Biotechnology Laboratory for Conservation

Triannual Report

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Basudha

Bio-cultural Diversity, Equity, Freedom

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Foreword

Basudha began its journey in 1997 with the pledge to conserve the nation's heritage of biological diversity, and to protect this heritage from biopiracy. To comply with this pledge, a demonstration and conservation farm was founded in 2001, where hundreds of indigenous varieties of rice, and other crops were cultivated *in situ*, and seeds were distributed among indigenous farmers of 12 States of India. Basudha's farm is now the largest repository of folk crop germplasms, including 1320 rice varieties, and dozens of varieties of aubergine, cucurbits, maize and millets. Basudha is also engaged in documenting the agronomic and morphological characters of the folk crop varieties, every year, for copyrighting in the name of farmers, as a tool to preventing biopiracy.

Since its inception, Basudha has never received any institutional support. Instead, it continues to thrive on personal donations of its founder-trustees and great friends. Owing to the paucity of resources, most of Basudha's research was, of necessity, confined to field surveys and on-farm experiments, with occasional support from academic colleagues. A new phase of Basudha's journey, however, began in August 2014, when Mr. Avik Saha of Kolkata gifted me with an amazingly modern laboratory for conducting cutting edge biotechnology research. His unwavering enthusiasm, support and advocacy for Basudha's work have enlisted a small group of committed donors, who continue to drive our laboratory research to complement our field experiments and conservation work.

Basudha's Laboratory is the *country's – and perhaps the world's – only* laboratory that receives no funding from either the government or corporate sector for dedicated research work. Our research now encompasses identifying and analyzing various morphological and molecular characters of rare and endangered wild plants and crop varieties. In addition to documenting and publishing the value of biodiversity over the past decades, Basudha is conducting biochemical and molecular analyses of agrobiodiversity and endangered wild plants.

Right after the establishment of the Laboratory, Basudha has received generous offers of assistance and collaboration from diverse institutions of national and international repute. Collaborative research on multiple fields – from metal profiling to nutritional analysis and electron microscopy of rice. An outline of our research activities can be gleaned from this year's second Work Report.

This Report serves to indicate the use value as well as intrinsic value of our astounding heritage of biodiversity, and the legion of scientific questions pertaining to biodiversity that remains to be explored. We have addressed some of these questions, the joy of which we are glad to share with you.

Chair,

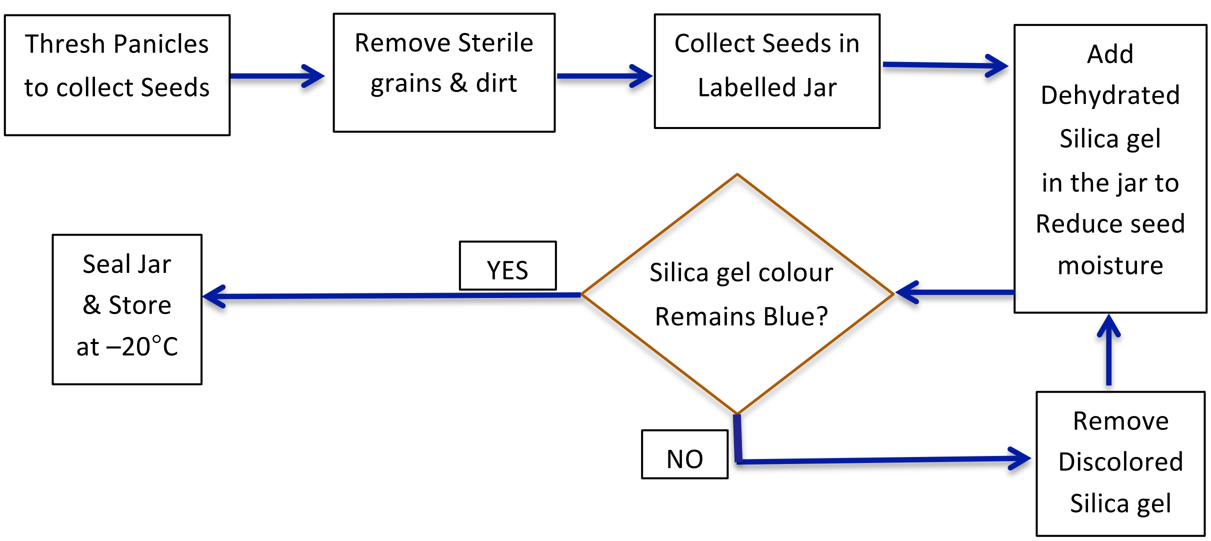
Basudha

1. Long-term Preservation of Rice Seeds

Rice seeds tend to lose capacity of germination over years of storage. Failure of germination entails the loss of a special farmer variety from farm fields. In this process, when a whole community of farmers stopped growing a variety for a few years, hundreds of rice varieties were lost. At present, Basudha is growing 1320 folk rice varieties on its farm for distribution of the seeds among indigenous farmers across 10 states. As an integral part of conservation, Basudha is also storing seeds of these folk varieties in the laboratory in preparation of the event of a failed harvest.

