmeric genotypes Amalapuram, Mananthavadi and

Anchal to callus induction and organogenesis of one turmeric genotype Mananthavadi were investigated. Callusing was successfully induced from pseudostem of all the three turmeric genotypes in MS full strength medium supplemented with 2, 4-D in all concentrations. But high amount of callus was observed in 'Mananthavadi' genotype with 2,4-D @ 1 mg/l and 2 mg/l. Genotype dependant callusing response was observed. 'Mananthavadi' genotype produced profuse callusing compared to other genotypes after eight weeks of culturing. In the treatment with BAP + NAA (1:1 mg/l) no callusing was obtained, 2, 4-D was found critical for callus induction. In the callus regeneration study, maximum number of shoots was produced in the medium having BAP + NAA @ 1: 0.5 mg/l where as the maximum number of roots were produced in the medium having BAP + NAA @ 1:1 mg/l. This indicates that hormone requirements for shoot and root regeneration from the callus is different.

6. **Molecular characterization of callus regenerated plants of turmeric (*Curcuma longa* L.) using RAPD markers.**(PR-8)

Riji, V. S / Periyar University / Guide: R. Ramakrishnan Nair

The present investigation is performed with the objective to assess the extent of somaclonal variation among the plants regenerated from seed derived callus of turmeric Acc. No. 399 using RAPD polymorphism. Various morphological characters recorded shown extensive variation between the soma clones as well as the mother plant. Specific chlorophyll variants were also observed among the regenerated plants. Characterization based on plant morphology is difficult in somaclones at juvenile stage. Conversely molecular markers such as RAPD can be utilized to compare even plants at juvenile stage as these are mainly based on the extractable DNA. RAPD profiling with the help of four operon primers viz., OPC-11, OPD-05, OPC-12 and OPD-08 revealed nine sets of variants among somaclones. None of the regenerated plants showed similarity towards the mother plant. This might be due to the fact that the plants were regenerated from the callus derived from the open pollinated seed, which might have increased the spectrum of variation.

7. A systematic study of the antimicrobial activity and chemical composition analysis of essential oils from turmeric (*Curcuma longa* L.) leaf and rhizome (PR 59)

Gobinath, P. / Bharathiyar University/ Guide - B.Champakam

The antimicrobial activity of turmeric leaf and rhizome essential oils were tested against 5 different bacterial strains, 2 yeast species and 4 fungal species. The chemical composition analysis of turmeric was also attempted. The turmeric leaf essential oil was more significant than turmeric rhizome oil in antimicrobial activity against bacterial species like *Bacillus*, *E.coli*, *Proteus* and *Staphylococcus*, yeast species like *C.albicans* and *S.cerevisae*. The *Klebsilla* species are resistant to both the turmeric essential oils. It was observed that there was low activity of turmeric essential oils against *Fusarium* sp. All other fungal species like *A.flavus*, *A.niger*, and *P.capsici* showed resistant to the turmeric essential oils. The turmeric essential oils contained a number of chemical compounds, from leaf (Phellandrene, Terpenolene, p-cymene, 1,8-cineole, a-Pinene) and turmeric rhizome having compounds as B-sesquiphellandrene, Ar-curcumene, curlone, Turmerone, Ar-turmerone.

8. Biochemical characterization and isoenzyme profile of selected *Curcuma* species (PR 60)

Ansil, P. N. / Bharathiar University / Guide - B. Chempakam

Among the turmeric species, the biochemical profile including the levels of primary and secondary metabolites are known to vary. Isoenzyme analysis by electrophoresis is an effective method to detect genetic differences among individuals. No common banding pattern was seen in any of the isozymes indicated the degree of variation between the species. The results could be used in developing biochemical markers for identifying individual species.

9. Comparative study of three curcuma oils and their antifungal activity (PR 67)

Sereena, K. / University of Calicut / Guide - N. K. Leela

Three curcuma species namely *C. amada*, *C. aromatica* and *C. zedoaria* were evaluated for their volatile oil content and chemical constituents. Camphor was the chief constituent of *C. aromatica* rhizome oil. All the rhizome oils contained ar-turmerone, camphor, terpinen - 4 - ol and 1,8-cineole. Among the oils *Curcuma amada* exhibited maximum inhibition of mycelical growth of *P. cajosin*.

10. Biochemical evaluation of turmeric (*Curcuma longa* L.) rhizomes and separation of curcuminoids by chromatographic techniques (PR-76)

Silja, K.V. / Bharathidasan University / Guide - N. K. Leela

The study was carried out to extract and isolate curcumin, DMC and BDMC from turmeric (*Curcuma longa* L.) rhizomes. Individual curcuminoids were separated by TLC using chloroform - methanol (95:5) as mobile phase. The purity of the compounds thus separated was tested by determining their UV-absorption maxima in ethyl alcohol.

11. Effect of sources of potassium on soil nutrient availability, yield and nutrient uptake of turmeric (PR 53)

Kadeeja, P / University of Calicut / Guide - V. Srinivasan

The chemical analysis of soil applied with different natural amendments (ash, rock powder etc.) indicated that addition of natural amendments significantly changed pH, available supplies of P, Mg, Ca and K. Application of different sources of K significantly increased all the major nutrients (N, P & K) secondary nutrients (Ca & Mg) status of the soil in the turmeric field. Among various natural amendments used, coconut ash and southern phosphate ash increased the rhizome yield of turmeric and potassium uptake by turmeric. The application of coconut ash and southern phosphate ash also increased the soil availability of K, N, Ca & Mg nutrients. Application of natural amendments has increased the yield, essential nutrients in soil and nutrient uptake of turmeric compared to the chemical fertilizer (muriate of potash) applied to turmeric.

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## NUTMEG

1. **Molecular characterization of male, female and bisexual lines of nutmeg, *Myristica fragrans* Hou (PR 57)**

**Girija, M.** / University of Calicut / Guide - *B. Krishnamoorthy & K. Nirmal Babu*

The present study was carried out to standardize protocol for isolation DNA and use RAPD for characterization of sex in different genotypes *viz.*, male, female and bisexual. Four accessions each in male, female and bisexual forms of nutmeg were used in the study. Isolation of genomic DNA was carried out using modified CTAB protocol with minor modification. 2% of PVP and 3% of b-mercaptoethanol was found to be optimal for the isolation of good quality DNA without considerable phenolic contamination. 20 random primers were screened for polymorphism. Most of the primers could detect the variability within the genotypes but failed to differentiate the band on sex differentiation. Primer OPA03 showed specific pattern among the bisexuals. Since clear cut polymorphism between male, female and bisexual lines, as groups, could not be detected and no consistent marker could be seen associated with sex differentiation in nutmeg. It may be indication of a multi-genic influence of sex segregation in nutmeg.

2. **Molecular characterization of male, female and bisexual lines of *Myristica fragrans* Hou and creation of a database for *Myristica* species (PR 20)**

**Sajitha Varghese** / Bharathidasan University / Guide-*B. Krishnamoorthy & K. Nirmal Babu*

DNA from three genotypes of male, female and bisexual types of *Myristica fragrans* were isolated, purified, quantified and RAPD profiling was conducted with three primers OPA 04, OPA 06 and OPA 17. Though low polymorphism was observed among the genotypes most of the primers could detect the variability among the genotypes. One specific marker with primer OPA 17 was found to be associated with female and the bisexual genotypes. This marker band (0.7 kb) associated with female genotypes using primer OPA-17 has to be confirmed and revalidated with more stringent experimentation. A data base on *Myristica* species was also created.



3. **Identification of sex-specific DNA marker in nutmeg (*Myristica fragrans*) using RAPD (PR 111)**

Najeebudeen, C. A. / Bangalore University / Guide - Johnson George K.

The study was undertaken in *Myristica* to identify sex specific DNA markers by using RAPD techniques. Good quality DNA was isolated by using CTAB method. Five primers (OPC-14, OPC-16, OPE-11, OPE-18, OPE-20) were selected for the RAPD reaction based on preliminary amplification trials. The random primer, OPE-11 produced a male specific band of 450 bp in length. While this result needs to be further confirmed, it provides an exciting possibility of being used to address different issues, including developing DNA markers to determine sex in nutmeg and to increase understanding of the evolution of dioecy in the primitive family *Myristicaceae*.

4. ***In vitro* propagation and molecular characterization studies of nutmeg (*Myristica fragrans*. Houtt)**

Unnimaya, G. / Bharathidasan University / Guide - T. E. Sheeja

Out of the different explants used nodal explants exhibited good establishment in both SH medium and WPM. Among the different basal media tried ¼ strength SH medium and ¼ strength WPM was found to be better for bud break from nodal segments. Shoot tips exhibited lower potential for emergence of bud while the axillary buds showed better response. The sub cultured nodal explants showed better growth in ¼ SH medium supplemented with BAP @ 2 mg/l in comparison with BAP with 2 mg/l BAP and 0.5 NAA. For the molecular characterization studies in nutmeg, six genotypes of nutmeg were taken. The DNA was isolated from the third leaves and the quality and quantity of DNA were checked by electrophoresis and spectrophotometer. Out of 10 primers, 3 showed amplification. The data obtained from the gels were scored for the presence and absence of the band. The dendrogram analysis showed two major clusters, the first comprising of the two elite accessions *viz.* A9/22 and A9/79. The second cluster was made of two sub clusters one at a similarity coefficient of 0.65 comprised of accession A11/29 and the male sibling of Viswashree and the seedling progeny of Viswashree and the mother plant of Viswashree showing ~ 80% similarity with each other. The JSI (Jaccard's Similarity Index) values ranged from 0.53 to 0.86 and there is considerable amount of genetic similarity existing among the individuals under study. The study shows

that good quality DNA could be isolated from nutmeg and this DNA can be successfully amplified by PCR.

#### 5. Microbial succession on nutmeg and mace during processing (PR 2)

**Sharmishta, K.** / Bangalore University / Guide - *M. Anandaraj*

Microbial profile of nutmeg and mace was analysed. Samples were collected from the field before harvest and during post harvest processing. Post harvest operations include separation of mace from the seed and drying both nutmeg and mace separately. The microbial population recorded during drying period included some of the toxin producing species such as *Aspergillus flavus*, *A. fumigatus* and *Penicillium* spp. Other organisms included *Pestalotiopsis*, *Geotrichum*, *Streptomyces* and *Trichoderma*. Among bacteria, both Gram +ve and Gram -ve strains were obtained. Among the market samples and samples from wholesale shop contained moisture of 11.1% had a high microbial load compared to nutmeg from retail shop having moisture of 8.9%. However the mace from retail shop having a moisture of 12.9% had a high microbial population, compared to mace from whole sale shop having a moisture of 9.5%. Among the three samples studied the mechanical dryer was found to be efficient in reducing moisture content and it was also found to be better over traditional drying in reducing fungal and actinomycetes populations.

#### 6. Evaluation of certain biochemical constituents in nutmeg (PR 107)

**Radhika, P.** / University of Calicut / Guide - *T. John Zachariah*

Nutmeg (*Myristica fragrans*) is an important spice used in culinary and pharmaceutical industry. There are many wild species, which comes under *Myristica*. The physical appearances of the nut as well as the chemical constituents of the secondary metabolites vary widely in the cultivated and wild. Leaf protein content varies widely between cultivated and wild *Myristica*. As protein was very tedious in *Myristica fragrans*, it was achieved only by using a high ratio of sample: butter. Fresh matured *Myristica fragrans* leaves contained 4% protein while wild *Myristica* contained 9% and dried *Myristica fragrans* leaves contain about 8% protein. Different solvent systems such as chloroform: methanol (9:1), benzene: ethanol (9:1), chloroform: acetic acid: water (30:15:2), hexane: ethyl acetate (8:2), acetic acid: hydrochloric acid: water (30:30:10) etc were employed for separating leaf flavonoids by Thin Layer Chromatography (TLC). Reliable spot of

flavonoid was detected in the hexane: ethyl acetate (8:2) system. UV and spraying with 10% H<sub>2</sub>SO<sub>4</sub> were found to be good visualization agents. Starch content in the wild *Myristica* leaf ranged from 10 to 13% and in *Myristica fragrans* it was 11.6%. Total free amino acid in the cultivated *Myristica fragrans* was 8.22 mg% while in the wild it was only 1.04 mg%. Isozyme studies indicated that peroxidase isozyme has similar bands in both and wild possess additional of bands at rf 0.33, 0.36, 0.47, 0.67, 0.93, 0.96. The study gave a good insight into the biochemical variability available in the cultivated and wild *Myristica*. These difference clearly demarcate the wild from the cultivated and it also give a tool to the researcher to exploit these properties for the industry and other related fields.

7. Chemical quality evaluation in nutmeg (*Myristica fragrans*) (PR 104)

Jashmira, N. / University of Calicut / Guide - T. John Zachariah

Nutmeg (*Myristica fragrans*) is an important spice finding application in food industry, pharmaceuticals and other industrial sector. In this study five nutmeg accessions were evaluated for oil, oleoresin, butter and lycopene, the colour pigment. The study established that good variability exist in the germplasm with regard to these constituents. The sabinene, and myristicin content in the oil are very critical to decide the end use. High myristicin is harmful while high sabinine is desirable. The study also established that we could identify samples with high oil with high and low myristicin.

8. Variability in nutmeg (*Myristica fragrans*) with reference to amino acid and carbohydrate (PR 65)

Sreejith, R. / Bharathiar University / Guide - T. John Zachariah

The carbohydrate content varied from 11.3 to 16.4 in the leaves of wild and cultivated *Myristica*. The carbohydrate value indicated similarity between wild and cultivated lines. *Myristica andamanica*, *M. fragrans* nutmeg and mace were relatively very low (3-4%) in reducing sugar concentration. The total free amino acid level is almost same in both wild and cultivated leaf as well as other plant parts. Some of the amino acids significantly varied between wild and cultivated includes glycine, alanine, isoleucine and histidine. Amino acids like glycine, alanine and leucine showed variability between the male and female lines of *M. fragrans*.

