भाकृअनुप
ICAR

Research Highlights
2015-16

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

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PREFACE

The highlights of research achievements of the institute during 2015 - 16 are presented in this publication. During the year, 193 *Piper* accessions including 134 cultivars and 59 wild types were collected. Besides the field gene bank at IISR, Pervannamuzhi, alternate field gene banks for black pepper are maintained at CHES, Chettalli, Karnataka and IISR, Chelavoor, Kerala. A high yielding, short duration turmeric line, Acc. 48, performed well under multi-locational (MLT) and All India Coordinated varietal (AICRPS) trials. Key genes and transcription factors with putative regulatory roles on curcumin biosynthesis were identified. Farmer participatory surveys were conducted in Thrissur, Idukki and Kottayam districts of Kerala and 19 germplasm accessions of nutmeg were collected

Strategy for management of virus affected black pepper gardens for yield sustainability was developed where marked improvement in the health of the vines was recorded. A renewable solar energy unit for turmeric curing was established at Experimental farm, Peruvannamuzhi. A QSAR model was developed to predict anti-oxidant properties of natural compounds in spices.

Application of *Lecanicillium psalliotae,* a entomopathogenic fungus, as soil drench was found to be promising for the management of thrips in cardamom. Comparative genomics of 10 Indian isolates of *Ralstonia solanacearum* was undertaken. Morphological and molecular characterization of eight *Phytophthora* isolates causing leaf and fruit fall of nutmeg revealed their close relatedness to *Phytophthora meadii.*

Advisory services of the Agricultural Technology Information Center were delivered to more than 1200 clients. Twelve training programmes were conducted by the institute targeting diverse stakeholder groups like farmers, youth, tribal beneficiaries and students. The institute participated in 14 exhibitions during the last year. In KVK, 88 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted. Nine front line demonstrations and five on farm trials on technology assessment and refinement were carried out.

During the year, the ITM-BPD Unit facilitated non exclusive licensing of ginger and nutmeg varieties. The Spice Processing Unit at Peruvannamuzhi farm became operational this year. Consequently, four license agreements were signed with clients for utilizing this facility. The novel method for delivery of PGPR *viz.*, "Biocapsule Technology" has attracted many biofertilizer manufacturing companies and the institute signed a non-exclusive agreement for its commercialization. Samples were provided to M/S. Mahyco Seeds for evaluation.

Overall, the efforts of the scientists, staffs, associates, farmers, planters and entrepreneurs have been unprecedented, overwhelming and encouraging. The relentless support and fair appraisal are what give me the verve to strive for betterment and strengthening of our research and extension programmes to trigger the change we want to witness in the spices sector.

I consider it a privilege to place on record the encouragement given by Dr. S. Ayyappan, Secretary, DARE and Director General, ICAR. I am also grateful for the strong support and necessary guidance received from Dr. N.K. Krishna Kumar, Deputy Director General (Hort. Science) and Dr. T. Janakiram, ADG (Hort. Science). 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 appreciate the editors for having compiled and brought out this publication.

Kozhikode
04.03.2016

(M. Anandaraj)

Contents

BLACK PEPPER

Genetic resources

Presently, 3213 *Piper* accessions are being maintained in the conservatory. A field gene bank with 200 accessions has been established at Peruvannamuzhi. The alternate field gene bank at CHES, Chettalli, Karnataka now comprises of 27 accessions including 200 accessions planted during the year. A field gene bank comprising of 223 cultivar accessions are maintained at Chelavoor campus.

The black pepper accessions were characterized and a catalogue was prepared. Passport details, morphological and reproductive characters, qualitative and quantitative characters and reaction to biotic and abiotic stress of each accession along with a photograph are included in the catalogue (Fig. 1).

Fig. 1. Specimen page of black pepper catalogue

During this year, a total of 193 *Piper* accessions including 134 cultivars and 59 wild types have been collected including an accession resembling Narayakodi with persistent stigma, ovate fruits and short spikes (Fig. 2).

Breeding

A trial involving 10 improved lines/selections was laid out at Peruvannamuzhi farm. One of the entries appears to be of early maturity. The entry, HP 117 x Thommankodi was ready for harvest by November with a mean fresh yield of 1.45 kg/vine.

Fig. 2. Black pepper accession with persistent stigma and ovate berries

Mining and identification of single nucleotide polymorphisms (SNP)

Unique SNP profiles have been developed for 14 cultivars *viz*., Panniyur 1, Panniyur 5, Panniyur 6, Panniyur 8, IISR Girimunda, IISR Malabar Excel, Subhkara, Panchami, Pournami, PLD2, Chumala, Acc 819, OPKM and Narayakkodi. Five different SNP patterns were found shared by two cultivars each, Panniyur 2 - Vadakkan, Panniyur 3 – Arka Coorg Excel, Panniyur 4 - Agali pepper, Panniyur 7 - Sreekara and Thevam - Shakthi (Fig . 3).

Fig. 3. Gel picture showing presence and absence of amplicons based on SNP in 24 black pepper cultivars.

Mining of antimicrobial peptides (AMP)

One hundred and twenty seven sequences of *P. colubrinum* transcripts showed significant similarity with the reference AMPs and 111 of the *P. nigrum* transcript sequences also showed

significant similarity. Eleven sequences with motif CX{3,30}CX{3}CX{3,30}CX{3,30} CXC and 48 AMPs with motif CX{3,5} CX{8,17}CX{4,6}C (where C is cysteine, X is any residue except cysteine and numbers in curly braces denote a range of variable residues) were discovered in *P. nigrum*.

Identified 24 signature peptides for the AMPs form black pepper using shot gun proteomics platform. The groups includes the cyclotides, defensins, thionins, lipid transfer proteins, snakins and hevein like proteins. These AMPs are expressed during black pepper – *Phytophthora* interaction and gives insight into the mechanism of resistance.

Crop management

Climate change and production

Recent studies in Madikeri, Karnataka indicated that low rainfall (73 mm), few rainy days (10) and low temperature (16°C mean during May) during pre-monsoon season (March-May) and very heavy rainfall (2917 mm), more number of rainy days (89), low light intensity (60-140 µ moles) with cloud cover, and low maximum and minimum temperatures during June to August (spike initiation period) in 2013 negatively influenced black pepper productivity. This resulted in very low and delayed (September) spike emergence. Moreover, there was a shift in flower formation from bisexual phase to female phase leading to poor pollination, very low number of berries per/spike which ultimately lead to very low yield.