Appropriate drying and storage conditions are very important to maintain the viability and vigor of the seeds over the long term. Lowering of seed moisture content enhances the longevity of the seeds. In our laboratory, seed dehydration is achieved by use of silica gel before final storage at –20°C. Seeds of 11 varieties from 2012, 88 varieties from 2013, 403 from 2014 and 518 from 2015 harvest are maintained in the sub-zero seed bank in our laboratory.

The Protocol of Dehydration and Storage of Rice Seeds



2. Genetic Base of Fragrance in *Indica* Rice

Our research revealed that at least eleven fragrant rice varieties (out of 55 examined) do not contain *badh2.1* gene, known to be responsible for producing aroma in Basmati-like rice varieties. This confirms that many fragrant rice varieties of Indica group may contain some other mutation(s) in a different part of the *badh2* gene or in the promoter region, unlike Basmati and its derivatives. We employed Applied Biosystem's VERITI PCR Thermal Cycler for this work. After the publication of specific gene sequences of 84 rice varieties in NCBI, as mentioned in our previous (Jan-Apr, 2016) Report, our article was subsequently published in the international peer reviewed journal *Genetic Resources and Crop Evolution* in its August 2016 issue. A copy of our paper is available for free on request.

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SHORT COMMUNICATION

An analysis of variation of the aroma gene in rice (*Oryza sativa* L. subsp. *indica* Kato) landraces

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Abstract The candidature of 8-bp deletion in *badh2* gene as the predominant cause for aroma development in rice was investigated in 84 subsp. *indica* rice landraces. Presence of this functional mutation was detected in 80 % of aromatic samples and in three non-aromatic landraces which were found to be heterozygous at this locus. However, 11 landraces did not show its presence despite being aromatic during qualitative assessment. None of the wild ancestors possessed this deletion. Finally, we have discussed implications of our findings in the broader context of aroma evolution.

Keywords Aroma · *badh2* · *Indica* landraces · *Oryza* · Rice · 2-AP · 8-bp deletion

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Introduction

Aroma is one of the most acclaimed traits of rice, not only because of its culinary value, but also because of its cultural value assigning local and national identity. Scientific investigations over decades have revealed the biochemical basis of aroma, identified the gene responsible for the expression of aroma, and its polymorphism. The principal compound responsible for rice aroma production is 2-acetyl 1-pyrroline (2-AP) (Buttery et al. 1983). The candidate gene for the expression of aroma, *badh2*, located on chromosome 8, encodes betaine aldehyde deydrogenase homologue 2 (BADH2), the key enzyme regulating 2-AP production (Ahn et al. 1992). An eight base-pairs (8-bp) deletion in the exon 7 of this gene causes the loss of gene function through untimely truncation of the *badh2* transcript, thus accumulating 2-AP in aromatic landraces (Bradbury et al. 2005a).

This recessive allele of *badh2* carrying three single nucleotide polymorphisms (SNPs) and 8-bp deletion is known as *badh2.1* and is proclaimed to be the most abundant causal mutation occurring in aromatic rice samples. Apart from Basmati or Jasmine varieties, a large number of landraces from diverse geographic areas have been documented to have the 8-bp deletion in the *badh2* gene (Kovach et al. 2009). Recently, a series of studies have described other novel alleles responsible for aroma (Bourgis et al. 2008; Shi et al. 2008; Kovach et al. 2009; Shao et al. 2013). However, landraces with these mutations are confined to certain

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3. Tracing the History of Fragrant Rice Varieties from South and Southeast Asia

The evolutionary origin of cultivated rice *Oryza sativa* L. has long been a topic of debate for decades. It is generally accepted that the wild species *Oryza rufipogon* and *O. nivara* were the ancestors of all rice populations of the subcontinent. There are three major distinct groups of rice: Indica, Japonica and Aus.

Each of the three groups of rice – Indica, Japonica and Aus - have distinctive characteristics. For example, purple stems and leaves, and stickiness of cooked rice, and 0 – 20% amylose content, low palmitic acid and oleic acid content are characteristics of the Japonica group. Early maturity and photoperiod sensitivity are mostly found in Aus varieties of eastern India. Slender grain, 23-31% amylose content, and high contents of oleic acid and palmitic acid are features of the Indica group. Although Aus may be considered a subgroup of the Indica, recent studies reveal significant difference between the two groups at the molecular level.

Presently, Basudha has a collection of 167 fragrant rice folk varieties, from the Indica, Japonica and Aus groups, collected from 4 countries of South Asia and 3 countries of South East Asia. We intend to examine all these rice varieties with an aim to understand the origin of the fragrance in Asian rice. Specifically we are aiming to understand the following:

1. To evaluate the contribution of the wild ancestral species towards the origin of fragrance in rice.
2. To assess the level of genetic diversity and population structure of rice with fragrance and anthocyanin pigments in rice samples using molecular markers.
3. To classify the landraces from Basudha's accession using molecular markers.

