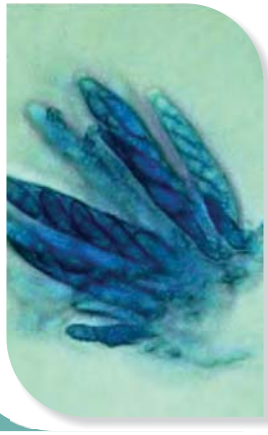
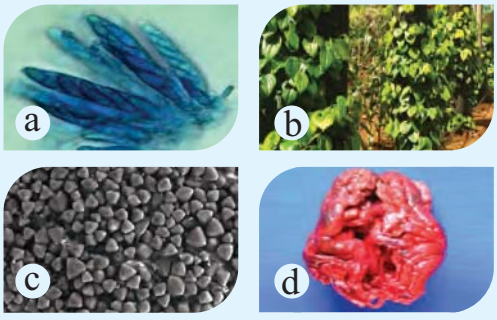
Research Highlights

2014/15



ICAR- Indian Institute of Spices Research
Kozhikode, Kerala



a. Microscopic view of *Colletotrichum gloeosporioides* perithecia
b. Intensifying black pepper production
c. A nutmeg collection with rudimentary sterile seed
d. Scanning electron microscopy of NPV infecting hairy caterpillar

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ICAR-Indian Institute of Spices Research
(Two times winner of Sardar Patel Outstanding ICAR Institution Award)
Kozhikode, Kerala, India

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PREFACE

The summary of research achievements of the institute during 2014/15 is presented here as Research Highlights. During this year, 255 accessions of black pepper were collected which includes 170 cultivars and 85 accessions of related taxa. A population of *Piper barberi* considered to be an endangered species was located in the evergreen forests of Anakulam forest range. In a farmer participatory germplasm collection, 31 nutmeg accessions including few farmers varieties and few unique germplasm were collected and conserved. A cardamom variety 'Appangala-2' developed through heterosis breeding has been recommended for release by AICRPS.

The fertilizer recommendations for cardamom were made for fixed target yield levels based on the soil test values for Appangala-1 and Green gold varieties. *In vitro* antioxidant activity and cytotoxicity of sequential extracts from selected black pepper varieties and *Piper species* indicated highest antioxidant activity in methanol extract of Malabar Excel followed by methanol extract of *P. colubrinum*. *In vitro* cytotoxicity indicated that chloroform extract of all the samples and hexane extract of *P. colubrinum* showed high cytotoxicity.

Screening of natural products and newer insecticides against cardamom thrips indicated that Spinosad, a natural product (derived from *Saccharopolyspora spinosa*), with low honey bee toxicity can be used for the effective management of cardamom thrips. Also, the field trials with the promising entomopathogenic fungus *Lecanicillium psalliotae* for the control of cardamom thrips indicated that basal application of *L. psalliotae* and as spray and basal application gave better control.

The institute conducted 17 training programmes of various durations for effective technology transfer to diverse stakeholder groups like farmers, youth, tribal beneficiaries and students. We have also embarked on empowering the tribal farmers under the aegis of the Tribal Sub-Plan of ICAR. In KVK, about 107 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted and 3263 trainees were benefitted. Nine front line demonstrations and five on farm trials on technology assessment and refinement were carried out. Non-exclusive license for commercializing designer micronutrient formulations have been given to four agencies through Business Planning and Development (BPD) unit.

I consider it a privilege to place on record the encouragement given by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR. We are also grateful for the strong support and guidance received from Dr. N.K. Krishnakumar, Deputy Director General (Horticulture), Dr S.K. Malhotra, former ADG (Hort. II) and Dr. T. Janakiraman, ADG (Hort.). I appreciate the efforts and zeal shown by all the project investigators in executing various programmes. The financial support for the projects received from ICAR is gratefully acknowledged. I also commend the editors for having compiled and brought out this publication.

M. Anandaraj
Director

Kozhikode
18.02.2015

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BLACK PEPPER

Genetic resources

Systematic surveys for collecting genetic resources of *Piper* were conducted at Anakulam, Virippara, Pettimudi and Kadalar forests of Mankulam forest division besides farmer's plots at Munnar, Idukki, Thodupuzha, and Kannur in Kerala and Coorg district in Karnataka. A total of 255 accessions were collected during the survey including 170 cultivars and 85 accessions of related taxa. A population of *Piper barberi* considered to be an endangered species was located in the evergreen forests of Anakulam forest range.

The present status of germplasm holding at the NAGS is 3181 (1669 cultivars, 1503 accessions of related taxa and 9 exotic species). A germplasm block consisting of 427 accessions was established at CHES, Chettalli as an alternate center. A field gene bank comprising 223 local cultivars was established at the Chelavoor main campus.

Breeding

A replicated yield trial involving 10 improved lines/selections and two controls was laid out at Peruvannamuzhi farm. The entries flowered are Hp780-5/30; OPKm-1/30; Thevam-7/30; Hp1411-1/30; Sreekara 6/30 and a back cross progeny-3/30 (Fig 1).

Fig. 1. Spikes of the back cross progeny [HP1117 x Aimpirian] x Aimpirian

Seedling populations derived from cv. Vadakkan x bold berried accession (Wayanadan bold) were raised. Lateral branches of Agali pepper, the accession having high bulk density were collected and rooted for breeding purpose.

Molecular biology

Drought stress

Ubiquitin gene, the most stable reference gene was identified using three different accessions of *Piper nigrum* under water deficit stress using Reffinder software. GAPD gene was found to be least stable under the above conditions. Among the genes tested for expression analysis in different accessions, Myb and NAC protein genes were found to be expressed three fold and above in susceptible cv. Sreekara under water deficit when compared to control. The increase in expression of these genes was low in drought tolerant line, Acc. 4216. Dehydrin gene was again found to be expressed many fold in Acc. 4216 compared to low expression in Sreekara.

Characterization of mentor grafted progeny

Seedling progenies grafted on to *P. colubrinum* showing characteristics of the root stock are being investigated for the presence of sequences from *P. colubrinum* using sequencing approaches (dd-RAD sequencing). Of the 1186604 million IlluminaHiSeq reads, 2000 reads of about 100bp in length were recovered. The variant calling was also performed in this sample from which, 4685 SNPs and 77 INDELs were identified at a minimum read depth of 10x.

Phytophthora capsici – *Piper colubrinum* interaction

Quantitative RT-PCR was employed to assess the level of expression of pathogenicity genes of *P. capsici viz.*, Glycoside hydrolase, NPP1, RXLR and Pectate lyase during *P. capsici* - *P. colubrinum* interaction (Fig. 2a-d). Glycoside hydrolase and RXLR genes showed high levels of expression during early stages of infection (up to 16 hpi), whereas the NPP1 gene showed maximum expression at later stages of infection (at 72 hpi). Pectate lyase gene showed high level of expression at early stages of infection but was down regulated during the

later stages of infection. The expression of these genes during initial phase of infection clearly indicated the importance of these pathogenicity genes during host colonization.

Phylogenetic analysis of these pathogenicity genes was also carried out to find the evolutionary relationship of these genes to other *Phytophthora* species. The predicted proteins of glycoside hydrolase, NPP1, RXLR and Pectate lyase were grouped into cluster belonging to *P. capsici* sequences in the database, except in the case of glycoside hydrolase, which was grouped along with *P. sojae*.

