Application for the grant of research project

Development of comprehensive SSR and SNP markers for the study of genetic diversity and association analysis in Curcuma

SUBMITTED TO

DEPARTMENT OF BIOTECHNOLOGY, New Delhi

By

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MARIKUNNU PO, CALICUT – 12
KERALA
### PART I: GENERAL INFORMATION

<table>
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<tr>
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<td>4</td>
<td>Name and designation of the Executive authority of the Institute/University forwarding the application</td>
<td>Dr. M. Anandaraj, Director, IISR, PO Marikunnu, Calicut-673012</td>
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12. **Scope of application indicating anticipated product and processes.**

This project proposal is visualized as the second phase of the Zingiberaceae network project funded by Department of Biotechnology, Govt. of India, which was operational at this Institute from 2006 to 2010. The scope of application of the present proposal is:

- The proposed study will enable to expand the already existing set of SSR markers and also identify a set polymorphic SNP markers for more accurate identification of germplasm for future conservation and improvement programmes in *Curcuma*
- Detection/development of SSRs and SNP markers associated to quality traits will aid MAS in turmeric

13. **Project Summary**

The genus *Curcuma* belonging to the family *Zingiberaceae* originated in the Indo Malayan region has a wide spread occurrence in the tropics of Asia to Africa and Australia (Purseglove *et al.* 1981). India is considered to be one of the centers of diversity for the genus as about 40 *Curcuma* spp. are reported from India (Velayudhan *et al.* 1999). The major problems in the taxonomic studies of the genus are lack of type specimens and illustrations of old species, lack of protologues with finer details in the earlier literature, absence of important floral parts in the herbarium specimens, incomplete description of the rhizome feature in the herbarium sheets, fleshy and perishable aerial portions etc (Sasikumar, 2005). It is also reported that some of the *Curcuma* spp. are synonyms (Syamkumar & Sasikumar 2005). ISSR markers were extensively used for diversity assessment and characterization in different plants. However, SSR markers due to their co-dominant nature, reliability coupled with high level of polymorphism and amenability to multiplexing are much preferred (Powell *et al.*, 1996; Milbourne *et al.*, 1997, McGregor *et al.*, 2000; Blair *et al.*, 2002; Mc Couch *et al.*, 1997; 2002) than ISSR. SSR markers have been used for genotype identification and diversity analysis in rice varieties (Prasad *et al.*, 2005; Saini *et al.*, 2004), for identification of hybrids in cotton, in identification and genetic purity testing of citrus (Oliveira and Garcia, 2002), cultivated and wild rice (Yashitola and Sonti, 2001; Shishido *et al.*, 2006), melon (Nakata *et al.*, 2005), maize (Smith *et al.*, 1997) and *Brassica napus* (Hasan *et al.*, 2006). In *Curcuma*
successful isolation and characterization of SSR markers were carried out in the DBT project (Siju et al., 2009, 2010 a&b).

An accurate identification of the specimen is very important from the point of bioprospecting of Curcuma as the genus is a treasure trove of many therapeutically important molecules. It is imperative to generate SSR fingerprints of the Curcuma species available at the IISR germplasm repository for establishing core collections and characterizing the example and extant varieties for authentic DUS testing. The present set of 37 genomic SSR markers and 17 EST markers available in Curcuma (Siju et al., 2010a) is not sufficient enough for carrying out marker assisted breeding in Curcuma. The markers identified are also insufficient to discriminate between the closely related cultivars/improved varieties. Hence there is a need to expand the sphere of SSR markers and identify SSRs with longer repeat tracts and spanning a larger region of the Curcuma genome.

Molecular marker systems such as SNPs (single nucleotide polymorphisms) derived from candidate genes are proving extremely useful in the genetic improvement of crop plants. SNPs are a marker system that can differentiate individuals based on variations detected at the level of a single nucleotide base in the genome and differs between members of a species (or between paired chromosomes in an individual. They help in the discovery of genes as a result of the differences in the nucleotide sequences.

The development of SNP markers allows automatizing and enhancing tenfolds the effectiveness of genotype analysis. SNP genotyping, provide for accurate genetic fingerprinting that is highly valuable in revealing species and population relationships of Curcuma far more accurately than method currently in vogue. Identification of markers linked to quality traits through a genome wide scan for search of QTLs is currently not feasible; hence a candidate gene approach with SNPs in select set of genes associated with phenotypes is an alternative.
PART II: PARTICULERS OF INVESTIGATORS

Principal Investigator

14. Name : Sheeja TE
Date of birth : 15.05.1969       Sex: Female
Indicate whether principal Investigator or co-investigator: Principal Investigator
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No. of projects handled at present: 1

Co Investigator I

15. Name : D Prasath
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No. of projects handled at present: 1

16. Name : Dr B Sasikumar
Date of birth : 21.1.1956       Sex: male
Indicate whether principal Investigator or co-investigator: Co Investigator

Designation: Principal Scientist
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(Indian Council of Agricultural Research),
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Pin: 673 012
Telephone: 0495-2731410
Email: bhaskaransasikumar@yahoo.com, sasikumar@spices.res.in
No. of projects handled at present: 1

PART III: TECHNICAL DETAILS OF THE PROJECT

16. Introduction:

16.1. Origin of the proposal

The genus Curcuma has a wide spread occurrence, extending from the tropics of Asia to Africa and Australia. The genus is believed to be originated in the Indo Malayan region by considering its great diversity and abundance in this region (Purseglove et al. 1981). Over eighty species of Curcuma are reported from Indo Malayan region and about forty of them are indigenous. (Velayudhan et al. 1999). It is now believed that at least some of the species may be synonyms and there may not be eighty true Curcuma species as reported earlier (Sasikumar, 2005). The scenario is true for the Chinese Curcuma species as well (Liu and Wu, 1999). A comprehensive global taxonomic revision of the genus is yet to be attempted. The major problems in the taxonomic studies of the genus are lack of type specimens and illustrations of old species, lack of protologues with finer details in the earlier literature, absence of important floral parts in the herbarium specimens, incomplete description of the rhizome feature in the herbarium sheets, fleshy and perishable aerial portions etc (Sasikumar, 2005) and also availability of limited number of molecular markers. At the Indian Institute of Spices Research, Calicut, a set of 17 EST SSRs and 37 genomic SSR markers were isolated and developed in Curcuma and 14 Curcuma species were characterized using these markers (Siju et al., 2010) besides 20
accessions of turmeric. Thus a feasible and economical technique of isolating genomic SSR markers was identified in *Curcuma*. However, the number of markers now available is insufficient for any further advanced study. Expanding the repertoire of SSR markers shall help in discriminating closely related cultivars/improved varieties, establishing unique fingerprints of the germplasm accessions and also help in marker assisted breeding of turmeric.

The recent advances in genomic resources, in particular SNP genotyping, provide for accurate genetic fingerprinting that is highly valuable in revealing species and population relationships of plant communities more accurately than existing molecular and taxonomic methods based on morphology. More broadly, such genetic fingerprinting will become an integral part of germplasm characterization information and management. Though several studies suggest that qualified candidate genes can be effectively converted into informative molecular markers by means of association studies, no such efforts have been initiated in *Curcuma*. The project proposes to identify potential SNP markers from *Curcuma*, to conduct a diversity analysis using SSR and SNP markers to characterize the accessions, and apply association mapping to analyze useful agronomic traits and high quality. The data from this effort will provide an opportunity to become familiar with SNP genotyping and the challenges and opportunities it presents.