4. Drought-Tolerance and Fragrance in Upland Rice

Objective: To investigate the relationship between drought tolerance and fragrance at the biochemical and molecular levels.

Work in progress: Two upland rice landraces DD01 and K04, are being grown in greenhouse. After transplanting, all rice plants rice were maintained under three different watering regime (a) standard (100% soil moisture), (b) Treatment 1 (moderately watered – 50% moisture) and (c) Treatment 2 (<20% moisture). We observed that some individuals of the Treatment 2 flowered earliest followed by Treatment 1 and the standard plants.

Replications of drought tolerant rice plants grown in Basudha's green house.

The rice plants have all produced panicles, and are now reaching maturity. Collection of tissues, and subsequent DNA and RNA extraction for analysis is afoot.

Task ahead: Two biochemical pathways in rice, involving the amino acid Proline, gamma-amino butyric acid (GABA), and Δ1-pyrroline-5-carboxylate (P5C), are involved both in adaptation to drought stress and in production of the fragrance molecule 2-amino 1-pyrroline (2AP). We intend to measure and monitor the synthesis of proline, GABA and P5C in the rice plant in different tissues at maturity of the rice plant, and determine if there is any trade-off between fragrance and the extent of drought adaptation. For this work, we shall use Applied Biosystem's (model 9902 VERITI) PCR Thermal Cycler and Jasco's V-730 BIO UV/ Visible spectrophotometer.

5. Rescue of a Rare Tree Now Extinct from West Bengal

A single mature specimen of *Vitex glabrata* was reported in 2001 by Dr. D Deb from a sacred grove of Bankura district. We attempted to germinate its seeds over a decade, until we resorted to tissue culture techniques in Basudha Laboratory in 2015. To our great grief, the tree was felled by local people in early April 2016. Dr. D Deb's survey over 10000 sq. km over the past 10 years has not recorded any other specimen in eastern India, the species is now extinct from West Bengal.

After 1.3 years of a series of trials and failures, we have now achieved success in germinating saplings of *Vitex glabrata*, and keeping them viable for a long time. This work is being conducted in two ways, in two laboratories.

(i) Germination from seeds: in our laboratory, we have grown in aseptic culture media and established two seedlings into artificial soil. One of these seedlings is now being exposed to open environment on normal soil, while the other was transferred to Prof. T. Bandyopadhyay's laboratory in Kalyani University.

(ii) Tissue culture from nodal tissues from the germinated seedlings: Murashige and Skoog media in combination with plant growth regulator like Auxin and Cytokinin, are used for this particular experiment. A germinated seedling of the plant has been transferred to the hardening unit, in the Green house of the Molecular Biology and Biotechnology Department of Kalyani University.

Task ahead: After establishing the new seedlings on normal soil, we will transplant them on Basudha farm in Odisha and distribute them to a willing farmer conservationist for nurturing them till maturity. In we intend to undertake hitherto unknown biochemical properties of the plant.

(A) *Transplanted seedling exposed to natural soil and light in Basudha Laboratory;* (B) *A nodal tissue in growth medium, showing rapid shoot growth in Kalyani University's MBBT culture room;* (C) V. glabrata saplings, *germinated from seeds (tube and cup) and embryonic tissue (jar), in Basudha Laboratory*

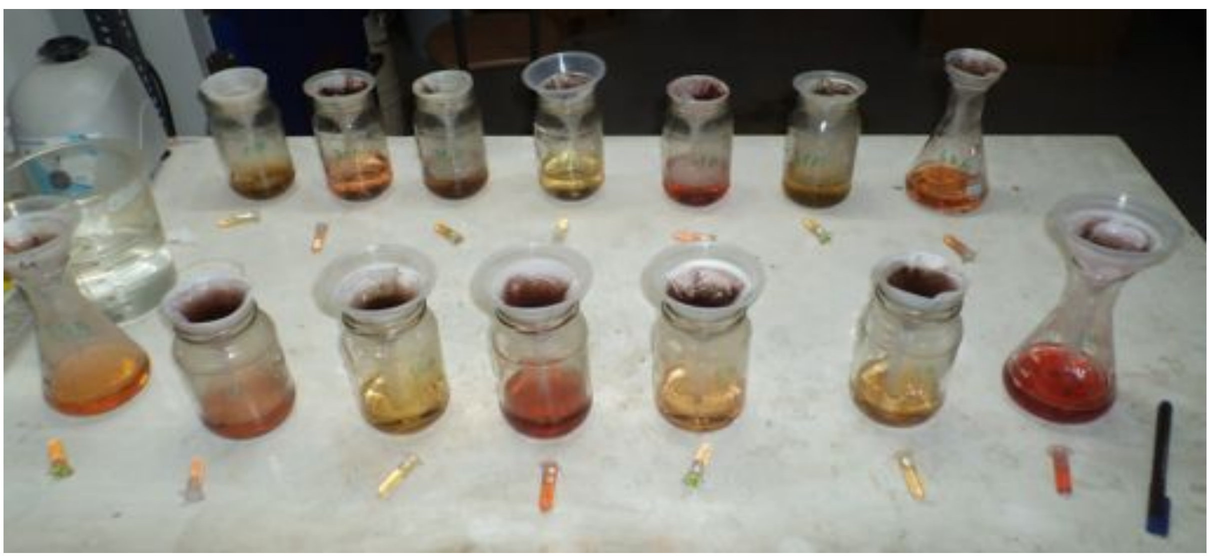
6. Estimation of Wood and Bark Lignin in 54 Tree Species

