Research Highlights 2007-08

Indian Institute of Spices Research

Calicut

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Front cover : GEB 17 - Endophytic bacteria from ginger effective against *Pythium*
Back cover : GEB 18 -Endophytic bacteria from ginger effective against *R. solanacearum*

FOREWORD

I am happy to present the research highlights for 2007-08. During the last one year, the institute continued to strengthen its activities. The Protection of Plant Varieties and Farmers' Rights Authority has accepted our proposal for developing the DUS (Distinctness, uniformity and Stability) guidelines for spices and constituted the task force VII with Dr.V.A.Parthasarathy, Director , as member Secretary. The DUS guidelines for turmeric, ginger, black pepper and cardamom are almost in final stage. The institute has been recognized as the test centre for accreditation of tissue culture facility for spices by Department of Biotechnology, GOI. Evaluation of bioconsortium against *Phytophthora* foot rot and slow decline diseases indicated the efficacy of rhizobacterial consortium in reducing the disease as well as survival of the plants. Also, evaluation of bacterial endophytes against *Phytophthora* foot rot and slow decline diseases in pepper and rhizome rot in ginger resulted in some promising strains for disease management. In cardamom, one hybrid (GG x NKE 19) is promising with bold capsules and field tolerance to rhizome rot and leaf blight. Cured turmeric samples took less time for drying but there was a considerable reduction in oil and oleoresin recovery in these samples. Clove, cinnamon and turmeric leaf oils inhibited the production of aflatoxins. In terms of infrastructure, a new block each for library, biocontrol research and farm have been inaugurated by the Deputy Director General (Hort.).

In order to refine the technologies, 18 front line demonstrations on black pepper have been laid out with new varieties developed at the institute along with the practices for control of foot rot of black pepper. Three accessions have been registered with NBPGR for their novelty. Patentable research on niche and utility area are underway with a strong Institute Technology Management Committee (ITMC). New Research Advisory committee under the chairmanship of eminent horticulturist Dr. N. Mohanakumaran, Retd. Director of Research, KAU, was constituted and held its first meeting to fine tune our research programmes. The KVK has intensified its training programme to the farmers' need besides operating the programme for developing skills to develop skilled gardeners under the State Horticultural Mission.

I take this opportunity to thank our honorable Director General Dr. Mangala Rai and our respected DDG (Hort.) for their interest in our institute. The help rendered by Dr. K.V. Ramana formerly ADG (Hort-II) and Dr. Umesh Srivastava, ADG (Hort-II) is gratefully acknowledged. I am grateful to all the staff of IISR, Calicut for the united support in running our programmes for the welfare of farmers.

Calicut
28-04-2008

V.A. Parthasarathy
Director

CONTENTS

Black pepper

Germplasm collection, conservation and evaluation

On exploration, 54 accessions from Shendurney WLS, 24 from Bisle Ghats forests and 32 from Karwar district were collected from the species *Piper nigrum*, *P. galeatum*, *P. trichostachyon*, *P. attenuatum* and *P. hapnium* including two local cultivars from farmers' fields. Ninety eight accessions were characterized based on IPGRI descriptor in addition to 1000 accessions. One hundred and fifty accessions were planted at the alternate germplasm centre at CPCRI-RS, Kidu, Karnataka.

Improvement in black pepper

For pollu beetle, nematode and drought resistance: A hybridization block was established with bush pepper var. IISR Subhakara as female parent and 'pollu' tolerant lines (Coll. No. 816, 841, 1084 and 1114), nematode tolerant lines (Acc. 820 and HP 39) and drought tolerant lines (Acc. No. 1495, 931 and 813) as male parents. Seedlings raised from these cross combinations are under multiplication for field planting.

For high yield and high caryophyllene: A total of 114 progenies of crosses involving IISR Subhakara (female) and high caryophyllene lines (male) were produced. Analysis of berry caryophyllene content of the elite lines (male lines) indicated variation. Location effect on beta caryophyllene content of IISR Subhakara was also observed in the samples collected from Ambalavayal, Peruvannamuzhi, Panniyur and Yercaud centres.

For *Phytophthora* resistance: To develop a second mapping population to tag *Phytophthora* resistance, 50 new crosses were made between IISR Shakthi x IISR Subhakara and the progenies were planted for germination. Twenty five new putative transgenics containing osmotin were hardened for testing.

Cloning of *Phytophthora* resistant gene from *Piper colubrinum*

To find whole gene sequence of the resistant gene from *Piper colubrinum*, 3' walking resulted in the amplification of 400bp and 800bp products using RNA as the template.

Effect of Sulphate of Potash (SOP) on yield and quality of black pepper

The soil fertility parameters like major, secondary and micronutrients and availability of K in different fractions were high in 125% of recommended K as SOP and recommended K as SOP & Muriate of Potash (MOP) treatments. The leaf nutrient status, biochemical parameters and yield were found to be on par in 50% of recommended K as SOP with or without foliar spray and magnesium supplementation. Recommended dose of K as SOP + 2% foliar spray recorded highest yield that was on par with 50% of the recommended K as SOP and recommended K as SOP + $MgSO_4$ treatments followed by recommended K as MOP and 125% of K as SOP treatments.

The net returns and benefit cost ratio was high in MOP treatment (1.72) followed by 50% of recommended K as SOP (1.59). When a premium price for the SOP treatments is considered, the recommended K or 50% of it as SOP with or without foliar spray recorded higher net returns and B: C ratio (1.73-2.10) as that of MOP treatment. Quality parameters like oleoresin and piperine contents were also high in these treatments. Hence, for the production of high quality organic pepper, SOP can be recommended.

Organic manures on P release

Effect of different organic sources on fractions of P and its availability was studied. Step wise forward regression analysis of P fractions showed Fe-P and Available P as the best predictors for yield (= 46.25 + 1.034 Fe-P + 0.570 Av P) with a R^2 of 0.611**.

Drought tolerance

SOD isozyme showed five prominent bands, which was common both for tolerant and susceptible accessions. Water stress induced an additional SOD band in some accessions. Tolerant accessions showed higher photosynthetic rate and higher stomatal conductance than the susceptible ones after five days of stress induction.

Physiological and biochemical basis of productivity

Nitrate reductase (NR) and malate dehydrogenase (MDH) activities were assayed in high and low yielding black pepper accessions during pre-bearing period. High yielders had higher NR and lower MDH activities compared to low yielders during this period. Measurement of carbohydrates and starch revealed that high yielders in general, had higher carbohydrates and starch during pre bearing period than low yielders.

Myristicin in *P. nigrum* volatile oil

Volatile oil composition of the leaf samples of eleven species of *Piper* with the help of GC and GC-MS showed 24 important components in varied concentrations. It is interesting to note that in the *P. nigrum* leaf samples collected from Dapoli and Karwar, a compound myristicin was present in good concentration, which is a general component of nutmeg and not recorded so far in *P. nigrum*.

Evaluation for quality

Black pepper samples from PRS Panniyur and Sirsi were evaluated for quality constituents and caryophyllene in oil. Caryophyllene content varied from 13.9 to 18.5%. HP-1411 had 3.6% oil, 9.8% oleoresin and 4.2% piperine.