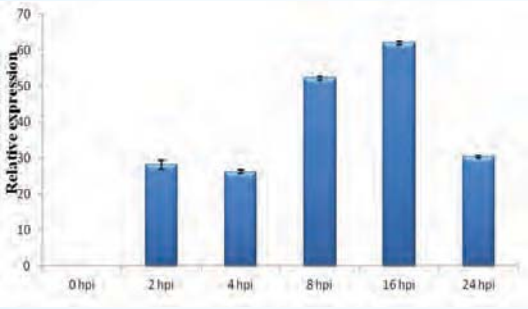


Fig. 2a. Relative expression of glycoside hydrolase gene

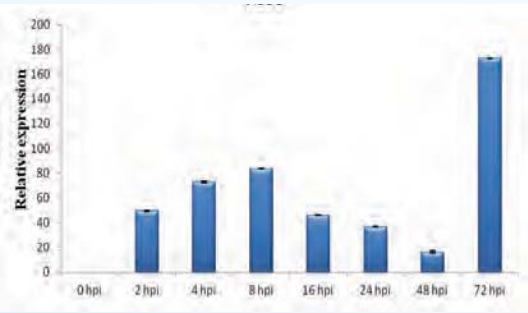


Fig. 2b. Relative expression of NPP 1 gene

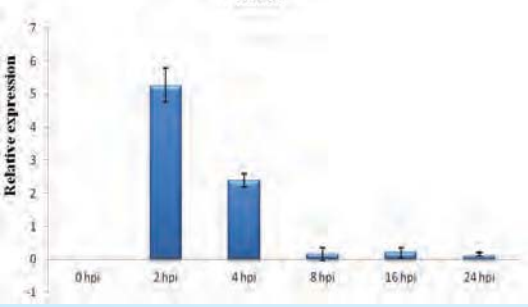


Fig. 2c. Relative expression of RXLR gene

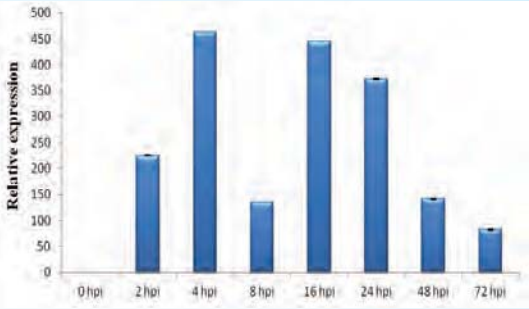


Fig. 2d. Relative expression of pectate lyase gene

In planta expression and docking studies of a glucanase inhibitor gene from *Phytophthora capsici* and beta 1, 3 glucanase gene from *Piper colubrinum*

The sequence characterization, *in planta* expression analysis and molecular docking studies of Glucanase inhibitor protein (GIP) and *P. colubrinum* endo beta-1,3 glucanase genes (pcEGase) based on sequence information derived from the *P. capsici* whole genome sequence data and *P. colubrinum* transcriptome data, respectively were carried out. The GIP gene from *P. capsici* have a 1059 bp ORF, encoding a putative peptide of 353 amino acids and the partial sequence of (pcEGase) gene from *P. colubrinum* had a 936 bp ORF, encoding a putative peptide of 312 amino acids. The expression of these genes was studied *in planta* at different time points by qRT-PCR. The *in planta* expression of GIP gene from *P. capsici* was at its peak during initial hours of challenge inoculation and the expression of pcEGase gene was at its peak at 16 hpi (hours post inoculation). The peak expression of pcEGase gene from *P. colubrinum* at 16 hpi and sharp decrease in later periods indicated the successful neutralizing activity of the pcEGase gene against the GIP gene in this incompatible plant- pathogen interaction. Three-dimensional model of GIP and pcEGase gene was constructed and molecular docking studies predicted sites on the surfaces of pcEGase gene and GIP that may be involved in high affinity binding. Molecular docking studies between pcEGase gene and GIP revealed that substrate inhibition is obtained by recognizing arginine and isoleucine residues in substrate molecule.

Identification and characterization of miRNAs in *P. colubrinum*

The *de novo* assembled *P. colubrinum* transcripts were analyzed for lncRNAs (long non-coding primary RNAs), microRNAs (miRNAs) and further their corresponding mRNA targets. Of the 4542 targets, 881 transcripts were predicted with putative functions which will help to understand the molecular basis of miRNA inhibition.

Phytophthora – P. nigrum interaction

Targeted expression analysis of two NBS-LRR gene loci/ beta 1, 3 glucanase locus was done in IISR Shakthi and Subhakara with the validated internal control genes from *P. nigrum* (GAPDH+UBCE). The R genes showed early expression in resistant variety than in susceptible. The glucanase gene showed constitutive expression in both the genotypes with the up regulation only in resistant variety upon infection with P. *capsici*. The susceptible variety recorded the down regulation of the gene. A full length cDNA library was generated for identification of the genes that are associated in defense mechanism against foot rot disease caused by *P. capsici*.

Host resistance

Hundred and forty progenies of Panniyur 1 x Subhakara were screened against *P. capsici* infection. Two progenies were found to tolerate stem infection. Fifty five open pollinated progenies of IISR Shakthi and 27 open pollinated progenies of 04-P24 were screened for *Phytophthora* resistance. Three progenies of IISR Shakthi were found to tolerate stem infection.

Post harvest technology

In vitro antioxidant activity and cytotoxicity of sequential extracts from selected black pepper varieties and *Piper* species

Antioxidant activity and cytotoxicity of four medicinally valued *Piper* species *viz., Piper nigrum, P. chaba, P. longum* and *P. colubrinum* were examined. Among all extracts investigated, methanol extracts showed highest antioxidant activity followed by chloroform extracts for all the four assays. Methanol extract of cv. Malabar Excel was found to be highest for all the assays followed by methanol extract of *P. colubrinum*. *In vitro* cytotoxicity was checked on cervical cancer cell line CaSki by MTT assay. Results indicated that chloroform extract of all the samples and hexane extract of *P. colubrinum* showed high cytotoxicity. Cytotoxicity increased with increase in the amount of extract as well as time of exposure of extract with CaSki. But chloroform extracts of *P. longum* and *P. colubrinum* were found to be highly toxic to CaSki than all other screened extracts for all three time intervals.

Disease management

Integrated management of *Phytophthora* foot rot and slow decline diseases

Field evaluation of bioagents integrated with chemicals effective against *Phytophthora* and nematodes showed that Metalaxyl-mz 0.125% + carbosulfan 0.1% + *Trichoderma harzianum* + *Pochonia chlamydosporia* was effective in reducing yellowing and decline of vines.

Evaluation of Actinomycetes against nematodes

An experiment to study effect of Actinomycetes on nematodes *in planta* showed that combined application of IISR Act 2 (*Ketosatospora setae*) with IISR Act 5 (*Streptomyces* sp.) or IISR Act 9 (*S. tauricus*) was effective in reducing the nematode population in the soil to an extent of 58 - 75%.

Biological control

In pot experiment with 15 *Trichoderma* isolates, highest growth promotion was observed

in PhytoFuRa-3 followed by PhytoFuRa-14 and highest biomass production was in PhytoFuRa-10. The isolate PhytoFuRa-10 consistently showed significantly higher biocontrol potential against *Phytophthora* foot rot.