***Ongoing work*:** In continuation of our previous work, we have completed the quantitative estimation of both acid soluble and acid insoluble (Klason) lignin content of all the 54 species of forest trees from Odisha and West Bengal. Bark and wood of a few species (*e.g.* Kendu, Mahua) showed pigments that interfered with the lignin. We therefore refined our method and repeated the analyses, which improved accuracy.

For estimation of ash content, we worked in the laboratory of Prof. S. C. Santra of Environmental Science Department, Kalyani University.

***Findings*:** Excepting Sal (*Shorea robusta*), no trees from Indian deciduous forests have ever been analyzed for lignin content in the wood and bark. This work is generating the first report of both Klason and acid-soluble lignin contents in wood and bark of 54 trees from the dry deciduous forest of southwestern Bengal and mixed deciduous forest of southern Odisha.

***Implications*:** Lignin imparts strength and durability of wood, and is important in determining the quality of timber. Thus, lignin content can indicate the economic value of wood from different species, and the adaptive significance of diverse forest trees in the face of various natural hazardous. Lignin content is also determinant of the quality of biochar which can improve the agricultural crop productivity.



Separation of Acid Soluble Lignin and Insoluble (Klason) lignin.

Klason (KL) and Acid Soluble Lignin (ASL) Content (%) of Wood and Bark of Selected Trees.

Species name	শাল *Shorea robusta*		তেঁতুল *Tamarindus indica*		মহুয়া *Madhuca indica*		আসন *Terminalia tomentosa*		বেল *Aegle marmelos*		কেন্দু *Diospyros melanoylon*	
Lignin Type	KL	ASL	KL	ASL	KL	ASL	KL	ASL	KL	ASL	KL	ASL
Wood	16	0.60	19.5	0.7	20.5	1.1	17	0.4	12	0.3	26	1
Bark	21	0.80	32	0.8	24.5	0.6	23	0.8	19	0.5	19.5	0.62

7. Identification of Sitapatra, A Rare Tree

Objective: Taxonomic Identification and Molecular Barcoding of Sitapatra Using Five Signature Genes (*matK*, *rbcL*, *rps16*, *trnLF*) and Gene Associate (*ITS*).

A handful of specimens of a rare tree, locally known as Sitapatra (সীতাপত্র), occur in the natural forest tracts of Bankura and West Medinipur districts. The name of this tree is not mentioned in taxonomic literature. Although the tree has many characters similar to two related species of *Cordia*, a few unique traits seem to defy an exact taxonomic identification. We have therefore sought to identify the plant through molecular barcoding method.

Work done: For molecular bar coding, we selected a set of 5 signature genes *(matK*, *rbcL*, *rps16*, *trnLF*) and internal transcribed spacer (*ITS*) upon kind advice from Dr. Bo Li of Beijing. We completed PCR amplification and sequencing of the five genes from Sitapatra. Available literature and NCBI database are inadequate to identify the tree. We therefore sequenced the same five genes of both *Cordia myxa* (collected from West Bengal) and *Cordia macleodii* (collected from Odisha), which are very closely matching the Sitapatra specimen.

Editing of the sequence data of five genes from each plant species has been completed. They have also been prepared for submission to NCBI/ Genbank. Our initial analysis with the molecular sequences of the three plants (Sitapatra, *Cordia macleodii* and *Cordia myxa*), indicates that Sitapatra is a subpopulation of *Cordia macleodii*. We have used Applied Biosystem's (model 9902 VERITI) PCR Thermal Cycler for this work.

Task ahead: In view of a few morphological differences between Sitapatra and *C. macleodii*, we intend to continue our analysis at a greater resolution, and also elucidate the evolutionary relationships among the three plants.