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## CINNAMON

1. **Molecular characterization of *Cinnamon* and related species using RAPD polymorphism (PR 95)**

Judy, E. P. / Periyar University / Guide - *K Nirmal Babu*

True cinnamon is the dried bark of *Cinnamomum verum* Presl. Seventeen species of cinnamon occurs in India. Eleven species of cinnamon – *C. verum*, *C. cassia*, *C. malabattrum*, *C. riparium*, *C. macrocarpum*, *C. perottitii*, *C. weightii*, *C. citronella*, *C. tamala*, *C. sulphuratum*, *C. glaucens* and *C. camphora* were characterized using RAPD polymorphism using 3 primers (OPA 1, OPB 5, OPC 15). Protocol for DNA isolation and RAPD profiling was standardized in cinnamon. The profiles developed were used to study molecular inter relationships. The dendrogram indicated that *C. malabattrum* is closely related to *C. verum*. The species like *C. cassia*, *C. weightii* and *C. citronella*, *C. camphora* are grouped together. Exotic species like *C. sulphuratum*, *C. glaucens* and *C. camphora* were also placed in single group indicating similarities between them. However some of the groupings do not agree with the present understanding of cinnamon taxonomy.

2. **Characterization of few accessions of *Cinnamomum cassia* using RAPD Markers (PR 11)**

Anjali Achuthan, V. / Periyar University, Salem / Guide - *J. Rema*

Assessment of genetic variability in cassia was based on both dendrograms and similarity indices. The study indicated that there is a considerable variation among the progenies of cassia as expressed by RAPD polymorphism. The amount of variation ranged from 0 to 40%. The genotypes C6 and D2, D3 and D8, B5 and B8 were exactly similar to each other. The dendrogram in general showed two major groupings indicating that these seedling progenies would have originated from two major sources of variability. No patterns were observed between the A, B, C, D groupings indicating that the naming is arbitrary. This coupled with phenotypic variations and variations observed in quality parameters indicate that there is reasonable genetic variation in the seed derived progenies of cassia available at IISR. This variation can be used for selection of improved lines especially in quality.

3. Studies on chemical constituents of four *Cinnamomum* species (PR. 68)

Sithara, S. / Bharathiar University / Guide - N.K. Leela

*Cinnamomum verum*, *C. cassia*, *C. tamala* and *C. camphora* were evaluated for leaf oil composition and fungitoxicity against *P. capsici*. The major compound in *C. verum*, *C. cassia* and *C. tamala* was eugenol, while *C. camphora* was dominated by camphor. Among the oils, *C. cassia* leaf oil caused maximum inhibition of mycelical growth of *P. capsici*.

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## GARCINIA

### 1. Molecular characterization of *Garcinia* using RAPD polymorphism (PR 52)

Venkata Pulla Rao Vendra / Acharya Nagarjuna University / Guide - K. Nirmal Babu & J. Rema

Molecular characterization of six *Garcinia* species namely *G. indicia*, *G. gummi-gutta*, *G. cowa*, *G. mangostana*, *G. tinctoria*, *G. hombroniana* was done to study their molecular taxonomy and species inter relationships. Protocol for DNA isolation and RAPD profiling was standardized in *Garcinia*. RAPD profiles developed using three polymorphic operon primers were analyzed for their similarity using NTSYS software and dendrogram were made to study their inter-relationships. The dendrogram showed 6 distinct groups. All collections of *G. gummi-gutta* formed one group and 2 collections of *G. indicia* formed another group. *G. cowa* showed similarity with *G. indicia* in molecular studies. *G. tinctoria* though a morphologically distinct genotype from *G. indicia* formed same cluster with *G. indicia* indicating their nearness. *G. indicia* was more similar to *G. cowa* and *G. tinctoria* than *G. gummi-gutta*. *G. mangostana* and *G. hombroniana* which are two morphologically distinct genotypes showed maximum distance from *G. indicia*, as they are placed in different and separate groups

### 2. Assessment of genetic diversity of *Garcinia* species using inter simple sequence repeat (ISSR) markers (PR 56)

Desly Mathew / Mahathma Gandhi University / Guide - Johnson George. K

Four ISSR primers were used to detect polymorphism among ten species of *Garcinia*. Primer ISSR02C2 generated a maximum of five unique polymorphic bands and differentiated four species viz., *G. hombroniana*, *G. tinctoria*, *G. cowa*, and *G. malabarica*. Primer ISSR02C3 showed three unique polymorphic bands and differentiated four species (*G. hombroniana*, *G. cowa*, and *G. gummi-gutta*). Primer ISSR02C4 generated four unique polymorphic bands and differentiated four species (*G. hombroniana*, *G. cowa*, *G. gummi-gutta*, *mangostana*). The primer ISSR02T2 generated two unique polymorphic bands discriminating *G. hombroniana*-1 and *G. malabarica*.

3. DNA profiling of selected species of *Garcinia* occurring in India using ISSR - PCR

Soumya, R. J. / Bharathiar University / Guide - Johnson George K

An attempt was made to characterize 5 Indian species of *Garcinia* using ISSR. Since the existing methods for DNA isolation did not yield high quality - DNA in sufficient quantities, a protocol suitable for *Garcinia* was developed. A yield of 200 mg/g fresh leaf tissue was obtained. The non anchored ISSR primers were tested for DNA profiling and two was found to be informative. Three species of *Garcinia* could be easily distinguished from other accessions studied.

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## VANILLA

### 1. RAPD Polymorphism in selfed progenies and inter-specific hybrids of vanilla (PR 87)

Jasmin, C. / Cochin University of Science and Technology / Guide - K Nirmal Babu

A population of segregating progenies, using ovule culture and four inter specific hybrids between *V. planifolia* X *V. aphylla* were developed at IISR. Phenotypic and isoenzyme studies earlier indicated variability among the selfed progenies and the interspecific hybrids. The present study attempted to standardize RAPD protocols and to use RAPD polymorphism to estimate genetic variability. RAPD protocols were standardized for vanilla. Seven selfed progenies of *V. planifolia* and three inter specific hybrids were compared among themselves and with their parents to estimate the genetic variability. Eight primers were screened and 2 of them OPC 09 and OPE 14 gave some amplification and were used for developing RAPD profiles. The RAPD profiles indicated that though the progenies of vanilla are more similar to their parent and to each other there is good variation in banding pattern between them indicating variations among the selfed progenies of *V. planifolia*. Thus, the RAPD profiles coupled with morphological characters indicate VH1, VH4 and VH5 are true interspecific hybrids between *V. planifolia* and *V. aphylla* since they are approximately equidistant from both the parents.

### 2. Micropropagation of vanilla and its genetic stability analysis using RAPD markers (PR 103)

Reshna, S. / Periyar University / Guide - K. Nirmal Babu

Micro propagation protocol developed at IISR was used for clonal multiplication of vanilla and 22 of the resultant plantlets were tested for their genetic fidelity using RAPD profiling. The protocol for RAPD profiling developed at IISR was used to study their molecular uniformity. No polymorphism was detected when 4 Operon primers (OPC-17, OPD-16, OPF-3, OPF- 20) were used indicating genetic uniformity of Micro propagated vanilla. Hence micro propagation can be used for clonal multiplication of vanilla planting material.



3. *In vitro* response of various explants of *Vanilla planifolia* Andrews on SH medium supplemented with different levels of 6 - Benzyl amino purine (PR 114)

Lisha, S. / University of Calicut / Guide - R. Ramakrishnan Nair

The present investigation was carried out to study the response of various explants such as nodes having auxiliary buds, internodes and leaf segments of vanilla (*Vanilla planifolia* Andrews) on SH medium supplemented with various levels of BAP. SH medium could be successfully used for shoot regeneration in vanilla. Multiple shoots could be induced from nodal segments having auxiliary buds. Multiple shoot formation was obtained by the proliferation of auxiliary meristem as well as *de novo* from the basal region of a explant. BAP found to be a suitable growth regulator for involving multiple shoots at a range of concentrations from 1.0 – 3.0 mg/l. Higher concentrations found to be inhibitory. Scope of using internodal segments for callus mediated shoot regeneration is indicated.

4. A study on *Colletotrichum* species causing yellowing and immature bean shedding of vanilla (PR. 29)

Sreerekha, M. / University of Calicut / Guide - R. Suseela Bhai

Studies on the biology of the pathogen *Colletotrichum* sp. causing yellowing and immature bean shedding of vanilla revealed that the fungus can survive in almost all pH levels tested as well as at a temperature range of 25-30°C which is the usual microclimate condition prevailing in the vanilla gardens during February-May months. Carbendazim was found very effective in inhibiting the fungus at 50 ppm concentration followed by carbendazim and mancozeb mixture. As carbendazim is systemic in nature, foliar spray with this fungicide is recommendable for this disease. The ED90 value for carbendazim was below 50 ppm which showed that very low concentration of carbendazim is sufficient to achieve maximum protection. Biocontrol agent such as *T. harzianum* was also found inhibitory under *in vitro* conditions. Around 58% inhibition was obtained under dual culture method, which extend its possibility for use as a bioagent against *Colletotrichum* sp.

5. A study on production, processing and marketing of vanilla (PR 35)

Abhilash, K. P. / University of Calicut / Guide - M. S. Madan

There is an immense growth potential for vanilla as our production is still less than 100 tonnes per annum. As the survey reveals, the crop is highly suitable for all the three farm sizes of small, medium and large. Without disturbing the standing crop in the field it can give a benefit cost ratio of more than 4.6, which no other plantation crops give under the present condition. If the crop is cured and sold the BC ratio obtained is 8.25. Since vanilla curing involves a very simple and cost effective method it is advisable to a vanilla grower to go for the processing of beans instead of selling them as raw beans. Overall analysis of vanilla industry in the region in particular and in the country as a general shows that there is huge potential for further development of the vanilla economy to earn enough foreign exchange, create direct and indirect employment to rural mass. Finally, the nature of the crop can allow a sustainable eco system in growing region.

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**BIO-CONTROL / BIO-FERTILIZER**

1. **Morphological characterization and classification of *Trichoderma* spp. from biocontrol repository (PR 46)**

**Betsy, K. Zachariah / Bharathidasan University / Guide - M. Anandaraj**

Sixty seven isolates of *Trichoderma* were obtained from the repository of biocontrol established at IISR, Calicut. They were identified and classified at the species level following the key proposed by Gams and Bisset, 1998. Most of the isolates were found to satisfy the characteristics exhibited by *Longibrachiatum*. But none of the isolates showed any character, which matched section *Hypocreanum*. Out of 67 isolates classified as *Longibrachiatum* 13 isolates were characterized as *Trichoderma* and *Pachybasium* isolates numbered upto 10. Under the section *Trichoderma* 2 species were identified namely *T. harzianum* and *T. koningii*. Under the section *Longibrachiatum* four different species were identified, namely; *T. longibrachiatum*, *T. pseudokonigii*, *T. ressei* and *T. parceramosum*. Out of 67, 16 isolates were collected from black pepper and the predominant one was *T. longibrachiatum*, 47 isolates were collected from ginger and 4 isolates were collected from anthurium. In these isolates the predominant species was *T. longibrachiatum*.

2. **Coconut coir pith as a mass multiplication and carrier media for the biocontrol agent, '*Trichoderma harzianum*' (PR 82)**

**Saluja, B. P. / Kannur University / Guide - M. Anandaraj**

The biocontrol agent must be produced in large quantities for use in commercial agriculture. The antagonistic activity of biocontrol agent (BCA) is mainly influenced by the availability of nutrients from the substrate or carrier medium through that the BCA is applied. *Trichoderma* being very versatile in utilizing several carbon sources, many agricultural wastes and bye products serves as food source. In the present work use of coconut coir pith as a carrier medium for *Trichoderma* was tested. Experiment was conducted using three types of coir pith. viz., raw coir pith, coir pith composts I and II. *Trichoderma* inoculum was produced by liquid fermentation using molasses as the medium in a fermenter was introduced in three different proportions, namely 1:10, 1:20, and 1:40. The study was made in both sterile and non-sterile conditions. Population of *T. harzianum* in these media was estimated immediately after inoculation and every 15 days interval up to 45 days. The population of *Trichoderma harzianum* was reduced in unsterilized media

except in the case of coir pith compost I, (1:10) where it was stabilized at cfu  $10^4$ . Sterilized coir pith compost I and II supported the growth of *T. harzianum*, than unsterilized raw coir pith. Nutrient analysis showed that, C/N ratio of coir pith compost I and II is less than 1:20, indicating that coir pith compost is suitable for the mass multiplication of *T. harzianum*.

### 3. Evaluation of agricultural waste products as carrier media for biocontrol agent *Pseudomonas fluorescens*. (PR 84)

Dhanya, P. T. / Kannur University / Guide - M. Anandaraj

An effective medium is necessary for the successful multiplication and application of these biocontrol agents. In the present study, easily available and cheap agricultural waste products such as, mature coconut water and molasses were tested for the suitability to be used as media for the mass multiplication of biocontrol agent *Pseudomonas fluorescens*, strain, IISR-51. IISR-51 was found to suppress root rot caused by *P. capsici* and enhance the growth of black pepper in the studies conducted earlier at IISR. Apart from testing coconut water and molasses for mass production of the bio-agent, coconut coir pith, and a waste from the coir industry have been tested whether it can sustain the population of IISR-51 acting as a carrier media. Population dynamics of *P. fluorescens* in three concentrations of molasses were tested, that is 0.05%, 0.1% and 0.2%. The population changes was observed at every 8 hrs of incubation. *P. fluorescens* was found to be showing a good growth in all the 3 concentration of molasses. Population dynamics was found to increase from  $10^6$  to  $10^{12}$  within 40 days. Since even 0.05% molasses supported  $10^{13}$  cells/ml at 48h of growth it is well proved that molasses is a cheap and best substrate for the mass production of IISR-51 for field release. From the present study, it is proved that coconut water not only supports but also enhances the growth of biocontrol agents *P. fluorescens*. In the experiment to test the suitability of coir pith as a carrier media for delivery of IISR-51, out of the 3 types of coir pith, viz. raw, decomposed I and decomposed II, decomposed I was found to be the best as carrier medium. Autoclaved and non-autoclaved samples were tested for the growth of IISR-51. Autoclaved and non-autoclaved samples showed almost same trend of growth of IISR-51. The nutrient analysis performed with all the three coir dust samples showed that the C, N, P and K levels in the samples can support the proliferation of the bio-agent.

4. Standardization of optimum conditions for mass multiplication of *Verticillium chlamyosporium*, a potential nematode biocontrol agent (PR 117)

Jissa N Varghese / Bharathidasan University / Guide - Santhosh J. Eapen

*Verticillium chlamyosporium*, a facultative parasite of plant parasitic nematodes, is capable of growing under a very wide range of temperature, pH and nutritional conditions. The optimum conditions required for mass multiplication of *V. chlamyosporium* were studied. The maximum growth and sporulation of this fungus were observed at pH 5 and even a highly acidic media did not inhibit the growth and sporulation of this fungus. The optimum temperature for mycelial growth and chlamyospore production was in the range of 26°C to 28°C. Among the four synthetic chemical media evaluated for growing the fungus, Czapek Dox Agar was the best. The highest mycelial production was observed on the starch medium followed by coconut water compared to the control PDB. Very little mycelial mass was produced in media containing molasses and there was absolutely no mycelial yield from media containing vermiwash. Yield of the fungus correspondingly increased with the increase in incubation period under liquid fermentation but no chlamyospore production was observed.