Comparative genomics of *Phytophthora* species

Secretome analyses of Phytophthora species (*P. capsici* (05-06 and 98-93), *P. sojae, P. infestans, P. ramorum*) were done using different softwares like SignalP, TMHMM and TargetP. Proteins with signal peptides were identified by sorting out proteins without trans-membrane domains and sub-cellular localization for further comparative genomics studies.

Anthracnose

Artificial induction of perfect stage of *Collecotrichum gloeosporioides* infecting black pepper

The perfect stage (perithecia) was artificially induced under *in vitro* conditions based on mating-test model, in which sterilized toothpicks, dried leaves and twigs of black pepper as well as split, unsplit twigs of silky oak placed between confronting inoculum sources (pathogen culture, infected young and dried leaves of black pepper) served as inert platforms for the induction of perithecia.

Under i*n vitro* conditions, production of perithecia was observed in all the combinations. While, formation of ascospores (indication of fertile perithecia) was observed only in the combination of dried black pepper twig + infected young and dried leaves. Exudate (Fig. 3) embedded with ascospores produced from fertile perithecia was observed in the combination; black pepper twig + infected young leaf even three months after inoculation indicating longevity and fertile nature of the perithecia. The twigs with exudate, partly or wholly, when tested for infectivity on variety Panniyur-1 under lab and field conditions resulted in the development of characteristic anthracnose symptoms 4-6 days after inoculation.

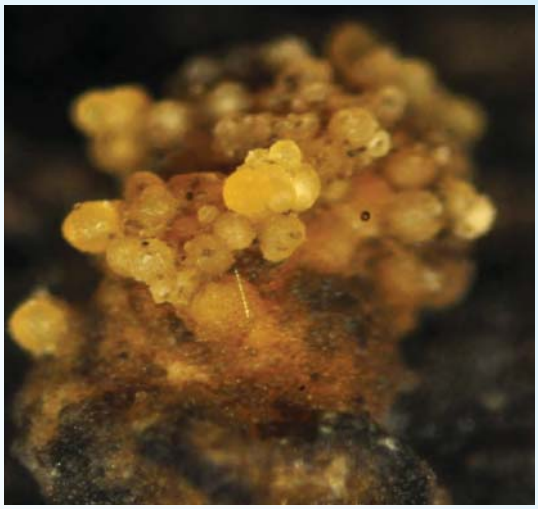


Fig. 3. Exudate embedded with ascospores produced from fertile perithecia

Sequential events in the colonization and proliferation of *C. gloeosporioides*

Sequential events involved in the infection process of *C. gloeosporioides* were studied under laboratory conditions. The leaf samples collected at 4, 6, 8, 12, 16, 20, 24, 48, 72, and 96 h after inoculation were subjected to staining, destaining and microscopically examined. Conidial germination (Fig 4a) was observed 4 h after inoculation. The germinating conidia were found congregating more towards stomatal region and 75 % of conidia germinated either with one (most cases) or two germ tubes after 10-12 h. Higher percentage of germination was noticed, when the conidia were in disaggregated condition which later produced melanized appressoria (Fig 4b). The infection hyphae originating from appressoria entered through stomata and subsequent intra/intercellular invasion was observed. Invading hyphae in the mesophyll cells and localized tissue death (Fig 4c) were noticed after 48 h. Acervulus initials were formed and mature acervuli with prominent setae (Fig 4d) were observed after 48 and 72 h, respectively. Several localized necrotic spots manifested on leaf surface after 72 h and the invaded epidermal cells turned brown, resulting in rapid collapse and death 72 h after inoculation.

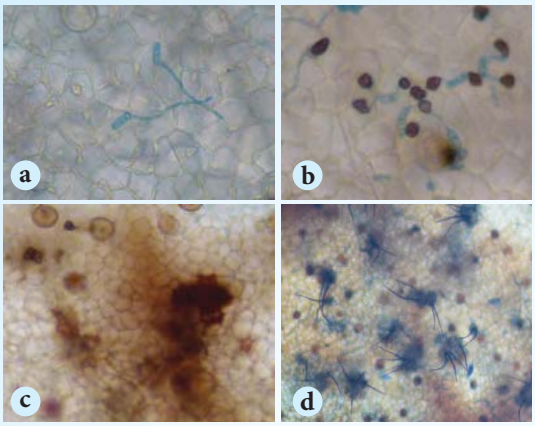


Fig. 4a. Conidial germination 4 h after inoculation, b. melanized appressoria formation, c. localized tissue death, d. acervuli with prominent setae

New target genes in *Radopholus similis*

Potential target genes of *R. similis* involved in parasitism such as FMR Famide-like peptides (nematode FLPs), β-1, 4, endoglucanase, transthyretin-like protein 3 precursor, serine-threonine phosphatases and survival such as glutathione-S-transferase(s), acetylcholinesterase, tetratricopeptide TPR-1, superoxide-dismutase and actin were amplified and sequenced.

Viral disease

Rapid identification of transgenic black pepper using loop-mediated isothermal amplification (LAMP) and real-time LAMP assays

A loop-mediated isothermal amplification (LAMP) and real-time LAMP based assays were developed for quick and sensitive detection of transgenic black pepper plants. Primers (six each) were designed based on the nucleotide sequence of two target regions [kanamycin and Cauliflower mosaic virus (CaMV) 35 S promoter] integrated into the genome of transgenic black pepper. The following conditions: 6 mM of magnesium sulphate, 0.4 M of betaine and 1h of reaction time proved optimal for amplification of the LAMP assay. Both assays successfully detected the transgenic plants whereas no cross-reaction was recorded with non-transgenic plants. The sensitivity of LAMP was up to 104 times that of conventional PCR while real-time LAMP was up to 103 times that of LAMP. The assays were validated by testing putative transformants of black pepper. The results presented clearly showed that LAMP and real-time LAMP assays developed in this study can provide a rapid and simple approach for screening transgenic black pepper and other plants transformed by using the above target gene sequences.

Sequencing of RNA2 and RNA3 of Cucumber mosaic virus infecting black pepper

Cucumber mosaic virus (CMV) is a tripartite ssRNA virus infecting large number of crops including black pepper. RNA1 of CMV codes for viral replicase while RNA2 and RNA3 each codes for two proteins namely RNA polymerase (2a), silencing suppressor (2b), movement protein (3a) and coat protein (3b). Cloning and sequencing of 2a, 2b, 3a and 3b gene of black pepper isolate of CMV showed that it consists of 2573, 337, 840 and 657 nucleotides respectively potentially encoding proteins with 857, 111, 279 and 218 amino acids respectively. Sequence comparison showed that black pepper isolate of CMV shared 92–95% and 70–71% identity in 2a with CMV subgroup I and II respectively, while it was 82–95% and 65% in 2b; 91–97% and 79% in 3a and 91–99% and 76–77% in 3b. In the phylogeny all the four genes (2a, 2b, 3a and 3b) showed close clustering with CMV subgroup I strains and distant relationship with subgroup II strains. Among the four genes, 3b showed high level of sequence conservation while 2b showed the least with other members in the subgroup.

Screening against Piper yellow mottle virus (PYMoV)

Out of 2437 germplasam accessions screened for resistance against Piper yellow mottle virus, four accessions showed resistance in the preliminary test.