Another investigation revealed that shade regulation and irrigation practices taken up during the month of March with continued irrigation till monsoon followed by a normal monsoon recorded higher spike initiation (65-70 spikes/m^2) with more number of berries (70-80 berries/spike) during the month of June-July, which ultimately resulted in higher productivity.

Management of virus affected gardens

Trials were taken on the management of virus affected black pepper gardens for rejuvenating its health and sustaining the yield at three estates in Madikeri district of Karnataka. The vines categorized as mild recorded significantly higher spike intensity, canopy development, health status and yield as compared to moderate infected vines. The health status of the vines scored 2.9-3.2 (on the scale of 1-5 from severely infected to very healthy) under both categories showing improved health. By the application of treatments (FYM, NPK, Micronutrients and PGPR) there was a marked improvement in the health of the vines (with a score of 3.1-3.25) as compared to control (2.8). The spike intensity (per 0.5 m^2) and yield were also significantly higher in treatments with nutrients and PGPR supplementation (3.19 kg/ std) as compared to control (1.9 kg/ std).

Post-harvest technology

Development of mechanical unit for production of white pepper

A mechanical unit was developed and evaluated for production of white pepper from dried black pepper (Panniyur 1). Fermentation took place inside a tightly closed vessel. Water was changed every alternate day for 12 days. The outer skin was removed and the white pepper obtained was dried in the solar tunnel drier. White pepper obtained had a dry recovery of 68.7% and the capacity of the pulping unit was 125 kg/h.

Plant health management

Variability in *Phytophthora* spp.

Comparative studies on colony morphology, sporangial ontogeny, sporangial morphology, mating and physiological studies of *Phytophthora* isolates revealed prevalence of two groups – *P. capsici* and *P. tropicalis* with wide variations within the group. However, a multi-locus sequence typing (MLST) involving nuclear and mitochondrial genes and whole genome sequencing (WGS) indicated the presence of a wide variety of genotypes with mixed characters indicating species diversification in black pepper agro-ecosystems.

Piper colubrinum - *Phytophthora capsici* interaction

Defense related genes, peroxidase and PAL showed highest level of expression at 24 hpi in *P.*

colubrinum challenged with *P. capsici*, with a fold increase of 450 and 18, respectively. Genes like *catechol oxidase*, *cinnamoyl coA reductase* and *polyphenol oxidase* showed the highest expression at 16 hpi with a fold increase of 350, 70 and 220. PR-1, PR-14, *chalcone isomerase* and EDS1 had a high expression at early stages of interaction *i.e.* at 4 hpi.

R genes in *P. colubrinum*

In silico analysis revealed 1371 unique *R* gene sequences in *P. colubrinum* transcriptome and transcripts belonging to all four major *R* gene classes were identified except TIR-NBS-LRR type. The expression patterns of 12 candidate *R* genes were evaluated at different hours post inoculation (hpi) on challenge inoculation with two isolates of *P. capsici,* 05-06 and 98-93. Eleven *R* genes showed similar expression pattern with both isolates and the expression of *R* genes was maximum in the initial hours of interaction.

P. nigrum - P. capsici interaction

Differential expression analysis of 11 resistant gene analogues (RGAs) in resistant (IISR Shakti) and susceptible (Subhakara) black pepper lines showed significantly higher transcript levels of PnRGA1, PnRGA8, PnRGA11 and PnRGA24 in IISR Shakthi, with the maximum expression level of 6 fold increase at 8 hpi for PnRGA24.

Evaluation of new fungicides against anthracnose disease

Among the seven fungicide molecules tested against *Colletotrichum gloeosporioides*, fenamidone + mancozeb (0.3, 0.2, 0.1, 0.05, and 0.025 %) and carbendazim + mancozeb (0.15, 0.1, 0.05, 0.025 and 0.0125 %) were found to completely inhibit the growth of the pathogen under *in vitro* conditions.

Metagenomics

To understand the rhizosphere microbiome in growth promotion and biocontrol activity of *Trichoderma harzianum* - black pepper interactive system, the metagenomic profile of black pepper-rhizosphere using shot gun whole genome sequencing (Illumina hiseq) was unraveled. The metagenomic datasets from rhizosphere with and without *Trichoderma* inoculation showed the taxonomic shift of rhizosphere bacteria, archae and eukaryote. The functional assignments and the metabolic pathway enrichment in *Trichoderma* amended soil showed the selective recruitment of organisms in terms of abundance in population and specific metabolic pathway functions in the soil.

Complete genome sequencing of *Cucumber mosaic virus*

The complete genome of *Cucumber mosaic virus* (CMV) was sequenced and compared with 27 CMV isolates reported worldwide from groups I and II. Percent identity and phylogenetic analysis clearly indicated that the current isolate belongs to subgroup IB. Sequence analysis also showed the presence of a rare deletion of nine nucleotides in the putative methyl transferase domain of *1a* gene which was observed only in one more isolate of CMV. The level of gene conservation among the CMV subgroups was highest in the *coat protein* gene and lowest in *2b*. The ratio of non-synonymous to the synonymous substitution of these genes was in the order *2a>2b>3a>3b>1a*.

Production and testing of somatic embryo derived plants for *Piper yellow mottle virus* (PYMoV)

Mature berries obtained from black pepper plants infected with PYMoV were used for somatic embryogenesis. For this the starting material was embryo scooped out along with surrounding micropylar tissue from the surface sterilized seeds. Primary somatic embryo was induced after 45 to 85 days and secondary somatic embryos were visible within 65 to 100 days in different black pepper varieties. Secondary somatic embryo gave rise to cyclic secondary somatic embryos from which plantlets were regenerated and hardened in the greenhouse. Out of 53 somatic embryo derived plants tested for PYMoV using primers specific for four ORF regions of the virus, nine plants showed freedom from PYMoV.

Occurrence of endogenous *Piper yellow mottle virus* (ePYMoV)

Studies based on polymerase chain reaction (PCR), reverse transcription (RT) PCR, and Southern hybridization of total DNA from PYMoV infected black pepper plants probed with PYMoV specific sequence indicated the occurrence of integrated PYMoV sequence in black pepper.

Studies on physiological parameters in virus infected plants

Physiological parameters such as stomatal conductance, photosynthetic rate, chlorophyll fluorescence, chlorophyll content and mid-day relative water content were studied in healthy and virus infected plants of three varieties of black pepper namely, IISR Malabar Excel, IISR Thevam and Subhakara. Results showed slightly higher values for all parameters in healthy plants of all three varieties compared to infected plants.