5. Comparative genomics of biocontrol genes present in *Pseudomonas* spp (PR 42)

Britto Cathrin / Bharathidasan University / Guide - Santhosh J. Eapen

*Pseudomonas* species plays a major role in controlling the pathogens. They produce several metabolites such as 2,4-DAPG, pyolluteorin, phenazine, phyochelin, pyrrolnitrin, alginate, pyoverdine and hydrogen cyanide. Functional and structural analysis of all these secondary metabolite protein sequences using bioinformatic tools such as Clustal W, Blast, bioedit, prosite, blocks, BLOCKMARKER and CODEHOP. Primers could be designed for HCN and alginate compounds while it was not possible for other compounds due to lack of any conserved regions. *In silico* designed primers should be tested in laboratory for PCR amplification.

6. *In silico* designing of PCR primers for locating nematode resistance genes (PR 41)

Vinaya Varghese / Bharathidasan University / Guide - *Santhosh J Eapen*

Six sets of nematode resistance genes (Mi, Hero, APS, Gra, Gre 3, RHG) were selected based on BLAST-P analysis. Based on multiple sequence alignment of these sequences, conserved regions were identified. Most of the nematode resistance genes were predicted to encode intracellular protein with nucleotide binding and leucine rich repeats domains. These domains are present in almost all genes among these six set of genes. *In silico* primers for each set of genes were designed from non gapped conserved BLOCKS.

7. Impact of copper oxychloride, carbon and nitrogen sources on the growth of *Verticillium* spp. (PR 116)

Mini Mani / Bharathiar University / Guide - *Santhosh J. Eapen*

Several *Verticillium* species are used as biocontrol agents of insect pests or nematodes. The growth of four isolates of *Verticillium* exposed to artificial media containing selected carbon and nitrogen sources in pure culture was compared. All the isolates used in the study were able to grow on most of the carbon sources used, except *V. lecanii* (Is.35) in dulcitol. In general, fructose was the preferred carbon source by all the isolates screened. Different isolates of *Verticillium* spp. had a varied response to various N sources. Except for isolate Vc. 32, asparagine was the best common source of nitrogen. The compatibility of two isolates of *V. chlamydosporium* with the broad-spectrum copper based fungicide, copper oxychloride, showed varied response. Growth of Vc. 32 was not adversely affected at any concentration and instead there was a slight growth promotion at lower concentrations of COC. Increasing concentrations of COC retarded the growth of Vc. 34.

8. Evaluation of nitrogen fixing and phosphate solubilizing bacteria for the control of root knot nematode (*Meloidogyne incognita*) (PR 118)

Shybi Sebastian / Bharathidasan University / Guide - *Santhosh J. Eapen*

Plant parasitic nematodes are suppressed by a range of bacteria. In this study the suppression of root knot nematodes by using two strains each of *Azospirillum* and

phosphobacteria (PB) were studied. A very high degree of nematode mortality was invariably observed in all the bacterial treatments with PB21 causing the highest mortality (78.45 %). The volatile and non-volatile metabolites of these bacterial isolates themselves were highly nematicidal in action. But growth promotion was observed only in non-sterile soil and only one of the isolate (PB19c) showed some improvement in growth of nematode infested tomato plants under greenhouse conditions. None of these biofertilizers were compatible with *Paecilomyces lilacinus*, a fungus commonly used as biocontrol agent for the management of root-knot nematodes.

9. Mass multiplication of *Verticillium chlamydosporium* on plant extracts and plant based solid substrates (PR 119)

Manju, S. / Bharathiar University / Guide- Santhosh J.Eapen

*V. chlamydosporium*, the effective biocontrol agent against nematodes could be exploited practically by the mass production of its chlamydo spores. In this regard seven plant extracts including *Lantana camera*, *Piper colubrinum*, *Naregamia alata*, *Strychnos nuxvomica*, *Chromolaena odorata*, *Glyricidia maculata* & *Azadirachta indica* and plant based solid substrates such as tapioca powder, neem cake, coir compost and rice bran were investigated for their efficiency to induce chlamydo spore production. The results revealed enhanced chlamydo spore production in solitary plant extracts, better induction when supplemented in synthetic media. Among solid substrates and synthetic media employed in the study, rice bran and CDA were proved to be promising substrates.

10. Isolation and PCR amplification of genomic DNA from plant parasitic nematodes (PR 55)

Srinivasa Rao Boyina/Acharya Nagarjuna University/Guide - Santhosh J. Eapen

Isolation of genomic DNA was attempted from two major plant parasitic nematodes (*Meloidogyne incognita* and *Radopholus similis*) using phenol extraction method. Four different population levels of nematodes were compared for standardizing the optimum number of nematodes required for DNA isolation. The study proved that 500 nematodes are sufficient for isolating good quality DNA. In RAPD analysis two primers viz., OPA-09 and OPA-10 produced scorable, prominent and reproducible bands in both *M. incognita* and *R. similis*. Ribosomal DNA of *R. similis* could be amplified using a set of universal primers.

11. Designing of species specific primers for plant growth promoting rhizobacteria (PGPR), *Bacillus* and *Pseudomonas* based on 16S rDNA polymorphism (PR 124)

Sreesmitha, V / Periyar University / Guide - Santhosh J. Eapen

This study was taken up to design species specific primers for plant growth promoting rhizobacteria (PGPR), belonging to the genera, *Pseudomonas* and *Bacillus*, based on the rDNA polymorphism for applications in biotechnology, bioremediation and biocatalysis. The rDNA sequences obtained from NCBI database for each species were aligned using Clustal X. From the alignment file the heterogeneous pattern within the 16S region were located using the options provided in the Clustal X. Unique patterns of each species were evaluated with the DNA CLUB, a primer evaluation program. Among the species specific ones, those sequences that satisfy various criteria viz., Tm, GC content, Hairpins, Dimers were selected. Patterns found at the beginning of the sequence were considered as forward primers and others as reverse primers. The corresponding primers of the selected patterns were picked out by another program PRIMER 3. The primer candidates obtained were checked with MFOLD for intra dimer and hairpins and also verified with DS GENE software. Inter dimer and hairpin formations were checked with AUTODIMER. Finally nineteen selected primer candidates were screened with BLAST which yielded species specific primer candidates for *B.pumilus*, *B. lentus* and *P.lini*. Primer candidates of *B. arbutinivoran*, *B. arvi*, *B. asabii*, *B.licheniformis* and *B.subtilis* can be used as genus level primers.

12. Designing of species specific primers for plant parasitic nematodes, *Radopholus* and *Pratylenchus* based on 16S rDNA polymorphism (PR 125)

Amina Sheharain, K. / Periyar University / Guide - Santhosh J. Eapen

The study was taken up to design genus level and species specific primers for facilitating the easy and accurate detection of nematodes belonging to the family *Pratylenchidae* based on polymorphisms seen in the DMNA. Nucleotide sequences were retrieved from NCBI database for various species of *Radopholus* and *Pratylenchus*. Conserved to be used at genus level and variable regions with respect to each species of the two genera were found out by subjecting the sequences to multiple sequence alignment using Clustal W and Clustal C programmes; respectively. Conserved region for *Pratylenchus* could not be found out. From the variable regions, only the unique ones



with respect to each species were selected. These species specific sequence patterns and the genus specific pattern have been evaluated with the DNA CLUB primer evaluation program. The primer candidates including the genus level ones obtained were checked with MFOLD for dimmers and hairpins. Finally seventeen selected primer candidates were short listed and screened with BLAST. Each of these primers were designed. Accurate identification of these agronomically important nematodes is particularly relevant in development of resistant cultivars and root stocks.

**13. Standardization of an organic base media for multiplication of *Azospirillum* and Phosphobacteria (PR 27)**

Siraj, N / University of Madras / Guide - V. Srinivasan

The cultural characteristics of *Azospirillum* and Phosphobacteria were studied in solid and liquid medium. The Phosphobacteria on Pikovskaya's agar medium exhibited well clearing zones indicating the presence. On semi solid N-free malate agar *Azospirillum* produced characteristic blue colour sub surface pellicle. Coir pith compost amended with rock phosphate and lime at 10:1 ratio and ash with 20:1 ratio were found to be very much suitable as carriers. For Phosphobacterial mass multiplication coir pith compost: ash was found to be highly efficient carrier medium under both sterile and unsterile conditions by supporting higher population for longer durations.

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## OTHERS

1. **Protoplast isolation, culture and micro callus development in chillies (*Capsicum annum* L.) (PR 88)**

**Kavitha, O.** / Cochin University of Science and Technology / Guide - *K Nirmal Babu*

Protocol for isolation and culture of protoplasts was standardized in chilli. Protoplasts were isolated from leaves of *in vitro* grown plants using a mixture of 1% each of macerozyme and cellulase. Mannitol 10% was used as osmoticum. A heterogenous mass of protoplasts of size 0.01mm to 0.026 mm with a density of  $2.3 \times 10^6$ /g of tissue was obtained. The protoplasts developed cell walls within 2 days on MS medium + 1 mg/l NAA + 1 mg/l 2, 4-D + 0.5 mg/l BAP. The first cell division was observed after 10 days and micro calli was formed after three weeks.

2. **Molecular characterization of few morphological variants of clove using RAPD markers (PR 12)**

**Dalia Jose** / Periyar University, Salem / Guide - *J. Rema*

11 accessions of clove, namely Dwarf clove, Zanzibar clove, Kallar-1, 2, 3, 4 and 5, Nagercoil-1, 2, 4 and 5 conserved at IISR were used to study the molecular characterization using RAPD markers. The study indicated that all the genotypes except Nagercoil-2 and 5 are different from each other. The dendrogram has shown clear separate groups between Kallar collection and Nagercoil collections except K6, which seems to be more closer to Nagercoil collection than Kallar from which it is originally collected. The dwarf clove is placed in separate group indicating a clear genetic difference. It is probable that the Nagercoil and Kallar collection might have been collected from different genetic stocks. However more primers may be screened for better understanding and genetic diversity in clove.

3. **Primer designing based on the conserved sequence motifs of disease resistance genes in plants (PR 48)**

Siju Senan / Bharathidasan University / Guide - Johnson George K.

PCR primer design was attempted based on oomycete resistance genes available in public databases. Three sets of resistance genes analyzed include blight resistance genes in *Solanum* species conferring resistance to *Phytophthora infestans*; downy mildew resistance genes in *Arabidopsis thaliana* providing resistance to *Peronospora parasitica*; *Bremia* resistance genes in *Lactuca sativa* inducing resistance to *Bremia lactucae*. Nucleotide and amino acid sequences of these oomycete resistance genes were retrieved and subjected to multiple sequence alignment program for detecting the conserved regions. Conserved regions were relatively more in *Bremia* resistance proteins and low in downy mildew resistance proteins. Eight forward and eight reverse primers were designed for blight resistance genes. No primers were designed for downy mildew resistance genes. Thirteen forward and eleven reverse degenerate primers were designed for *Bremia* resistance genes. Three forward and four reverse primers were designed for the amplification of blight and *Bremia* resistance genes.

4. **Morphology and taxonomy of *Fusarium* species affecting spice crops (PR 17)**

Shimna, C. / Bharathiar University / Guide - R. Suseela Bhai

Different spice crops such as black pepper, ginger, vanilla, cardamom which were infected with *Fusarium* species were collected and studied for morphology and growth character. *Fusarium* species were characterized based on the standard morphological characters and growth rate. Effect of temperature on the growth of *Fusarium* species was studied using potato sucrose agar and their microscopic analysis also done.

5. **Biochemical profile of fruits and bioactivity studies of *Annona squamosa* (PR 58)**

Deepa Varghese / Bharathidasan University / Guide - N. K. Leela

The study was carried out to identify the pesticidal fractions in the extracts of

*Annona Squamosa* seeds. The seeds were extracted with hexane and methanol and the extracts were fractionated by column chromatography and divided into 29 groups. Out of these, the major groups were screened for antifeedant and nematicidal activities. The fraction from hexane extract of seeds, eluted with hexane-ethyl acetate (95:5) showed 92.98% antifeedant activity against 'pollu' beetle at 2% concentration. This fraction also caused nematicidal activity (32%) against root knot nematode (*Meloidogyne incognita*) at 0.25% concentration. Residue from the methanol extract also exhibited 39% nematicidal activity against the test nematodes.

#### 6. Identification of proteinase inhibitors from *Piper* species (PR-72)

Nitha, M. P. / Bharathidasan University / Guide - Johnson George K.

Presence of trypsin and chymotrypsin inhibitors in three *Piper* species were investigated under the study. Inhibitory activities of these proteins towards casein were detected in considerable amounts in two wild *Piper* species, namely *P. colubrinum* and *P. chaba*, whereas in the cultivar *P. nigrum*, the activity was detected at low concentrations. These inhibitor proteins were tolerant to heat, acid and alkali but were denatured at extreme conditions of pH and boiling temperature. Optimum activity was detected at neutral conditions of pH. The heat treated samples retained up to 98% of their inhibitory activity towards casein. Preliminary studies by western blotting indicated the presence of a trypsin inhibitor of approximately 58 KDa.