CARDAMOM

Genetic resources

A total of 618 accessions are being maintained at NAGS, which consist of 442 accessions from IISR Regional Station, Appangala; 73 accessions from Pampadumpara; 47 accessions from Mudigere and 56 from Sakleshpur. About 117 accessions were characterized for morphological and yield characters. FGB-13 and FGB- 82 recorded maximum yield and more number of capsules per plant.

Breeding

The accession, IC 547167 (Appangala 1 x NKE 19) with potential yield of 1393.12 kg/ha (three years after planting) and mean yield of 456.79 kg/ha over locations, with mosaic resistance and good quality characters has been recommended for release in Karnataka as new variety under the name Appangala 2 (Fig. 5) by XXV AICRPS meeting held at UBKVV, Pundibari, West Bengal in September 2014.

Fig. 5. Appangala-2, a high yielding cardamom mosaic virus resistant hybrid

Crop management

Standardizing the parameters for target yield

Based on the previous year's crop yield under different treatments and the nutrient uptake data the nutrient removal for producing 100 kg of capsule was worked out and fertilizer recommendations were made for fixed target yield levels based on the soil test values for Appangala-1 and Green gold varieties. In both the varieties, the recorded yield parameters were higher in target specific applications as compared to the blanket recommendations.

In green gold, recorded yield levels per plant basis was 0.7, 0.9 and 0.9 kg/plant for the targets 0.4, 0.6 and 0.8 kg/plant with a positive mean deviation of 72, 55 and 15%, respectively. Similarly, in Appangala-1 yield per plant has shown a positive mean deviation of 83, 76 and 14% for the fixed target levels. The mean bias error and root mean square deviation for the prediction model and the recorded (projected) yield were also minimum, indicating better fitness of the target yield equation

Soil carbon pools buildup under cropping systems

The total and particulate organic carbon (POC) and nitrogen pools were quantified under different spice based cropping systems and high density multiple cropping system. The POC and PON pools were higher in coffee + pepper system (56.7 & 16.8 mg/ha) with highest total organic C & N (TOC & TON) pools (90.1 & 33.4 mg/ha). POC constituted 63% of TOC in this system. The non particulate carbon and nitrogen (NPOC & NPON) pools were higher under cardamom alone and coffee + pepper + cardamom cropping system (67.3 and 58.3 mg/ha) constituting 73-78% of TOC pools.

Among different management systems in black pepper, organic management has accumulated higher POC, NPOC and TOC pools as compared to integrated and conventional management systems. In HDMCS, black pepper basin has accumulated highest TOC, NPOC and POC pools (106.8, 71.6, 35.2 mg/ha, respectively) and coconut and nutmeg systems had higher NPON and PON (7 & 0.8 mg/ha) as compared to other component crops.

Quality evaluation of cardamom varieties

Eighteen varieties *viz.,* Njallani, Pannikulangara-1, Pannikulangara-2, Thiruthali, Elarajan and Wonder cardamom collected from Idukki district were analyzed for essential oil profile. The

essential oil content ranged between 5.8-7.4% on capsule weight basis. Pannikulangara-2 recorded highest essential oil content. Among the 21 components identified, the chief constituents of the oil, 1,8-cineole and α-terpinyl acetate varied between 18.1-32.7% and 36.9-48.5%, respectively. Concentration of pinene, sabinene, myrcene, α-terpineol, 4-terpineol, nerol, neryl acetate and nerolidol ranged from 1-5%.

Quality evaluation using E-nose

Hand-held electronic nose was modified with suitable sensor array for determining quality. Samples were analyzed using the modified hand-held electronic nose for essential oil content and could be graded into low (<4.0%), medium (4.0-6.0%) and high (>6%).

Pest management

Management of thrips (*Sciothrips cardamomi*)

Screening of natural products and newer insecticides for three years at Appangala indicated that Spinosad, a natural product (derived from *Saccharopolyspora spinosa*), with low honey bee toxicity can be used for the effective management of thrips. The product can also be used in organic system.

Evaluation of entomopathogenic fungus

Field trials with the promising entomopathogenic fungus *Lecanicillium psalliotae* for the control of thrips were conducted at Kodagu, Wayanad and Idukki. The trials indicated that basal application of *L. psalliotae* and as spray and basal application gave better control than other treatments at Wayanad.

Studies on *Wolbachia*

Studies on removal of the endosymbiont *Wolbachia* from thrips as a strategy for its management indicated that when the thrips were fed with tetracycline (20 mg/mL) treated leaves for 5 days, *Wolbachia* was completely eliminated from the insect system which was confirmed by molecular studies.

Root grub (*Basilepta fulvicorne*)

Infectivity of entomopathogenic nematodes (EPNs) against root grub, *Basilepta fulvicorne* was tested *in vitro*. Among the test EPNs, *Heterorhabditis* sp. (IISR-EPN 01) and *O. gingeri* (IISR-EPN 07) were more pathogenic as they caused 100% mortality to the insect within 72 h post exposure, followed by *Steinernema* sp. (IISR-EPN 03), *S. carpocapsae* (IISR-EPN 06) and *Oscheius* sp. (IISR-EPN 08). *Steinernema* sp. (IISR-EPN 02) and *Oscheius* sp. (IISR-EPN 04 and 05) took 120 h to kill the test insect.

Anthracnose

Occurrence of perfect stage of *C. gloeosporioides*

Surveys carried out in cardamom plantations revealed manifestation of different types of foliar symptoms *viz.*, spot, blight and shredding. The cultures isolated from these symptomatic samples exhibited variations in colony morphology and colour. Among the cultures, greyish white culture appeared puffy with a faster growth rate (14 mm/day) and produced dark brown-black, globose perithecia, four weeks after incubation. Microscopic examination of perithecia (Fig. 6a) revealed the presence of narrow, cylindrical, unitunicate asci (Fig. 6b) with hyaline, aseptate, cylindrical ascospores (Fig. 6c).

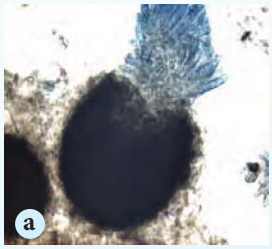


Fig. 6a. Perithecia

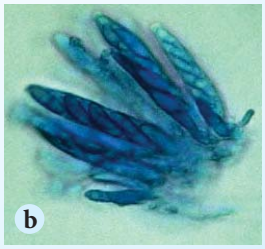


Fig. 6b. unitunicate asci

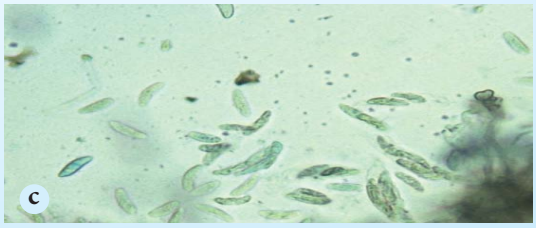


Fig. 6c. ascospores

Differential reaction of *C. gloeosporioides* isolates on varieties

Differential reaction of 20 *C. gloeosporioides* isolates was studied on cardamom varieties *viz*., Appangala 1, IISR Vijetha and IISR Avinash by employing prominence of yellow halo and streak as well as lesion area as criteria for recording observation. The isolates exhibited differential reaction as indicated by prominence and non-prominence of yellow halo and streak. The area of lesions developed on young leaves varied between 4.91 – 40.82, 7.85 – 60.45 and 11.78 – 38.47 mm^2 in IISR Avinash, IISR Vijetha and Appangala 1, respectively.