16.2a. Rationale of the study supported by cited literature

Conventional taxonomic tools have its own limitations in *Curcuma* taxonomy. The major problems in the taxonomic studies of the genus are lack of type specimens and illustrations of old species, lack of protologues with finer details in the earlier literature, absence of important floral parts in the herbarium specimens, incomplete description of the rhizome feature in the herbarium sheets, fleshy and perishable aerial portions etc (Sasikumar, 2005). Taxonomic revision of the genus is in progress now. It is believed that the Indian species *C.zoedoaria* is synonym of *C.xanthorrhiza*, similarly the Chinese species *C.albicoma* and *C.chuanyugin* are synonyms of *C.sichuanenesis* and *C.kwangsiensis*, respectively. *C.wenyujin* is now recognized as *C.aromatica*.

Among the traditional taxonomic tools, spike position is an important trait in *Curcuma*. Position of spikes in *Curcuma* is either terminal or lateral and is one of the
major discriminatory traits of the species along with presence of coma bract, bract color. However, the position of the spikes and the color characters has been a subject of controversy (Larsen and Smith, 1978). Roxburgh (1910) pointed out that this difference is seasonal, the early spikes been lateral and the latter ones central. Santapau (1945, 1952) added that in *C. pseudomontana* at the beginning of the rainy season the plant has a large spike coming out from the side of the leaves. Gradually by beginning of August, this lateral spike decays and the central one appears surrounded by leaves, resulting in both central and lateral spikes in the same plants. Santapau (1945) also reported that the color characters shows much variations within the species and that in *C. pseudomontana* the color of the bracts of the coma is green, pink, rose, purple or pure white. Bract color variation is also noted in *C. ecalcarata* (Sasikumar, 2005; Sabu, M. Unpublished). Hence both these characters are undependable for the delimitation of species in the genus. Thus it is clear that relying much on the morphological characters alone in species delimitation has its own limitations in the genus. Correct taxonomic identification of the *Curcuma* species will go a long way in bioprospeting as the genus is credited with molecules having a variety of therapeutic and other properties such as insect repellant, antivenomous, antiinflammatory, antimicrobial, antiviral, anticancerous, antidiabetic etc. (Sasikumar, 2005). Incorrect taxonomic identification also leads to inappropriate conservation priority.

The number of genomic resources such as ESTs/SSRs and SNPs for key traits of interest is too meagre in the public domain; hence, there is an urgent need to develop these resources to provide an impetus to molecular analysis of *Curcuma*. In the Phase I of this project we identified a robust set of 17 EST and 37 genomic SSR markers from *Curcuma* and these markers were found to be highly polymorphic and able to distinguish clearly the 14 different species of *Curcuma*. However, the markers could not discriminate among the closely related accessions; hence there is a need for identifying more number of SSR markers.

SNP genotyping, studies in the recent past has proved to complement SSRs and provide for accurate genetic fingerprinting, are highly valuable in revealing species and population relationships more accurately than existing molecular and taxonomic methods. Moreover, identification of SNP markers is also reported to be effective for the genetic
characterization and association analysis of markers linked to important agronomic traits. So far such studies in *Curcuma*, is rather nil. Lack of such markers is the major lacuna in marker assisted breeding in *Curcuma*. Moreover it is felt essential that accurate genetic fingerprinting should form an integral part of germplasm collection, characterization, and management for fixing appropriate conservation priority as well as bioprospecting in *Curcuma*. Identification of markers with significant associations to useful traits of interest is also an utmost necessity for marker assisted breeding in *Curcuma*.

16.2 b. Hypothesis:

- In the first phase of the project it has been possible to identify 17 EST and 37 polymorphic genomic SSR markers from curcuma for discrimination of the different species of *Curcuma*. However, some of the closely related accessions and cultivars were not distinguishable. Also there are no markers that are currently available that can be tagged for a particular trait. It is hence hypothesized that a detailed investigation involving a larger set of SSR markers will help to solve this problem. It is also expected that SNPs, can help in broadening the scope for meeting the above objectives of informative markers for cultivar identification and taxonomic revision in *Curcuma* and also markers having strong associations with quality traits like curcuminoids content.

16.2 c. Key Questions:

- What is the extent and frequency of polymorphic SSR and SNP markers in the genome of *Curcuma*?
- What is the possibility of locating species specific markers in the genus?
- Can we have markers tightly linked to quality/agronomic traits in turmeric?

16.3 *Current status of research and development in the subject* (both international and national status)

**International status**

Molecular marker studies are limited in *Curcuma*. Apavatjrut *et al.* (1999) studied isozyme polymorphism in seven early flowering and two unidentified *Curcuma* species...
of Thailand. Comparative isozyme polymorphism of the cultivated and natural populations of *C. alismatifolia* from Thailand and Japan revealed that the cultivated population is more uniform genetically (Paisooksantivatana *et al.* 2001). A novel attempt to identify the genuine *Curcuma* species traded as drug in China and Japan based on sequence analysis of 18 S rRNA gene and trnK gene coupled with amplification refractory mutation system (ARMS) analysis was done by Sasaki *et al.* (2002). Though designed to identify the spurious *Curcuma* Spp. in the marketed drugs, this method also would help in molecular taxonomy of *Curcuma*.

Molecular analysis based on polymorphisms of the chloroplast DNA (cpDNA) using the intergenic spacer between *trnS* and *trnM* (*trnSfM*), distinguished four *Curcuma* species include *C. longa*. The number of AT repeats in the *trnSfM* region was predictive of the curcumin content in the rhizome of *C. longa*. (Minami *et al.*, 2009)

High intra-populational genetic diversity and low inter-populational diversity was observed in the RAPD analysis of *C. zedoaria* analysed by RAPD (Islam 2004)

Significant genetic variations by RAPD markers have also been reported in *Curcuma* species at cultivar level (Das *et al.*, 1998). Utility of rice microsatellite markers in genetic diversity analysis of *Zingiberaceae* from eight Asian countries was established by Jatoi *et al.* (2006).

The phylogenetic analysis conducted by Nagamriabsakul *et al.*, 2004 using the ITS regions of ribosomal and chloroplast DNA in the tribe Zingiberaceae confirmed the monophyletic status of the tribe with the *Curcuma* clade and *Hedychium* clade. Kress *et al.*, 2002 also suggests the utility of molecular markers for phylogenetic studies in *Curcuma* species.

A high polymorphism of >91% displayed existence of genetic variability in the germplasm of *C. amada*. Neutral regions of the mango ginger were more variable compared with the functional regions using rice SSR based RAPDs and functional genomic (P450 based analogue) markers (Jatoi *et al.*, 2010). Seventeen polymorphic microsatellite loci were developed using a CT/GT/CTT enriched genomic library from *C. longa* (Sigrist *et al.*, 2010).

Molecular marker systems such as SNPs (single nucleotide polymorphisms) derived from candidate genes are proving extremely useful in the genetic improvement of
crop plants. They are excellent markers for association mapping of genes controlling complex traits and provide the highest map resolution (Brookes, 1999; Bhattramakki et al., 2002). SNPs are robust in usage and polymorphisms are identifiable and there are several methods that can be used to detect them. SNPs are the most frequent type of variation found in DNA (Brookes, 1999; Cho et al., 1999) and their discovery together with insertions/deletions has formed the basis of most differences between alleles. Though, studies on the occurrence and nature of SNPs are beginning to receive considerable attention in plants reports from genus *Curcuma* is confined to that of Sasaki et al. (2004) for identification of plants and drugs derived from *Curcuma* species including *C. longa*. Based on the difference in the nucleotide positions at 177, 645, 724 and a 4 base indel on the *trnK* gene obtained using three different lengths of (26 mer, 30 mer and 34 mer) reverse primers helped to identify the four *Curcuma* species studied.