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## Ph.D. Dissertation Abstracts

### BLACK PEPPER

1. *In vitro* plant regeneration in black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.): shoot organogenesis, somatic embryogenesis and ontogeny of shoot buds and somatic embryos. (TH 111)

Ramakrishnan Nair, R / Indian Institute of Technology, Kharagpur / Guide -  
S. Dutta Gupta

Efficient micropropagation protocols were developed for black pepper (*Piper nigrum* L.), a popular spice and long pepper (*Piper longum* L.), an important medicinal plant. Plant regeneration was achieved through shoot organogenesis and somatic embryogenesis in both the species. SH medium supplemented with 6.0 and 8.0 mg/l BAP was optimal for direct shoot regeneration in black pepper using juvenile internodes and apical buds respectively. E1 medium supplemented with 3.0 - 4.0 mg/l BAP was optimal for long pepper using nodal explants. Callus induced on E1 medium supplemented with 4.0 mg/l NAA and 2.0 mg/l BAP from juvenile apical buds of black pepper regenerated shoots on SH medium containing 1.0 - 3.0 mg/l BAP. In long pepper callus induction and regeneration was obtained as a single-step process on E1 medium containing optimal concentration of 3.0 mg/l BAP from internode explants. *In vitro* grown shoots were rooted on hormone-free or auxin supplemented basal medium in black pepper and on induction medium itself or hormone-free basal medium in long pepper. Plantlets resulted were acclimatized under green house conditions with 90% survival. Direct somatic embryogenesis and plant regeneration were obtained for the first time in black pepper from the germinating seeds on SH medium containing 1.5 - 3.0 % sucrose under 24 h dark period. Callus mediated somatic embryogenesis was induced in long pepper from petiole explants cultured on E1 medium containing 1.0 mg/l BAP under 16 h photoperiod. Maturation and germination of somatic embryos occurred on the induction medium itself in both the cases. Secondary cyclic embryogenesis was observed in black pepper at high frequency. The secondary embryos were formed from the suspensor region of primary embryos. Germination and conversion of somatic embryos were achieved in high frequency in solid as well as liquid medium. The nature and orientation of explant,

genotype, amount of available nitrogen, sucrose concentration and media supplements markedly influenced *in vitro* propagation of black pepper and long pepper. Among these genotype found to be most critical. Cultivar Karimunda of black pepper and Viswam of long pepper found to be highly morphogenic. At concentrations above 150 mg/l adenine sulphate increased the number of shoots induced from apical bud explants of black pepper. Increase in myo-inositol concentration in the medium influenced morphogenesis in long pepper. Effects on embryogenesis were evident only at higher concentrations (1000 mg/l). Growth regulators were ineffective in inducing somatic embryogenesis from seeds of black pepper. Addition of ABA (1.0 - 3.0 mg/l) increased the proliferation of secondary embryos in black pepper. Embryogenic suspension culture was successfully established in black pepper and plant regeneration was achieved by plating as well as directly in the liquid medium. Ontogeny of shoot buds and somatic embryos in black pepper and long pepper was traced. Shoot buds originated *de novo* as well as by axillary bud proliferation in both the species. Somatic embryos have a single cell origin in black pepper and multicellular origin in long pepper. A marked similarity in the developmental pattern of zygotic embryos and somatic embryos were observed right from the globular stage. The differences observed were the larger size of somatic embryos during early stages and smaller size of cotyledons during later stages.

2. **Bioefficacy of systematic fungicides against *Phytophthora* infections in black pepper (*Piper nigrum* L.) (TH - 105)**

Ramachandran, N. / University of Calicut / Guide - Y. R. Sarma

The objective of the present investigations was to evaluate the effect of some of the available anti-Oomycete fungicides against *P. capsici* both *in vitro* and *in vivo*. The inhibitory effects of five anti-Oomycetous fungicides namely ethazole, fosetyl-A1, metalaxyl, Oxadixyl and propamocarb on different developmental stages of the foot-rot pathogen, *Phytophthora capsici* were studied. Ethazole and metalaxyl were the most effective fungicides on mycelial growth of the fungus. Among the hyphal abnormalities induced by the fungicides the most prominent were, thickening, excessive branching and coiling of hyphal tips. In some cases vacuolization of the hypha was also noticed. However with propamocarb, these abnormalities were not seen. The activities of two of the fungicides namely fosetyl-A1 and propamocarb were pH dependent. Fosetyl-A1 was less effective in the alkaline medium. All the five fungicides showed good antispore activity.

In the presence of 100 µg/ml each of metalaxyl, fosetyl-A1 and ethazole, the fungus failed to produce any sporangia. However, the subsequent stages of the fungus namely indirect germination of sporangia and germination of encysted zoospores were less sensitive. Even at 1000 µg/ml Fosetyl-A1 which was less effective on mycelial growth was found to completely inhibit zoospore germination at 100 µg/ml. Besides the quantitative changes discussed earlier, the fungicides could induce many qualitative changes (like indirect germination and rupturing of sporangia). No loss of properties of either metalaxyl or the insecticides when mixed and used. Metalaxyl on the other hand showed synergistic interaction with endosulfan at 1:8 ratio, with regard to fungitoxicity whereas the effects were additive with quinalphos. Metalaxyl at 100 µg/ml (constituting 20 µg/ml of dry soil) offered total protection to black pepper seedlings planted in fungicide-treated soil heavily contaminated with *P. capsici*. The persistence of activity of fungicides as shown by the seedling bio-assay was dose-dependent. Experiments were conducted to evaluate the performance of metalaxylziram, fosetyl-A1 and ethazole in comparison with Bordeaux mixture in controlling *Phytophthora* infections in black pepper both in pure crop and arecanut back pepper mixed cropping systems during 1984-'86. The intensity of foliar infection and mortality of the vines were the lowest in plants treated with metalaxyl-ziram and fostyl-A1. The activity of the fungicide could be detected for a longer period in leaves compared to that in soil. When applied at 40 g/vine, the activity of the fungicide could be detected even after 50 days in the leaves whereas in soil, it was negligible after 20 days. Metalaxyl has shown good mobility both vertically and horizontally in the lateritic soil. Taking into consideration, the low frequency of application, high efficacy in reducing the infections and absence of metalaxyl residues in detectable levels in the black pepper, the use of metalaxyl-ziram or a similar combination of metalaxyl has been suggested as a major component in the integrated management of *Phytophthora* infections in black pepper.

3. Ecology of *Phytophthora capsici* (Leonian 1922, Emend A. Alizadeb and P.H. Tasao) causal organism of foot rot disease of black pepper (*Piper nigrum* L.) (TH 57)

Anandaraj, M. / University of Calicut / Guide - Y. R. Sarma

The fungus *Phytophthora capsici* could be isolated from the soil using *Albizia falcataria* leaflets as baits and the disease potential index (DPI) could be calculated in order to quantify the inoculum load. *P. capsici* survives in the form of mycelium and chlamydospores during

the inter monsoonal drought period. Soil moisture has a positive correlation with the activity of *P. capsici* in the soil and its population drops to undetectable levels when the soil moisture is reduced. Infection by *P. capsici* on black pepper occurs non-randomly in a plot and subsequent spread also tend to cluster around the previously infected vines, showing the role of initial infection serving as source of inoculum for its secondary spread. Thus, removal of infected vines would reduce the initial inoculum load and further spread of the disease. The population of *P. capsici* increased under weed cover, probably due to the availability of nutrients in the root exudates of weeds which supported its population build up, removing weeds from the gardens after the monsoon would reduce the population build up of *P. capsici*. The competitive saprophytic ability of *P. capsici* is very low and it could not compete with saprophytes. Amending the soils with organic matter promoted saprophytic microflora and suppressed *P. capsici* population. Addition of organic matter to the soil encourages saprophytes to grow and these saprophytes checked *P. capsici* populations. VAM could be used to enhance growth and also to protect roots against *P. capsici* infection in black pepper. The ecological investigations carried out on *P. capsici*, brought out important leads which are of great practical value for effective disease management. The population fluctuation of *P. capsici* based on DPI during different periods of the year indicated its positive correlation with soil moisture. Spatio-temporal distance class analysis of foot rot incidence using STCLASS analysis showed the non random occurrence and clustering of secondary spread around previously infected vines. As the infected plants serve as sources of inoculum, removing the infected vines should be reduce the inoculum load. Increased populations of *P. capsici* under weed cover and consequent increased disease incidence necessitates to implement post monsoon clean cultivation. Though agrochemicals are important components of IDM, the study emphasizes the potential of organic amendments for an effective disease management, as *P. capsici* has a low competitive saprophytic ability and the saprophytic colonization of organic amendments by saprophytes checks the population build up of *P. capsici*, the protection offered by VAM against *P. capsici* would be of practical importance to protect the root system of black pepper from nursery onwards. Thus, integrating the leads obtained in the present study would lead to an effective disease management strategy to contain *phytophthora* foot rot disease of black pepper.



4. Physiological, biochemical and molecular studies on the root rot (caused by *Phytophthora capsici*) suppression in black pepper (*Piper nigrum* Linn) by rhizosphere bacteria (TH 116)

Diby Paul / University of Calicut / Guide - Y. R. Sarma

Rhizobacteria isolated from the rhizosphere of different spice crops were screened *in vitro* against *Phytophthora capsici*, the root rot pathogen of black pepper. The short-listed strains were again screened in plants based on their ability for disease suppression, growth promotion and induction of systematic resistance (ISR) against *Phytophthora* root rot of black pepper. Five strains of *Pseudomonas* spp. viz., IISR-6, IISR-8, IISR-11, IISR-13 and IISR-51 that were efficient in root rot suppression (>70%) also promoted growth of the black pepper vines (>50%) apart from inducing systematic resistance in the host plant against the rot pathogen. The strains also reduced the Disease Potential Index (DPI) of the soil. The selected strains were intrinsically resistant to antibiotics, Rif<sup>100</sup>, Kan<sup>50</sup>, Na<sup>140</sup> a property, which could be used for their effective tracking from soil. The different phase of growth of *P. capsici* viz., mycelial growth (72%), sporangia (98%) were inhibited by the metabolites produced by the bacteria. The fluorescent pseudomonad strains induced systematic resistance in black pepper as evidenced by the reduced lesion diameter in the leaves upon challenge inoculation of root bacterized black pepper plants. The rhizobacterial strains brought this about mainly through the induction of elevated quantities of defence related enzymes viz., phenylalanine ammonia lyase (PAL), peroxidase (PO), Poly phenol oxidases (PPO) and catalases as estimated by biochemical methods. The growth promotion in black pepper brought about by the *Pseudomonads* spp was attributed to the phytohormones produced viz., IAA and GA as detected in TLC. The other determinants were found to be the enhanced production of fibrous roots in the plant and also the increased absorptive surface area of the roots. *Pseudomonas* sp, IISR-6 effectively colonized the root of black pepper, multiplied and proliferated reaching a population size above log-8 cfu/g. Up to 44.7% increase in number of planting material was generated in plants treated with *Pseudomonas* strain IISR-51 in the black pepper nursery due to the growth promotory effects of these bacteria. The selected strains also brought about the rejuvenation of black pepper vines (66.7% survival) already infected with *P. capsici*. This rejuvenation efficacy was enhanced (100% survival) when the application of rhizobacteria was supplemented with a recommended dose of the systematic fungicide, Metalaxyl-Mancozeb. An economical and ecofriendly multiplication medium was

developed for the large-scale production of this bacterial biocontrol agent. Molasses (0.1%) and coconut water supported growth of the bacteria to log 14 cfu/ml in 32h. On the mode of application of PGPR, soil drenching coupled with foliar spray was found effective compared to either drenching or spraying alone. Dose response studies revealed that a bacterial population as low as  $10^4$  cfu/g of soil can bring about root rot suppression. The present investigation carried out resulted in identification of efficient strains of rhizobacteria, which could effectively protect black pepper from root rot caused by *P.capsici* apart from improving the total vigor of the vine. The varied modes of antagonism by the strains, their ability to induce systematic resistance in the host plant and their endophytic nature rendered them as ideal biocontrol agents in black pepper - *P.capsici* pathosystem.

5. Factors affecting the biological control of *Phytophthora capsici* infections in black pepper (*Piper nigrum* L.) (TH-115)

Saju, K. A. / University of Calicut / Guide - Y. R. Sarma

*Trichoderma* spp. were hyperparasitic on the mycelia and sporangia of *Phytophthora capsici* infecting black pepper *in vitro*. They exhibited growth promotion of black pepper and reduced root rot caused by *Phytophthora capsici* *in vivo*. More rhizosphere competent isolates of *Trichoderma* were identified. *Trichoderma* spp over a wide range of climatic and soil conditions were highly adoptable. Many non-volatile compounds inhibitory to mycelial growth of *P.capsici* were isolated from *T.harzianum* IISR 1369, *T. virens* IISR 1370, *T. aureoviride* IISR 126, *T. aureoviride* IISR 140 and *T. aureoviride* IISR 148. They also inhibited sporangia formation, indirect germination of sporangia and germination of zoospores. Seven new strains capable of fast growth and sporulation and production of higher levels of non-volatiles were obtained by protoplast fusion between *T. harzianum* IISR 1369 X *T. aureoviride* IISR 140. Non-volatiles of fusants inhibited sporangial formation, indirect germination of sporangia and germination of zoospores at low concentration. Based on the comparative growth promotion of black pepper vines in the field by *Trichoderma* and *P. fluorescens* isolates and glass house experiments with challenge inoculation with *P. capsici*, combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41 can be recommended as a strategy for disease management of foot rot of black pepper. Protection of black pepper from *P. capsici* in the present study indicated growth mediated defense coupled with suppression of *P. capsici* propagules by *Trichoderma* through its

various mechanisms as outlined earlier. Chemical characterization of volatiles and non-volatiles that inhibit *P.capsici* would be useful for refining screening systems to select further useful antagonists. The leads obtained in the area of strain improvement need to be exploited for the development of highly efficient biocontrol agents to develop new formulation for biocontrol. Combination of *T. harzianum* IISR 1369 + *P. fluorescens* IISR 41 need further large scale field evaluation/ demonstration before advocating the consortium to farmers.