Modified atmospheric storage and quality

Black pepper (Panniyur-1) was stored in three layered metalised polyester cover under vacuum, 100% nitrogen and 90% nitrogen + 10% oxygen for a period of 120 days. Moisture, essential oil, oleoresin and piperine were monitored at 60 days interval. The levels of these constituents remained unchanged throughout the period.

Phytophthora foot rot and slow wilt diseases

Molecular characterization of nematodes: Four populations of *Radopholus similis*, collected from banana (Kayamkulam, Ernakulam and Kasaragod) and coconut (Kayamkulam), were characterized through ITS-PCR using species-specific primers. Extraction of nematode genomic DNA from formalin fixed specimens preserved for more than 10 years was standardized.

Resistance: Forty one hybrids, six cultivars (short-listed as moderately tolerant in the preliminary screening) and three *Piper* spp. were subjected to root inoculation of *Phytophthora capsici*. The mortality of the plants ranged from 0-100 % in 100 days. The hybrid HP-449 showed the least mortality (20%), followed by HP-1375 (30%) indicating their resistance to *P. capsici*. All the cultivars tested showed 100% mortality within 13-21 days of inoculation. Among the wild accessions, Acc. 3177 (*P. sylvaticum*) showed resistance to *P. capsici*.

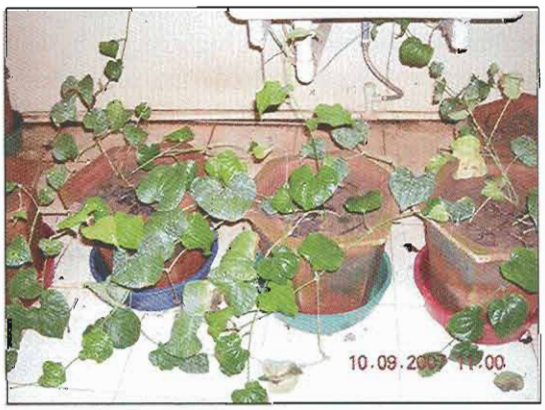


Fig. 1. Acc 3177- a wild accession showing resistance to *P. capsici* infection

Three open pollinated progenies namely, 04-P24-1, 04-HP 1533-2 and 04-HP 1533-3 that

were short-listed based on all methods of screening during 2005-06 were planted in a sick plot at Peruvannamuzhi for evaluating their field reaction to *Phytophthora* and nematodes. No *Phytophthora* infection could be noticed in these plants.

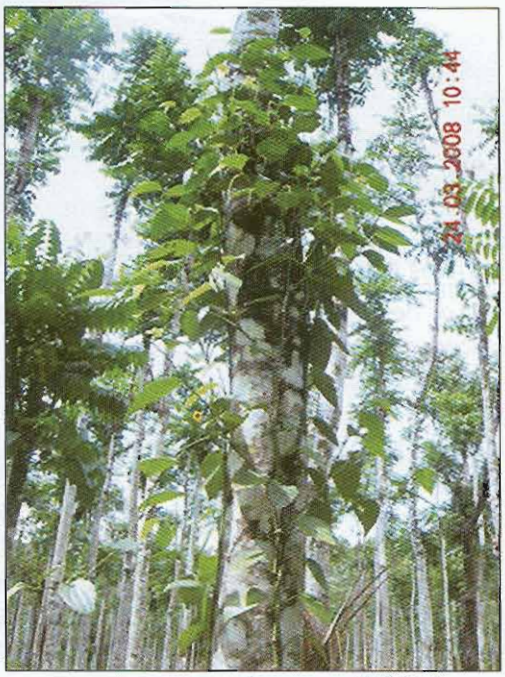


Fig. 2. Open pollinated progeny 04-P24-1

The cultivated accessions C 820 and 1041 and wild accessions Acc. 3177 (*P. sylvaticum*) and Acc. 5225 (*P. ribusoids*) were subjected to *Phytophthora* and nematode inoculation prior to being used as root stock for grafting with var. IISR Sreekara. IISR Thevam (Acc. 1041) was found susceptible to both the pathogens. Acc. 3177 was highly susceptible to both the nematodes *viz.*, *Radopholus similis* and *Meloidogyne incognita* but was resistant to *P. capsici* while Acc. 5225 showed high susceptibility to *R. similis* alone. C5/12, an open pollinated progeny of the polyploid was resistant to *R. similis*.

Out of the nine black pepper accessions under field evaluation, only one each *R. similis* resistant line (HP-39) and *Meloidogyne* resistant line (Acc. 1090), were free from nematode infestation after four years of field evaluation.

Effect of NaCl and biocontrol agents on *P. capsici*

Sodium chloride 4M was found effective against soil population of *P. capsici*. However, the concentration was detrimental to black pepper rooted cuttings. The soil treated with NaCl for 5 days was washed several times with water to remove traces of NaCl and then fortified with bioagents (IISR-853, *P. chlamydosporia*, *T. harzianum* and consortium of bioagents (IISR-6, 8, 13, 151, 853) to study their effect on survival and plant growth in comparison with solarized and ordinary potting mixture. Ninety per cent survival of plants was noticed in NaCl treatment when compared to 92% in solarized soil and 96% in ordinary potting mixture.

Evaluation of antagonists

The promising isolates of fungal and bacterial antagonists namely, *Pochonia chlamydosporia*, IISR-859 and IISR-853 were evaluated under green house conditions in comparison with recommended fungicides copper oxychloride (0.25%) and potassium phosphonate (0.3%) and biocontrol agents (*T. harzianum* + *Pseudomonas fluorescens*) against *P. capsici*. The study indicated the efficacy of IISR-853 and IISR-859 in controlling *P. capsici* infection and was on par with copper oxychloride.

Field evaluation of bioconsortium

A field trial to evaluate the effect of six different combinations of rhizobacteria, in the form of consortia, against *Phytophthora* foot rot and slow decline diseases of black pepper was conducted for five years. The results indicated the efficacy of the rhizobacterial consortium containing IISR-6, 8, 13, 51, 151, 853 (rhizobacteria) + PB-21C (P-solubilizer) in reducing the disease incidence as well as increasing the survival of the plants.

Evaluation of endophytic bacteria

The endophytic bacteria promising against *P. capsici* and nematodes were identified as *Pseudomonas aeruginosa* (IISR BP35), *Pseudomonas putida* (IISR BP25), *Bacillus megaterium* (IISR BP17) and *Curtobacterium luteum* (IISR TC10) based on 16s rDNA analysis. These cultures have been registered with IMTECH, Chandigarh, under Budapest Treaty.

Among the two strains of *B. megaterium* (IISR BP17 and IISR 522), BP17 possessed higher nematicidal activity and better adaptability to temperature and pH. When evaluated in the greenhouse for disease management, the isolates BP35, BP25 and BP17 recorded over 70% disease suppression irrespective of the variety. However, disease suppression was marginally better on Panniyur-1 type (80%) compared to Karimunda type (60-70%). The endophytes could offer protection even with the pathogen population size of 6-7 Log cfu g^{-1}.