Evaluation of microbes for antagonistic potential against rhizome and root rot pathogens under *in vitro* conditions

The endophytic fungal isolates from varieties IISR Vijetha, IISR Avinash and Appangala-I were evaluated *in vitro* for antagonistic efficacy against *Fusarium oxysporum, Rhizoctonia solani* and *Pythium vexans*. Among the isolates tested, Va 4-2 (IISR Vijetha), Cb 4-1, Cb 6-2 (Appangala 1) and Aa 1-1 (IISR Avinash) were found promising against *F. oxysporum*. While, Cb 4-1, Cb 6-2 (Appangala 1) and Ab 6 (IISR Avinash) were effective against *P. vexans* and Cb 2 (Appangala 1) was inhibitory to *R. solani*.

GINGER

Genetic resources

Six hundred and sixty eight *Zingiber* accessions have been maintained in the field gene bank. Germplasm conservatory was enriched with 10 ginger accessions including two extra bold local ginger from Kerala and West Bengal (Fig 7a,b).

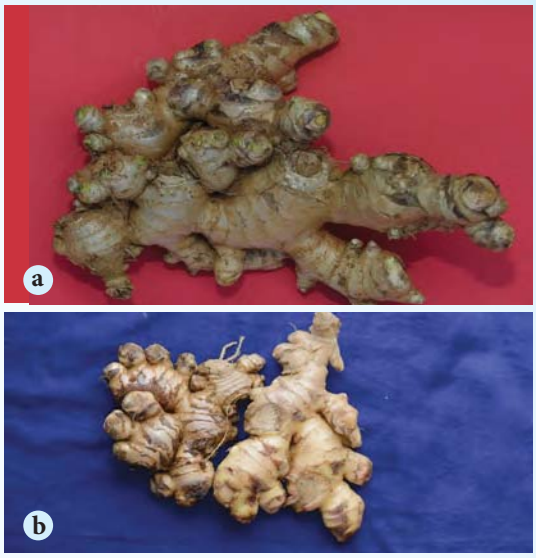


Fig. 7. Unique bold ginger collection from a. Kerala b. West Bengal

Breeding

Among 13 extra bold ginger accessions evaluated, maximum fresh and bold rhizomes were recorded in Acc. 723, Acc. 247 followed by Acc. 713

Four genotypes (IISR Varada, IISR Mahima, Acc. 182 and Acc. 247) were subjected to Yirradiation (900 buds each) at different doses of 0.80, 1.00 and 1.20 kR at Mangalore University, Mangaluru, Karnataka. Differential response was observed for germination (Fig 8). The M1V1 mutants were established in the green house for screening against *Pythium sp.*

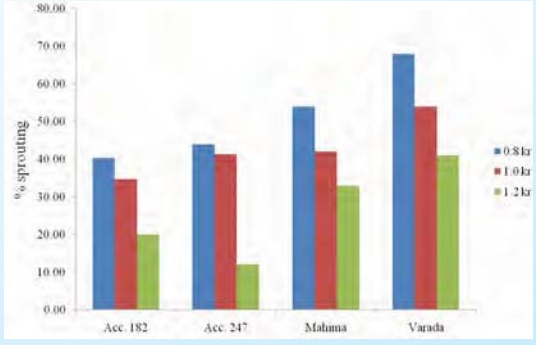


Fig. 8. Radio sensitivity of ginger cultivars exposed to different doses of gamma rays

Three potential mutants against *Ralstonia solanacearum* (HP 0.5/2, HP 0.5/15 and M 0.5/1) were clonally multiplied for further screening and evaluation. Also, three potential mutants identified against *Pyhtium* sp. (V 0.5/2, R 0.8/1 and R 1.25/4) were multiplied for further screening.

Genetic diversity in *Curcuma amada*

Thirty accessions of *C. amada* have been screened for resistance to race 4 strain of *R. solanacearum* by soil and pseudostem inoculation methods. Two accessions were found to be resistant by both soil and pseudostem inoculation. Bright field and fluorescence microscopic work was carried out with inoculated and uninoculated samples of *C. amada* and *Z. officinale*. It was noticed that the stelar portion of *C. amada* had extensively thick cell walls compared to *Z. officinale*. The casparian thickenings were clear and thick compared with the endodermal cells of *Z. officinale*.

Tissue specific expression analysis of shortlisted genes/ESTs using qPCR

Among the candidate genes LRR-NBS, ABC transporters, 4-coumarate: coenzyme A ligase (4-CL), WRKY transcription factor 8 and callose synthase were studied for their expression level in ginger and mango ginger at different time intervals (0, 1, 4, 8, 16, 24, 48, 72, 96 and 120 hpi) in leaves and pseudostem. In general, the expression patterns of the genes were higher in *C. amada* compared to *Z. officinale*.

Crop management

Fertigation scheduling

Fertigation schedule is being standardized under soil less ginger production using coir pith and farm yard manure (1:1). Five treatments with varying doses of fertilizers (75-125 %) and 75 % recommended dose + PGPR was laid out. Sampling at 120 DAP showed that the maximum dry matter was partitioned into stem (43-50 %) followed by rhizome (25-32 %) and control (solid fertilizer at monthly interval) followed by recommended dose had the maximum rhizome fresh weight.

Whole genome sequencing of *Ralstonia solanacearum*

Two strains of *R. solanacearum* (GRs-SIK and GRs-MEP) were Illumina sequenced and the raw data has been assembled using A5-miseq. Both the strains have been annotated using Prokka (a software tool for the rapid annotation of prokaryotic genomes). In GRs-MEP there are 5120 CDS, 80 tRNA, and 1tmRNAwhile GRs-SIK possesses 5080 CDS, 63 tRNA and 1 tmRNA. To better classify the predicted proteins from Prokka, a refined annotation has been done using Blast-2GO with 1.0E-3 as e-value cut off and 33 as HSP cut off length. The genomes were mined for various effector proteins and other virulence factors.

Pest management

Hairy Caterpillar (*Spilarctia obliqua*)

A new tetrahedral shaped, multiple nucleocapsid nucleopolyhedrovirus (IISR-NPV-02) (Fig. 9) isolated from *S. obliqua* was characterized based on sequencing of conserved baculovirus genes and restriction endonuclease analysis. Polyhedrin and lef-9 gene sequencing and phylogenetic analyses revealed that SpobNPV is a new addition to the group I NPVs and is very closely related to other NPVs infecting Arctiidae. Restriction endonuclease analysis with *Pst I, Xho I* and *Hind III* indicated that the approximate genome size of SpobNPV as 131 kb. In laboratory bioassays the LD50 value of the NPV against third instar of *S. obliqua* was 43 OBs/larva. The median lethal time for third instar larvae was 181.01 h at a dose of 1×10^6 OBs/ mL and 166.99 h at a dose of 1×10^8 OBs/ mL, respectively, indicating high virulence of the isolated new strain of SpobNPV.