Barcode generated with intron sequence of trnL & trnF gene

Barcode generated with partial sequence of rbcL gene

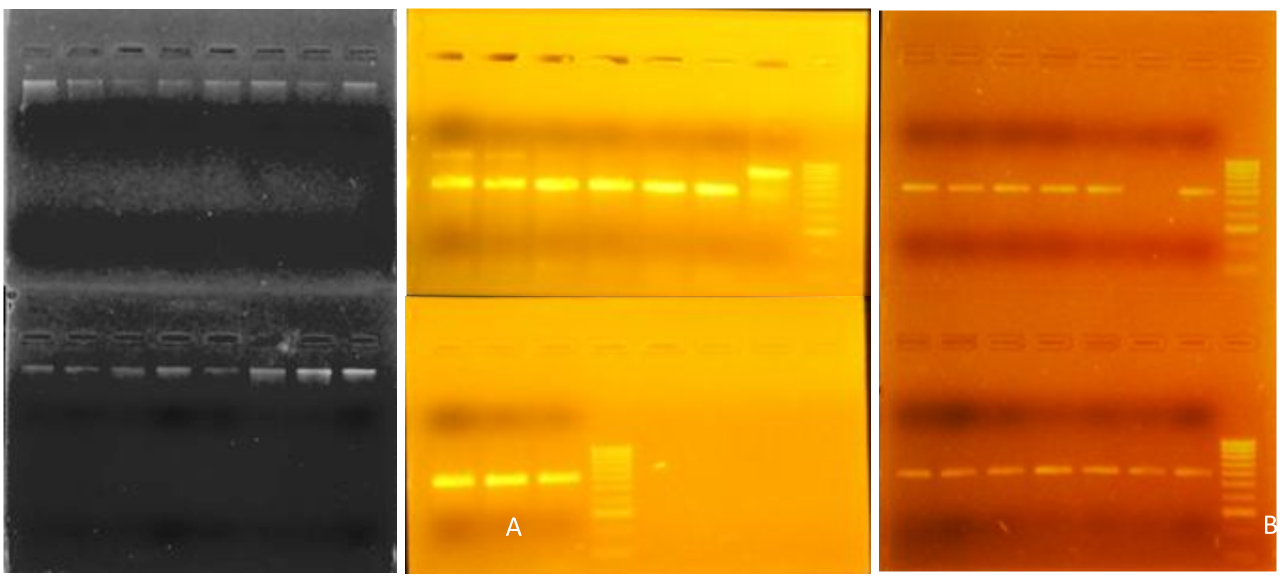
Barcode generated with intron sequence of rps gene

8. Molecular Phylogeny of 50 Moss Genera from Eastern Himalaya

Objective: To elucidate the phylogenetic relationship among approximately 50 moss genera (under bryophytes), from the Eastern Himalayan Biodiversity Zone (a Biodiversity Hotspot), on the basis of the plastidial *rbcL* gene, mitochondrial *nad5* gene and nuclear genes.

This study is being conducted in our laboratory, by Ms Anashuya Biswas-Raha, under joint supervision of Dr. Mousumi Poddar-Sarkar of Calcutta University and Dr. Avik Ray of Basudha.

Ongoing work: Genomic DNA of the mosses have been isolated, PCR amplification has been standardized for selected primers of *rbcL* genes and checked for desired bands. We are using Applied Biosystem (model VERITI) PCR Thermal Cycler for this work.



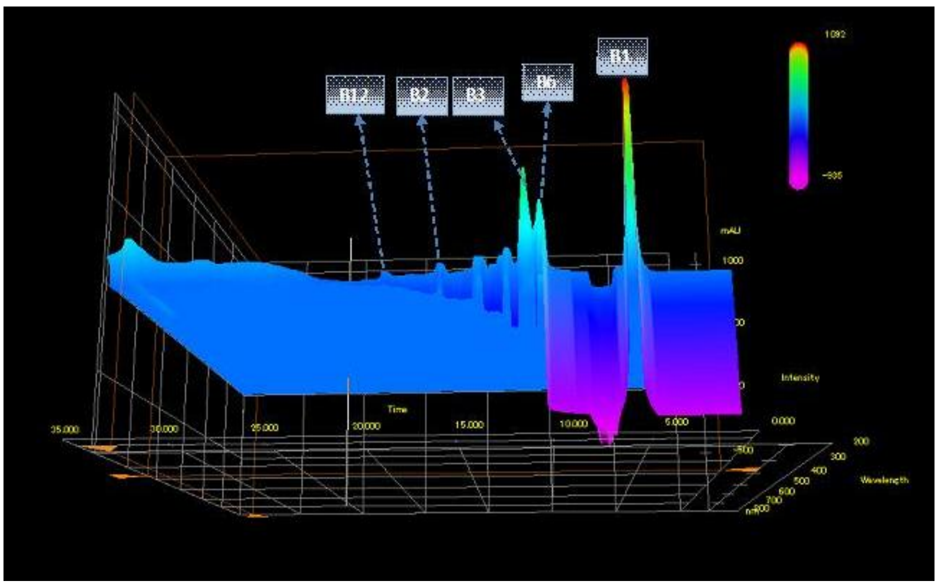
Gel electrophoresis of moss DNA showing (A) bands for gDNA concentration; (B) standardisation of RT PCR amplification of rbcL at different temperatures; (C) bands for rbcL amplified for sequencing.