Differential protein expression in PYMoV infected plants under temperature stress

The LC-MS analysis of a few selected differentially expressed proteins showed down regulation of membrane kinase, NBD sugar kinase, rubisco activase, plastocyanin and heat shock protein 60 family suggesting that catalytic activity, photosynthesis and metabolism were affected during virus symptom expression. Unique proteins expressed in symptomatic plants included thylakoid acid phosphatase which has a role in symptom development, 2 cys-periredoxin BAS1, SOD (Copper/zinc binding family), Miraculin like protein with trypsin activity (Increased protease inhibitor in CTV infected libraries). In addition, symptomatic plants also showed many unique peptide signatures of badnavirus in general, and ORF 3 and ORF 4 of PYMoV, in particular.

New pesticides against nematodes

Nematicidal activity of five new pesticides *viz*., thiamethoxam, flubendiamide, cartap hydrochloride, carbosulfan (granular formation) and chlorantraniliprole were tested under greenhouse conditions. Carbosulfan and flubendiamide were found promising in suppressing *Radopholus similis*. Field trials for evaluating efficacy of carbosulfan (liquid formulation) and fipronil were laid out at four locations : ICAR-IISR Experimental Farm, Peruvannamuzhi, CPCRI, Kasargod and at two farmers' plots in Wayanad and Kodagu districts. Initial results indicated that application of carbosulfan/fipronil at quarterly intervals or twice a year (pre- and post-monsoon) significantly reduced *R. similis* population in soil.

Differential display of genes on colonization with endophytic bacterium

Differential display of gene expression consequent to colonization of black pepper roots (Sreekara) with *Pseudomonas putida* BP25 was studied through Suppression Subtractive Hybridization technology. Analysis of ESTs revealed the differential expression of genes responsible for different defence responses such as pathogenesis related proteins (PR 1 and PR 4), glutathione S-transferase, catalase, metallothionein-2, WRKY transcription factor 40 etc.

Field evaluation of endophytic bacteria

Maximum growth of plants and least incidence of disease were observed in black pepper plots treated with *Trichoderma-Pochonia* combination followed by endophytic bacterium (*Curtobacterium luteum*) + Metalaxyl-Mz and *P. putida* + Carbosulfan combinations.

EPNs suppress leaf feeding caterpillars

Infectivity of eight native entomopathogenic nematodes was tested against semi-looper (*Synegia* sp.) under *in vitro* conditions. Among the test EPNs, *Steinernema* sp. (IISR-EPN 02) and *Oscheius gingeri* (IISR-EPN 07) were found more pathogenic to the insect causing 100% mortality within 72 h.

CARDAMOM

Genetic resources

Four hundred and five cardamom accessions conserved at the germplasm repository were replanted and 105 accessions were characterized for

morphological and yield characters. FGB 75 recorded maximum yield and more number of capsules per plant. Field screening of cardamom accessions for leaf blight and rhizome rot resistance resulted in identifying highly resistant accessions to rhizome rot (FGB 63, FGB 70, FGB 82, FGB 83, FGB 85 and FGB 108) and one highly resistant accession to leaf blight (FGB 130).

Breeding

In a Preliminary Evaluation Trial (PET III), 23 inter-varietal F1 hybrids were evaluated for morphological and yield characters. The hybrid, Mudigere 2 x IISR Avinash recorded maximum plant height with more number of leaves, whereas the hybrid Mudigere 2 x Appangala 1 registered more number of capsules with highest fresh weight. The disease intensity of leaf blight and rhizome rot disease in these hybrids ranged between 3.33 – 43.33% and 0 – 8.88%, respectively.

Crop management

Organic Farming

Organic composts, FYM, and neem cake combinations are being evaluated including bio agents like *L. psalliotae*, Spinosad (a natural insecticide derived from actinomycetes *Saccharopolyspora spinosa*) and *Trichoderma harzianum* at AVT Plantations, Wayanad and IISR Regional Station, Appangala, Karnataka. The yield recorded at RARS, Ambalavayal, Kerala in pest management trial was higher in all the organic treatments than in control.

Plant health management

Evaluation of potential *Trichoderma* isolates against rhizome rot pathogens

Nine shortlisted *Trichoderma* isolates *viz.*, TN-3, KA-1, KA-20, KA-3, KL-3, KL-10, KL-19 and

Fig. 4. a *Trichoderma* isolate (KA-3) against *P. vexans*; b. *Trichoderma* isolate (KA-20) against *R. solani*; c. *Trichoderma* isolate (KA-3) against *F. oxysporum*

KL-17 were evaluated against *Pythium vexans*, *Rhizoctonia solani* and *Fusarium oxysporum* under greenhouse conditions. The isolate KA-3 was found to be the most effective isolate against *P. vexans* (Fig 4a) and *F. oxysporum* whereas KA-20 was effective against *R. solani* (Fig 4 b,c).

Screening of endophytic fungi and bacteria

Endophytic fungi and bacteria isolated from *Alpinia mutica*, *A. galanga* and *Amomum microstephanum* were evaluated for their antagonistic efficacy against *R. solani*, *F. oxysporum* and *C. gloeosporioides*. Among the isolates, AmL 1C, AgR 5A, AgR 5D and AmiPs 4C were found promising against *R. solani*. AmL 1B, AgR 5D and AmiPs 4A were effective against *F. oxysporum* while AmL 1B and AmiPs 4A were inhibitory to *C. gloeosporioides*.

Evaluation of chemicals against rhizome rot pathogens

Efficacy of four shortlisted fungicides *viz*., fenamidone + mancozeb (0.2%), captan + hexaconazole (0.2%), metalaxyl + mancozeb (0.125%) and tebuconazole (0.05%) against *P. vexans*, *R. solani* and *F. oxysporum* was studied in a glasshouse. Among the fungicides tested, tebuconazole proved to be effective against *R. solani* and *F. oxysporum*, whereas metalaxyl + mancozeb was effective against *P. vexans*.

Standardization of spray schedule against cardamom thrips

Four promising insecticides and natural products (fipronil 0.005%, imidacloprid 0.009%, quinalphos 0.05% and spinosad 0.135%) were evaluated in the field for the management of cardamom thrips (*Sciothrips cardamomi*) at Appangala, Kodagu. The spray schedule included three sprays during March, May and August along with standard spray schedule (five sprays during March, April, May, August and September). The trial indicated that reduction of damage by thrips by three sprays was equally effective as five sprays and all the treatments were on par with each other and significantly superior over control.