In *Arabidopsis* over 37, 000 SNPs have been identified through the comparison of two accessions (Jander et al., 2002). It has been reported in maize that there occurs a frequency of one non-coding SNP per 31 bp and 1 coding SNP per 124 bp in 18 maize genes assayed in 36 inbred lines (Ching et al., 2002). In a related transcriptome-based molecular marker technique. Development of new SNPs include re-sequencing of PCR amplicons with or without pre-screening, electronic SNP (eSNP) discovery in shotgun genomic libraries and eSNP discovery in expressed sequence tag (EST) libraries (Rafalski, 2002). A number of EST collections have also been used to describe and detect SNPs in maize (*Zea mays* L.) (Ching et al., 2002) and Soybean (*Glycine max* L. Merr.) (Zhu et al., 2003). In another studies using cassava Lopez et al. (2005) have developed strategies for detecting SNPs from ESTs. The estimated frequency for intra-cultivar SNPs was one per 905 bp and one per 1,032 bp for the inter-cultivar SNPs. For SNPs detection derived from 3’ ESTs, they detected a total of 136 SNPs and the frequency of one per 66 bp. The number of SNPs in the coding regions was low compared to outside the coding regions and the non-coding regions can accumulate a greater number of polymorphisms and that not all genes accumulate SNPs at the same time. They observed two groups of genes: 1) those containing a relatively high number of SNPs (more than 6) and those with few or no SNPs. SNP haplotypes (the specific pattern and order of alleles on a chromosome) may be detected in genomes and this may give us information as to
whether they are in Linkage Disequilibrium (LD), that is if alleles at one locus are not randomly assorted with alleles at another locus (Borecki and Suarez, 2001).

SNP technology may allow us to locate and detect candidate ESTs associated with agronomic traits and obtain a transcript map, which can be directly compared with an earlier detected quantitative trait loci. Several candidate genes in relation to cell-wall digestibility and forage quality are meanwhile available (http://www.polebio.scsv.ups-tlse.fr/MAIZEWALL/index.html). Varying levels of LD have previously been observed between genes of the phenylpropanoid pathway, decaying within few hundred bps for CCoAOMT2 and COMT (Guillet-Claude et al. 2004a, b, Zein et al. 2007; Fontaine et al., 2003) while spanning more than 3.5 kb at the PAL locus (Andersen et al. 2007). The PAL gene was investigated in a set of 32 European elite inbred lines (Andersen et al. 2007). A one-bp deletion in the second exon of PAL, introducing a premature stop codon, was associated with high IVDOM. In conclusion, availability of qualified candidate genes can be effectively converted into informative molecular markers by means of association studies. It is envisaged that the knowledge gained from the understanding of plant functional genomics, ESTs and SNPs may find important application in breeding, agronomic practice and ecosystem research, especially in developing countries.

National status

Shamina et al (1998) studied isozyme polymorphism in C. longa accessions. The study revealed that C. longa accessions collected from same geographical area are genetically more uniform. Salvi et al (2001) reported the RAPD analysis of eight plantlets derived from the leaf base callus of turmeric using 14 random decamer primers. Sasikumar et al (2004) developed a PCR based method to detect extraneous Curcuma species contamination in marketed turmeric powder. Syamkumar et al (2003) developed a PCR technique to discriminate Curcuma longa varieties from DNA extracted from fresh rhizomes. Recently Syamkumar & Sasikumar (2005) characterized 16 Curcuma species based on ISSR/RAPD markers and indicated the existence of synonymous entities. RAPD analysis in a set of 13 morphotypes of C. longa and wild species of Curcuma was adjudged to be highly informative in discriminating the germplasm of Curcuma (Hussain et al., 2008).
Random amplified polymorphic DNA (RAPD) analysis clearly revealed genetic variation among 17 cultivars of turmeric showing differential polymorphism using 20 primers (Nayak et al., 2006). MaturaseK gene (*MatK*) of chloroplast from Zingiberaceae was identified as a good candidate for DNA barcoding of plant family Zingiberaceae (Selvaraj et al., 2008).

Though in many crop plants, SNPs are present with sufficient frequency to offer an alternative for genetic mapping and marker-assisted selection, reports in the family Zingiberaceae is little. Identification of EST derived SNPs in ginger has been done basing on *In silico* studies through bioinformatics (Chandrasekhar et al. 2009). These e-SNPs are not yet validated for characterization and identification of different cultivars of *Zingiber officinale*.

Expressed sequence tags (ESTs) from turmeric were used for screening of type and frequency of Class I (hypervariable) simple sequence repeats (SSRs). A total of 231 microsatellite repeats were detected from 12,593 EST sequences of turmeric after redundancy elimination. The average density of Class I SSRs accounts to one SSR per 17.96 kb of EST. Mononucleotides was the most abundant class of motifs followed by trinucleotides. Genetic diversity of 30 turmeric accessions collected from different geographical locations of India was carried out (Siju et al., 2010b). Isolated 37 genomic microsatellite markers from *Curcuma* (Siju et al., 2010a; communicated). The nucleotide sequences of the polymorphic microsatellite markers were deposited in GENBANK. Biologically active peptide turmerin was isolated from 13 Indian *Curcuma* and 15 turmeric varieties and cultivars.

**Literature cited**


12. Das A B, Rai S and Das P. Karyotype analysis and 4C DNA content in some cultivars of ginger (Zingiber officinale Rosc.). Cytobios. 1998; 93:175-84


24. Mc Couch, S.R., Leonid Teytelman, Yunbi Xu, Kartarzyna B. Lobos, Karen Clare, Mark Walton, Bining Fu, Reyceel Maghirang, Zhikang Li, Yongzhong Xing, Qifa Zhang, Izumi Kono, Masahiro Yano, Robert Fjellstrom, Genevieve Declerck, David Schneider, Samuel Carithour, Doreen Ware and Lincoln Stein (2002). Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.)
16.4. Relevance and expected outcome of the proposed study

1. Help to develop species specific markers for the identification of different Curcuma species, and also help in genetic diversity studies and aid in taxonomic revision of the genus

2. Lay out correct conservation priority and aid in better management and conservation of Curcuma germplasm nationally/globally

3. Generate an exhaustive SSR/SNP based marker data base in Curcuma
4. Identification of potential markers associated to quality traits will help in future introgression of this novel allelic variation from genebank accessions into elite germplasm through breeding

16.5.1 Preliminary work done so far

In the first phase of the project on development of microsatellite markers from *Curcuma* the team has successfully isolated 17 EST and 37 genomic SSR markers from *Curcuma* and all these sequences have been deposited in the NCBI. Two full length research papers, one poster presentation and one short communication on EST and Genomic SSR development and characterization in *Curcuma* are published/communicated.

Relevant publications of the PI & Co-PI in the subject are given below
4. Siju, K. Dhanya, S. Syamkumar, T.E. Sheeja, B. Sasikumar, A.I. Bhat and V.A. Parthasarathy. Expanding the repertoire of genomic microsatellite markers in *Curcuma longa* L. (Communicated to Scientia Horticulturae)
17. Objectives (should be written in bulleted form, a short paragraph indicating the methods to be followed for achieving the objective and verifiable indicators of progress should follow each specific objective).

- Expanding the repertoire of EST and genomic SSRs and SNP markers

SSRs and SNPs will be identified from the EST database of *Curcuma* using popular SSR and SNP search analysis tools. Primers for SSR and SNP analysis of specific gene regions will be designed using appropriate softwares and PCR conducted as per available protocols for SSR. Protocols for SNPs need optimization.