Colonization of endophytic bacteria

The endogenous population and spatiotemporal colonization of *P. aeruginosa* IISR BP35 in bacterized shoot was analysed. Minimal concentration of bacterium required for suppression of *P. capsici* was 10^{12}-10^{13} cells ml^{-1}. At this concentration the endogenous population of the bacterium was 10^{5}-10^{6} cells g^{-1} of tissue. Duration of bacterization *vis-à-vis* the endogenous population of bacteria and suppression of *P. capsici* was optimized. The minimum duration of bacterization for suppression of lesion on shoot was 15-20 min. At this duration endogenous population of *P. aeruginosa* was 10^{6} cells g^{-1} of tissue with significant lesion inhibition (96%-100%) on the excised shoots.

Anthracnose disease

Monitoring of anthracnose disease of black pepper and leaf blight in three locations revealed the rapid spread of disease during early monsoon period (June) through rain splash of the pathogens in the soil.

Viral diseases

Preparation of *Piper yellow mottle virus* construct : A 409 bp coding region from open reading frame (ORF) III of *Piper yellow mottle virus* (PYMoV) infecting black pepper was used for the preparation of plant transformation vector construct in the binary vector, pBI121 both in sense and antisense orientation. Each of the identified sense (pBI121-PYMoVS) and antisense (pBI-121PYMoVAS) clones were then mobilized into *Agobacterium tumefaciens* strain EHA 105 by triparental mating with the help of helper plasmid pRK 2014. The constructs would be used to transform black pepper for getting transgenic plants resistant to PYMoV.

Pollu beetle

Fifty-seven cultivated accessions and 61 hybrids were screened against the pollu beetle (*Longitarsus nigripennis*) in the field for identifying sources of resistance to the pest. Among the cultivated accessions, Accs. 4052, 1472, 4093 and 4095 were free from the pest attack. Among the hybrids, HP 1357 was free from pest infestation .

Cardamom

Germplasm collection

Total germplasm in the repository of cardamom was raised to 442 by adding six accessions of land races from Idukki, Kerala. Setting varied from 48 to 82 %, when 18 high yielding selections were selfed. Accessions, IC – 547146 and IC – 547219 were short listed for high yield, green and bold capsules.

Evaluation through PET and CYT

Evaluation of F_1 hybrid progenies of PET I and PET II resulted in short-listing of 12 high yielding selections and one hybrid (GG x NKE –

19) with more than 70 per cent having 8 mm capsules and field tolerance to rhizome rot and leaf blight.

Fig. 3. GG x NKE -19, hybrid with field tolerance to rhizome rot and leaf blight

Under CYT III and IV, three high yielders of Malabar selections and 2 high yielders of Vazhukka selections yielding 10 and 35 per cent higher crop than their respective checks (CCS 1 and Njallanil Gold) were identified.

Drought tolerance

In a study to evolve drought tolerant type, RR1 x 893 was found superior for both quality and yield (4200 green capsules/ plant) and one of the selections in Njallanil Gold x 893 for compound panicles and green bold capsules.

In another trial, two best yielders (APG 497 and 498 with 4800 and 4906 capsules per plant) with more than 80% bold capsules were short listed for further evaluation.

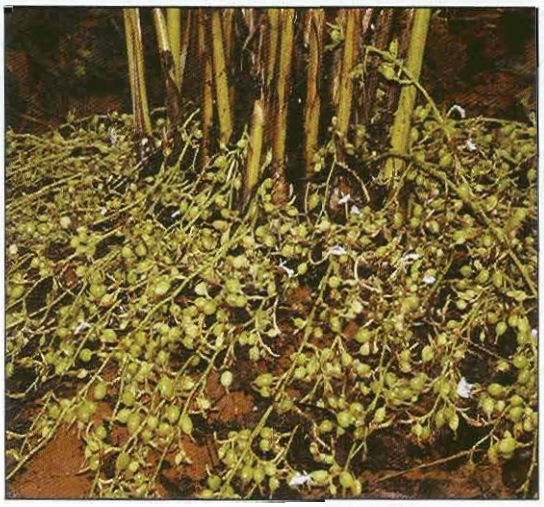


Fig. 5. APG 498, accession with 80 % bold capsules

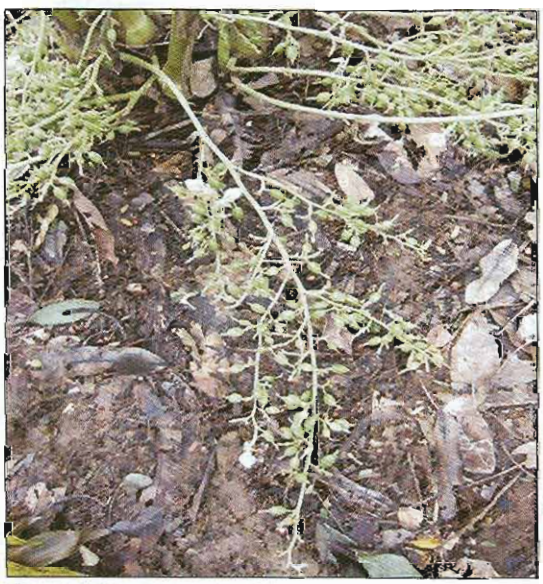


Fig. 4. GG x 893, hybrid having compound panicles

Crop protection

Under *in vitro* evaluation, *Trichoderma hamatum* was found more effective against rhizome rot (*Pythium vexans* and *Rhizoctonia solani*) of cardamom, which is evident from the tropical movement of *T. hamatum* hyphae towards *R. solani* followed by penetration and parasitization.

Considerable diversity was observed in *Colletotrichum gloeosporioides* isolates with regard to colony, conidial and appressoiral characteristics from the infected specimens of cardamom, black pepper, turmeric and other crops, collected from cardamom ecosystem of Kodagu, Hassan and Chickmagalur districts of Karnataka and Valparai (Tamil Nadu). All isolates from cardamom and black pepper were identified as *C. gloeosporioides*.

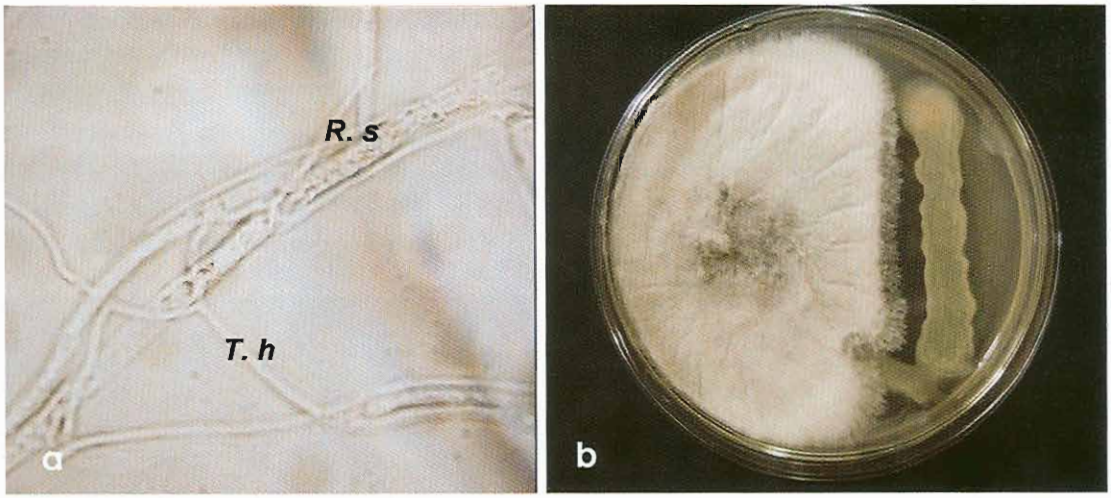


Fig. 6. *Trichoderma hamatum*, an effective organism against rhizome rot
a). *Rhizoctonia solani* x *Trichoderma hamatum*
b). *Rhizoctonia solani* x *Pseudomonas fluorescens* (IISR 6)

Turmeric

Conservation and characterization

In the *ex-situ* conservatory, 1040 *Curcuma* accessions are being conserved and characterized.