Effect of insecticide spray schedules on pollinators

The effect of insecticide spray schedules on honey bee pollinators was studied in insecticide treated plots from 6.00 am to 5.00 pm at Appangala, Kodagu. Honey bee activity in all the treatments including control was higher during 1.00 pm to 3.00 pm and there were no activity after 5.00 pm. The mean bee visits per flower during 6.00 am to 5.00 pm was maximum in quinalphos treated plots (35.1), followed by imidacloprid (28.6), control (20.9), fipronil (16.8) and spinosad (14.2).

Evaluation of entomopathogenic fungus

Evaluation of the entomopathogenic fungus *L. psalliotae* in the field at Wayanad, Idukki and Kodagu districts for the management of cardamom thrips indicated that application of *L. psalliotae* in soil as well as spray + soil application was promising for the management of thrips in all locations.

Role of *Wolbachia* in physiology of cardamom thrips

Studies on the effect of tetracycline treatment in removal of *Wolbachia* from cardamom thrips indicated that *Wolbachia* was removed from the test insects and tetracycline treatment in laboratory-bred populations of thrips reduced egg hatchability to 15.3% whereas in control egg hatchability was 53.7%. In F1 generation, the survivability of thrips from egg to adult was 36.6% and in control it was 53.7%.

Evaluation of insecticides against shoot borer

Evaluation of four insecticides and natural products (fipronil 0.005%, imidacloprid 0.009%, quinalphos 0.05% and spinosad 0.135%) in the field at Appangala, Kodagu in two spray schedules namely, three sprays during March, May and August and five sprays during March, April, May, August and September, indicated that reduction in shoot borer damage on the capsules was promising in plots treated with fipronil (3 and 5 sprays) and spinosad (3 and 5 sprays).

GINGER

Genetic resources

Six hundred and sixty eight ginger accessions have been maintained in the field gene bank. The ginger germplasm conservatory was enriched with 24 ginger accessions collected from Nagaland, Kerala and Karnataka. The characteristic collections includes, extra bold ginger from Nagaland and putative wild types from Periyar Tiger Reserve, Kerala, red ginger (*Z. officinale* var. *rubra?)* and Ellakallan (Fig 5a, b) from Santhan Para, Idukki, Kerala.

Fig. 5a. Red ginger, a unique ginger germplasm collection

Fig. 5b. Ellakallan, a germplasm collection from Kerala

Breeding

Evaluation of extra bold ginger accessions resulted in shortlisting of accessions Acc. 723, Acc. 247 and Acc. 713 for high yield and bold rhizomes. Chromosome indexing of bold rhizome type ginger accessions confirmed that all the accessions are having 2n=22, indicating that boldness is not due to polyploidy but genotypic in nature. Two bold accessions showed aneu-somatic variation with 2n=21 and 2n=23.

In order to induce resistance to rhizome rot, five genotypes (Gorubathane, Rejatha, Acc. 578, HP 0.5/16 and M 0.5/12) were subjected to gamma irradiation (500 buds each) at different doses of 0.80 and 1.00 kR. The M1V1 mutants were established in the green house for screening against *Pythium* sp. Two genotypes (Gorubathane and M 0.5/12) were also tried against chronic exposure of gamma rays (0.1 and 0.2 kR for 4 h) for inducing more variability and needs further standardization to fix the dosage and exposure time.

Crop management

Fertigation scheduling

A soil less culture consisting of coir pith and farm yard manure in 1:1 proportion with fertigation is being standardized for ginger production. Two fertigation frequencies, once in three days and once in six days with five treatments were tried. Micronutrient spray and calcium nitrate drenching were given in alternate weeks. Results showed that 75% recommended dose of fertilizers through fertigation had the maximum partitioning to rhizome (35%) at 120 days after planting. Rhizome yield was maximum in the same treatment (15% higher compared to recommended dose as solid fertilizers). In general, fertigation once in three days had better response than fertigation once in six days.

Influence of coloured shade nets on yield and quality

Ginger was grown under red, green, white and black shade nets with open as control. Light (PAR) intensity in shade nets varied from 58-63% of open light intensity. Sampling just before harvest revealed

that rhizome dry weight was about 10-12% higher in ginger under red shade net compared to open condition.

Plant health management

New isolate of *Ralstonia*

A new isolate of *R. solanacearum* biovar 3 was collected from wilted *Gomphrena globosa* plant from Ambalavayal, Kerala.

A new diagnostic tool for *Ralstonia*

Real Time LAMP was found as a quick diagnostic tool for on-farm detection and quantification of *Ralstonia* in soil as well as seed rhizomes of ginger. The detection was found to the limit of 10^3 CFU/g of soil or rhizomes.

Apoplastic bacteria suppress bacterial wilt

Treatment with apoplastic bacterium, IISR GAB 107, reduced bacterial wilt of ginger by 60% under greenhouse conditions. The study has showed that individual treatments are better than consortia of bacteria in suppressing disease.

Comparative genomics of Indian isolates of *R. solanacearum*

Whole genome sequences of 10 isolates of *R. solanacearum* were collected, reassembled and compared. The *de-novo* assembly of ten *Ralstonia* strains yielded 5.6 to 6.2 Mb genome size at an N50 contig length of 58,400 to 68073 bp. While 1463 genes are common in all the strains of *R. solanacearum,* a number of unique genes present in each strain were identified. Maximum numbers of unique genes were found in Rs-2 and Rs-75. Cell wall degrading enzymes like polygalacturonases (PehA, PehB and PehC) were not present in majority of the isolates. On the other hand, pili-driven twitching motility factors (pilQ, pilT or pliA), endoglucanases and most of the metabolism and stress related factors were present in all the isolates.

Gene expression analysis in ginger vs. mango ginger

Tissue specific expression analysis of candidate genes/ESTs in *Curcuma amada* and *Z. officinale* showed significantly higher expression of Ethylene Response Factor, HMG-CoA Reductase, HMG-CoA Synthase and WRKY transcription factor 8 in both leaf and rhizome tissues of *C. amada*.