Verifiable indicator of progress: Development of additional number of robust set of SSRs and SNPs

- Genetic diversity analysis and characterisation of *Curcuma* species using SSR and SNP markers and development of fingerprint database of major cultivar/varieties and other *Curcuma* species of importance using SSR and SNP markers.

*Curcuma* species are already available in the germplasm repository of IISR that houses about 1070 accessions of *Curcuma longa* and other important species of *Curcuma*. DNA analysis shall be done as per the protocols available with us. Detailed taxonomical analysis of collected germplasms will be made and documented. Cultivar specific amplicons will be eluted and custom sequenced. Informative SSRs and SNPs will be assigned to the cultivars.

Verifiable indicator of progress: Assigning informative markers to identify important accessions and development of fingerprints of important accessions

- Association analysis and identification of markers linked to quality traits using SSR and SNP approaches

A set of accessions that include accessions varying in curcumin levels will be selected. The validation of marker trait association will be done using atleast 100 accessions and genotyped with the identified marker. Concurrently, we will sequence portions of genes that are candidate loci involved in curcinoid biosynthesis. The single nucleotide polymorphisms (SNP) if detected will be developed into markers for those specific genes.
All plants will be genotyped with the SNP markers. We will test for associations based on both SSR molecular markers, as well as on SNP markers for candidate genes. 

Verifiable indicator of progress: Identification of markers associated to quality traits

18.1. Work Plan

First Year
a) Appointment of staff
b) Purchase of equipments
c) Developing SSR enriched libraries and identification of microsatellites

Second year
a) Sequencing of clones and primer designing and synthesis
b) Identification of useful polymorphic SSR markers
c) Developing SNP marker system in curcuma (EST mining, sequence analysis of genes of key enzymes of curcumin biosynthetic pathway)
d) Developing DNA fingerprints using polymorphic SSR and SNP markers of important accessions of the germplasm (including DUS example varieties and extant varieties)

Third year
a) Characterization of *Curcuma* species and cross species transferability studies using selected markers
b) Genetic diversity analysis of important accessions with the newly developed markers
c) Association analysis and identification of markers linked to quality, if any
d) Compilation of final report

18.2. Connectivity of the participating institutions and investigators (in case of multi-institutional projects only): NA
18.3 **Alternate strategies** (if the proposed experimental design or method does not work what is the alternate strategy): Already the protocols are optimized for SSRs, in case of SNPs alternate strategies will be adopted if needed based on the observations from the experiments

1. **Timelines**

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<td>April 2012</td>
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<td>2</td>
<td>Identification of polymorphic SSRs, characterization of selected turmeric accessions using microsatellite markers and cross species amplification studies</td>
<td>April 2012</td>
<td>Nov 2012</td>
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<tr>
<td>3</td>
<td>Identification of SNPs from ESTs in public domain and candidate genes</td>
<td>Aug 2013</td>
<td>Feb 2014</td>
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<td>Validation of SNP and genotyping</td>
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<td>Association analysis and identification of markers linked to quality traits</td>
<td>Mar 2015</td>
<td>Aug 2015</td>
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<td>6</td>
<td>Compilation of final report</td>
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<td>Dec 2015</td>
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20. **List of experts in India in the proposed subject area**

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Designation</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dr. C. Aswath</td>
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<td>Division of Biotechnology, IIHR, Hessaraghatta, Bangalore- 20. E mail: <a href="mailto:aswath@iihr.ernet.in">aswath@iihr.ernet.in</a>.</td>
</tr>
<tr>
<td>2</td>
<td>Dr. P. Ananda Kumar</td>
<td>Project Director</td>
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</tr>
<tr>
<td>3</td>
<td>Dr. J.L. Karihaloo</td>
<td>Co-ordinator</td>
<td>Asia-Pacific Consortium on Agricultural Biotechnology, C/o ICRISAT, NASC, DPSM, ND- 12 Tel. ph. 91-11-32472305 E mail: <a href="mailto:j.karihaloo@cgiar.org">j.karihaloo@cgiar.org</a></td>
</tr>
<tr>
<td>4</td>
<td>Dr. P. Das</td>
<td>Former INSA Senior Scientist</td>
<td>C-122, HIG Orissa State Housing Board Colony, Baramuda, Bhubaneswar, 751003, Orissa Tel. 0674 2557970 Email: <a href="mailto:das_premananda@yahoo.co.uk">das_premananda@yahoo.co.uk</a>, <a href="mailto:profpdas@yahoo.co.uk">profpdas@yahoo.co.uk</a></td>
</tr>
<tr>
<td>5</td>
<td>Dr. H S Gupta</td>
<td>Director</td>
<td>Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, Delhi <a href="mailto:director@iari.res.in">director@iari.res.in</a></td>
</tr>
</tbody>
</table>
PART IV – BUDGET PARTICULARS

17. Budget

A. Non Recurring (equipments, accessories, etc): Nil

B. Recurring

B1) man power

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Position No.</th>
<th>Consolidated Emolument</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SRF (3)</td>
<td>16000+20% HRA for 1 &amp; 2 years 18000+20% HRA for 3rd year</td>
<td>691200</td>
<td>691200</td>
<td>777600</td>
<td>21,60,000</td>
</tr>
</tbody>
</table>

Justification for the staff in terms of work content and expertise required
The proposed project work involves exhaustive work in both SSR and SNP markers besides the morphological data collection and hence three research fellows are needed.

B2) Consumables

<table>
<thead>
<tr>
<th>S1.No.</th>
<th>Item</th>
<th>Year 1 (Rs)</th>
<th>Year 2 (Rs)</th>
<th>Year 3 (Rs)</th>
<th>Total (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chemicals/Solvents/kits/sequencing</td>
<td>700000</td>
<td>950000</td>
<td>700000</td>
<td>2350000</td>
</tr>
<tr>
<td>2</td>
<td>Glass/Plastic wares</td>
<td>50000</td>
<td>50000</td>
<td>-</td>
<td>100000</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>750000</td>
<td>1000000</td>
<td>700000</td>
<td>2450000</td>
</tr>
</tbody>
</table>
Justification for consumable items
The consumables include routine chemicals used for the molecular biology/biochemical experiments such as enzymes, primers, vectors, cloning kits etc. and other consumable items including microtips, PCR plates, microtubes/vials, petriplates, glasswares etc. needed for the proposed work. A good amount of custom sequencing is involved in the project.

B3) Travel

<table>
<thead>
<tr>
<th>Travel</th>
<th>Year 1 (Rs)</th>
<th>Year 2 (Rs)</th>
<th>Year 3 (Rs)</th>
<th>Total (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50000</td>
<td>50000</td>
<td>50000</td>
<td>1,50000</td>
</tr>
</tbody>
</table>

Justification for travel
Annual reporting, attending seminars, workshops/training, presentation of research findings etc.

B4) Contingencies

<table>
<thead>
<tr>
<th>Contingencies</th>
<th>Year 1 (Rs)</th>
<th>Year 2 (Rs)</th>
<th>Year 3 (Rs)</th>
<th>Total (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200000</td>
<td>100000</td>
<td>100000</td>
<td>4,00,000</td>
</tr>
</tbody>
</table>

Justification for other contingencies
Minimum amount has been requested under contingencies for miscellaneous expenditure such as stationery, postage, preparation of annual/final reports, photocopying/scanning/printing and other miscellaneous purchases and expenditure etc.