Task ahead: Sequencing of PCR products, followed by subsequent editing and alignment of the sequences in order to construct a phylogenetic tree of the moss species under study.

9. Vitamin B Content of Rice Landraces: Phase II

Objective: Exploration of nutritional value of folk rice varieties. This work will constitute the first authentic analysis of water soluble vitamins in folk rice varieties.

Ongoing work: As mentioned in the previous Report, we quantified five B vitamins in 28 rice varieties at IIT-Madras. Phase II of this work, with at least 50 more landraces, began from August 17 in Prof. Pradeep's laboratory at IIT-Madras. We are following a cutting-edge quantification method developed by Dr. Mousumi Poddar-Sarkar of the University of Calcutta, and using Shimadzu (model Prominence DGU) HPLC system (with CTO-20A Column Oven, and the SPD-M20A Diode array detector).



HPLC chromatogram of B vitamins in Nagaland Habeh rice (Code N20)

B-Vitamin Concentration (mg/gm) in Selected Folk Rice Varieties

Variety code	**B1**	**B2**	**B3**	**B6**	**B12**
B86	3.46	BDL	22.06	3.86	0.07
DD11	4.26	BDL	20.9	4.41	0.03
DD30	0.98	0.06	0.05	0.02	0.09
K38	4.98	0.18	25.01	6.45	1.39
M77	3.49	0.04	7.79	0.08	0.04
N20	4.61	0.15	5.61	5.72	BDL
S35	4.57	BDL	22.28	6.23	0.06
SH12	5	0.03	24.72	6.24	0.1
T05	4.78	0.01	3.91	0.05	0.07
Z16B	3.24	0.03	21.76	18.12	0.81

10. Heavy Metal Profiling of Rice Varieties: Phase III

Objective: Heavy Metal Profiling of additional 500 Folk Rice Varieties, in continuation of Phase I and II work.

Ongoing work: In continuation with our previous screening of 130 rice varieties (already published in *Current Science* Aug 16 2015 issue), we completed a second phase of metal profiling of another 315 varieties. A manuscript containing the result of this work has been submitted to an international journal.

In the Phase III, analysis of 200 additional varieties will begin from August 17, 2016 in the Nanoscience lab, IIT-Madras. This study is being conducted in the Department of Chemistry, IIT-Madras, under supervision of Prof. T Pradeep. In this work, we are using Perkin Elmer (model NexION 300X) Inductively Coupled Plasma Mass Spectrometer.

Metal Concentrations (mg/kg) in Grains of Selected Folk Rice Varieties.

Variety code	Cr	Mn	Cu	Zn	Ag	Fe
R41	23.5	25.6	2.8	16.5	0.003	92.7
Q09	29	48	2.7	20	0.003	135
H25	76.5	67	5	135	0.14	416
S17	42.3	72.7	2.8	29	0.006	92
TT15	40	60	9	53	0.05	124
K123	24.9	35	2.1	18	0.008	62
J16	31.4	64.1	2.5	27.4	0.01	91
M24	14.44	17.64	0.9	17.6	BDL	40.84
B27	20.65	37.04	1	13.07	0.01	61.03
B37	16.14	54.92	2.33	23.5	0.01	49.63
L03	39.89	59.29	1.49	40.74	0.01	123.32
K82	50.51	85.92	5.28	39.13	1.64	132.32
B91	69.97	106.78	5.53	37.38	0.4	243.77
B32	19.13	31.77	2.48	18.42	0.006	56.00
P03	23.87	44.01	1.98	28.65	0.01	70.80
B27	20.65	37.04	1	13.07	0.01	61.03
B37	16.14	54.92	2.33	23.5	0.01	49.63