TURMERIC

Genetic resources

One thousand four hundred and four *Curcuma* accessions have been maintained in the field gene bank. The germplasm conservatory was enriched with six new turmeric accessions. Eighty seven turmeric accessions have been characterized for morphological and flower characters as per DUS guidelines. Also, 10 genotypes of turmeric comprising of three pre-release selections (Accessions 48, 79, 849) and seven released varieties (IISR Prabha, IISR Prathibha, IISR Kedaram, Suguna, Suvarna, Rajendra Sonia and Megha turmeric -1) were characterized for quality.

Breeding

A multi-location trial with three promising accessions (Acc. 48, Acc. 79 and Acc. 849) along with IISR Prathibha and local check was laid out in Kerala (Peruvannamuzhi), Andhra Pradesh (Vijayawada), Tamil Nadu (Erode) and Karnataka (Chamrajanagar and Chettalli). The long duration genotype, Acc. 849 recorded maximum yield of 9.10 t/ha (mean across locations and years, 2013-15 and 2014-15) followed by the short duration genotypes Acc. 48 (7.95 t/ha). The performance of the Acc. 48 was also evaluated in AICRPS, CVT trials across nine locations during 2013-14 and 2014-15. The Acc. 48 recorded 26.6 and 20.6 increase in yield over local and national checks, respectively.

Curcumin biosynthesis

R2R3-MYB, WRKY transcription factors with putative regulatory roles on curcumin biosynthesis genes were identified. Expression analysis of key genes (*pal, c4h, 4cl, c3h, hct, comt, dcs, curs1, curs2* and *curs3*) and a novel *pks* (*clpks11*) was completed under different growth conditions and developmental stages and gene to curcuminoid

correlations were analysed. Two genes *c4H* and *clpks 11* showing positive correlation and *hct* showing negative correlation to curcuminoid content were identified (Fig. 6).

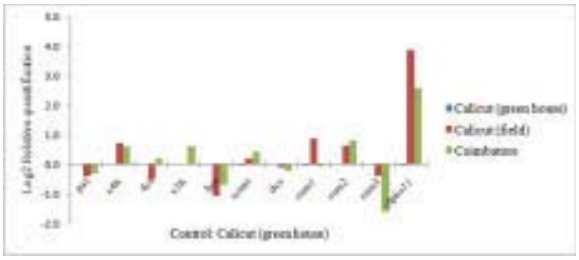

Fig. 6. Relative gene expression profiling of curcuminoid biosynthetic pathway genes in rhizomes of IISR Prathibha under different environments

Crop management

Influence of coloured shade nets on yield and quality

Turmeric was grown under red, green, white and black shade nets with open as control. Light (PAR) intensity in shade nets varied from 58-63% of open light intensity. Sampling just before harvest revealed that rhizome dry weight was 13-15% higher in turmeric under red shade net compared to open condition. The oleoresin content under red shade net was 12.9% (20% higher compared to open and also black, white and green nets) and essential oil was 2.1% (5% higher compared to open).

Post-harvest technology

Turmeric curing with solar cooker

A renewable solar energy unit for turmeric curing was installed at ICAR-IISR farm, Peruvannamuzhi. It has solar thermal collectors with curved parabolic mirrors which concentrate solar radiation on to a central pipe called as the receiver. The receiver is coated with solar selective coating to absorb 90% of the incoming rays and insulated with air jacket to save the collected heat. The collected heat is transferred to the water circulated in the pipe which in turn generates steam. A micro controller based single axis tracking system with GPS is employed to achieve continuous and automatic tracking. The unit has a cooking vessel of capacity 50 kg turmeric/batch. The initial trial indicated that complete cooking of turmeric could be achieved in 45 min.

Supercritical fluid extraction (SFE)

Dried turmeric (IIISR Kedaram) rhizomes were extracted with compressed CO_2 at two different flow rates namely, 30 and 40 g/min. and pressure range of 20-30 MPa using methanol as modifier. The yield of SFE extract varied from 3.5 - 4.7%. The yield and composition of SFE extracts varied with extraction pressure and flow rate. The volatiles and non-volatile constituents of the extract were analyzed by gas chromatography and HPLC. GC-MS profile of SFE extracts was compared with that of essential oil. The general constituents in turmeric oil, such as α-pinene, α-phellandrene, limonene, α-terpinolene and t-caryophyllene were not detected in SFE extracts.

Plant health management

Molecular characterization of shoot borer populations

PCR conditions for the amplification of mitochondrial cytochrome-c oxidase subunit 1 (COI) gene region of *Conogethes* spp. infesting ginger, turmeric and cardamom using the primer pairs, Lep F1/LepR1 were standardized.

Seasonal incidence of shoot borer in relation to crop phenology

The incidence of shoot borer infesting ginger and turmeric in relation to crop phenology was studied by recording the incidence of the pest at fortnightly intervals. On ginger, infestation by the shoot borer on shoots was first observed during the second fortnight of July and was high during the second fortnight of September to the second fortnight of October. On turmeric, the pest infestation was first observed on the shoots during the second fortnight of July and was high during the first and second fortnights of October.

Screening of insecticides for shoot borer management

Ten insecticides (malathion 0.1%, lambda-cyhalothrin 0.0125%, quinalphos 0.05%, fipronil

0.003%, imidacloprid 0.009%, thiamethoxam 0.0125%, spinosad 0.135%, flubendiamide 0.02%, chlorantraniliprole 0.01% and cyantraniliprole 0.005%) were screened for their efficacy against shoot borer of ginger and turmeric. Plots treated with chlorantraniliprole had minimum pest infestation on the shoots that was on par with lamda-cyhalothrin, flubendiamide and cyantraniliprole.

Field evaluation of EPNs against shoot borer

Among the EPNs, *Steinernema* sp. (IISR-EPN 02) treated plants showed less shoot damage (34.5 %) compared to Malathion treatment (33%) and control (65.8 %).

NUTMEG

Genetic resources

Farmer participatory surveys were conducted in Thrissur, Idukki and Kottayam districts of Kerala and 19 germplasm accessions of nutmeg were collected (Fig. 7).