B5) Overhead Charges (Institutional charges)

<table>
<thead>
<tr>
<th>Institutional charges</th>
<th>Year 1 (Rs)</th>
<th>Year 2 (Rs)</th>
<th>Year 3 (Rs)</th>
<th>Total (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125000</td>
<td>125000</td>
<td>125000</td>
<td>3,75,000</td>
</tr>
</tbody>
</table>

Grand total (A + B) = Rs. 55,35000
Part V. Existing Facilities

B. Equipment available with the Institute/ Group/ Department/ Other Institutes for the project:

<table>
<thead>
<tr>
<th>Equipment available with</th>
<th>Generic Name of Equipment</th>
<th>Model, Make &amp; year of purchase</th>
<th>Remarks including accessories available and current usage of equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI's Department/PI and his group</td>
<td>Cold Centrifuge</td>
<td>Biofuge</td>
<td>All the equipments are housed in a Central Instrumentation Facility within the Department and open to use by all Scientists of the Division</td>
</tr>
<tr>
<td></td>
<td>Gel Electrophoresis Unit (vertical)</td>
<td>Biorad</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hot Air Oven</td>
<td>NSW-labex instruments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water Bath</td>
<td>Remi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Laminar Air Flow</td>
<td>Klenzaids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gel documentation system</td>
<td>Alpha Imager</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water bath with temp control and water circulation</td>
<td>Julabo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybridisation oven</td>
<td>Biorad</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCR machine</td>
<td>MJ Research, Eppendorf</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein Purification System</td>
<td>LKB</td>
<td></td>
</tr>
</tbody>
</table>

List of facilities being extended by parent institution(s) for the project implementation.

A) Infrastructural Facilities:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Infrastructural Facility</th>
<th>Yes/No/ Not required Full or sharing basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water &amp; Electricity</td>
<td>Yes</td>
</tr>
<tr>
<td>2.</td>
<td>Laboratory Space/ Furniture</td>
<td>Yes</td>
</tr>
<tr>
<td>3.</td>
<td>Power Generator</td>
<td>Yes</td>
</tr>
<tr>
<td>4.</td>
<td>AC Room or AC</td>
<td>Yes</td>
</tr>
<tr>
<td>5.</td>
<td>Telecommunication including e-mail &amp; fax</td>
<td>Yes</td>
</tr>
<tr>
<td>6.</td>
<td>Transportation</td>
<td>Yes</td>
</tr>
<tr>
<td>7.</td>
<td>Administrative/ Secretarial support</td>
<td>Yes</td>
</tr>
<tr>
<td>8.</td>
<td>Information facilities like Internet/ Library</td>
<td>Yes</td>
</tr>
</tbody>
</table>
PART VI: DECLARATION/CERTIFICATION

It is certified that

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b) the same project proposal has not been submitted to any other agency for financial support.

c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)

d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.

e) if the project involves the utilization of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.

f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.
h) We agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i) The institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.

j) The Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of executive Authority of Institute/University with seal
Date:

Signature of Principal Investigator

(On deputation till October 2011)

Signature of Co-Investigator
Date:

Signature of Co-Investigator
Date:
PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS – 1

Annexure I

Biodata of Principal Investigator

Name: Dr. Sheeja TE
Address: Senior Scientist (Biotechnology), Indian Institute of Spices Research, Marikunnu PO, Calicut- 673012
Home Phone: 0495 2382655
Mobile: 91 9495760661

E-Mail: teshee@rediffmail.com
sheeja@spices.res.in

QUALIFICATIONS:
MSc Biotechnology
Title of dissertation work: Production of cyclodextrin glycosyl transferase enzyme by batch, fedbatch and continuous fermentation approaches

PhD (Plant Biotechnology)
Title of thesis- Studies on in vitro culture response of tomato (Lycopersicon esculentum mill.) and development of drought tolerant varieties through biotechnological approaches

EMPLOYMENT BACKGROUND:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Position held</th>
<th>From</th>
<th>To</th>
<th>Employer</th>
<th>Institute/Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Scientist (Biotechnology)</td>
<td>27.01.1998</td>
<td>04.03.1999</td>
<td>ICAR, New Delhi</td>
<td>Indian Institute of Spices Research (Indian Council of Agricultural Research, Govt. of India), Calicut-12</td>
</tr>
<tr>
<td>2.</td>
<td>Scientist (Biotechnology)</td>
<td>05.03.1999</td>
<td>28.02.2003</td>
<td>Do</td>
<td>Central Agricultural Research Institute, Port Blair</td>
</tr>
<tr>
<td>3.</td>
<td>Scientist Sr. Scale (Biotechnology)</td>
<td>28.02.2003</td>
<td>26.06.2004</td>
<td>Do</td>
<td>Do</td>
</tr>
<tr>
<td>4.</td>
<td>Scientist Sr. Scale (Biotechnology)</td>
<td>26.06.2004</td>
<td>27.01.2008</td>
<td>Do</td>
<td>Indian Institute of Spices Research (Indian Council of Agricultural Research, Govt. of India), Calicut-12</td>
</tr>
<tr>
<td>5</td>
<td>Sr. Scientist (Biotechnology)</td>
<td>27.01.2008</td>
<td>Till date</td>
<td>Do</td>
<td>Do</td>
</tr>
</tbody>
</table>
## Responsibilities/Achievements (Projects undertaken so far)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Title of the project</th>
<th>Duration</th>
<th>Total approved cost in the project (in Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>In vitro</em> propagation and molecular characterization of valuable medicinal plants of Bay Islands-PI</td>
<td>2000-2004</td>
<td>16.0</td>
</tr>
<tr>
<td>2.</td>
<td>Physiological approaches for improved abiotic stress tolerance in solanaceous vegetable crops-Co-Investigator</td>
<td>2000-2004</td>
<td>6 lakh</td>
</tr>
<tr>
<td>3.</td>
<td>Biodiversity characterization, conservation and bio-prospecting of four economically important medicinal plant species of Bay islands-Co-Investigator (National Medicinal Plant Board)</td>
<td>2003-2006</td>
<td>16 lakh</td>
</tr>
<tr>
<td>6.</td>
<td>Tolerance to drought- sub project under the mega project Breeding black pepper- PI</td>
<td>2004-2008</td>
<td>6 lakh</td>
</tr>
<tr>
<td>8.</td>
<td>Development of microsatellite markers and characterization of <em>curcuma</em> Co Investigator</td>
<td>2006-2009</td>
<td>85 lakh</td>
</tr>
</tbody>
</table>

### Achievements in brief

- Conducted extensive surveys and collected important endemic, endangered and indigenous medicinal plants of the A&N islands.
- Technology developed for mass multiplication of selected medicinal plants indigenous to the islands. Standardized techniques for biochemical and molecular characterization of some of the important endemic, endangered and indigenous medicinal plants of A&N islands.
- Public awareness creation among the locals and other inhabitants towards conservation and use of indigenous medicinal plants and products.
- Developed protocols for indirect and direct regeneration from *Lycopersicon esculentum*. Optimised micropropagation protocols and identified promising somaclones tolerant to drought.
- Optimised protocols for developing drought tolerant regenerants of *L. esculentum* using *in vitro* screening methodology using Polyethylene glycol stress. Putative tolerant somaclones identified and were found to perform well in field.
• With a view to generate drought tolerant *Lycopersicon esculentum*, developed *Agrobacterium* mediated transformation techniques using *osmotin* and *sod* genes. The transformants were confirmed using PCR, dot blot and southern blotting.