6. **Biochemical studies on host pathogen interactions of black pepper (*Piper nigrum* L.) infected with *Phytophthora capsici* (TH. 52)**

Shamina, A. / University of Calicut / Guide - Y. R. Sarma

The best used carbon sources by *Phytophthora capsici* were sucrose and glucose followed by starch, fructose, maltose, cellulose and xylose. The other pentoses, hexoses, di- and poly saccharides, carboxylic acids, polyols and organic acids were poor sources of carbon. Of these sodium citrate did not support any growth at all. Of the organic and inorganic nitrogen sources studied, the organic sources were distinctly superior. The amino acids able to serve as sole nitrogen sources were Glu, Pro, Asp, Asn, His, Ser, Arg and Gly. The less effective amino acids were Gin, Phe, Thr and Val and the least effective Cys- cystine, Ile, Leu, Lys, Met and Trp. Of the B vitamins studied only thiamine was required by all the five isolates for their vegetative growth. In host pathogen interactions of foliar infection, no significant difference was noticed between the two selections (KS-27 and P 24) in foliar lesion size on infection. Both selections recorded significant increase in PPO and PRX activities on infection, increasing with the increase in DAI, the maximum being noted in tender leaves. Higher PAL and  $\beta$ -1, 3-glucanase activities were observed in the control P 24 leaves, compared to KS-27. While the total protein content increased on infection, the total free amino acid content decreased in both P 24 and KS 27. The total carbohydrate content also increased in both selections on infection. However the response of the two selections to infection, in their reducing sugar content was erratic. The total phenol content decreased in KS 27 on infection on all DAI, whereas in P 24 an initial increase on the 1<sup>st</sup> DAI was followed by a decrease on subsequent days. Electrical conductivity of the leachates of the infected leaves increased with increase in lesion size and decreased with maturity of leaves, in both KS 27 and P 24. In host pathogen interaction studies with root infection, all the enzymes studied PPO, PRX, PAL and  $\beta$ -1, 3-glucanase increased in activity on infection, the increased efficiency of P 24 in its defence response

and therefore its tolerant P 24 compared to KS 27. The isozyme profiles of PPO and PRX also revealed differences – a few induced bands and disappearance of existing ones. On infection, carbohydrates and phenol contents of the roots increased, while the reducing sugar content decreased, the protein content increased till the 10<sup>th</sup> DAI and thereafter decreased in both selections. The free amino acid content decreased in KS 27 and increased in P 24 on infection. Electrical conductivity of the root leachates increased up till the 10<sup>th</sup> DAI and later fell. This was greater in KS 27 compared to P 24, indicating greater membrane integrity in the latter. The concentration of piperine and oleoresin were estimated in the vegetative parts of KS 27, P 24 and *P. colubrium*. In both KS 27 and P 24 maximum piperine content was noticed in the roots, followed by the stem and least in the leaves, it was not detected in leaves of KS 27. The primary metabolites such as enzymes and metabolites and the membrane integrity of this pathosystem seem to be vital factors in determining the susceptibility / tolerance/ resistance to *P. capsici*. Though evidence is inadequate, indications point to the fact that the mechanism of tolerance/ susceptibility in foliar and root system might be different. The secondary metabolites seem to be of secondary importance in this issue.

7. Approaches towards the integrated disease management of *Phytophthora* infection of black pepper (*Piper nigrum* L.) (TH. 61)

Rajan, P.P. / University of Calicut / Guide - Y. R. Sarma

Twenty-seven isolates of *Trichoderma*, 104 isolates of other fungi, 16 isolates of bacteria and 12 isolates of actinomycetes were short listed as antagonists by dual culture technique. Short listed antagonistic fungal and bacterial isolates were tested in pot culture for their bio-efficacy on root rot suppression. Three *Trichoderma* spp. (10, 12 & 19) and three other fungal isolates (63, 71 & 74) were found very effective against *Phytophthora* infection in black pepper. Out of 16 bacterial isolates tested two fluorescent pseudomonad (Pf2, Pf5) and one non-fluorescent pseudomonad (BN) was found effective on root rot suppression. The suppressive effect of *Verticillium teneum* on *Phytophthora* induced root rot is reported for the first time in India. Four locally available organic soil amendments (coffee pulp, poultry manure, neem cake and farm yard manure) were tested for their disease suppressive effects on *Phytophthora* in comparison with recommended dose of NPK (140:55:270). Least root rot was noticed in plants treated with coffee pulp followed by neem cake compared to NPK treated plants. Aqueous garlic and mustard extracts

were tested against *P. capsici*, both *in vitro* and *in vivo*. Four important phases of *P. capsici* viz., growth, sporulation, sporangial germination and zoospore germination were inhibited by aqueous garlic and mustard extracts at low concentrations and their effects were synergistically inhibitory when they were used in combination. Pot culture studies indicated the suppressive effect of these extracts on root rot incidence, increased multiplication and proliferation of *Trichoderma*. Three systematic fungicides viz., dimethomorph, heptaene antibiotic (aurefungin) and potassium phosphonate were tested against *P. capsici* both *in vitro* and *in vivo*. Dimethomorph was found very effective on suppression on growth, sporulation, indirect sporangial germination and zoospore germination of *P. capsici*, but aurefungin and potassium phosphonate were found effective on these phases only at higher concentrations. Out of these chemicals evaluated in pot culture studies, potassium phosphonate at 1200 ppm (three applications) was found most effective on disease suppression with 17.78% root rot compared to 23.33% in dimethomorph (400 ppm) and 47.78% in aurefungin (300 ppm). Nine species of *Trichoderma* and *Verticillium tenerum* were found compatible with potassium phosphonate at 1200 ppm. Three levels of ( $N_0P_0K_0$ ,  $N_1P_1K_1$ ,  $N_2P_2K_2$ ) of fertilizers and their combination were evaluated for their effect on disease incidence. Least amount of root rot incidence (24.44%) was noticed in plants treated with recommended dose of phosphorus without the application of nitrogen and potash. Phosphorus application showed disease suppressive effect. In IDM study conducted by incorporating all the leads coupled with a disease tolerant (P24) and susceptible (KS27) black pepper, P24 showed minimum root rot (1.66%) compared to KS 27 (13.3%).

8. **Bioecology of leaf gall thrips *liothrips karnyi* bagnall infesting black pepper (TH-95)**

Devasahayam, S. / University of Calicut / Guide- U. V. K. Mohamed

Surveys conducted in Kerala, Karnataka and Tamil Nadu indicated that the incidence of leaf gall thrips (*Liothrips karnyi*) on black pepper was higher in Waynad, Idukki, Shimoga and Kodagu districts. There was a positive and significant correlation between the incidence of the pest and altitude. The pest infestation was also significantly higher in plantations where there was heavy shade and on vines that were < 3 years old. The pest infestation resulted in the formation of hypophyllous marginal leaf galls, thickening of leaf lamina in the gall region, loss of flaccidity, crinkling, malformation

and reduction in leaf area. The changes in biochemical constituents of galled and healthy leaves were studied. The pest infestation affected the growth of black pepper cuttings in the nursery and 1-year old vines in the field. The length of spikes, number of berries and fresh and dry weights of berries were reduced in spikes that were formed opposite to galled leaves. The life cycle and duration of various stages of leaf gall thrips was studied. There was a positive and significant correlation between pest population and rainfall, minimum temperature, relative humidity and populations of major predators. The major natural enemies of leaf gall thrips recorded in the field include *Montandoniola moraguesi* (Anthocoridae) and *Androtrips flavipes* (Phlaeothripidae). The life cycle and predatory potential of these two predators were also studied. There was a positive and significant correlation between predator populations and rainfall, minimum temperature, relative humidity and population of leaf gall thrips. The toxicity of insecticides used for the management of leaf gall thrips to the predators was also studied. Thirty-five species of arthropods were recorded from leaf galls of black pepper which included primary consumers of gall tissues, natural enemies of primary consumers, detritus feeders and *uccessori* which colonized the galls after it was free of primary consumers and natural enemies. All the 471 accessions of black pepper available in the Germplasm Conservatory of Indian Institute of Spices Research, Peruvannamuzhi, were susceptible to leaf gall thrips, though there was wide variability in their reaction to the pest. The pest infestation was also observed on wild species of *P. nigrum* and *P. sugandhi*.

#### 9. Studies on zinc and molybdenum nutrition of black pepper in relation to yield and quality (TH 93)

Hamza Srambikkal / University of Calicut / Guide - A. K. Sadanandan

Experiment was conducted with the objective of studying the response of zinc and molybdenum to black pepper on yield, quality and economics. As part of the objective a field survey, adsorption desorption studies, fractionation study, field and plot experiments were conducted. The results are summarized below. The survey in the major pepper growing areas showed that 57% of the soils and 13% of the leaf samples are deficient in Zn. Thirty eight percent of soils were deficient in Molybdenum and 22% of the gardens had yield less than one kg per vine. Soil site evaluation of major black-pepper growing areas showed that high yielding areas have pH near neutral, higher sand and silt fractions, exchangeable base, base saturations, CEC, organic carbon, major, secondary and micro nutrients except sulphur, and clay content compared to low yielding locations. Zinc

fractionation studies showed that there exists a dynamic equilibrium among different forms of zinc in soil solutions. They are correlated positively among themselves and with soil available zinc. Adsorption and desorption studies showed that zinc adsorption more confined to Langmuir equation than Freundlich equation. Through sand culture study, zinc deficiency in black pepper identified as interveinal chlorosis of young leaves, retardation on the growth of terminal shoots, shortening of internodal length resulting in chlorotic little leaves appearance, which on advanced stage give rosetted appearance. Response study showed that In zinc deficient soils application of Zn @ 6.2 kg ha<sup>-1</sup> as zinc sulphate was optimum producing a yield increase of 25%. In molybdenum deficient soils, application of Mo @ 0.9 kg ha<sup>-1</sup> as sodium molybdate was optimum. Studies on the use efficiency of zinc showed that, soil application of Zn as Zn EDTA chelate was better than application of ZnSO<sub>4</sub> alone and produced 56% increase in soil and 20% increase in leaf Zn over ZnSO<sub>4</sub>. Studies on the effect of different amendments to increase Mo availability in soil showed that application of half tone lime followed by Mo @ 0.5 kg ha<sup>-1</sup> produced maximum soil Mo availability.

10. **The effect of organic fertilizers on soil quqlity,nutrient availability and quality of Black pepper (TH - 122)**

Rubina, M. R /University of Calicut / Guide- A. K. Sadanandan

Experiment was conducted with the objective of studying the influence of different organic fertilizers (Farm yard manure, vermicompost, leaf compost, neem cake) on soil physical parameters, soil availability and uptake pattern of nutrients, on P- adsorption. Soil dehydrogenase and acid phosphatase activities, on growth, yield and quality of black pepper. A decrease in bulk density with an increase in water holding capacity of soil was obtained with the addition of organic fertilizers to soil. The incorporation of organic fertilizers ameliorated the physical properties of soil. Annual application of organic fertilizers over the years significantly increased the soil pH, organic carbon, microbial biomass and nutrient status. The humic acid contributed by the soil organic matter on the application of organic fertilizers not only complexed Al<sup>3+</sup> and Fe<sup>2+</sup> ions in the acidic soil but also increased the soil pH. Adsorption of P was significantly reduced with the increased levels of P- application in the presence of FYM, compared to other organic sources. The soil enzyme activities were highly correlated with the soil organic carbon, total N and-microbial biomass carbon. The yield and quality of black pepper was significantly increased due to application of organic fertilizers. Among the

sources, vermicompost and FYM treatments were superior. Among the organic treatments, the benefit /cost ratio was highest on FYM application followed by vermicompost treatment.

11. **Physiological and biochemical characterization of yield and quality in black pepper with special reference to nitrogen nutrition (TH 53)**

Shaukathali, T. M. / University of Calicut / Guide - A. Ramadasan

A pot culture study was conducted to determine nitrogen utilization in pepper cultivars. Four months old rooted cuttings of three pepper cultivars viz., Aimpiriyan, Karimunda and Panniyur-1 were used for the experiment. The experiment was laid out in a completely randomized design with four replicates, each of two different nitrogen doses (zero and 2.1 g N/pot) applied thirty days after planting. Four plants were cut of at soil level randomly from each treatment at 14, 28; 42 and 56 days after treatment (DAT). Physiological and biochemical characters viz., number of leaves, leaf area, dry matter production, leaf nitrate reductase (NR) activity, total nitrogen, nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and total non-structural carbohydrate (TNC) were studied. The result showed that Panniyur-1 was superior compared to other cultivars. A field study was conducted to characterize the yield and quality in black pepper. Pepper cultivars viz., Karimunda and Panniyur-1 grown under high (40 MSL) and low (974 MSL). Highest yields were obtained for cultivars grown under HE and Panniyur-1 recorded the highest yield irrespective of elevations. Analysis of nitrogen in the leaves and stem revealed higher content in the month of June, followed by a gradual reduction up to harvest. Karimunda recorded higher nitrogen content than Panniyur-1 throughout the experiment. Estimation of leaf and stem nitrate nitrogen content revealed higher content in the month of July for both the elevations for two years. While stem  $\text{NO}_3\text{-N}$  content was lower at the time of fruit set, it increased rapidly and attained a peak in October and then decreased gradually up to harvest. In leaves, the level of TNC was maximum at the time of harvest and minimum in the month of December. The CN ratio was quite stable during the first three stages of sampling at both locations, thereafter the ratio increased. Significantly higher CN values were obtained in Panniyur-1 at both the locations than Karimunda. Estimation of EO content revealed higher content in the month of November, which gradually decreased until harvest. Berry OR content showed a steady increase from July and reached maximum value in October and LE and in November under HE, then declined slowly up to harvest.



Karimunda recorded higher EO content in berries than Panniyur-1, while the trend was reverse for OR.

**12. Physiological and biochemical studies on drought tolerance in black pepper (*Piper nigrum* L.) (TH 62)**

**Vasantha, S** / University of Calicut / Guide - *A. Ramadasan*

The objective of the present study is to record responses of various physiological growth and biochemical parameters to moisture stress and to arrive at drought index (DI) for screening large germplasm lines. Based on the above study five promising (high yields) lines were screened for drought tolerance (Acc. No 1495m, KS 69, KS 88, Panchami and Acc. No. 931). The shortlisted characters were studied for the above genotypes, subjecting them to moisture stress in pot culture experiment. The results established the usefulness of these characters. Among the genotypes studied Acc No. 1495 and KS 69 performed better. Hence, these two lines were planted in the field and their field tolerance was studied. Genotypes 1495 (Kottanadan) and KS 69 (Karimunda) responded in the similar fashion in the field also as in the pot culture experiment. Stomatal resistance increased gradually from the month of September and continued to increase up to April corresponding with the depleting moisture content. A reverse trend was observed in the case of transpiration rate while leaf water potential reached more negative values during summer months.

**13. Influence of soil moisture regimes on growth and yield in bush pepper (TH 88)**

**Thankamani, C. K.** / Kerala Agricultural University / Guide - *P. K. Ashokan*

Five experiments were conducted to study the influence of soil moisture regimes on growth and yield in black pepper. Among the varieties evaluated Panniyur-5 possesses the best morphological, physiological, biochemical and anatomical adaptations to tolerate water stress and Panniyur-1 has poor adaptations. Translocation of photosynthates in a drought tolerant (Panniyur-5) and drought susceptible (Panniyur-1) bush pepper variety was studied. In bush type of panniyur-5, 14 CO<sub>2</sub> fixed in the leaf were translocated to the spike on the corresponding node as well as to other spikes of the plant under mild and moderate water stress. In Panniyur-1, all the 14 CO<sub>2</sub> from the leaf was translocated to the

spike on the corresponding node and there was no translocation to other spikes in water stressed as well as in daily irrigated plants. The intensive root zone of bush pepper was observed around 30 cm lateral distance and 40cm depth. Optimum depletion of available soil moisture is 50% for bush pepper variety Karimunda grown in pots of 60X40 cm size. Number of spikes, length of spikes, number of berries, 100 berry weight, berry volume, dry berry yield, oleoresin and piperine content were maximum in bush pepper plants irrigated with 8l drip in coconut garden.