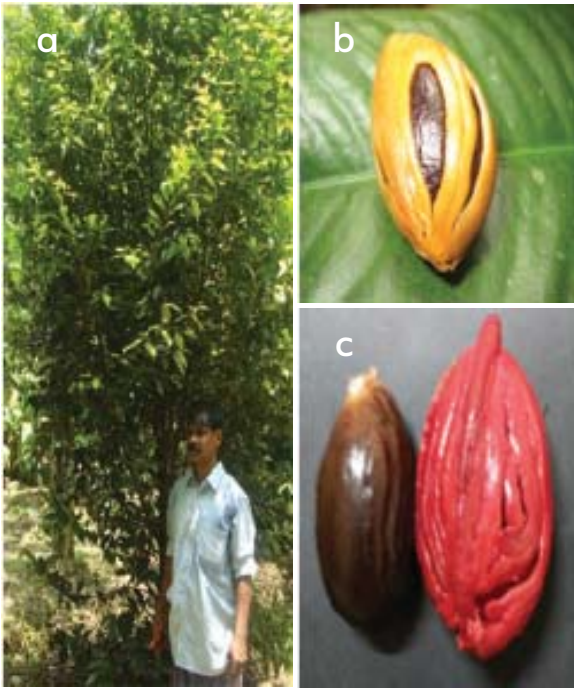

Fig. 7. Nutmeg collections, a. erect canopy nutmeg; b. bold nut with yellow mace; c. whole mace type

Monoecious trees were recorded across the nutmeg tracts and three types of flowers *viz*., male (75%), female (19%) and hermaphrodite (6%) were noticed in the monoecious trees studied (Fig. 8).

Fig. 8. Variability in nutmeg flower composition, a. pistilate; b. hermaphrodite; c. staminate flowers

ANTIOXIDANT PROPERTIES OF SPICES

Antioxidant properties of natural compounds: a QSAR model using semiempirical descriptors

In this study multiple linear regression (MLR) and kernel-based partial least square regression (K-PLS) methods with semi empirical, topological and a combination of both descriptors were employed to generate QSAR models to predict the antioxidant activity of natural compounds. For this, the training set comprised of 31 compounds while the test set included 10 compounds already reported in the literature. The models generated were internally and externally validated with 19 and 16 additional compounds, respectively. The results showed highest correlation with results of the wet lab experiment and is recommended as a robust model for predicting antioxidant property.

Antioxidant potential of cinnamon and turmeric

The study was used to evaluate the antioxidant potential of sequential extracts of these spices in relation to total phenols. DPPH radical scavenging activity of these extracts as determined by IC_{50} values, ranged from 11.9 to 1500µg/mL. Antioxidant activity by PM method and FRP varied from 0.30-2.99 MAAE/g and 0.27 - 1.56 MAAE/g of extract, respectively. By PM method chloroform extract of turmeric showed highest activity (2.99 MAAE/g) which was followed by methanol extract of cinnamon (2.34 MAAE/g).

Antioxidant potential of *Myristica* species

Studies on antioxidant potential of leaf extracts of *Myristica fragrans*, *M. malabarica*, *M. andammanica* and *M. prainii* indicated that methanol extracts of all four species had higher antioxidant activity compared to petroleum ether extract. Among the methanol extracts the *M. fragrans* and *M. malabarica* showed higher antioxidant activity which was comparable with that of the synthetic antioxidant BHA.

Plant health management

Phytophthora meadii infects nutmeg

Eight *Phytophthora* isolates causing leaf and fruit fall of nutmeg in Kerala were characterized using morphological and molecular tools. ITS sequencing, restriction analysis using MSP1 and MLST analysis nuclear and mitochondrial genes revealed that these isolates are closely related to *P. meadii.*

Dimethyl trisulfide, a new compound for soil fumigation

Soil fumigation assays with different concentrations of dimethyl trisulfide resulted in 100% inhibition of *P. capsici*, *P. myriotylum*, *R. solani*, *Gibberella moniliformis*, *Athelia rolfsii, C. gloeosporioides* and *R. similis* at different concentrations.

MVOCs suppress a range of phytopathogens

Microbial volatile organic compounds (MVOCs), identified from *Pseudomonas putida* BP25R, such as 2, 5-dimethyl pyrazine, methyl pyrazine, dimethyl trisulfide, 2-ethyl 5-methyl pyrazine, 2-ethyl 3, 6-dimethyl pyrazine were evaluated at different concentrations under *in vitro* conditions and their EC_{50} values were calculated. All the tested compounds showed significant inhibitory activity against oomycete pathogens, *P. capsici* and *P. myriotylum*; fungal pathogens *R. solani, C. gloeosporioides, A. rolfsii, G. moniliformis* and *M. oryzae,* bacterial pathogen *R. solanacearum* and plant parasitic nematode *R. similis*.

New synthetic media for production of EPNs

A new artificial media was developed for the mass production of entomopathogenic nematodes. By this technique around 23 lakh infective juveniles of EPNs can be multiplied from a single flask (250 mL). The media is suitable to multiply infective juveniles of *Steinernema* spp., *Heterorhabditis* sp. and *Oscheius* spp. Ingredients of this media are cheaper and are locally available.

Documentation of natural enemies of spice crop pests

Surveys for incidence of natural enemies of spice crop (black pepper, cardamom, ginger, turmeric, nutmeg, allspice and clove) pests were conducted in 22 locations in Idukki, Wayanad and

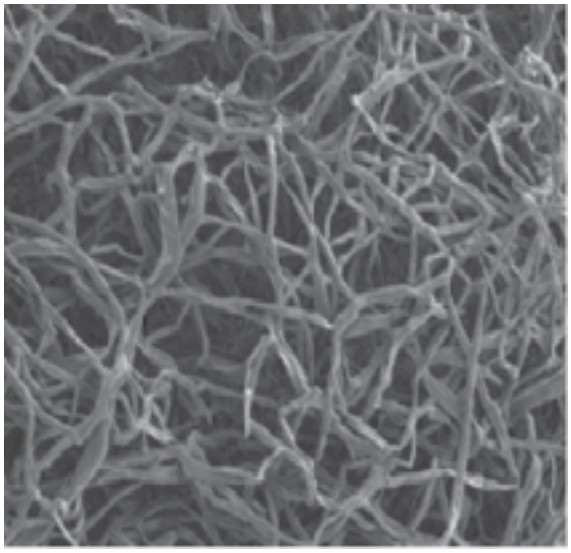

Fig. 9. Ramification of *Beauveria bassiana* mycelia on adult *S. anale*

Kozhikode districts of Kerala. Six entomopathogens and three mermithid nematodes (IISR-MN-01 to IISR-MN-03) were documented from different spice crop pests (*Sinoxylon* spp., *Aspidiotus destructor*, *Aphis craccivora*, *C. punctiferalis* and *Udaspes folus*). The fungus infecting *S. anale* has been identified as *Beauveria bassiana* (IISR-EPF-04) (Fig. 9). The entomopthoralean fungus recorded from *A. craccivora* has been tentatively identified as *Neozygites* sp. (IISR-EPF-12). An NPV (IISR-NPV-03) infecting *Pericallia ricini*, a pest of cardamom was isolated.