• Efficient protocols developed for isolation of DNA from nine wild and related genera of *Myristica* sp. rich in polysaccharides and polyphenols. Parameters were optimized for amplification of DNA from *Myristica* sp. by ISSR, RAPD and 18S rDNA ITS primers. Developed a molecular method for determining the genetic diversity in closely related accessions of large germplasm repositories.

• Molecular characterization of wild and related genera of *Myristica* using RAPD, ISSR, ITS-RFLP and sequence analysis of 18S rDNA regions was carried out.

• The major objective is to transfer drought tolerance character into a superior black pepper variety through inter varietal hybridization and identification of markers linked to drought. The project is ongoing and shall utilise techniques such as molecular markers and map based cloning. The hybridisation programmes were successfully completed and about 300 seedlings derived from the cross was planted. These progenies were screened for drought tolerance by withdrawal of irrigation. Putative tolerant lines were identified and planted in field.

• Isolated 57 genomic and EST microsatellite markers from *Curcuma longa* L. a popular medicinal spice plant otherwise known as turmeric, adopting the selective hybridization method using biotinylated probes. The characterisation of turmeric accessions and wild species of *Curcuma* using microsatellite markers completed. The sequences were deposited in the NCBI.

**Other responsibilities undertaken**

• I am actively involved in HRD activities. During the past five years I have guided 25 MSc students and 8 post MSc students for their project work on various aspects of Molecular Biology. I have been involved in organizing training programmes in Biotechnology as course co-ordinator and as resource person in the training programmes on Biotechnology and Bioinformatics conducted by the Institute.

I am acting as the Member Secretary/Nodal Officer of the Institute Technology Management Unit (ITMU) looking after the IPR related issues at our institute level. The ITMU is a very active unit under the control of ICAR functioning as a nodal agency to facilitate services to the Scientists of the institute and farmers and NGOâ€™s of the area in imparting IPR rights to their innovations and commercial products. So far assisted in several issues like granting GI to Malabar pepper, GI to cardamom, obtaining trademark to commercial products of voluntary organizations in the area, registration of patents for innovations made by scientists and clarifying the doubts of farmers and NGOs regarding IPR. ITMU has mediated the job of training all Scientists of the institute in the area of IPR with the aim to educate them about the various facets of IPR. I have delivered several lectures on IPR to farmers/lecturers/students with the aim to awareness generation. Also prepared documents for registration of plant varieties under the

❖ Research papers published in approved journals


23. VA Parthasarathy, Sheeja TE. and Sajesh VK. 2009. Geographical Indicators in agri-horticultural crops. Lead lecture to be delivered at the symposium on Recent Global Developments in the Management of
SKILLS/ TRAINING:

(Details of relevant training courses attended)

<table>
<thead>
<tr>
<th>Course Description</th>
<th>Dates</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR amplification and cloning of gene</td>
<td>29th Mar-18th April 2000</td>
<td>CAS for Biochemistry, IARI, New Delhi</td>
</tr>
<tr>
<td>Molecular markers (SSR)</td>
<td>16th-19th Aug, 2004</td>
<td>CPCRI, Kasargod</td>
</tr>
<tr>
<td>Bioinformatics and Biotechnology Applications in</td>
<td>26th Sept to 16th Oct, 2004</td>
<td>IISR, Calicut</td>
</tr>
<tr>
<td>Agricultural research</td>
<td></td>
<td>Regional Research Laboratory (CSIR), Trivandrum, for Public R&amp;D Labs under EU-India Trade and Investment Development Programme</td>
</tr>
<tr>
<td>&quot;Workshop on Intellectual Property Rights held at</td>
<td>1-2 March 2007</td>
<td>Regional Research Laboratory (CSIR), Trivandrum, for Public R&amp;D Labs under EU-India Trade and Investment Development Programme</td>
</tr>
<tr>
<td>IP and Technology Management in ICAR System</td>
<td>May 28-30, 2007</td>
<td>ICAR, NAARM, Hyderabad</td>
</tr>
<tr>
<td>Recombinant DNA Techniques</td>
<td>August 16 to Sept 5, 2008</td>
<td>CAS for Biochemistry, IARI, New Delhi</td>
</tr>
<tr>
<td>Programme on IPR&amp; WTO related issues</td>
<td>Feb 18-22, 2008</td>
<td>ASCI, Hyderabad</td>
</tr>
</tbody>
</table>

International Training: I have attended a 10 days training programme in Plant Variety Protection, conducted by Naktuinbouw in collaboration with WUR, Wageningen during 14-25th June 2010 at Netherlands

Course attended
Successfully completed the online course on "Introduction to the UPOV System of Plant Variety Protection under the UPOV Convention (DL-205)" by UPOV

PROFESSIONAL MEMBERSHIPS

1. Life member Indian Society of Spices
2. Life member Indian Society of Plantation Crops
AWARD

Dr. H.S. Mehta award for the best paper (poster) 2009, in the National symposium on spices and aromatic crops (SYMSAC V) held at CIH, Nagaland, India for the paper ‘A SCAR marker based method for sex determination in dioecious betel vine (Piper betel L.)’ by Sheeja et al

Chapters in books


Bulletin


Reports

Popular Articles

2. **Sheeja T.E. (2005).** *Stevia rheubaudiana* a splash of sweetness. Spice India, Pg 53-54.

Seminars/symposia attended

1. **Sheeja, T.E., Sheeba and Asit B. Mandal (2003).** Medicinal uses of mangroves: A report from Bay Islands. Submitted for presentation in the workshop on Mangrove Biodiversity, CARI, Port Blair.
3. **Mandal A.B., D. Chattopadhyay, Sheeja, T.E., R. Senthil kumar (2003).** Characterisation and bioactive compound profiling of three valuable ethnomedicinal plants of Bay Islands. Presented in National Seminar on production and Utilization of Medicinal Plants held at Annamalai University, TamilNadu
7. **Sheeja T.E., Martin AB., Praveen K. (2006).** Medicinal uses of the weeds commonly found in Bay Islands. National Seminar on Recent Trends in Crop Science Research (Gregor Mendel Foundation) held at Calicut University on 21st and 22nd January, 2006 pg 39
8. **NBA workshop on Biodiversity and Bioresources Conservation Awareness, 21 Jan 2006, IISR, Calicut.**
9. **PLACROSYM VII held at Kochi during 5-10 Dec, 2006**
10. **SYMSAC III held at Lalbagh, Bangalore during 8-10 Nov, 2006**
11. **Two day Seminar cum Workshop on Capacity building programme for Indian Agricultural Research, extension, Development organization in Globalized Economy, sponsored by ICAR at NAARM Hyderabad on 29-30 April.**
12. **One day Sensitization Programme on Business Opportunities in Biotechnology on 19th January 2005 at Hotel Hyson Heritage, Kozhikode organized by Small Industries Service Institute (Ministry of SSI, Govt. of**
India) jointly with Indian Institute of Spices Research, (Govt. of India, Marikkunnu, Kozhikode) District Industries Centre Govt. of Kerala, Kozhikode.

Training Imparted to Post MSc, MSc and BSc students (from 2003)- 30 students guided in all
Phd Students- one.