Characterization of Indian *Curcuma* species

Comparative sequence analysis of the 18 S rRNA regions of three Indian *Curcuma* species indicated that the entities recognized as *Curcuma aromatica*, *C. longa* and *C. aeruginosa* in India and China are the same, however, the entity recognized as *C. zedoaria* in India and China may not be the same. The entity recognized as *C. zedoaria* in India and Japan may be the same, while *C. zedoaria* of China is distinct from the Indian and Japanese populations.

Development of microsatellite markers and characterization of *Curcuma* spp.

Good quality genomic DNA was isolated and purified from 15 *Curcuma* species available in the field genebank and *Rsa* I was selected as the best restriction enzyme on the basis of the fragment size (2.5 Kb – 100 bp). A total of 700 clones from the enriched libraries (microsatellite containing fragments) were screened by colony PCR and selected clones were sequenced which resulted in 82 microsatellite repeats of variable length. Suitable primers to amplify these regions are being designed.

Cytological analysis of mother plant and seedling progenies of turmeric

Among the 13 mother plant accessions, a chromosome number of 2n=63 in 10 accessions (Acc. No.18, 20, 65, 69,126,138, 399, 435, 426, 434) and 2n=84 in 3 (Acc. No. 300, 415, 449) were observed, while in seedling progenies, 2n=84 in four accessions (20/8, 20/9, 65/12, 65/24) and 2n=77 (65/23) in one accession was recorded.

Flowering and seed set in seedling progenies of turmeric

Flowering was recorded in about 50 seedling progenies. Seeds were recovered from 11 of them and were put for germination to establish advanced generation of seedling progenies. Germination started in two lots after 100 days of sowing.

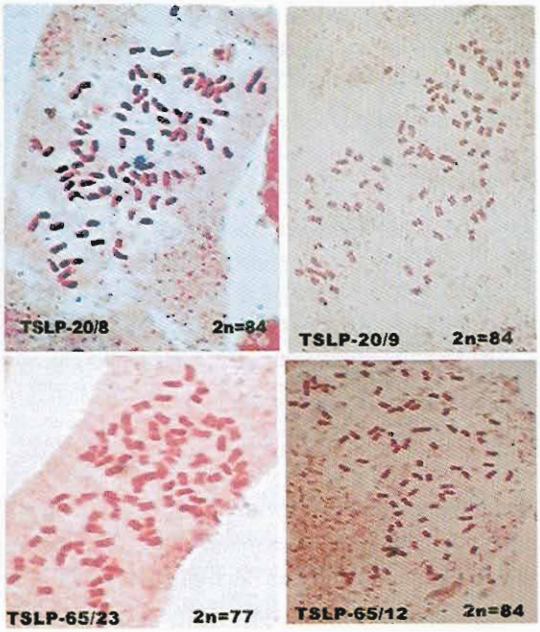


Fig. 7. Chromosome number variation among turmeric seedling progenies

Phenology of turmeric

Phenological events in turmeric varieties viz., IISR Prabha, IISR Prathibha, Alleppey Supreme, Kedaram and Suguna were noted under different times of planting treatments and the same is summarized below in Table 1.

Targeted yield response

Based on the initial soil fertility levels of N, P, K and Zn the fertilizer doses for obtaining 15, 20 and 25 kg/bed yield targets in turmeric were worked out and applied. Zinc was supplemented as foliar spray twice. The achieved turmeric rhizome yield was 14.1, 19.4 and 20.9 kg/bed with a deviation of -5.8%, -3.0% and -16% from the target. Through targeted nutrient application 4-54% increased yield over recommended dose could be achieved in var. IISR Prathibha.

Input use efficiency in turmeric in relation to quality

The role of micro nutrient elements *viz.*, Boron (B) and Zinc (Zn) is being studied in a pot culture experiment at IISR Experimental Farm, Peruvannamuzhi. Turmeric was raised under open and shade condition (under shade net and polyhouse). Photosynthetic rate was higher under open than in shade. While open condition recorded higher plant height and photosynthetic rate, poly house condition recorded more number of leaves and tillers. Plant height, number of leaves and tillers were more with Zn (0.25% spray) and were comparable with no spray treatment. Higher concentration reduced the growth of turmeric.

Biochemical characterization of market samples of turmeric

Biochemical characterization of different traded varieties of turmeric ('Alleppey', 'Rajapuri' and 'Wyanadan') revealed a high content of curcumin (3.11%) in Alleppey finger turmeric (AFT), as compared to Rajapuri (2.26%) and Wyanadan (2.42%) turmeric.

Post harvest technology

Modified atmospheric storage : Dried turmeric rhizomes (IISR Prathibha) were stored in three layered metalised polyester cover under vacuum, 100% nitrogen and 90% nitrogen + 10% oxygen for a period of 120 days.

Table 1. Phenological events in turmeric

Planting time	Emergence (DAP)	First tiller appearance (DAP)	Last tiller appearance (DAP)
30th April	32	81	156
15th May	32	71	139
30th May	23	63	122
15th June	24	54	110

Moisture, essential oil, oleoresin and curcumin were monitored at 60 days interval. The levels of these constituents remained unchanged throughout the period

Mechanical drying and curing: Turmeric var. IISR Prathibha was dried in hot air oven, reverse flow drier, agriwaste drier and also sun dried. Both raw (non- cured) and cured rhizomes (boiled for one hour) were subjected to same process. Cured sample took less time (40-50%) for completion of drying (to a moisture level of 10%) in all the drying treatments. However, cured samples showed a reduction of 40% in oil recovery and 25% reduction in oleoresin recovery.

Soil microbial community structure in soils under turmeric

The microbial community structure in soils under different nutrient management practices (fully organic, integrated and chemical) of turmeric was studied by PCR RFLP and denaturing PAGE using seven different primer sets for multi and single copy genes. The community profiles resolved better under denaturing conditions, than in case of PCR RFLP under non-denaturing condition. The touch down protocol adopted improved the resolution and number of bands detected. Maximum bands were obtained in integrated treatment for ITS1, ITS2, prokaryotic IGS and 16S rDNA primers. Chemical and control treatments registered maximum bands for ITS fungal primers. In case of fully organic treatment maximum bands were observed for prokaryotic IGS primers. Maximum number of nematodes was found in case of IPNM and fully organic treatments.

Soft rot

Forty five of the 81 isolates of *Pythium* sp. collected from different turmeric growing regions of South India showed pathogenicity. Among them 32 isolates were identified as *P. aphanidermatum*, and 8 as *P. myriotylum*. *P. aphanidermatum* collected from Gulburga (Karnataka), Jagtial (Andhra Pradesh) and Chittoor (Kerala) were the most virulent.