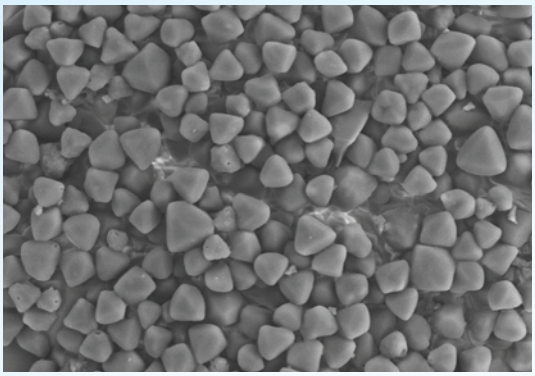


Fig. 9. Scanning electron microscopy of NPV infecting *S. obliqua*

TURMERIC

Genetic resources

One thousand four hundred and four *Curcuma* accessions are being maintained in the field gene bank. Germplasm conservatory was enriched with five *Curcuma* accessions.

Breeding

A multi-locational trial with three promising accessions (Acc. 48, Acc. 79 and Acc. 849) along IISR Prathiba and local check was laid out in Kerala (Peruvannamuzhi), Andhra Pradesh (Vijayawada), Tamil Nadu (Erode) and Karnataka (Chamrajanagar and Chettali). The short duration genotypes *viz.,* Acc. 48 and Acc. 79 performed well under different locations.

Molecular biology

Amplification of full length cDNA

A simple protocol for cloning of full length gene was optimized by inverse PCR combined with SMART system using gene specific primers. Full length cDNA of curcumin synthase 3 (curs3) with 137 bp of 5' UTR and 299 bp of 3' UTR was amplified from normalized cDNA library constructed from pooled tissues of turmeric using curs3 specific outward primers.

Cloning of specific miRNAs

Four miRNAs *viz.,* miR156, miR167, miR172 and miR396 were cloned and sequenced by stem loop RT-PCR method. Among these, targets of two miRNAs *viz.*, miR156 and miR172 were predicted and identified as squamosa promoter binding like genes and floral homeotic protein AETALA 2 like isoform X1, respectively. Targets were also identified for miRNAs identified through deep sequencing which mainly included conserved transcription factors. Important targets identified were growth regulating factors (GRFs), NAC domain containing proteins, F-box family proteins, GAMYB transcription factor like proteins, homeobox leucine zipper proteins, TCP transcription factors and three auxin response factors were targeted by miR396, miR164, miR394, miR319, miR166, miR171 and miR160, respectively.

Table 1. Details of miRNAs identified by cloning

miRNAs	Reference miRNAs	Abundance	Sequence length	Targeted gene
clo-miR156	osa-miR156	82	20	SPL
clo-miR167	ath-miR167	281	21	NA
clo-miR172	ath-miR172	13	21	ethylene responsive TF-AP2 like
clo-miR396	ath-miR396	751	21	NA

Mining for genomic SSRs

MultiNA analysis of 10 polymorphic genomic SSR primers (CLM 2, CLM 25, CLM 33, CLM 34, CLM 61, CumiSat 8, CumiSat 18, CumiSat 20, CumiSat 22, CumiSat 28) in 96 turmeric accessions was performed (Fig. 10). MultiNA is a microchip based electrophoresis system with high sensitivity detection that uses LED excited fluorescence detector. Although major differences among the released varieties could not be detected, the varieties Suvarna, Suguna and Sudarshana could be distinguished from other released varieties and also Suvarna showed a distinguishable pattern from the rest.

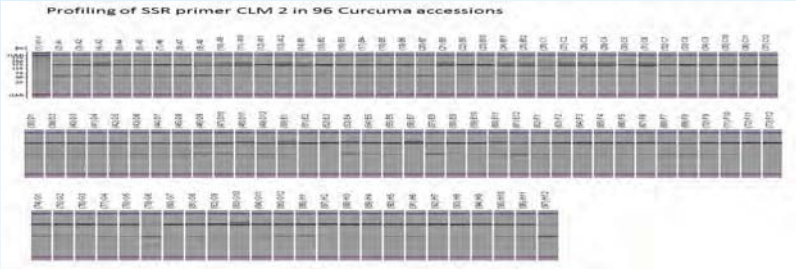


Fig. 10. Profiling of SSR primer CLM2 in 96 accessions of Curcuma

Influence of coloured shade nets on ginger and turmeric production

Ginger and turmeric were grown under red, green, white and black shade nets with conventional planting as control. Light (PAR) intensity in shade nets varied from 58-63% of open light intensity. Sampling at 140 DAP revealed that partitioning to rhizomes varied from 45-53% in ginger and 28-31% in turmeric. Total fresh weight and partitioning to rhizomes were maximum under red shade net in ginger but were almost similar in turmeric.

Pest management

Shoot Borer (*Conogethes punctiferalis*)

Studies on symbiotic bacteria of EPNs

The symbiotic bacterium associated with *Heterorhabditis* sp. (IISR-EPN 01), promising against shoot borer of ginger and turmeric was identified as *Photorhabdus luminescens* (IISR-EPN BC 09) on the basis of morphological, biochemical and molecular characterization.

Evaluation of EPNs

The efficacy of four promising EPNs such as *Heterorhabditids* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 02), *O. gingeri* (IISR-EPN 07) and *Oscheius* sp. (IISR-EPN 08) was tested against shoot borer infesting ginger and turmeric under field conditions. Among the test EPNs, *O. gingeri* (IISR-EPN 07) treated plants showed minimum shoot damage in ginger and turmeric (19.4 and 28.4 %, respectively) in comparison to control (36.9 and 51.9%, respectively) which was on par with malathion treatment (18.4 and 24.6%, respectively).

TREE SPICES

Genetic resources

A farmer participatory nutmeg germplasm collection was made in Idukki, Kottayam, and Malappuram districts of Kerala and 31 nutmeg germplasm including few farmers varieties were collected and conserved (Fig. 11a-e). The unique germplasm collected include a nutmeg with rudimentary sterile seed; nutmeg with bold nut; thick and entire mace type; high yielding monoecious nutmeg and *Punnathanam Jathi*, a farmers variety which had very bold nut and thick mace.

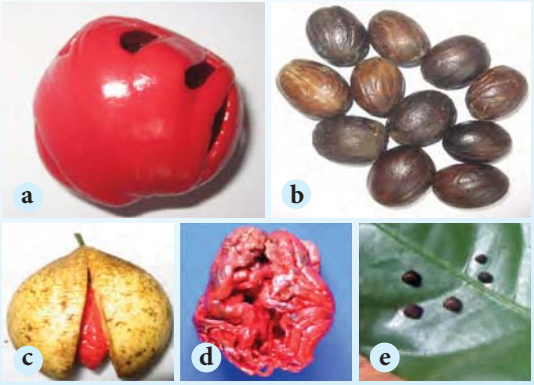


Fig. 11a. Nutmeg collection with thick mace covering entire seed

Fig. 11b. The bold nut collection

Fig. 11 c-e. Nutmeg with rudimentary sterile seed

Molecular biology

Isolation and amplification of genomic DNA from nutmeg mace

A protocol was developed to isolate high quality DNA from nutmeg mace (Fig. 12). The purity of the DNA was checked by qualitative and quantitative estimation, restriction digestion, RAPD and amplification of the barcoding loci rbcL and ITS.