New species of *Spilarctia obliqua NPV*

A new species of group I tetrahedral shaped multiple nucleopolyhedrovirus isolate, belonging to the genus *Alphabaculovirus* of the family *Baculoviridae*, infecting *Spilarctia obliqua*, a polyphagous pest of ginger, turmeric and other crops was isolated and characterized based on morphological (Fig. 10) and molecular data. The isolate showed high virulence against the pest based on LC_{50} and ST_{50} data.

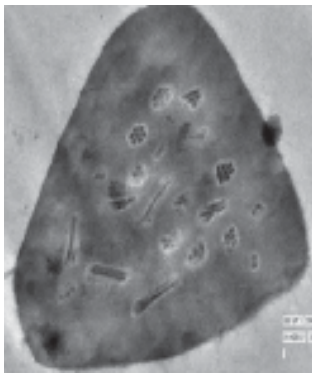

Fig. 10. Transmission electron micrograph of SpobNPV

New databases

The Bioinformatics Centre has developed and hosted three new databases *viz.*, SpiceComDB, PiperPepDB (Fig. 11 a,b)and Radobase. SpiceCom comprises of plant based compounds and their bioactivity. It allows the user to search using compound name, plant name as well as based on activity. Facility to download compounds individually as well as in batch mode is provided. Literature information will be incorporated. PiperPepDB is a database of experimentally MS/MS generated peptides from black pepper while Radobase is a database on burrowing nematodes.

Fig. 11a. User interface of SpiceCom

Fig. 11b. User interface of PiperPep

INSTITUTE TECHNOLOGY MANAGEMENT AND BUSINESS PROCESS AND DEVELOPEMNT

During the year ITM-BPD Unit has facilitated the non exclusive licensing of nutmeg variety, "IISR Keralashree", the first variety of its kind developed through a farmer's participatory research approach. The progressive farmer Mr. Mathew Sebastian, Malappuram, Kerala who was involved in developing the variety will be eligible for benefit sharing with ICAR-IISR on equal terms. The variety was licensed to another progressive farmer, who will attempt large scale commercial production and marketing of this variety (Fig. 12a). One of the elite ginger varieties, "IISR Mahima" was licensed to a progressive farmer, Medak District, Telangana. Owing to the consistent performance of the high curcumin turmeric variety, IISR Prathibha, one of the licensees, has renewed the license for a second term.

Fig. 12a. Signing of MOU for non exclusive licensing of nutmeg variety, "IISR Keralashree"

The novel method for delivery of PGPR, "Biocapsule Technology" has attracted many biofertilizer manufacturing companies and IISR signed a non-exclusive agreement for commercialization with Codagu Agritech, Karnataka (Fig. 12b). The same company has availed the technology of "Microbial consortium for black pepper" and *Trichoderma harzianum*. Another private firm from Kottayam, Kerala, M/s. Agri Life Biotech have also availed the license for commercial production of *T. harzianum*.

Fig. 12b. Signing of MOU with Codagu Agritech, Karnataka for commercialization of biocapsule technology

The Spice Processing Unit at Peruvannamuzhi, Farm started functioning this year. Four license agreements were signed with clients this year for utilizing facilities. The first agreement was with M/s SUBICSHA, the Coconut Producer's company, which comprises of 532 women self help groups. M/s Abhiruchi Food Products, a unit of Kudumbasree, a cluster of women self help groups and two private entrepreneurs, M/s Maaloos Pure Food Mix and M/s Cookway foods, Kozhikode are the other clients. Curry powders like turmeric, coriander, chilli, chicken masala, garam masala, rasam mix etc are the major products. M/s SUBICSHA has already launched their product in the market on 1st January, graced by the presence of the Hon'ble Mayor, Kozhikode (Fig. 12c).

Fig. 12c. The product launch ceremony of 'SUBICSHA Spice Powders'

EXTENSION AND TRAINING

The advisory services of the Agricultural Technology Information Center were delivered to more than 1200 clients including visitor advisory services to more than 3000 visitors (Fig. 13a,b). Twelve training programmes were conducted by the institute targeting diverse stakeholder groups like farmers, youth, tribal beneficiaries and students. The scientists from the institute served as resource persons in more 33 training programmes across various states of the country benefiting more than 6000 participants. The institute also facilitated the monthly technology advisory meeting of the district Agricultural Technology Management Agency (ATMA) by providing expert support for resolving field problems. The institute was also featured in a special educational programme as leading destination for higher education in the field of agricultural research.

Fig. 13a. Sri K P Mohanan, Kerala state minister for agriculture, visiting ICAR-IISR exhibition stall during organic agriculture summit at Angamaly.

Fig. 13b. Farmers from North Eastern states visiting institute

A total of 67 exhibition days across 14 exhibitions were arranged by the institute during the last year. The institute facilitated a total of 31 group visits for educational institutions to provide exposure to research and development activities in spice crops. About 17 farmer groups from within and outside the state visited the institute for learning about the technologies developed for improved spices productivity.

Other activities included training on production technology for yams and turmeric, exposure visit to tribal farmers and introduction of improved varieties of tuber crops and spices benefitting more than 20 tribal hamlets in two districts. Technology awareness initiatives have also been undertaken in North Eastern state of Tripura with close coordination and support of other ICAR institutes and Krishi Vigyan Kendras. Technology inputs worth more than 10 lakh rupees have been sold through the sales counter functioning at the Agricultural Technology Information Centre.

Varietal dissemination

IISR Prathibha, a popular turmeric variety developed at ICAR-IISR is establishing as a popular turmeric variety in major turmeric growing regions of the country. The field visits conducted at Gundalpet in Karnataka state and Medak district of Telangana state (Fig. 14) confirmed this fact. The story of Mr. Ramprasad Reddy (34), MCA, an IT professional turned turmeric farmer, is one of the several instances offering anecdotal evidence for this fact.

Fig. 14. ICAR-IISR scientists and a group of farmers from Telangana/Andhra Pradesh in Prathibha plot of at Zahirabad.