Bacterial wilt

Evaluation of *Curcuma* sp. against soft rot disease (caused by *Pythium aphanidermatum*) indicated that *Curcuma longa*, *C. aromatica*, *C. zedoaria* and *Z. officinale* were susceptible while *C. amada and C. caesia* did not take up the infection.

Shoot borer

Bioecology: The seasonal population of shoot borer (*Conogethes punctiferalis*) on turmeric in the field was studied at Peruvannamuzhi. The symptoms of pest infestation were first observed during August (0.17% new shoots infested) and was high during December (10.9% new shoots infested).

Apanteles sp. (*A. taragammae*?) (Braconidae) was recorded to parasitize larvae of shoot borer especially during the post monsoon season. However, the level of parasitism was low (2.1%) when compared to that on ginger.

Resistance: Screening of 891 turmeric gremplasm accessions against shoot borer in the field indicated that 109 accessions remained free from pest infestation.

Ginger

Conservation and characterization

In the *ex-situ* conservatory, 700 *Zingiber* are being conserved and characterized.

Phenology of ginger

On an average, ginger varieties *viz.*, IISR Varada, IISR Mahima, IISR Rejatha, Maran and Himachal took 27, 23, 17 and 12 days for germination in 30 April, 15 May, 30 May and 15 June planted crops, respectively. Corre-

spondingly degree days also varied from 412.9 degree days at first planting to 143.6 degree days for last planting. The time taken to produce first tiller was 73, 61, 51 and 42 days in 30 April, 15 May, 30 May and 15 June planted crops, respectively. The degree days for the events were in the order 1029.8, 833.3, 650.1 and 501.6. The last tiller was produced 140, 144, 121 and 114 days after planting in 30 April, 15 May, 30 May and 15 June planted crops, respectively and this event happened at 1849.5, 1854.3, 1518.7 and 1403.6 degree days.

Molecular characterization

Genotypes could be easily discriminated using RAPD and ISSR markers. Cluster analysis placed the ginger genotypes into four separate groups, in which the grouping of elite genotypes with the putative wild types implies some phylogenetic relationship between the putative wild types and modern cultivars. The exotic type from Japan, resembling the putative types in rhizome features, shared high similarity with the four indigenous putative types.

Morphological variation in M2 generation

In the M2 generation progenies, chlorophyll variegation appeared in the M1 generation due to gamma irradiation disappeared from most of the plants. Variation in plant height and number of tillers was evident among M2 plants treated with specific dose of radiation.

Chemical quality

Among the high oil types Acc. 209, 50, 57,162, 99, 156 and 197 had above 2% oil and Acc. 50,156 and 57 had more than 7% oleoresin. Acc. 57 and 162 contained high limonene in oil. Nepal ginger collections had 1.5-1.9% oil and 3.8-6.1% oleoresin. Acc. 592, 593 and 598 contained 1.9% oil and Acc. 578 contained 6.1% oleoresin.

GCMS profiling of essential oils of traded ginger

A total of 20 compounds in Indian (Cochin) ginger and 18 in Chinese ginger were detected through GCMS. The major compounds identified are camphene, α phellandrene, 1, 8 cineole, linalool, zingiberene, farnesene, and β sesquiphellandrene. High zingiberene was recorded in Chinese ginger (27.14%) compared to the Indian produce (23.75%).

Yield evaluation of ginger

Yield evaluation of 12 selected Nepal ginger accessions revealed the superiority of Acc. 581(16.6 kg per 3 m^2 bed) over others.

Micropropagation of ginger

A method using petri plates containing MS basal medium with 0.8% agar, 30g/l sucrose to control apical dominance and thus activating axillary bud development with minimal or no use of growth regulators was developed. *In vitro* shoot-tip explants of ginger and cardamom were induced to produce multiple shoots (18- 25) with stimulated root growth and 80% establishment during hardening. It is ideally suitable for *in vitro* studies on disease progression and reducing the effect of growth regulators on the somaclonal variation.

Fig. 8. Micropropagation of ginger using petri plate system

Cryo preservation

Cryo preservation protocols were standardized in ginger, pepper and cardamom shoot buds/ somatic embryos using encapsulation and nitrification methods with over 70% success. The conserved materials are genetically stable as evident by molecular profiling.

Soft rot

Pythium myriotylum and *P. aphanidermatum* were associated with soft rot of ginger and turmeric respectively. Four other species of *Pythium* namely, *P. ultimum*, *P. vexans*, *P. splendons*, and *P. delaines* were also associated with ginger soft rot in India, among which *P. myriotylum* was predominant. PCR based method was suitable for identification of *P. myriotylum*.

Bacterial wilt

Among the species of zingiberaceae such as *Globba* sp, *Alpinia galanga*, *Maranda* spp, *Costus* sp, *Curcuma caesia*, *Amomum subulatum* and an unidentified *Curcuma* sp. evaluated for resistance to bacterial wilt, only three of them namely, *A. galanga*, *Globba* sp. and *C. caesia* were found to be the hosts for ginger strain of *Ralstonia solanacearum*. *C. amada* the Indian mango ginger was resistant to bacterial wilt.

Evaluation of endophytic bacteria

Nineteen endophytic bacteria were isolated from ginger rhizomes collected from Wyanad and Kozhikode districts in Kerala using modified protocols (an enrichment method was adopted where the rhizome was incubated in the nutrient rich medium) for isolation of endophytes. These bacteria were characterized using morphological criteria such as colony character, shape, elevation, motility and gram reaction, and all of them belonged to gram negative group and were short rods. These isolates are being evaluated in green house for their potential against soft rot and bacterial wilt diseases of ginger. Among the isolates GEB 7, GEB 9, GEB 13, GEB 17, GEB 18, and GEB 19 were promising for suppression of bacterial wilt and soft rot diseases.

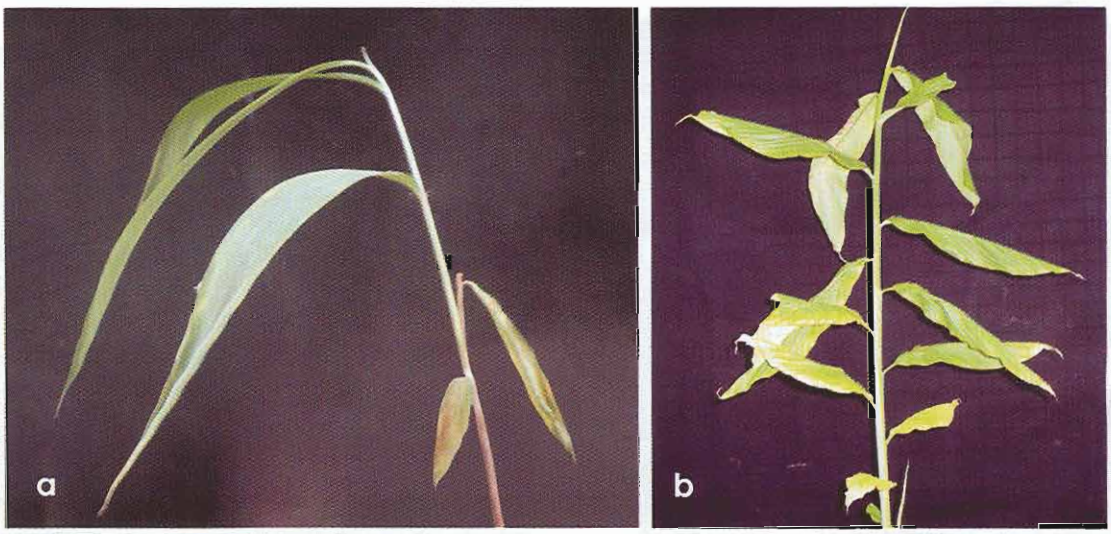


Fig. 9. a) *A. galanga* and b). *Globba* sp. - hosts for ginger strain of *Ralstonia solanacearum* Biovar 3