**14. Improvement of black pepper (*Piper nigrum* L.) through induction of stress tolerance *in vitro*" (TH 114)**

Anuradha, Y. / University of Calicut / Guide - V. J. Philip

The present study involved two aspects; one involving studies on abiotic stress and the other involving studies on biotic stress. Under abiotic stress, studies on the responses of black pepper cells to abiotic stress included the effect of polyethylene glycol, the water stress inducing agent, on the growth and differentiation of the black pepper cultures, analysis of modulation of different antioxidant enzymes, accumulation of organic and inorganic solutes and induction of water stress proteins. *In vitro* selection of black pepper cells for water stress tolerance and characterization of water stress adapted cells was also carried out. Among the various biochemical responses of black pepper cells to water stress, it was observed that the black pepper cells accumulated significant concentrations of reducing sugars, free amino acids and K<sup>+</sup> ions during PEG imposed water stress. In addition, the water stressed cultures also recorded moderate increase in proline levels in response to water stress. The activity of antioxidant enzymes increased in black pepper cultures in response to water stress. In the cultures under severe and lethal water stress, the enzyme activities recorded an initial increase after which they declined and failed to maintain high levels till the end of the stress period. Studies on the effect of water stress on the protein profile of the black pepper cultures revealed the induction of certain water stress induced polypeptides. *In vitro* selection studies carried out to select cell lines tolerant to water stress revealed that the step wise and direct selection methods adopted for *in vitro* selection did not yield truly water stress tolerant seedlings/ cell lines. Thus black pepper cells could survive prolonged exposure to water stress, probably through physiological adaptation. Work on biotic stress was carried out by treating the black pepper cultures with hyphal wall components of *Phytophthora capsici* and *P. meadii* to

study the compatible and incompatible interactions respectively between *Phytophthora* and black pepper cells. The most characteristic response of black pepper cultures to elicitation was hypersensitive cell browning and phenolics exudation. The cells elicited by the hyphal wall elicitors characteristically exhibited blue autofluorescence under UV epifluorescent light. Elicitation also induced aggregation of cytoplasm and deposition of physical barriers like callose and lignin on the cell walls. Other immediate responses of cells to elicitation were extracellular alkalization and oxidative burst. It was observed that all these defense responses were induced faster and were higher in magnitude in PmE (*P. meadii* elicitor) treated cultures compared to the PcE (*P. capsici* elicitor) treated cultures. It was observed that the antioxidant enzymes were depressed during the initial phase of elicitation in the PmE-treated cultures compared to the PcE-treated cultures. The activities however increased later and remained high by the end of 72 hrs. Peroxidase activity in particular was significantly high in PmE-treated cultures, which could be responsible for the rapid triggering of various defenses in the PmE treated cultures than in PcE treated cultures. Among the other defence enzymes studied,  $\beta$ -1, 3 glucanase and phenylalanine ammonia lyase were found to increase significantly in the elicited cultures over the controls, the increase being rapid and more pronounced in PmE treated cultures. All the defenses studied were found to be triggered more rapidly and were more pronounced in the incompatible interaction than in the compatible interaction.

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## GINGER

### 1. Cytogenetical studies in ginger (*Zingiber officinale* Rosc.) (TH. 12)

Ratnambal, M.J. / Bombay University / Guide - K. A. Patel

The present investigation to predict the yield of rhizome, based on morphological characters of pseudostem like the height, number of leaves and breadth and length of last fully opened leaf. Multiple regression analysis using morphological characters showed that the final yield could be fairly accurately estimated. Path coefficient analysis has revealed that the phenotypic correlation between yield of rhizomes and height of pseudostem is quite high and also the direct effect of height of pseudostem is quite high and also the direct effect of height towards the correlation is very high. Wilk's statistic from multivariate analysis of variance showed difference between cultivars of *Z. officinale* with respect to rhizome characters like number of nodes, length, breadth and internodal length of mother rhizomes, number of nodes, length and breadth at base, middle and top, internodal distance of fingers. Karyotypes of 32 cultivars of *Z. officinale* and three species viz., *Z. zerumbet*, *Z. macrostachyum* and *Z. cassumunar* revealed considerable difference in their gross morphological characteristics. The karyotype analysis also showed that cultivars of *Z. officinale* have more asymmetrical karyotype than the three wild species and it is concluded that the trend of evolution in the genus *Zingiber* has been towards increasing karyotype asymmetry. Interspecific variability for meiotic behaviour has been observed in 256 cultivars of *Z. officinale* investigated. The presence of inversion heterozygosity in *Zingiber* has been indicated by chromatin bridges at anaphase-I and II. It is concluded that the univalent in various cultivars of *Z. officinale* and other species have mostly originated from precocious separation of bivalents. Chromosomal stickiness has been observed in many of the cultivars of *Z. officinale* and species investigated and it is suggested that the abnormality is more or less genetically controlled. A significant positive linear regression between pollen sterility and chromosome aberration at anaphase-II and aberrant quartets has been established. Quartet survival was calculated based on a ratio of 1/4<sup>th</sup> of the total quartet cells, divided by the prophase cells and it was found to influence fertility levels in cultivars of *Zingiber*. Considerable changes in the height of pseudostem, number of leaves, length and breadth of leaves and breadth of stomata and epidermal cells were observed in the tetraploid plants. Majority of the tetraploid were stunted with dark green leaves and increased number of epidermal cells and stomata per unit area. While the

diploids flowered, induced tetraploid completely failed to flower and hence it is too early to state whether the mechanism sterility could be circumvented through the induction of polyploidy. An increase in the size of rhizome has been observed in the tetraploids in comparison with the diploids.

## 2. *In vitro* studies in *Zingiber officinale* Rosc (TH 56)

Nirmal Babu, K / University of Calicut / Guide – M. K. Nair

Ginger, *Zingiber officinale* Rosc. is one of the most important medicinal spices of the world. Protocols were developed in ginger cv. Maran from vegetative bud, leaf, ovary and anther explants for micro propagation by direct multiplication, plant regeneration from callus through organogenesis and embryo genesis, production and evaluation of soma clones, for their genetic variability, isolation of useful genotypes, development of synthetic seeds, *in vitro* conservation of ginger germplasm by slow growth, establishment of cell suspension and protoplast cultures, isolation of DNA and gene delivery system for transient expression of  $\beta$ -glucuronidase (GUS) gene. Micro propagation was achieved on Murashige and Skoog (MS) medium supplemented with 1 mg l<sup>-1</sup> NAA and 4 mg l<sup>-1</sup> BAP. Plants could be regenerated from all the explants tried on MS medium supplemented with 10 mg l<sup>-1</sup> BAP and 0.2 mg l<sup>-1</sup> 2,4-D medium supplemented with 1 mg l<sup>-1</sup> NAA and 4 mg l<sup>-1</sup> BAP. The pathway for plant regeneration was by organogenesis in leaf and anther explants while it was both by organogenesis and embryogenesis in vegetative bud and ovary explants. Histological studies also confirmed their ontogeny. Plant regeneration from ovary derived callus was the most efficient for plant recovery (100-300) plants per culture tube. Conversion of floral buds into vegetative buds and *in vitro* development of fruit was also successful on MS medium with 10 mg l<sup>-1</sup> BAP and 0.2 mg l<sup>-1</sup> 2,4-D. The micropropagated (TC) plants were established in soil with 85% success. The size of the plant determined the success in hardening and establishment. Variations were observed in somaclones of ginger for plant height, number of tillers, number of leaves per tiller, rhizome yield, rhizome size, number of internodes per finger, dry recovery, oleoresin and essential oil contents reaction to *Pythium aphanidermatum* and *Pseudomonas solanacearum* infection etc irrespective of the mode of plant regeneration. Protocol for conservation of ginger genetic resources in *in vitro* gene bank by slow growth was also developed. Slow growth was induced in ginger and subculture intervals could be extended to 12 months with 80% survival on half strength MS medium with 10-15 g l<sup>-1</sup> mannitol and sucrose in culture tubes to minimize evaporation loss. Synthetic seeds were made by

encapsulating shoot buds and somatic embryos of ginger in calcium alginate (5%) matrix and they can be stored up to 9 months. Micro rhizomes were also induced in ginger for generating disease free planting material and germplasm exchange. Cell suspension cultures were successfully established with 5 percent of the cells were found to store oil for possible production of ginger essential oils through cell cultures in bioreactors. Protoplasts were isolated with an protoplast yield of  $2.5 \times 10^5$ /g by digesting leaf tissue. Genomic DNA isolated from the sprouting buds and leaf tissues of ginger using modified CTAB method. Transient expression of  $\beta$ -glucuronidase (GUS) gene was successfully induced.

### 3. *In vitro* technology of genetic conservation of some genera of Zingiberaceae (TH 112)

Geetha S Pillai / University of Calicut / Guide - K. Unnikrishnan

The present investigation as undertaken to develop an efficient *in vitro* conservation strategy as a safe alternative in the field gene bank and also for germplasm exchange in zingiberaceous plants. Two methods viz., cryoprotective dehydration and encapsulation dehydration were tried in ginger shoot buds. In cryoprotective dehydration, ginger shoot tips could be successfully cryopreserved with limited success (40% of the cultures) while that with encapsulated shoot buds was 50% of the cultures. *In vitro* regenerated shoot buds of cardamom, ginger and turmeric were successfully encapsulated in 3% calcium alginate matrix. Encapsulated synthetic seeds were stored up to 6 to 9 months in sterile environment at  $22 \pm 2^\circ\text{C}$ . Micro rhizomes were induced in *in vitro* cultures of ginger and turmeric by using high concentrations of sucrose and mannitol. Anatomically the micro rhizomes resembled the normal rhizomes and they could be directly planted in the field and established with 90-100% survival. The *in vitro* multiplied cultures did not exhibit any deficiency symptoms or deformities and were similar to the control in their appearance and growth. In cardamom, the tissue cultured plants performed similar to those of seedlings and hence can be directly used for commercial planting within 6 months of hardening. But in ginger, turmeric and *Kaempferia* spp, where the commercially useful part is the rhizome, tissue cultured plantlets cannot be directly used for commercial cultivation. Genomic DNA was successfully isolated from leaves of cardamom, ginger and *Kaempferia* spp. using modified CTAB method. In cardamom, genomic DNA of Cl-37 and APG 50 were used as template. Twenty OPERON primers were tested and only three of the primers namely OPA 12, OPC 19, OPE 02 were polymorphic between genotypes viz., Ci-37 and APG 50. In ginger, two cultivars viz., Maran and Varada were used for primer

screening. Eleven OPERON primers were tested and 8 of them viz., OPA 12, OPB 2, OPC 4, OPC 5, OPC 7, OPD 01, OPE 02 gave good amplification and were polymorphic between the genotypes. In *Kaempferia* spp six primers were tested and 3 of them viz., OPA 06, OPA 08 and OPE 01 were polymorphic between the two species. The primers, which were polymorphic between the genotypes, were used to develop RAPD profiles of *in vitro* conserved lines. No polymorphism was detected between the conserved lines in any of the primers tested. Thus RAPD profiles confirmed the genetic stability of *in vitro* conserved genotypes of spice crops.

4. **Studies on somaclonal variation produced by *in vitro* culture in *Zingiber officinale* Rosc. (TH 54)**

Samsudeen, K. / University of Calicut / Guide - M. K. Nair

A protocol was standardized for shoot development with disc as the explant and with following combinations of growth regulators -0.1 mM BAP with 10 mM NAA or 0.1 mM BAP with 1.0 mM NAA. The protocol developed for callus formation had disc as the explant and the following combinations of growth regulators -10 mM 2,4-D with 0.1 mM kinetin or 50 mM, 2,4-d with 0.1 mM kinetin. In this second experiment protocol was standardized for regeneration of plants from callus. Two cytokinins (BAP and Kinetin) at five levels (0, 0.1, 1.0, 10 and 50 mM) and two auxins (NAA and 2,4-D) at three levels (0, 0.1 and 1.0 mM) were tried. Most of the combinations gave profuse rooting. However, presence of 2, 4-D was inhibitory to root formation 2, 4-D at 0.1mM BAP or kinetin resulted in callus proliferation. Embryoid and shoot development was obtained in presence of 0.1 mM 2, 4-d with 50mM BAP or kinetin. To develop a method for *ex vitro* establishment of *in vitro* regenerated plants, two types of containers (Polythene bags and thermacol cups), three different types of planting media (Sand, soil mixture and vermiculite) and two conditions (covered with polybags and not covered) were used. Polythene bags filled with soil mixture and covered with another polythene bag gave maximum survival rate and establishment of *in vitro* regenerated plants. Using the above protocol, plants were produced directly from disc explant (micropropagation) and through callus regeneration and established in soil. Variation in morphological characters were observed among micropropagated plants, callus regenerated plants and conventionally propagated plants. Significant variation for all the traits viz., no.of tiller/plant, no.of leaves/plant, average height of tillers/ plant and rhizome weight/plant was observed among micropropagated, callus regenerated and conventionally propagated plants. Biochemical

characters viz., dry recovery, oleoresin content and fibre content varied significantly among all plant groups. The results indicated that tissue culture induced variation i.e., Somaclonal variation can broaden the genetic base of ginger, which can be used for crop improvement programmes.