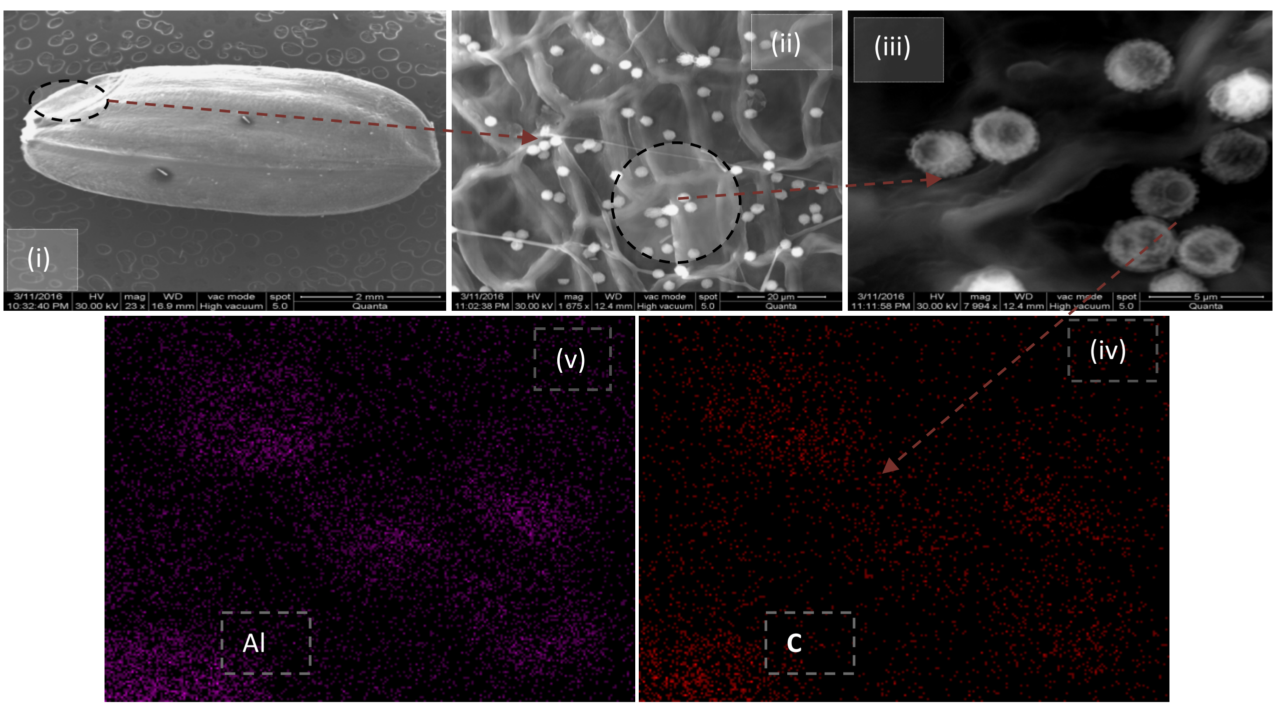
Task ahead: After the metal analyses of a total of 700 varieties, we intend to statistically examine the association of metal content with morphological traits, to understand the physiological consequences of specific heavy metal uptake in rice.

11. Morpho-Structural Characterizaion of Rice Grain : Surface Imaging and Elemental Mapping Phase II

Objective: Identification of rice grain surface pattern using Scanning Electron Microscopy (SEM), to find correlation with other morphological and agronomic traits.

Ongoing work: In collaboration with Dr. Shubhra Mukherjee of Barasat Govt. College, surface pattern imaging of 60 rice varieties was already accomplished (reported in previous Report). In addition, SEM imaging of another 22 varieties has been completed at IIT Madras in Prof. T. Pradeep's lab. Furthermore, and SEM-EDAX and mapping of elemental depositions on rice aleurone surface of 20 more varieties are being conducted.

Implications: The relationship of seed surface topology with other morphological and agronomic properties of rice varieties is yet unknown. It seems plausible that diverse patterns of the surface topography are indicative of drought or salt tolerance, specific pest resistance, or the size and anthocyanin contents of leaves and grains. Our study aims to explore such relationships, if any, and find the evolutionary and biomolecular significance of such relationships.



SEM images of a grain of rice variety E01:(i) *Scale- 2 µm, Mag. 23X;* (ii) *Scale- 20 µm, Mag. 1675X;* (iii) *Scale- 5 µm, Mag. 7994X. Elemental mapping of the variety on the* (iii) *position, showing (iv) carbon (C) deposition and (v) aluminium (Al) deposition on the aleurone surface.*

12. Volatiles and Fatty Acids in Rice

After a previous study of the contents of fatty acids in 25 rice landraces, we have proceeded further to assess the volatiles (including the fragrance molecule 2AP) in 13 landraces. This work is being conducted in collaboration with Sri Sandipan Ray in Dr. Mousumi Poddar-Sarkar's laboratory in the Botany Department of the University of Calcutta.

Using headspace solid phase microextraction gas chromatography, we detected the presence of 6-methyl-5-hepten-2-one, a C8 ketone having lemon grass flavour, the distinctive apecranoid, produced by oxidative cleavage of catotenoid by CCD in some varieties. For analyzing the fatty acid methyl ester (FAME), we used GCMS (Agilent Technologies, 7890A GC system with 5975C triple axis detector MS) attached with HP5-MS. Subsequently, FAME was quantified by using GC-flame ionizing detector (FID) attached with 15mm x 0.25mm x 0.25μm Factorfour™ capillary column.

We detected a significant amount of **omega-3 fatty acids** in a large number of folk rice varieties, in contrast with none in the modern high-yield rice varieties. Omega-3 fatty acids are known to prevent breast cancer, suppress inflammation in patients with rheumatoid arthritis and have ameliorative effect on asthma.