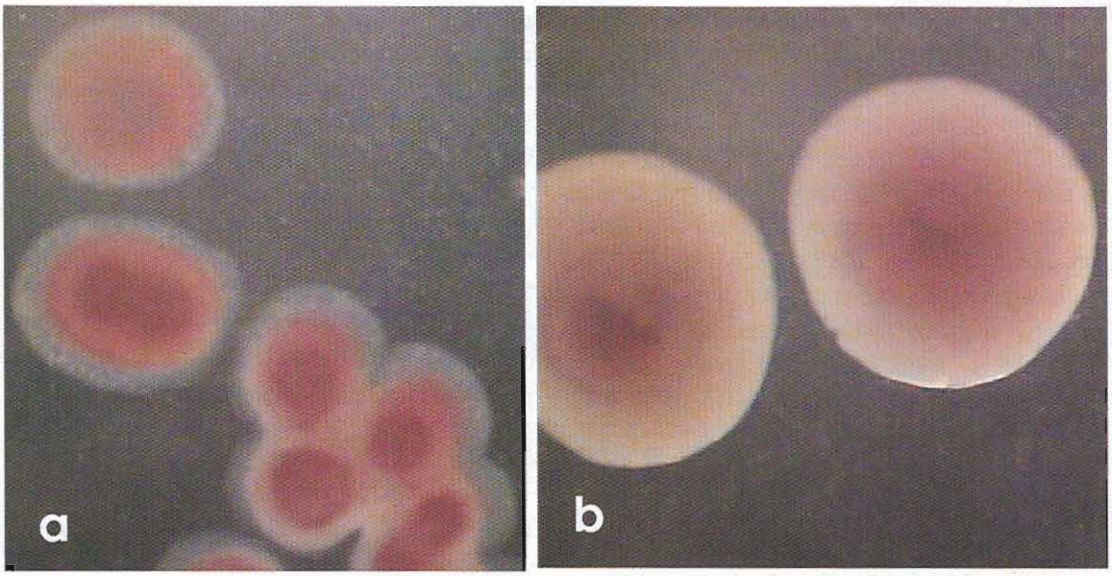


Fig. 10. a). GEB 17 and b). GEB 18 - Endophytic bacteria from ginger effective against *Pythium* and *R. solanacearum* respectively

Paprika and paprika like chillies

Collection, evaluation and maintenance

A high pungent and high colour accession from North East *'Bhut Jolokia'* was added to the germplasm. Fifty four germplasm accessions including 21 Byadagi collections and six exotic collections were raised and multiplied.

Moderate to high variability was recorded for plant characters. Among the indigenous germplasm, ICBD-10 registered the highest color value (302.12 ASTA units), followed by ICBD-11 and ICBD-23. The EC-171 recorded the highest color value, followed by EC-71 and EC-490, among the exotic lines. The capsaicin content varied from 0.0071 to 0.166. The lines, ICBD-10, Kt-Pl-19 and EC-71 were found promising with low pungency and high colour value.

DNA profiling of Capsicum (*Capsicum* spp.) collections

ISSR profiles have been developed for nine *Capsicum* collections and 10 of the primers gave reliable markers for distinction of the accessions tested.

Vanilla

Inter-specific crosses

Inter-specific crosses performed between *Vanilla planifolia, V. aphylla, V. pilifera and V.* sp. from A&N islands revealed successful fruit setting in all cross combinations indicating cross compatibility. However, maximum fruit set was observed in the cross combination of *V. pilifera* x *V. aphylla* (12), followed by *V. aphylla* x *V. pilifera* (10) and *V. planifolia* x *V. aphylla* (9).

Pollen germination

Culturing fresh pollen by sitting drop method at 25±2°C under light using B&K medium containing 10% sucrose gave maximum germination in *V. aphylla* (58%) and longer pollen tube in *V. pilifera* (91.25 µm) after 4 hours.

Viral disease

Vanilla plants showing typical symptoms of

chlorosis and leaf deformation collected from Madikeri district of Karnataka when subjected to RT-PCR using primers specific for five different potyviruses, gave amplification with primers specific to *Bean yellow mosaic virus* (BYMV). The PCR product was eluted, cloned and sequenced. The cloned region contained portion of NIb and coat protein gene. The sequenced region of coat protein contained 778 bases potentially coding for 259 amino acids. Sequence analysis based on per cent nucleotide and deduced amino acid identity, and

Fig. 11. Occurrence of *Bean yellow mosaic virus* on vanilla

phylogenetic analysis clearly showed that vanilla isolate is a strain of BYMV.

Tree spices

Evaluation of nutmeg

In a study to evaluate the performance of various genotypes, A4-17 was found to be the best with high germination (80%), growth and cent per cent grafting success.

Growing media for nutmeg

Among the various growing media combinations, seedling growth was best in the combination of soil:granite:FYM (2:1:1) with 86% germination.

Sex determination in nutmeg

Out of 165 RAPD primers and 25 ISSR primers used for screening sex pooled DNA from male and female *Myristica fragrans*, seven random primers showed polymorphism between them and four primers (OPAM 13, OPAF 16, OPAM 16 and OPAF 5) showed sex specific polymorphic bands.

Molecular profiling in *Garcinia* species

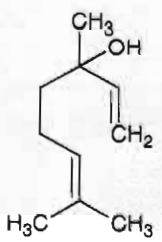
Nine species of *Garcinia* were used in DNA profiling based on ISSR and RAPD markers. A total of nine ISSR primers and twelve RAPD primers could successfully distinguish them.

Natural food colors and pigments in *Garcinia* and nutmeg

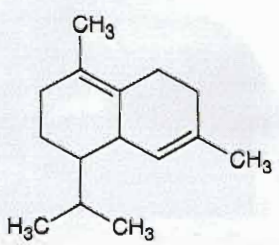
Rinds of *Garcinia cowa, G. gummi-gutta, G. hombroniana, G. indica and* mace of nutmeg (0.1 g) were used to extract color in two solvents – acetone and ethanol (100 ml). The absorption maxima was read in a spectrophotometer and the reaction of the color to changes in pH (by addition of 1N HCl and 1N NaOH) and temperature (heat) were studied. Heat did not alter the color of the extracts.

Composition of *Cinnamomum* essential oil

The fruits of *Cinnamomum verum* and *C. malabatrum* contained 0.3-0.6% essential oil. The chief constituent of the essential oils of fruit of *C. verum* and *C. malabatrum* was linalool during the initial stages. However the concentration of linalool was higher in *C. malabatrum* (60.5%) compared to that of *C. verum* (25.77%). During the later stages of development, linalool content decreased and



Linalool



δ-Cadinene

both the oils were dominated by δ-cadinene (19.6% and 24.9% respectively).