#### 5. Bio-Ecology of rhizome rot pathogen(s) of ginger and disease management (TII-94)

**Balakrishnan Panayanthatta** / University of Calicut / Guide – *Y. R. Sarma*

Rhizome rot is caused predominantly, by *P. aphanidermatum* (Edson.) Fitzp and occasionally by *P. myriotylum* Drech. in major ginger growing areas of Kerala. Bacterial wilt caused by *P. solanacearum* is equally important in Wynad district but sporadic in Ernakulam and Calicut districts. *P. solanacearum* consists of both biotype 3 and 4. Root knot nematode (*M. incognita*) also infects ginger. Interaction of *P. aphanidermatum* and *M. incognita* results in increased severity of the disease. Onset of rhizome rot occurs during July – August and becomes more severe in September- October. Survival structures of the pathogen are oospores in scales of infected juvenile fingers. In soil it might survive in host plant debris, for eg., infected roots or decayed rhizomes in the form of oospores. Solarisation enhances soil temperature up to 8.53°C in the upper soil layers, and eliminates at least partially, inoculum of *Pythium* in infested soil, protects ginger from rhizome rot and enhances yield of rhizome. Biocontrol agents viz., *T. harzianum*, *A. niger*, *A. terreus*, *Penicillium* sp. and rhizosphere bacteria suppresses *P. aphanidermatum* and protects ginger from rhizome rot. Among the biocontrol agents, *T. harzianum* is also tolerant to fungicides viz., metalaxyl, mancozeb and captafol which makes it an ideal candidate for integrated management. Among 148 varieties of ginger, varieties viz., 72, 74, 79, 215 & 250 showed numerically less incidence of rhizome rot as compared to other varieties. This might be a reflection of some amount of tolerance of these varieties. However, wild varieties viz., Indonesian wild, *zingiber* species (accessions 335 & 336), Kanyakumari and Karakkal did not succumb to rhizome rot infection. Fungicidal seed treatment with supplementary soil drenching protects ginger from rhizome rot by reducing pre-emergence rot and disease incidence. Among fungicides, captafol, metalaxyl-mancozeb and mancozeb are most effective. Integrated management practices consisting of soil disinfestations by solarization, seed treatment with fungicide which is compatible with biocontrol agent (*T. harzianum*) and soil application of neem oil cake in combination with *T. harzianum* were found promising for effective management of rhizome rot of ginger.



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## TURMERIC

### 1. Variability in somaclones of turmeric (*Curcuma longa* L.) (TH 120)

Praveen, K. / University of Calicut / Guide - K.V. Peter, Co-guide - T. John Zachariah

Turmeric, the underground rhizome of *Curcuma longa* L. (syn: *C. domestica* Val.) is fifth among the most important spices of the world. It is used as a spice, confectionary, dye and in medicine and cosmetics. Turmeric belonging to the family Zingiberaceae is a sterile triploid with relatively shy flowering nature and rare seed set. Though turmeric has variability for various morphological and rhizome characters, its variability for resistance to biotic and abiotic stress is limited. There is an urgent need to identify varieties resistant/tolerant to various biotic and abiotic stresses in turmeric. Being vegetatively propagated, the induced variability can be fixed immediately and the true to type lines established in turmeric. Micropropagation, callus induction, regeneration embryogenesis and *in vitro* mutagenesis. Various growth regulators like kin, BA, TDZ, putrescine, Dicamba, picloram were studied for their efficiency in inducing multiple shoots and induction, proliferation and regeneration of plantlets from callus using axillary bud and leaf explants of turmeric. Embryogenesis was observed on MS medium supplemented with 0.1  $\mu$ M TDZ. The *in vitro* derived shoots were rooted on MS basal medium supplemented with IAA, IBA and NAA. NAA (liquid) at 8.7  $\mu$ M gave the best response with 11 roots per plant in Prabha and 12 in Suvarna. The rooted plantlets were successfully hardened before transferring to polybags. *In vitro* plantlets were grown on MS medium with high concentrations of sucrose and placobutrazol for induction of microrhizome. EMS and gamma irradiation was to increase the spectrum of variation. The one year old putative somaclonal plantlets were studied for morphological, cytological and molecular characterization to detect variants or mutants.

2. Influence of varieties, times of planting, spacings and nitrogen levels on growth, yield and quality, crop-weather and growth simulation modelling and yield forecast in turmeric (TH 104)

Kandiannan, K./Tamil Nadu Agricultural University /Guide - K. K. Chandragiri

The objectives were to study the influence of varieties, times of planting, spacings and nitrogen levels on growth, yield and quality of turmeric. Besides, studies on turmeric-weather relationship, yield forecast, and growth simulation modelling were also undertaken. Field experiments were laid out 2000 - 2001 and 2001 - 2002 in split plot design with three replications. The combinations of two varieties (BSR 1 and BSR 2) and three times of planting (May 15<sup>th</sup>, June 15<sup>th</sup> and July 15<sup>th</sup>) constituted the main plot treatments. The combinations of three spacings (30 x 15, 45 x 15 and 60 x 15 cm) and three nitrogen levels (125, 150 and 175 kg ha<sup>-1</sup>) formed the sub plot treatments. Planting turmeric variety BSR 2 during May 15<sup>th</sup> at 30 x 15 cm spacing and application of 175 kg N ha<sup>-1</sup> was the best treatment combination. The turmeric-weather relationship indicated that magnitude of relation of weather variables with turmeric yield was in the order: minimum relative humidity > solar radiation > maximum relative humidity > rainfall > growing degree day > minimum temperature > wind speed at eight feet height > sunshine hours > evaporation > maximum temperature. The coefficient of determination of the crop-weather models ranged between 0.29 and 0.99. The multiple regression models developed to predict turmeric yield from weather. In phenology, the thermal time requirement from planting to emergence, first tiller appearance and maturity was calculated and the thermal time from planting to maturity (50 per cent leaf drying) for the varieties BSR 1 and BSR 2 at May 15<sup>th</sup>, June 15<sup>th</sup> and July 15<sup>th</sup> plantings were 3442.2, 3029.8 and 2806.8d°C and 3686.8, 3294.8 and 3020.5d°C, respectively. The partition of assimilate among root, rhizome and shoot at different developmental stages indicated that at emergence proportion of allotment was only between shoot and root (0.72: 0.28). The dry matter production of shoot and root increased initially up to the peak vegetative growth and declined thereafter whereas, the rhizome became a major sink and its weight steadily increased up to maturity. The coefficient of partition between root, shoot and rhizome at maturity was 0.09:0.20:0.71. Daily potential crop assimilation for turmeric based on solar radiation at 11°. 29' latitude was calculated from the published information by linear interpolation and the information on assimilation rate, phenology and dry matter partitioning to various plant parts at different developmental stages utilized to develop

simple growth simulation model for potential production of turmeric excrete that the predicted and observed yields did not differ much as evident from verification with chi-square test and the model is found valid.

3. **Investigations on the biosynthesis of curcumin in turmeric (*Curcuma longa* L.)** (TH. 118)

Neema Antony / University of Calicut / Guide - B. Chempakam

The present study mainly emphasis on the *in vitro* synthesis of the coloring pigment in the crop. As a preliminary step, the relative concentration of other metabolites – essential oil, oleoresin and phenolic acids as well as starch during plant growth were determined which gave a basis information on the rate of biosynthesis of the compounds during rhizome development and provided an indication on the availability of other precursors required for biosynthesis. Along with secondary metabolites, the total curcuminoids also showed higher levels during the initial growth of the plant. As rhizomes matured, there was an enhancement of starch synthesis resulting in the relatively lower percentage of curcuminoids. However 3-fold increase in the level of the pigment was seen in 3 to 4 month seedlings. Tracer studies involving labeled precursors/ intermediates indicated higher levels of enzymes phenylalanine ammonia lyase during initial phase. The incorporation of the label was seen in phenolic acids, the intermediary precursor in biogenesis, indicating the conversion of a part of  $^{14}\text{C}$ -phenylalanine to phenolic acids in the vegetative parts. In addition, the presence of  $^{14}\text{C}$ -label in curcuminoids confirmed the inclusion of phenolic acids into curcumin molecules. This comes out as an evidence for the phenyl propanoid pathway involving phenylalanine as the primary precursor and phenylalanine ammonia lyase as the rate-determining enzyme. The major phenolic acids identified through TLC and HPLC were found to be *p*-coumaric acid, caffeic acid and ferulic acid. This gave an additional evidence for channelization of the major portion of ferulic acid towards curcumin biosynthesis. Results on HPLC analysis of curcuminoids in a few high and low curcumin varieties/ accessions could be correlated with PAL levels. However, the percentage distribution of curcuminoids followed a general pattern 65:20:15 for curcumin. I, II and III even though slight variations were observed between the accessions. Curcumin I was found to be a good source at maturity from all varieties studied except var. Prathibha, which showed high values in the immature stages. Varieties Alleppy, Prabha and Suguna appear to be better source of curcumin II (DMC). As for

curcumin III (BDMC), Suguna and Sudarsana were found to be good sources. PAL could be purified from turmeric leaves to 157 fold after ammonium sulphate fractionation followed by anion exchange chromatography over DEAE cellulose and gel filtration chromatography using sephacryl S-200. The purified enzyme was subjected to electrophoresis, which gave a single thick band corresponding to a molecular weight of approximately 39,000 Kda. This suggests the holoenzyme to be a tetramer of similar or identical subunits, which is in agreement with the tetrameric nature of PAL in all organisms examined to date. The purified enzymes was found to have an optimum pH of 8.8 with temperature optima at 25°C ambient. The Km value of the enzyme was found to be 0.33 nM. Cinnamic acid and its hydroxyl and methyl derivatives, especially coumaric, caffeic ferulic and chlorogenic acids were found to be strong inhibitors of PAL. Rate of inhibition were in the order cinnamate > *p*-coumarate > caffeate > ferulate > chlorogenate.

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## VANILLA

### 1. Seedling and somaclonal variation and their characterization in vanilla (TH-110)

Minoo Divakaran / University of Calicut / Guide - K. V. Peter

The main objectives of the present investigation were to study the existing variability in some of the vanilla cultivars and species available in India, to broaden the spectrum of variations in vanilla gene pool using conventional as well as biotechnological approaches, to characterize the extent of variability generated using morphological, cytological, biochemical and molecular markers, and to standardize protocol for *in vitro* conservation of vanilla germplasm. Few progenies were screened for reaction against *Phytophthora meadii* and *Fusarium oxysporum*, the two major pathogens affecting vanilla. Murashige and Skoog's medium (MS) was used for *in vitro* studies. Successful seed germination from hand pollinated fruits was achieved in MS medium with BA (0.5 mg l<sup>-1</sup>) and shoot multiplication in media with BA (1 mg l<sup>-1</sup>) and IBA (0.5 mg l<sup>-1</sup>). Conversion of root tips into shoots in *V. planifolia* and *V. aphylla* and micropropagation responses were standardized for *V. planifolia*, *V. aphylla*, *V. andamanica* and *V. pilifera* in the same medium. Plant regeneration from callus was induced in MS medium fortified with NAA (0.5 mg l<sup>-1</sup>) and BA (1.0 mg l<sup>-1</sup>). Production of colchipooids was attempted and seed were germinated in MS medium supplemented with colchicine (0.002%). The regenerated shoots produced roots in growth regulator free medium. Interspecific hybridization between *V. planifolia* and its related species *V. aphylla* was successful and the hybrids exhibited segregation of both the parental characters. This is the first report of successful interspecific hybridization and production of hybrids between *V. planifolia* and *V. aphylla*. The selfed progenies, somaclones and interspecific hybrids of *V. planifolia* obtained through seed culture have shown variations in morphological characters. Molecular characterization using RAPD and AFLP profiles indicating that the percentage of similarity ranged from 98.8–42.7 % among the different species. The population was screened against the infection of *Phytophthora meadii* and *Fusarium oxysporum*, the causal agents of foot rot and wilt diseases in vanilla. High degree of variability in resistance to fungal pathogen was noticed among them and lines expressing resistant reaction were identified. Various approaches for conservation were attempted. Synthetic seeds were produced using *in vitro* regenerated shoot buds, which could be stored for ten months, and recovered thereafter for further

multiplication. Shoot cultures of *Vanilla* species could be stored under slow growth conditions for 1 year, and recovered later facilitating conservation of endangered species like *V. aphylla*. The technologies developed during the present investigation can be used for broadening the spectrum of genetic variations in vanilla. The protoplast isolation and fusion technology developed can be used in transfer of useful traits through the production of somatic hybrids thus, making way for genetic manipulations in vanilla. The *in vitro* conservation methods, through synthetic seed, slow growth and cryopreservation, standardized in the present study will form an integral and important part of overall conservation strategy in genetic resources management of vanilla germplasm

## 2. Studies on genetic variability in open pollinated progenies of vanilla (TH 119)

Vimala Jose / University of Calicut / Guide - K. Nirmal Babu

The present work was taken up with the objectives such as induction of variations in segregating progenies of vanilla and enhancing the spectrum of variability of induction of polyploidy and mutations. Interspecific hybridization was also attempted. This experiment was planned to find out the 56 days after pollination was found to be optimum stage at which the *Vanilla planifolia* embryo could be rescued and grown *in vitro* to get maximum germination. MS + BA 1 mg/L + IBA 0.5 mg/L + Tryptone 2g/L resulted in a relatively higher embryo germinating percentages (> 80%) when compared to the control treatment (MS media without hormones). The seedling progenies differed among themselves in their external morphological characters such as leaf shape, leaf size, internodal length, root number, root colour etc. The cytological examination of the progenies produced by selfing showed the expected number of  $2n = 32$  in most of the *V. planifolia* progenies. Apart from the diploid chromosome number some aneuploids with chromosome number less than  $2n = 32$  were also observed. Of the six primers screened to detect RAPD polymorphisms among the seedling progenies, four primers showed faintly developed DNA fragments showing polymorphism, while two primers showed little or no polymorphism. Interspecific hybridization between cultivated vanilla (*V. planifolia*, *V. tahetensis*) and wild vanilla (*V. aphylla*) was done to produce fertile progeny which may carry economically important genes. Results showed that hybridization influenced cytological, anatomical and molecular characteristics of the progenies in addition to morphological characters. Most of the hybrid plants showed a phenotypic similarity to the maternal parent. The chromosome number of interspecific

hybrids resulting from a mating between *V. planifolia* ( $2n=32$ ) and *V. aphylla* ( $2n=64$ ) would be expected to be  $2n=4x=48$ . This prediction was confirmed by chromosome counts employing root tip meristematic cells recovered from some of the allopolyploids. The anatomical characters were found to be specific based on which a key was prepared. The anatomical key to the species is the first of its kind in *vanilla*. Twenty features of vegetative anatomy of the three species and eighteen hybrids were scored and used as the basis for analyzing the segregation pattern among the hybrids. Based on the scored values, a dendrogram was constructed which was used to study the interrelationship between the hybrids and their parents. The production of auto polyploidy by colchicines treatment was reported for the first time in *V. planifolia*. Tetraploid plants were characterized by slow growth, greener stem and narrower and thicker leaves and could be first selected based on these morphological characters. Induced mutation breeding was initiated to augment the spectrum of variation and select desirable mutants. Attempts were made to standardize a micro technique for plant regeneration from mutated tissue of seed, stem node, callus and PLB. The primary effect of treatment includes changes in leaf shape and a reduction in growth vigour. The EMS concentration of 75% was most effective in inducing variation. Among the different types of explants used, the protocorms and seeds were equally efficient in producing variations.