Training Manual Edited

- B. Chempakam and Sheeja T.E., 2005. "Techniques in Biochemistry and Biotechnology" for Summer Training (May-June 2005) conducted for MSc students at IISR, Calicut

Training organized

1. Course Co-ordinator for the Summer Training of 30 days duration on "Techniques in Biochemistry and Biotechnology" organized for MSc. Students at the Institute during May-June, 2005

Recognised guide
- Acharya Nagarjuna University, Andhra Pradesh
- Kannur University, Kannur
- External Examinar, Kannur University, Kerala
Annexure II

Biodata of Co Investigator 1

Name : D. Prasath  
Designation : Senior Scientist  
Department/Institute/University : Indian Institute of Spices Research , Calicut  
Date of Birth : 30th May 1973  
Sex (M/F) : Male  
SC/ST : NA

**Education** (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>SL No</th>
<th>Name of the University/Place</th>
<th>Degree</th>
<th>Year</th>
<th>Division/Class</th>
<th>Subjects taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tamil Nadu Agricultural University, Tamil Nadu</td>
<td>M.Sc</td>
<td>1997</td>
<td>First</td>
<td>Horticulture  (Vegetable Breeding)</td>
</tr>
<tr>
<td>2</td>
<td>Ph.D</td>
<td>2005</td>
<td>First</td>
<td>Horticulture  (Vegetable Breeding)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>University of Guelph, Canada</td>
<td>Post doc.</td>
<td>2010</td>
<td>-</td>
<td>Applied genomics</td>
</tr>
</tbody>
</table>

**A. Position and Honors**

**Position and Employment** (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>SL No</th>
<th>Institution / Place</th>
<th>Position</th>
<th>From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indian Institute of Spices Research, Calicut</td>
<td>Senior Scientist</td>
<td>2007</td>
<td>Till date 2007</td>
</tr>
<tr>
<td>2</td>
<td>Indian Institute of Spices Research, Research Centre, Kodagu, Karnataka</td>
<td>Scientist Senior Scale Scientist</td>
<td>2005</td>
<td>1999</td>
</tr>
<tr>
<td>3</td>
<td>Indian Institute of Spices Research, Research Centre, Kodagu, Karnataka</td>
<td>Scientist</td>
<td>2005</td>
<td>1999</td>
</tr>
</tbody>
</table>

**Honors/Awards**

<table>
<thead>
<tr>
<th>Year</th>
<th>Award</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Gold medalist (Kunnam Ramamurthy Iyer Award), M.Sc., (Horticulture), Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu</td>
</tr>
<tr>
<td>1995-1997</td>
<td>TNAU Merit scholarship, M.Sc., (Horticulture), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu</td>
</tr>
<tr>
<td>1997 &amp; 1998</td>
<td>National Eligibility Test (NET) certificate holder ICAR, ASRB, New Delhi</td>
</tr>
</tbody>
</table>
2005 | Topper in PhD (Horticulture), Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu
---|---
2006 | Finalist, Young Scientists Award Programme, 93rd Indian Science Congress, 3-7, January, Hyderabad
2007 | Dr J S Pruthi Award, for the best research paper
2008-09 | BOYSCAST Fellowship, Department of Science and Technology, Government of India

### Professional Experience and Training relevant to the Project

<table>
<thead>
<tr>
<th>Training</th>
<th>Duration</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foundation course for Agricultural Research Service</td>
<td>June 2 to Sep 29 2000</td>
<td>National Academy of Agricultural Research Management, Hyderabad</td>
</tr>
<tr>
<td>Short-term training course on Micropropagation of Horticultural and Forestry species (ICAR-NATP)</td>
<td>Sep 3-23 2001</td>
<td>Regional Plant Resources Centre, Bhubaneswar, Orissa</td>
</tr>
<tr>
<td>Training on use of HORTIVAR: Horticulture Cultivars Performance Data base</td>
<td>Nov, 12 2002</td>
<td>FAO, Rome, Italy at Bangalore</td>
</tr>
<tr>
<td>BTIS training programme on Protein and DNA sequence Analysis</td>
<td>Mar 24-26 2004</td>
<td>Centre for Plant Molecular Biology (CPMB), TNAU, Coimbatore -3</td>
</tr>
<tr>
<td>ICAR winter school on Trade oriented exploitation of Horticulture in humid tropics: Opportunities and challenges</td>
<td>Dec, 1-21 2005</td>
<td>College of Agriculture, KAU, Vellayni, Thiruvananthapuram</td>
</tr>
<tr>
<td>Testing of plant varieties for DUS-Principles and procedures</td>
<td>March, 5-9 2007</td>
<td>IARI, New Delhi</td>
</tr>
<tr>
<td>Graduate course on Intellectual Property Management</td>
<td>May, 19-22 2007</td>
<td>Society for Technology Management, Goa</td>
</tr>
<tr>
<td>Advances in Biometrical Techniques</td>
<td>Feb, 8-28 2008</td>
<td>IASRI (ICAR), New Delhi</td>
</tr>
</tbody>
</table>
B. Publications (Numbers only) ..................

Patents : Nil    Others (Please specify) :    4*  Data Bases : - Symposia seminar articles: 24

*Promising genetic stock registered with NBPRG, New Delhi

Selected peer-reviewed publications (Ten best publications in chronological order)

<table>
<thead>
<tr>
<th>S. No</th>
<th>List of publications during last five years</th>
<th>*Impact factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Prasath D and V Ponnuswami (2008) Screening of chilli (Capsicum annuum L.) genotypes against Colletotrichum capsici and analysis of biochemical and enzymatic activities in inducing resistance. Indian Journal of Genetics and Plant Breeding 68(3): 344-346.</td>
<td>4.0</td>
</tr>
<tr>
<td>8</td>
<td>D Prasath, I El-Sharkawy, S Sherif and S Jayasankar (2010). Cloning and expression analysis of CaPR5 and ZoPR5, the main genes involved in defence response in Curcuma amada and Zingiber officinale (submitted to Planta).</td>
<td>9.0</td>
</tr>
</tbody>
</table>
**NAAS impact factor (out of 10)**

**List maximum of five recent publications relevant to the proposed area of work**
1. Prasath D, I El-Sharkawy, S Sherif and Jayasankar S (2010). Cloning and expression analysis of *CaPR5* and *ZoPR5*, the main genes involved in defence response in *Curcuma amada* and *Zingiber officinale* (submitted to *Planta*).

**C. Research Support**

**Ongoing Research Projects**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Project</th>
<th>Investigator status</th>
<th>Funding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gen. XIX (813): Conservation, characterisation, evaluation and improvement of <em>Zingiber</em> and <em>Curcuma</em> Spp (2007-2012)</td>
<td>Principal Investigator</td>
<td>ICAR</td>
</tr>
<tr>
<td>2</td>
<td>Gen. XV (813). Investigations on the reasons and solutions for the absence of seed set in ginger (<em>Zingiber officinale</em> Rosc.)</td>
<td>Co-Investigator</td>
<td>ICAR</td>
</tr>
<tr>
<td>3</td>
<td>DBT-CIB 4. Development of microsatellite markers, molecular characterization of small and large cardamom, identification of core collection and development of database of important genotypes</td>
<td>Co-Investigator</td>
<td>DBT</td>
</tr>
</tbody>
</table>

**Completed Research Projects** (State only major projects of last 3 years)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Project</th>
<th>Investigator status</th>
<th>Funding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gen. IX (813). Collection, conservation, cataloguing and evaluation of cardamom germplasm</td>
<td>Principal Investigator</td>
<td>ICAR</td>
</tr>
<tr>
<td></td>
<td>Project Description</td>
<td>Role</td>
<td>Organization</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>2</td>
<td>Gen. X (813). Breeding cardamom for high yield and resistance to <em>katte</em> disease</td>
<td>Principal Investigator</td>
<td>ICAR</td>
</tr>
<tr>
<td>3</td>
<td>Phy. VI (813). Characterization of drought tolerance in cardamom</td>
<td>Co-Investigator</td>
<td>ICAR</td>
</tr>
<tr>
<td>4</td>
<td>Gen. VI (813). Collection, conservation, cataloguing and evaluation of tree spices</td>
<td>Co-Investigator</td>
<td>ICAR</td>
</tr>
<tr>
<td>5</td>
<td>Collection, characterization, evaluation and maintenance of paprika and paprika alike chillies</td>
<td>Co-Investigator</td>
<td>ICAR</td>
</tr>
</tbody>
</table>