Antioxidant levels in spice extracts

The spice extracts of black pepper, ginger, turmeric, curry leaves, *Garcinia indica*, *Garcinia gummi-gutta* and tamarind were analyzed for their total antioxidant capacity *in vitro*. Ethanol extracts of *Garcinia gummi-gutta* and water extracts of turmeric and tamarind had the most total antioxidant capacity as analyzed by the phosphomolybdenum method. The 1,1-diphenyl-2-picrylhydrazyl radical scavenging ability was greater in the essential oil of black pepper and ethanol extracts of black pepper, curry leaves and tamarind. Fe (III) to Fe (II) reducing activity was highest in the ethanol extracts of turmeric and the two Garcinia species, much more than the food additives BHA and BHT; black pepper essential oil exhibited the most Fe (II) chelation activity. Ethanol extracts of *Garcinia gummi-gutta* and turmeric had higher phenol content compared to others.

Inhibition of aflatoxin production by spice essential oils

Extent of fungitoxic and fungicidal effect by spice essential oils and their major components on *Aspergillus* has been quantified in terms of aflatoxin B_1. Clove oil, cinnamon oil and turmeric leaf oil had complete inhibition of aflatoxin production. Both the aflatoxins (G_1 and B_1) could be inhibited by cinnamon bark oil as well as the active ingredient – cinnamaldehyde. Minimum inhibitory concentration (MIC) of cinnamon bark oil was found to be 0.05%.

Rhizobacteria in spices

One hundred and nineteen rhizobacteria have been isolated from black pepper (50) and ginger (69). Out of these, 53 produced IAA, 38 solubilized phosphate and only 4 isolates produced HCN. Two isolates BRB 28 and BRB 37 were positive for all and 16 isolates were positive for IAA and phosphate solubilization. *In vitro* screening of these isolates was done against *P. capsici*, *Pythium and Fusarium*. Twelve rhizobacterial isolates were shortlisted based on *in vitro* tests and those showing more than 50% inhibition against *P. capsici*, *Pythium* and *Fusarium*.

Extension and training

The satellite technology based Village Resource Centre(VRC) scheme sponsored by the Kerala State Planning Board under the RSVY / VSAT programme has been commissioned by ISRO. The scheme envisages interactions between experts in identified knowledge centers

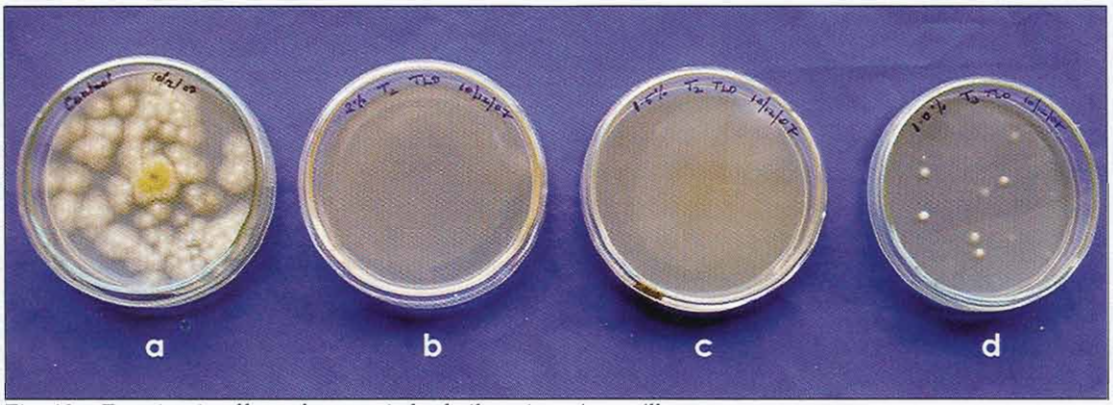


Fig. 12. Fungitoxic effect of turmeric leaf oil against *Aspergillus* sp.
a) Control, b) Leaf oil (2%), c) Leaf oil (1.5 %), d) Leaf oil (1.0 %)

and farmers enrolled in resource centres in Wyanad district of Kerala through video conferencing. Four VRCs are functioning in Wyanad district. The identified expert centres are IISR, KAU, Kerala Institute of Local Development,. Kerala State Planning Board and KVK, Amabalavayal. During the period, six conferencing sessions were held involving experts from the institute and other institutions in Calicut.

Four institute on campus training courses sponsored by various schemes under the Spices Board and State Horticulture Mission (SHM) were organized for the benefit of extension personnel in which 60 trainees participated.

A summer training programme on Biochemistry, Biotechnology and Bioinformatics was organized from 07th May to 06th June 2007 in which 42 M.Sc. students participated. Another training course on "Information Security for Government Officers" was conducted during 12-13 November, 2007 in collaboration with DOEACC Centre, Calicut.

Under the Central Sector Scheme, Technology Mission for Integrated Development of Horticulture in North Eastern States including Sikkim, one state level workshop on 'production and processing in major spices in NE states was organized in Dimapur, Nagaland in which 60 farmers/ extension personnel representing 10 districts of Nagaland participated. The programme included interactive lecture sessions on spice production systems in Nagaland on crops like black pepper, large cardamom, ginger, turmeric and chillies and field visits and method demonstrations on turmeric cultivation. The progarmme was organized in collaboration with National Horticulture Mission (NHM) for NE states.

All India Coordinated Research Project on Spices

The National group meeting (XIX workshop) of research workers of AICRP on Spices was held at Orissa University for Agriculture and Technology (OUAT), Bhubaneswar, Orissa during 23-25, November, 2007. The varietal release proposal of leafy type coriander DH – 228 from CCS HAU, Hisar was recommended for Haryana state release. The promising 11 technologies emanated from different research projects were identified for adoptive trials. Twelve new technical programmes were formulated on different spice crops.

Trials at Panniyur and Sirsi on black pepper revealed maximum yield per vine with the application of biofertilizers (*Azospirillum* @50g/ *Phosphobacteria* @ 50g) + FYM 10 kg along with recommended dose of NPK. Combined application of *Azospirillum* and *Phosphobacteria* along with VAM enhanced the yield under organic farming. Application of neem cake @ 0.5 or 1.0 kg/plant significantly reduced the shoot and capsule borer damage and significant increase in yield of cardamom both at Pampadumpara and Mudigere. The CVT trial at Pundibari on ginger showed maximum yield of 20.34 t/ha in Gorubathan compared to the check Acc. 117 (12.95 t/ha). The biofertilizer experiment at Coimbatore on turmeric revealed that inorganic N (50%) + *Azospirillum* (5 kg/ha) + 5 t FYM recorded the highest yield. A total of 37 clove, 119 nutmeg, 39 cinnamon and 6 cassia germplasm have been maintained under AICRPS centres, Dapoli and Peechiparai.

Others

Bioinformatics databases

The bioinformatics centre has developed two databases during the period under report.