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## CHILLI

### 1. Selection of promising lines, production of somaclones and their utilization in paprika (TH 96)

Anu Augustine / University of Calicut / Guide - K. V. Peter

Forty paprika genotypes were evaluated for their morphological and biochemical characters for three seasons at the Indian Institute of Spices Research, Calicut, Kerala. Among the forty genotypes, Paprika King had the highest colour value (268 ASTA), followed by PBC 828 (258 ASTA) and Kt-pl-19 (225 ASTA). There were quite a few genotypes under the high colour group. Among the lines studied, PBC 436 had the lowest capsaicin percentage (0.05) and the highest was in 517-1 (0.63%). Seed protein analysis of the different lines of *Capsicum* was performed by SDS-PAGE electrophoresis. It was found that the average similarity was highest between PBC 436 (0.91%) (Originated in Portugal) and Round ornamental (collected from KAU), suggesting that Round Ornamental could have originated in Portugal. Most of the paprika lines had high seed protein content and they also clustered together. Fruit length, fruit colour or percentage of capsaicin did not show correlation with the clustering of the genotypes; but in general lines with common places of origin clustered together, low pungency high colour lines (paprika) also showed clustering together. With the objective of studying the effect of Somaclonal variation in the improvement of paprika lines, callus regeneration was attempted. For callus regeneration, different explants like leaf, stem, shoot tip and nodal explants were used. Auxins like 2,4-D, NAA, IBA and IAA and cytokinins like BAP and Kinetic were used. The combination BAP (3mg/lit) + IBA (1mg/lit) was found to be the best combination and nodal explant was found to be the most responsive. Elongation of the regenerated plants was achieved using MS medium containing 1mg/lit IBA. Rooting occurred in MS medium containing activated charcol. Hardening was done in plastic cups containing sand and potting mixture in 3:1 ratio and covered with polythene bag to conserve humidity. The somaclones were transferred to field after hardening and their morphological and biochemical characters were recorded. From the observations, it was found that maximum variation was found in fruit morphology and colour value. In this way it may be possible to get a plant with high colour value by producing somaclones in large numbers and screening them. Anthers collected from somaclones of the five genotypes were used for cytological studies. Abnormalities like quadrivalent formation,



lagging chromosomes, bridge formation and hexavalent formation indicated that there are abnormalities in pollen formation of the somaclones. To find whether the variation found in somaclones of *C. annuum* were continued in the next generation, seedling progeny of the somaclones were also evaluated for their morphological and quality characters. It was found that there was significant variation in the morphological and biochemical characters of all the five lines evaluated. Variations were found in characters like stem pubescence, plant growth habit, leaf colour, flower position, fruit colour and fruit shape. Colour value also varied among the somaclones seed progeny.

2. **Studies on chilli pepper (*Capsicum annuum* L.) I. Transfer of certain specific agronomically desirable characters into popular chilli cultivars. II. Induction of mutations and autopolyploidy. III. Assessment of phylogenetic relationships by seed protein analysis. IV. Induction of haploids (TH 92)**

**Dhamayanthi, K.P.M / Bharatiar University / Guide - V. R. K. Reddy**

With an object to improve the five popular South Indian chilli varieties, four agronomically desirable characters were transferred from semi wild chilli stocks into Indian chilli varieties viz., G-4, K-2, Byadagi, MDU-1 and Co-2. The four agronomical characters viz., compact plant type (cpt'), upright fruit form (up'). Cluster fruit form (çl') and destalkness ('dst') were obtained from four donor parents namely KAU cluster, Kanthari local, Ujwala and Dharwad cluster. The genes controlling the above four characters were found to be recessive in nature hence, they were transferred by selfing and screening the backcross progeny. All the 20 F<sub>1</sub> hybrids were backcrossed to respective recurrent chilli parents to produce BC<sub>1</sub> and BC<sub>2</sub> hybrids. Screening, selfing and selections were made in these generations to obtain BC<sub>1</sub>F<sub>2</sub> populations and desirable lines were constituted from these populations. All the constituted lines were compared with the control under the field conditions. The constituted lines carrying desirable genes showed variable results. Lines carrying genes 'up' and 'çl' were found predominance indicating the rate of expression of these two genes are higher than the other two genes. Induced mutation work was carried out in four chilli varieties namely G-4, K-2, Byadagi and Co-2 using one physical mutagen (gamma rays) and two chemical mutagens (EMS and MMS). Mutagens were applied both solo and in combination. Studies were made in M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> generations. A total of seven agronomically desirable mutants were isolated in M<sub>2</sub> generation. The mutants include compact, dwarf, mutants with increased number of branches, increased number of fruits per plant, early flowering/fruitletting, cluster fruit

bearing and male sterile lines. The mutants showed better performance both in  $M_2$  and in  $M_3$  generation over control in not only the character for which it was selected but also in other yield contributing characters. Using colchicines, induced autotetraploids were produced in chilli by treating the seeds and seedlings of *Capsicum annuum* var. *grossum* (sweet pepper) and *C. frutescens* var. *Kandhari* local (hot pepper). Seedling treatment was found to be effective and reliable method than the seed treatment for induction of tetraploids. Among the various concentrations with different duration tried, 0.4% with 16<sup>th</sup> duration in seed treatment and 0.5% with 12<sup>th</sup> duration in seedling treatment was found more effective in inducing autotetraploids. Tetraploids were relatively slower in growth in the earlier stages, but later they become tall, robust with large, thick dark green leaves. The capsaicin content and the colour values were significantly higher in tetraploids than the diploids. The electrophoretic seed protein profiles of the representative species, commercial varieties, sweet pepper lines, exotic cultivars and local cultivars have been outlined in the form of electrophorograms. Altogether 76 protein bands have been identified. The densitographic analysis of the protein fraction showed distinct quantitative difference among the species, commercial varieties, sweet pepper lines, exotic cultivars and locally grown cultivars. The gross protein fraction suggest that the spices / commercial varieties / sweet pepper lines/exotic cultivars/ local cultivars differ considerably in their mobility pattern.

3. **Studies on the development of  $F_1$  hybrids in paprika type chilli (*Capsicum annuum* L.) with high yield, colourant and resistance to anthracnose (TH-121)**

Prasanth, D. / Tamil Nadu Agricultural University / Guide- V. Ponnuswami

An investigation was undertaken to develop  $F_1$  hybrid in paprika type chilli with high yield, colourant and resistance to anthracnose. Twenty seven accessions were chosen. Based on 13 quantitative traits, 27 genotypes were grouped into two major clusters and selected six genotypes three each from a major cluster. The selected parental genotypes were Acc 11 (P1), Acc 22 (P2), Acc 5 (P3), Acc 12 (P4), Acc 23 (P5) and Acc 14 (P6). Diallel crosses among six chosen parents were attempted and subsequent analysis was carried out in hybrids and parents for important component characters with a view to obtain information about heterosis, combining ability, gene action and for identifying potential parents and superior cross combinations. The magnitude of GCA variances for almost all the characters studied except leaf area index, dry yield per hectare and capsaicin were higher than their corresponding SCA variances in all the 30 crosses, suggesting that

the traits studied were controlled by additive gene action. The traits *viz.*, leaf area index, dry yield per hectare and capsaicin were found to be under the control of non-additive gene action. The parent P5 followed by P2 had high *per se* coupled with high *gca* effects for most of the economic traits studied. So, the parent P5 could be employed in breeding programmes for overall crop improvement. The parent P2 would be a better choice when the aim is primarily to increase the fruit and quality characters. Low heritability was observed for leaf area index, fresh yield per plant and dry yield per hectare. Graphical analysis indicated the role of partial dominance for days to first flowering, individual green and dry fruit weight and total extractable colour where as over dominance was observed for the traits *viz.*, leaf area index, fruits per plant, fruit length, fruit girth, fresh yield per plant, dry yield per hectare and capsaicin. The estimate of covariance was found to be lesser than corresponding variance components for all the 11 characters studied in all the six parents suggesting that the non-allelic interactions were of complementary type. With respect to biochemical basis of resistance, phenol content was highest in all the resistant hybrids and least in susceptible crosses. The results on the changes in peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase indicated that the activity of all the enzymes was higher in the resistant genotypes than the susceptible one. From the present study, the potential  $F_1$  hybrids identified with high extractable colour coupled with low capsaicin and high yield per hectare are P4 X P5, P6 X P5 and P2 X P6. The anthracnose resistance in P1 X P3 could also be exploited since it had other improved economic traits like number of fruits, dry fruit yield and moderate extractable colour content besides resistance factors such as high phenol content and least disease incidence. The hybridity of two promising  $F_1$  hybrids *viz.*, P4 X P5 and P2 X P6 were confirmed by molecular marker (RAPD) analysis. These hybrids are worth test verifying in different seasons for assessing their stability in extractable colour and yield.

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## OTHERS

### 1. Studies on capsule rot ('Aazhukal') disease of cardamom (TH-59)

Suseela Bhai, R. / University of Calicut / Guide - Y. R. Sarma

Based on the detailed investigation, it was found that two species of *Phytophthora* viz. *P. meadii* A2 mating type and *P. nicotianae* var. *nicotianae* A2 mating type were associated with capsule rot ('Aazhukal') disease of cardamom (*Elettaria cardamomum* Maton) of which *P. meadii* A2 mating type was the predominant species. *P. nicotianae* var. *nicotianae* was mainly associated with foliar infection of cardamom viz. leaf blight occurring during the post monsoon season. However there was certain amount of overlapping of these in initiating disease in cardamom. As reported earlier, *P. palmivora* could not be isolated from the infected plant parts at any time during the investigation. The potential of *Trichoderma harzianum* as an important biocontrol agent against capsule rot disease of cardamom has been brought out very clearly in the present investigation. The fungicide Potassium phosphonate (Akomin-40) was found compatible with the bioagent *Trichoderma* and hence this was also included in the integrated management strategy. Though conspicuous differences were noticed with respect to *T. harzianum* and disease potential index, the overall difference in terms of disease incidence were not conspicuous and are on par. During the period of two years of experimentation in the field, a negative correlation was obtained between *Trichoderma* population and Disease Potential Index. A clear cut distinction might be discernible in terms of disease suppressiveness and consequent yield increase only after a few years in view of the perennial nature of the plantation crop and also the soil borne nature of the disease. However, biocontrol agent has got an edge over the chemical control in terms of its adaptability and eco-friendly nature. Hence use of bio control as an effective management strategy has been well brought out in the present investigation.

### 2. Biological control of plant parasitic nematodes of spices (TH-109)

Santhosh J. Eapen / University of Calicut / Guide - M. A. Haq.

Root-knot nematodes (*Meloidogyne* spp.), burrowing nematodes (*R. similis*) and

lesion nematodes (*Pratylenchus* spp.) are the most important plant parasitic nematodes, causing damage and yield losses on most of the spices. Fungi and bacteria from rhizosphere soil and roots and also directly from root-knot nematode egg masses or individual eggs or females were isolated. Some promising isolates were obtained from IISR repository and various other sources. Altogether there were 5 isolates of *Verticillium* spp., 8 isolates of *Paecilomyces* spp., 88 isolates of *Trichoderma* spp. and 48 isolates of various other fungi. Among the 57 bacteria isolated, 17 isolates belonged to different species of *Bacillus* while 12 were *P. fluorescens* isolates. The test fungi were evaluated for their ability to parasitize various life stages of the nematode viz. eggs, juveniles, females and egg masses. Their modes of action have been studied. Egg and female parasitism was the most prominent mechanism of many of these fungal facultative parasites. Out of the 149 fungal isolates screened, 115 isolates colonized the gelatinous matrix of root-knot nematode egg masses (77.18%). Fifty-nine isolates showed 50 - 90 % inhibition in egg hatch. Further, the biocontrol potential of these short-listed microbes was assessed through a series of greenhouse and field trials. Among the 5 isolates of *Trichoderma* evaluated, isolates C.22 and C.23 were found superior in suppressing root-knot nematode populations than the other isolates. Though not significant, most of the isolates, especially the fluorescent pseudomonads, induced some degree of growth promotion. The egg parasitic fungus *V. chlamydosporium* and the obligate bacterial parasite *P. penetrans* were able to check the root-knot nematode multiplication in cardamom nurseries. Significant reduction in nematode population was observed in plots where *V. chlamydosporium* was applied. In black pepper, the reduction in yellowing was statistically significant only in the case of vines treated with biocontrol agents. The studies also threw light on the varied ecological requirements like temperature and pH of some of the promising fungi. Compatibility of these bioagents with some of the commonly used pesticides and their mass multiplication on locally available substrates were also studied.

### 3. Quantitative analysis of Indian research out put on spices since 1970. (TH 117)

Sushama Devi, C. K / Annamalai University / Guide - M. Nagarajan

The study made an attempt to analyze the performance of research output on spices in terms of content coverage growth rates and authorship pattern, performance of research institutions in promotion of spices research is also given due emphasis. The study revealed that around 90% of spices literature was published in journals followed by

conference proceedings. Language wise analysis showed that English accounted for 70% of world spices literature. Study of authorship pattern revealed the increase in multiple authorship papers when numbers of authors increase number of output performance decreases. Extent of collaboration was found to be a function of time. Author wise distribution revealed scientific productivity is concentrated in a limited number of individuals and authorship pattern follows negative binomial distribution. Relative growth rate of literature showed a declining trend. A crop wise growth of literature output study was conducted and relative growth rate for each crop was calculated. Study of institutional distribution of publication output revealed outstanding performance by agricultural universities. UAS Bangalore, IISR Calicut, Banaras Hindu University, College of Horticulture, Trichur etc. Top various categories of institutions in terms of spices research output area wise analysis revealed predominance of crop protection aspects. Capsicum and Turmeric outstands all other crops in research output in the national and international level. A comparative study revealed that major portion of world research output on pepper, cardamom, ginger and turmeric were done in India. A study of scattering of literature revealed a total number of 13 journals account for 46% of the scientific output on spices. Application of Bradford's law also confirms this fact. Analysis of relative growth rate of pages showed a declining trend. GNP (Gross National Product) and RGR (Relative Growth Rate) during the period were found to be the same. A study of the journals published from G8 countries were made. A comparison of Indian research output with some selected countries were also carried out.

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