We have also identified some fatty acid-derived C6 'green leaf volatiles' as the end product of LOX-mediated reactions. These are: 1-hexanal, 1-butanol, 2-hepatone, furan 2-pentyl, 2,4-nonadienal, cyclohexanol, 1-methyl- 4-(1- methylethyl), 2-furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl, Pterin-6- carboxylic acid, 12,15-Octadecadiynoic acid, methyl ester, longifolene, isophorone, L-α-Terpineol, naphthalene etc.

The results of this study has been accepted and will be presented in the Conference "Shaping the Future of Food Quality, Health and Safety" held in Amsterdam, the Netherlands (30 October – 1 November 2016).

In addition, we are assessing the aromatic volatile compound 2-acetyl 1-pyrroline (2 AP) in 165 fragrant rice varieties from Basudha's accession. The work of quantitative estimation of 2AP has begun in Prof. T Pradeep's Nanoscience laboratory at IIT-Madras from 17 August. We are using Perkin Elmer's Gas Chromatography (model Clarus 680) and Mass Spectrometer (model Clarus 600 C) with auto-sampler for this work.

13. Total Flavonoids in Rice

Objective: Exploration of metabolic pathways in rice plant.

Flavonoids, and their *in vivo* metabolites, may exert modulatory actions in cells through actions at protein kinase and lipid kinase signalling pathways. Flavonoids, and more recently their metabolites, have been reported to act at phosphoinositide 3-kinase (PI 3-kinase), Akt/protein kinase B (Akt/PKB), tyrosine kinases, protein kinase C (PKC), and mitogen activated protein kinase (MAP kinase) signalling cascades. Inhibitory or stimulatory actions at these pathways are likely to affect cellular function profoundly by altering the phosphorylation state of target molecules and by modulating gene expression.

Ongoing work: We began an assay of total flavonoids in 47 landraces, and quantified the various flavonoids, including rutine, quercetine, myricetine. This study indicates that a greater amount of flavonoids in landraces with red, purple and black decorticated grains contain more flavonoids –than those with white and ivory coloured grains. This work is being conducted by Sandipan Ray, under supervision of Dr. Mousumi Poddar-Sarkar in the Department of Botany, University of Calcutta. High pressure liquid chromatography, using Agilent (model 1260 Infinity) HPLC system, and spectrophotometry using Jasco (model V-630) spectrophotometer were employed to quantify the flavonoids and related compounds.

Task ahead: We aim to assess total flavonoids of 1000 rice landraces from Basudha's accession and examination of the association of flavonoids with micronutrients in rice.

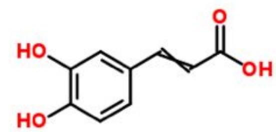
Estimation of flavonoids and phenolic compounds in rice grains using HPLC.

14. Total Phenol and Antioxidant Activity in Rice Landraces

Objective: Identification of medicinal rice varieties based on their nutraceutical properties.

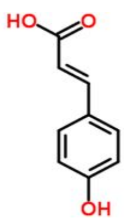
Phenolic antioxidants are found only in plants. These antioxidants carry out their protective work by suppressing the oxidation needed for cells to die and for tumours to grow and spread. A principal benefit of these antioxidants is that they prevent swelling and tissue inflammation, which is the source of most pain and infection. At the cellular level, antioxidants guard against DNA damage and cell deterioration.

Consumption of foods with antioxidants is reported to reduce risks of cardiovascular disease, cataracts, cancer, and a range of neurological diseases including Alzheimer's disease. Alternative medical practitioners recommend phenolic antioxidants for diabetes.



4-Coumaric acid

Ongoing work: After a previous study of anti-oxidant activity (AOA) and total phenol contents in 25 rice landraces at Dr. Mousumi Poddar-Sarkar's laboratory in the University of Calcutta, we have proceeded further to analyse another 47 landraces. The work is continuing with more landraces, and is being conducted by Sandipan Ray, under supervision of Dr. Mousumi Poddar-Sarkar of the University of Calcutta. Phenolic compounds tend to be in high concentrations in coloured grains (brown, purple, black).



Caffeic acid

For detection and quantification of phenolic compounds, we used a Jasco (model V-630) spectrophotometer. Phenolic compounds from a large number of rice landraces with colored grains (with high anthocyanin in the bran) include: *trans-p*-coumaric acid, 4-hydroxybenzoic acid, and caffeic acid, all of which are known to have antioxidant activity.

Total Flavonoids, Phenolics and Anti-Oxidant Activity Profiles of Selected Rice varieties

Rice Variety Code	Decorticated Grain Colour	Total Flavonoids (µg quercetin eqvt/ 100 g)	Total Phenolic Compounds (µg gallic acid eqvt/ 100 g)	Anti-Oxidant Activity (mg/ml)
A07	Light Brown	13.33	22.94	43.96
B09	Brown	148.75	81.87	4.08
B12	White	20.67	43.69	37.09
H24	Black	299.58	190.00	3.19
K81	Purple	182.08	220.00	2.19
TH01	White	16.83	29.87	59.74