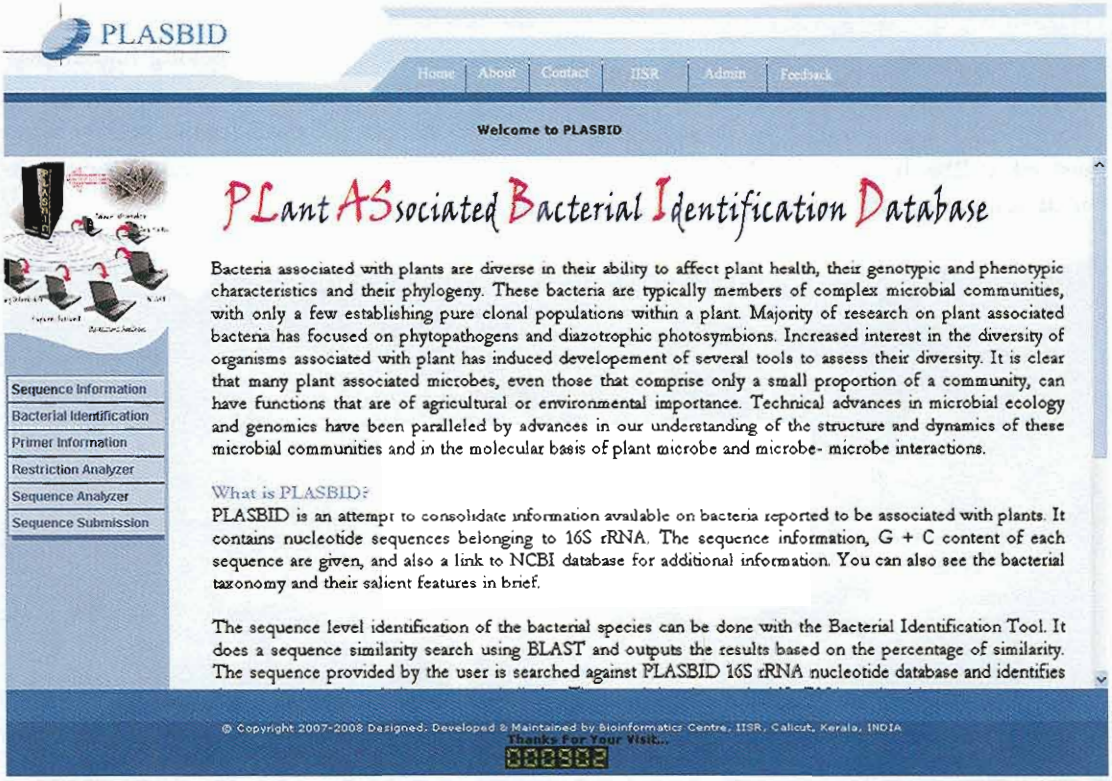


Fig. 13.PLASBID- a database on plant associated bacteria

PLASBID: A database on plant associated bacteria integrated with several tools like sequence editing, primer analysis etc.

PASSCOM: A database on predicted activities of spice compounds is being developed. The module on black pepper has been completed.

The databases PIR and PHYDISH were integrated to serve as a National Repository of *Phytophthora*.

Development of new websites and other e-resources: A new website for Agriculture Scientists Recruitment Board, New Delhi was developed. The site was launched by Dr. Mangala Rai, DG, ICAR on 5 November 2007 at New Delhi. HortWeb, the website of Horticulture Division of ICAR was developed and updated. An e-book on completed research projects of the institute (RPF III) was prepared. ARISoft, the IISR office automation software was fine tuned and modified to incorporate several new features.

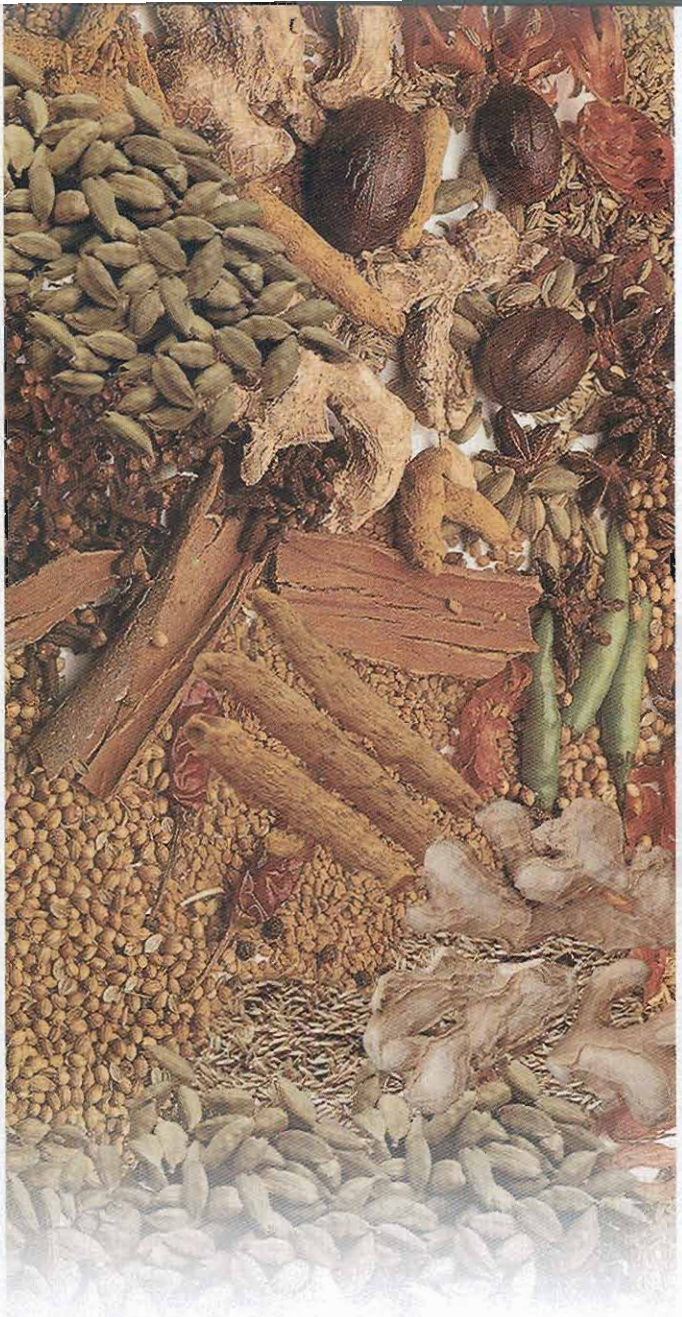
DUS test guidelines for black pepper, ginger, turmeric and cardamom

The institute under the guidance of Technical Advisory Group (Task Force – 7/2007) appointed by the Protection of Plant Varieties & Farmers' Rights Authority (GoI) is engaged in the development of DUS (Distinctness, Uniformity and Stability) test guidelines for major group of spice crops and a series of sensitization programmes were arranged at IISR and CPCRI for the benefit of farmers.

National Workshop on Zingiberaceous Spices

Dr H. P. Singh, Deputy Director General (Hort.), ICAR inaugurated the workshop organized at IISR from 19th to 20th March 2008 for disseminating the latest developments in the research on ginger, cardamom and turmeric with the aim of achieving higher productivity in these crops in a sustainable manner. More than 100 participants, mostly farmers from different states attended the workshop.

Fig. 14. H.P. Singh, DDG (Horticulture) inaugurating the National Workshop on Zingiberaceous Spices



Indian Institute of Spices Research
Post bag 1701, Marikunnu p. o.,
Calicut-673012, Kerala, India.
Phone: 0495-2731410, Fax: 0091-495-2731187,
E-mail: mail@spices.res.in, Web site: www.spices.res.in