Standardisation of barcoding loci rbcL and ITS for *Myristica* species (*M. fragrans, M. malabarica, M.andamanica, M. fatua, M. beddomei, M. amygdalina*)

The PCR temperature profiles were optimized with annealing temperatures of 52.5°C and 56°C for rbcL and ITS loci respectively. The rbcL and ITS amplicons yielded products of 600bp and 500bp respectively (Fig 12).

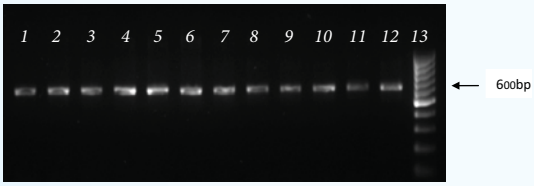


Fig. 12. Amplification of rbcL locus. Lanes 1-4 – *Myristica fragrans*, lanes 4-8- *M. malabarica*, lane 9- *M. andamanica,* lane 10*A*- *M. fatua*, lane 11- *M. beddomei*, lane 12- *M. amygdalina.*

Generation of barcode sequences

The matK barcode sequences for some *Cinnamomum* species (*C. verum, C. glaucescens, C. sulphuratum*) were generated and submitted to GenBank nucleotide database of NCBI.

Post harvest technology

Phytochemical analysis of *Myristica* species

Essential oil profile of nut, mace and pericarp of *M. fragrans* was studied. Nut and mace had similar composition, the chief components being sabinene, pinenes, myrcene, γ- terpinene, 4- terpineol, safrole, myristicin and elemicin. The pericarp of *M. fragrans* was dominated by 4-terpineol, α-terpineol, γ-terpinene, α-terpinene, pinenes and myrcene. Seeds of *M. prainii* and *M. fragrans* yielded 40% and 32% butter, respectively. Fatty acid profile of nuts of *M. prainii* and *M.fragrans* indicated that both were dominated by myristic acid (>80%).

Antioxidant activity of volatiles of nutmeg

Antioxidant activity of major essential oil constituents of *M. fragrans viz.*, myristicin, 4- terpineol and α-terpineol were compared by DPPH and Phoshomolybdenum methods. Results showed that myristicin had higher antioxidant potential.

Documentation of natural enemies

Surveys for entomopathogens and other natural enemies of spice crop (black pepper, cardamom, ginger, turmeric, garcinia and nutmeg) pests were conducted in Idukki, Wayanad and Kozhikode districts of Kerala, Coimbatore and Nilgiris Districts in Tamil Nadu and Dimapur District in Nagaland. The host insects included black pepper scale, *Aspidiotus destructor*, cardamom thrips, *S. cardamomi,* cardamom scale, *Aulacaspis* sp., garcinia hopper, *Busoniomimus manjunathi*, and nutmeg shoot borer, *Sinoxylon anale*. The fungus infecting *B. manjunathi* was identified as *Metarhizium flavoviridae* (IISR-EPF-03) and the fungus infecting *S. cardamomi* as *Isaria sp.*(IISR-EPF-03) based on morphological and molecular studies. The identity of the four larval and pupal parasitoids of ginger shoot borer collected during the surveys were confirmed as *Eriborus ricini, Xanthopimpla stemmator, Trathala flavoorbitalis* and *Apanteles sp*.

INSTITUTE TECHNOLOGY MANAGEMENT UNIT (ITMU)

During the year, four licenses have been issued to Rainbow Agri Life, Kadapa, Andhra

Pradesh thorough NRDC for the commercialization of micronutrient mixtures for black pepper, ginger, turmeric and cardamom. The license for turmeric variety, IISR Prathibha was renewed. A non exclusive license for the commercial production of *Trichoderma harzianum* was issued to District Agricultural Farm, Thaliparamba, Kerala. The office and incubation facilities were licensed to a private company.

Four consultancy visits were carried out to deliver technical advices to plantations. As a part of BPD activities, the scientists of the institute participated and delivered lectures on processing of spices and business incubation facility of IISR during the workshops organized by the District Industries Centre Wayanad, Kerala, State Small Industries Association etc. A Spice Processing Unit with facilities for cleaning and grading of black pepper, white pepper and curry powder production has been installed at IISR Experimental Farm, Peruvannamuzhi (Fig. 13).

Fig. 13. Spice processing unit at IISR farm, Peruvannamuzhi

EXTENSION AND TRAINING

Transfer of technology programmes

The institute conducted 17 training programmes of various durations for effective technology transfer to diverse stakeholder groups like farmers, youth, tribal beneficiaries and students. The training programmes included both on-campus and off campus trainings. Six training programmes were conducted exclusively for the benefit of tribal farmers. The training programmes and strategies for technology transfer were deployed across a wide geographical region through leveraging the linkages with other institutions in public, NGO and cooperative sector. Apart from this, the Agricultural Technology Information Center facilitated and organized group visits and short orientation programmes for organized groups of students and farmers. The visitors included farmers, farm women and students from other states also. The customized advisory services offered by the ATIC remain popular and was availed by more than 3500 stakeholders during the last year. The advisory services was provided across categories like varietal selection, plant protection, facilitating input delivery, nutrient management etc.

Rehabilitation package for Wayanad

Soil samples collected from hot spot areas in four Panchayats (180 nos) were analyzed for the pathogen load of which 10 were found to be *Phytophthora* positive and advisories were given for control. The soil and leaf samples were also analyzed for major, secondary and micro nutrients and results with crop specific recommendations passed on to the farmers. Five visits were made by team of scientists to hot spot areas to educate on soil health and disease problems including three farmers' seminars on black pepper nursery/ cultivation. Seventy five FLD plots spread across Poothadi, Mullankolli, Pulpally, Thirunelli and Meppadi Panchayats to demonstrate combating yellowing of black pepper are being maintained by supply of inputs like neem cake, vermicompost, bio control agents and micronutrient mixtures. The plots with moderate to high yellowing have become healthy by the adoption of site specific technology package involving soil acidity correction, biocontrol application and micronutrient management.

Study on trends in export of spice oils and oleoresins from India

The export of value added commodities derived from spices have shown a sustained positive

trend over the last several years. The increase has occurred across the commodities, which indicates a general shift in the preference for value added products over the raw materials in the major export markets for Indian spices. The fact that food industry has evolved as the major consumer of spices, replacing the retail household consumer segment, has aided this shift in favor of value added products like spice oils and spice oleoresins. The processed food industry is one of the major segments which has shown robust growth during the immediate past. It is expected that this segment shall witness further growth and consolidation in the medium term. This means that the demand for value added spice derivatives in the export market shall remain strong in the coming years.

Quick estimate of potential of technology interventions in black pepper

The district wise data on district wise area production and productivity of black pepper in Kerala and Karnataka for the period corresponding to the 11th five year plan was used to estimate the yield gap and the potential for yield enhancement through technology adoption in these states. The technology backed yield potential was calculated for individual states based on the yield of pepper obtained in the varietal trials conducted in the state under All India Coordinated Research Project on Spices (AICRPS) during the period 2007-08-2011-12. The average yield gap of black pepper in Kerala and Karnataka was estimated to be 309 kg/ha and 634 kg/ha respectively. The total production gap in quantity terms due to non-adoption of technology was estimated to be about 50,000 tonnes at the national level. The technology generated by the public funded research institutions offer tremendous scope for enhancing the productivity levels of pepper in the country.