Trade competitiveness of black pepper

The trends in trade competitiveness of black pepper exports from India were examined through a time series of Revealed Comparative Advantage over the period 1988-90 to 2011-14. A sharp and steady decline is revealed comparative advantage (RCA) in black pepper trade could be identified from the analysis. The RCA declined from 26.8 during 1988-90 to 7.9 during 2001-05 and further

declined to 4.6 for the period 2011-14. The instability in area, production and yield was higher during the period 2002-13 than the previous period of similar duration (1991-2001).

Comparison of ginger production in Kerala and Karnataka

The commercial high input intensive cultivation practices followed by commercial ginger farmers in Karnataka yields higher level of profits in the short run. The cost benefit ratio was 1: 3.39 for the sample ginger farms in Karnataka whereas it was significantly lower in Kerala. The study also identified key intervention points for enhancing sustainable production of ginger. The cost of cultivation of ginger in commercial production system was found to be Rs. 412837/ ha.

Study on nutmeg production in Kerala

The data indicated wide diversity in practices related to tree management, nutrient use, plant protection practices, labour use, harvesting practices and marketing of the produce. There was a predominance of low input management strategy among the sample farms with low usage levels of inorganic fertilizers and plant protection chemicals. Thread blight and fruit rot was estimated to cause about 20% of yield loss.

Integrated black pepper research and development in North Kerala districts

Four hundred and thirty soil samples from farmer's plots were analysed for macro and micro nutrients and issued with soil health card advisories. Demonstrations on site specific nutrient management were taken up in five farmer's plots. Three trainings on soil health management, black pepper production technology and disease management were given to 420 farmers. Twenty four FLDs on improved technologies and 20 participatory nurseries that were initiated at farmer's plots in four panchayats of Kozhikode district during last year continued. In nurseries, 500-980 cuttings were produced and sold by the farmers through participatory mode.

Enhancing the economic viability of coconut based land use systems

Soil (profile) and leaf samples (370 samples each) were collected from experimental and

Fig. 15a. Demonstration on released varieties at farmers plot: Mr. Thomas, Kallanode, Koorachundu

Fig. 15b. Participatory nursery at Mr. Sajeev Joseph, Edamana, Nanminda

demonstration plots of coconut, pepper (in Naduvannur and Arikulam panchayats of Kozhikode) and nutmeg (at Mookanur panchayat of Ernakulam). Experimental plots of coconut had low OC, P, C, Mg, Cu, Zn and B and high Al content in the soil profile. Similarly black pepper soils showed high P, low K, B and Mo and that of nutmeg soil showed very low K, low Cu, B, Zn and Al and high P. Based on these fertility levels, site specific nutrient doses (fertilizers, dolomite) were applied.

Establishment of integrated organic farming system model plot

A farming system was established with spices (turmeric), fodder (hybrid Napier grass *viz*., CO3, CO4, Congo signal grasses, DHN-6) and vegetables at Chelavoor farm. A diary unit was also started with two cows (Jersey and Jersey cross) and two calves.

KRISHI VIGYAN KENDRA

During the period, KVK conducted total of 88 on-campus and off-campus training programmes. A total of 3332 trainees were benefitted. Two on job trainings (OJT) to the vocational students (60 numbers) of Government Vocational Higher secondary School, Balussery were organized. Paid trainings on bee keeping, broiler goat rearing, plant propagation, ornamental fish culture and dairy management were also organized. The Kendra conducted two field days, two seminars, participated in ten exhibitions, broadcasted nine radio talks and conducted two study tours for farmers to various research institutes.

Nine Front Line Demonstrations and six On Farm Trials on technology assessment and refinement in 195 farmers' fields were carried out. Among these, technologies on micronutrient formulation for ginger, transplanting technique of ginger using pro-trays, column method of pepper propagation and utilization of kasthuri turmeric (*C. aromatica*) for herbal cosmetics production were well received by the stakeholders. KVK also documented two success stories and farmer innovations during this period which were also published in print media. One technology (using banana pseudo stem for mushroom production) is being selected for further refinement and application through farmer's innovation programme of ATMA.

KVK also provided soil health cards to farmers. A hatchery unit started functioning at KVK with the total hatching capacity of 30,000 eggs at time. More than 35000 chicks were supplied to farmers and other agencies. A NABARD funded project on "LEAD farm development" for one year with the funding of 7. 3 lakhs and a DBT funded project on "Empowerment of rural women and youth in Kozhikode district through ornamental fish culture applying biotechnologies" for three years at a total cost of 30.6 lakhs have started operation.

The goat unit established during 2008 has been strengthened with the addition of goats and presently the unit has 31 goats including three as broiler goats. A high tech diary unit was established

Fig. 16a. View of hatchery unit at KVK

Fig. 16b. Study tour for farmers at KVK

at ICAR-IISR, Kozhikode comprising of two milch animals with the technical support of KVK.

ICAR-ALL INDIA CO-ORDINATED RESEARCH PROJECT ON SPICES

New varieties

The following five varieties developed by different centres were recommended for release

- Coriander: Susthira (LCC-219) with high essential oil suitable for rain fed conditions in Andhra and Tamil Nadu.
- Fennel: AF 2 (moderate resistance to *Ramularia* blight and higher 1.9 % essential oil content) and RF 157 (high volatile oil content).
- Fenugreek: Narendra methi 2 (tolerant to *Cercospora* leaf spot and Downy mildew) and RMt 354 (bolder seed, tolerant to downy and powdery mildew)

Technologies developed

- Standardization of water requirement of turmeric through drip irrigation
- Micro irrigation management in fennel and fenugreek
- Management of *Phytophthora* foot rot of black pepper
- Management of seed midge in fennel
- Management of pseudostem rot of small cardamom

HUMAN RESOURCES DEVELOPMENT

Trainings conducted

DBT funded national training on Bioinformatics for whole genome sequencing, January 27 - 30, 2016 (Fig.17).

Fig. 17. Faculty members and trainees of national training on Bioinformatics for whole genome sequencing

AWARDS

Rajbhasha Shield Award was granted to IISR for significant contribution to Hindi correspondence, organizing Hindi workshops, OLIC meetings, publications like Annual Report, Masala Samachar, Research Highlights, official language magazine Masloon Ki Mehak, Popular articles and extension bulletin in Hindi and OL implementation during 2014-15.

Masloon Ki Mehak (best official language magazine award)

Best OL magazine award was granted for official language magazine Masloon Ki Mehak, among the 76 central government organizations and members of the TOLIC, Kozhikode.

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