**Investigator**

Signature of

(D Prasath)

Place: Calicut

Date: 15.11.2010
Annexure III

Biodata of Co Investigator 2

Name: Dr B Sasikumar
Indian Institute of Spices Research
P.B. No. 1701, P.O Marikunnu
Calicut ï 673 012, Kerala
Fax: 0901 ï 0495 2731187
Phone: 0495 ï 2731410/2730294/2730906 (O)
0471 ï 2726432 (R) 9496178142.(Mob)
Grams: ☐Research ☐Calicut
E-Mail: bhaskaransasikumar@yahoo.com
☐sasikumarsooranadu@gmail.com

Born on January 21, 1956 at Sooranadu, Quilon (Dt.) Kerala, India

Education

Degree/ Specialisation/ Year/ University/ OGPA

- M.Sc (Plant breeding & Genetics) 1979, Guj.Agril. University, Anand, Gujarat 3.56/4.0
- Ph.D (Plant breeding & Genetics) 1983, Guj.Agril. University, Anand, Gujarat 3.92/4.0
- Post doctoral training in Plant Molecular Biology, School of Biotechnology, Madurai Kamaraj University, Madurai/Plant Biotechnology Institute, NRC, Canada

Short Courses Attended

3. Two days Residential Workshop on ΩForging Strategic Linkage between Local self Govt. and R&D Institutions.1-2 March,2005.State Planning Board. Trivandrum
7. Fourteen days training on cDNA cloning at CAS, Division of Biochemistry, IARI, New Delhi, September 1999.
8. Twenty one days training in PCR Amplification and Gene cloning at CAS, Division of Biochemistry, IARI, New Delhi, 29 March to 18 April, 2000.

Employment

<table>
<thead>
<tr>
<th>Year</th>
<th>Position and Institute/Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 June-2010 June</td>
<td>Spices Expert (ITEC Expert) National Agricultural Research Institute, Mon Repos, ECD, Guyana, S.America</td>
</tr>
<tr>
<td>2006-2008 May</td>
<td>Principal Scientist (Plant breeding). Division of Crop Improvement and Biotechnology, Indian Institute of Spices Research, P.O. Marikunnu, Calicut-673012, Kerala, India.</td>
</tr>
<tr>
<td>1998-2006</td>
<td>Sr. Scientist (Plant breeding) Division of Crop Improvement and Biotechnology, Indian Institute of Spices Research, (ICAR), P.O. Marikunnu, Calicut-12</td>
</tr>
<tr>
<td>1990-1998</td>
<td>Scientist Sr. Scale (Plant breeding) Division of Crop Improvement and Biotechnology, Indian Institute of Spices Research, P.O. Marikunnu, Calicut-12</td>
</tr>
<tr>
<td>1984-1985</td>
<td>Res. Asst./Jr. Scientist (Botany/Plant breeding) Division of Botany, Rubber Research Institute of India, Rubber Board, Kottayam -686 001, Kerala, India</td>
</tr>
</tbody>
</table>

Current Research

My research interest primarily relate to improvement of black pepper, ginger and turmeric through conventional and molecular approaches; collection, conservation and characterization of germplasm of ginger, turmeric and black pepper using morphological and molecular tools.

I have been associated with different Institute projects such as:
- Breeding black pepper for high yield, quality and resistance to pests (Institute project-Principal Investigator)
- Collection, conservation, cataloguing and evaluation of germplasm of ginger and turmeric (Institute project-Principal Investigator)
- Collection, conservation, cataloguing and evaluation of germplasm of black pepper (Institute project, Co-Principal Investigator)
- ISSR marker for black pepper improvement (Institute project, Co-Principal Investigator)
Major Research Grants as Principal Investigator or Co Principal Investigator

1. Development of microsatellite markers and characterisation of Curcuma. (PI, DBT, Rs. 85.95 lakhs)
2. Organization of ginger and turmeric germplasm based on molecular characterization (PI, ICAR Adhoc scheme. Rs. 14.41 lakhs)
3. Biochemical characterization of ginger and turmeric germplasm (Co-PI, ICAR Adhoc scheme, Rs. 8.4 lakhs)
4. Determination of purity of market samples of spices using PCR, protein profiling and HPLC techniques. (PI, DBT Rs. 26 lakhs)
5. Strengthening the cause of geographical Indication in spices (PI, ICAR Adhoc scheme Rs. 29.26 lakhs)
6. Immunoserological approaches to pathogen detection, defense protein and disease management in ginger and cardamom (Co-PI, DBT Rs. 28 lakhs)
7. Technology mission in black pepper (Co-PI, Govt. of Kerala Rs. 85 lakhs)

Awards/Honours/Rewards

i. ICAR Team Research Award 94-96 in Horticulture.
ii. J.S. Pruthy Award ’97 of Indian Society of Spices for the best research paper published in J. Spices and Aromatic Crops, Vol. 6, 1996
iii. Dept. of Atomic Energy (Govt. of India) Awarded Dr. K.S. Krishnan Memorial Fellowship for Ph.D Studies.
iv. Dept of Biotechnology (Govt. of India) awarded DBT National Associateship for post doctoral training in Biotechnology.
v. University of Calicut, Kerala, India has given recognition as an out campus Research Guide in Botany (Plant breeding, Genetics, Cytology and Biotechnology)
vi. Nagarjuna University, Guntur, Andhra Pradesh recognized as Ph.D guide in Biotechnology
vii. Mangalore University, Mangalore, Karnataka recognized as research guide in Biotechnology, Genetics and Plant breeding.
ix. Served as Judge, Young Scientist Award Contest in Biotechnology Kerala Science Congress, Kannur, 29-31 Jan.07.& 29-31 Jan.05, Trissur
x. Served as an invited expert in the Interactive Workshop on Management of ginger diseases and pests in the North Eastern and Himalayan Region. ICAR Res. Complex, Tadong, Gangtok, Sikkim, 4-5 Jan. 07.organised by DBT, New Delhi.
Important Assignments International

2. Visiting scientist, Plant Biotechnology Institute, National Research Council, Saskatoon, Canada (Aug. 1994 ñ Nov.1994)

Major Research Contribution

a) Conventional Breeding/Germplasm conservation

* Developed / released ginger varieties Varada, Mahima & Rejatha
* Developed / released turmeric varieties Prabha & Prathiba (first ever True turmeric seedlings derived turmeric varieties) besides IISR Alleppey Supreme and IISR Kedaram.
* Developed black pepper varieties IISR Thevm (tolerant to foot rot diseases) IISR Girimunda and IISR Malabar Excel
* Produced and characterized first ever inter specific hybrids in Piper
* Responsible for building up of the largest germplasm collection of ginger (653 accessions), turmeric (890 accessions) besides black pepper (3000 accessions).
* It is shown scientifically for the first time that black pepper is self pollinated (geitonogamy).
* Acc.657 (a turmeric accession with high yield and high curcumin) and Coll.1041 (a black pepper line tolerant to Phytophthora foot rot) are got registered with ICAR.
* Prepared draft DUS guidelines for turmeric, ginger and black pepper.

Publications

a) International


**b) Indian**


12. Lincy, A.K., Remashree, A.B. and Sasi


c). Review Articles


d) Chapters in Books


e) Books