KRISHI VIGYAN KENDRA

During the period, KVK has conducted 107 training programmes for practicing farmers and farm women, rural youth and extension functionaries in the disciplines of agronomy, horticulture, animal sciences, home science, fisheries, plant protection and allied fields. A total of 3263 trainees were benefitted out of the programmes. Nine Front Line Demonstrations and five On Farm Trials on technology assessment and refinement were carried out during this period. Among these, technologies on upland rice cultivation (Fig. 14a), IISR nutrient mixture for ginger, cultivation of Renusree variety of amaranthus, transplanting technique of ginger using pro-trays were well received by farmers. One gardeners' training programme of six months duration was organized under the sponsorship of State Horticulture Mission empowering 25 rural youth. Out of these, 12 trainees started self-employment units in various nursery activities. The Kendra operates a Plant and Animal Clinic offering various services to farmers, in which 570 consultancy services, 12700 vaccinations of poultry birds and animals and two animal health campaigns (Fig. 14b) were conducted. KVK also gives soil health cards to farmers after testing soils from their plots. Participatory seed production on high yielding varieties of ginger and turmeric was also taken up in four farmers plots. The Centre is providing Short Message Service (SMS) to all registered farmers on latest updates in agriculture and allied field over their mobile phones. KVK has so far sent twelve SMS and five voice messages benefitting 743 farmers and 151 Extension functionaries. The Kendra conducted nine seminars, participated in nine Kisan Mela cum exhibitions, broadcasted six radio talks and conducted three study tours for farmers to various research institutes. During this period an amount of Rs.5.19 lakhs has been realized through sale of various technological inputs benefiting 8572 farmers. KVK started a mobile sales unit to supply planting materials and other inputs to farmers of remote areas of the district. KVK also documented eight farmer innovations during this period which was selected by ATMA for further refinement. A hatchery unit with a production capacity of 20000 day old layer chicks per month was developed at KVK during this period.

Fig. 14 a. Harvesting upland rice, b. animal health campaign

ALL INDIA COORDINATED RESEARCH PROJECT ON SPICES

Genetic resources

This year over 75 new collections were collected in black pepper, ginger, turmeric, nutmeg, and clove.

New varieties

Developed five high yielding spices varieties, which were recommended for release:

- Two cardamom varieties –Appangala 2 (first hybrid resistant to Katte virus) and PV 3 (moderately resistant to drought)
- Two coriander varieties – RCr 475 (bushy and erect plant type) and Narendra Dhania 2 (dual purpose variety)
- LFC 103: A high yielding Fenugreek variety – suitable for both irrigated and rainfed conditions

Safer new molecule for integrated disease management in black pepper

New fungi toxicant molecule Fenomidone (10%) + Mancozeb (50%) @ 2 L/vine as spray and 3 L/vine as drenching along with *Trichoderma harzianum* 50 g with 1.0 kg of neem cake as soil application during 1st week of June and 3rd of August reduced leaf infection, yellowing, defoliation and death of vines and recommended for adoption in Karnataka state.

Fertigation in cardamom

Technology for fertigation in small cardamom was developed which saves 44% water and 25% fertilizer by application of 9 L/clump with 75% of recommended dose of fertilizers.

Nutrient supplementation through organic manure in ginger

For integrated nutrient management in ginger the fertilizer dosage of FYM, 30 t/ha + NPK 80:50:50 kg/ha under Bihar conditions was recommended.

Control of rhizome rot of ginger by biofumigation

Crop residues of mustard and cabbage incorporated in soil (biofumigation) and rhizome treatment with Metalaxyl + Mancozeb 1.25 g/L of water for 15-20 minutes is recommended for management of rhizome rot of ginger.

Micronutrients for yield enhancement in turmeric

For iron deficient soils of Bihar foliar application of ferrous sulphate @ 0.5% at 60 and 90 days after planting is beneficial for yield enhancement.

Nutrient management in off-season coriander leaf production was standardized

Application of NPK @ 30:40:20 kg/ ha along with spraying of GA, 15 ppm at 20 DAS was recommended to get maximum leaf yield of coriander (4824 kg/ha). Yield increase of 25% over control was observed.

PGPR in fenugreek, coriander and fennel

Seed pelletizing with IISR PGPR strains either FK-14 (*Pseudomonas putida*) or FL-18 (*Macrobacterium paraoxydans*) or combination of both was found as effective as talc formulation @ 1.5 kg/ha seed treatment. The treatment has increased the yield on average by 10-15% in Andhra Pradesh, Rajasthan, Gujarat, Haryana and Uttar Pradesh conditions

Micro irrigation in Fennel

Micro irrigation technology was developed for higher seed yield (30.8 t/ha) in fennel when fennel was planted in paired rows with drip irrigation at 0.8 IW/CPE ratio.

HUMAN RESOURCE DEVELOPMENT

Trainings conducted

- DBT sponsored short term training course on Genomics and proteomics in plants and microbes towards translational research, January 21 – February 10, 2015 (Fig. 15).

Fig 15. DBT Short term training on 'Genomics and proteomics in plants and microbes towards translational research'

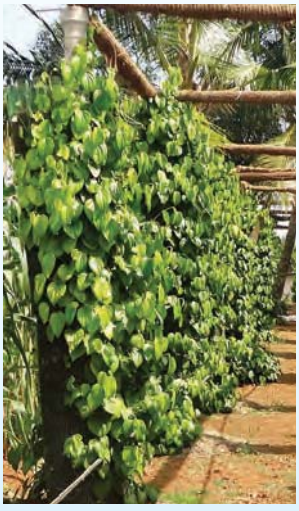
NEW NETWORK PROJECTS

ICAR has approved two new network projects *viz.*, High Value Compounds and Organic Horticulture in XII plan with ICAR-IISR as the Nodal Institute. The projects started functioning by November 2014.

The total budget of High Value Compounds and Phytochemicals is Rs. 2560 lakhs for the XII plan period with nine ICAR partner institutes. Prediction and validation of nutraceuticals and functional properties of phytochemicals/ and high value compounds identified from selected crop plants, developing knowledge base, *in silico*, *in vitro* and *in vivo* validations, and developing formulations are the major objectives of the project.

The broad objectives identified for Network Project on Organic Farming in Horticulture Crops are, evaluation of suitable organic amendments for meeting the nutrient requirement and pest and disease management, developing an organic package for different horticulture crops. This network includes nine ICAR research institutes with a budget allocation of Rs. 300 lakhs for the XII plan period.

Intensifying black pepper production



Traditionally pepper is trailed on live support trees in India, whereas, in few other pepper producing countries non-living support mainly wooden poles are also used. A new and novel idea of providing support with rooting media was conceptualized and six months old rooted top shoots with one or two lateral branches were planted during April 2014. The vertical column (3 m height, 50 cm width) was made with a plastic coated welded wire mesh filled with composted pasteurised cocopeat and powdered dry cow dung @ 3:1 ratio. The column was irrigated regularly with drip system; nutrient was applied in liquid form through media and foliar. As and when vines put-forth new node, it was firmly fixed along the rooting medium filled in the vertical column and this facilitated in converting the clinging root to absorbing root, which in turn accelerated the growth of pepper. In this way the pepper vine covered the entire column within 10 months time and started producing spike in the same year.

Spicing up the nation's progress

हर कदम, हर डगर
किसानों का हमसफर
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